Results: There were five males and four females; mean age = 63 years, range 39-87 years. The patients presented with abdominal pain, weight loss, and jaundice for an average of 4.3 months. Seven tumors were located in the head and two in the tail; the average size of tumor was 4.7cm (1.0–12.5 cm). The histology of the nine primary pancreatic leiomyosarcomas (7 spindle and 2 epithelioid) was identical to leiomyosarcomas of other sites. An immunohistochemical battery including smooth muscle actin, desmin, CD117 and CD34 confirmed the diagnosis. Pancreatico-duodenectomy was performed in four cases; three patients had palliative procedures and two had biopsies only. No lymph node metastasis was identified in the four resected tumors, but liver metastases were present in 4/9 patients. All nine patients died and five deaths were known to be disease-related. The overall mean survival was 31 months (ranging from 5-98 months).

Conclusions: Primary pancreatic leiomyosarcoma is the most common primary pancreatic sarcoma, though primary pancreatic sarcomas were exceedingly rare overall. Pancreatic leiomyosarcoma occurs in older adults with no gender preference. It is often located in pancreatic head and more likely to metastasize to liver but not regional lymph nodes. The prognosis is poor with a mean survival of 31 months.

Table1. 54 primary pancreatic sarcomas at Mayo Clinic (1994-2006)

Diagnosis	Number of cases
Leiomyosarcoma	22 (40.7%)
Gastrointestinal stromal tumor	14 (25.9%)
Undifferentiated high grade pleomorphic sarcoma	7 (12.5%)
Spindle cell sarcoma, NOS	7 (12.5%)
Liposarcoma	2 (3.7%)
Malignant peripheral nerve sheath tumor	1 (1.9%)
Ewing sarcoma	1 (1.9%)
Total	54 (100%)

648 Calretinin Is a Valid Marker To Assist Diagnosis of Hirschsprung Disease (HD) in Rectal Suction Biopsies without Use of Acetylcholinesterase (Ach) Staining

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Background: Varying success has been reported for the identification of HD using Ach enzymatic stains. Also frozen tissue for such stains causes artifact on permanent sections and sacrifices limited biopsy tissue. A recent study, using 10 full thickness of colon with HD, reported positive staining of calretinin in ganglion cells and their nerve trunks in normally innervated colon, but negative staining in hypertrophic nerve trunks (HNT) in HD segmments (J Clin Pathol 2004;57:712-716). The current study was to test validity of calretinin in assisting diagnosis of rectal suction biopsies and to compare it with neural specifc enolase (NSE) and vasoactive intestinal peptide (VIP).

Design: Over past 9 months, 22 pediatric patients (2 weeks old to 5 years old) underwent rectal suction biopsies. In each patient, there were one to three biopsies labeled "3 to 5 cm". Each biopsy was serially sectioned into 6 levels with H&E staining for light microscopy. The levels with either ganglion cells (16 controls) or HNT (6 HD cases) were destained and immunohistochemically re-stained for calretinin, NSE and VIP. Full thickness sections of pull-through colonic specimens of 6 patients, with biopsy diagnoses of HD, were also stained using the 3 markers.

Results: All 16 control cases showed adequate biopsies with ganglion cells (including immature ganglion cells in neural units) in at least one biopsy; two out of 16 cases (2/16) had one biopsy at 3 cm lacking both ganglion cells and hypertrophic nerve trunks (possibly from distally physiological hypo-ganglion zone), but ganglion cells were found at 5 cm in the two cases. Cytoplasmic and nuclear stains of calretinin were positive in all ganglion cells (16/16 controls) but negative in HNT (6/6 HD). This staining pattern was confirmed in all 6 pull-through colonic specimens (6/6). In addition, there was positive calretinin staining in mucosal nerve branches of 15/16 controls but in none of HD cases (biopsies or surgical specimens). NSE and VIP stains were positive in both ganglion cells and HNT.

Conclusions: We confirmed the role of calretinin in HD (as cited) and found that the mucosal calretinin expression was correlated with the presence of ganglion cells. Using this method, we propose to have 3 categories of adequate rectal suction biopsies: No HD (ganglion cells present), HD (no ganglion cells but HNT negative for calretinin) and non-diagnostic (no ganglion cells or HNT).

649 Expression of Cell Cycle Proteins p53, p21, p27, p14 and p16 and Proliferation Index Ki67 in Gastric Carcinomas

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Background: Gastric carcinomas (GC) are devastating and aggressive human tumors and molecular pathogenesis has been under intense investigation as a part of the effort to develop more effective therapeutic strategies for these tumors. This study investigates the role of the expression of 5 essential cell cycle molecules (p53, p21, p27, p14 and p16) and proliferating index (Ki67) as prognostic indicators in GC.

Design: The study included 116 gastrectomy specimens obtained from 116 patients with gastric cancer. Seven tumors were TNM-stage I, 35 II, 56 III, and 18 IV, whereas 92 tumors were low-grade (grade I and II intestinal type adenocarcinomas) and 18 high-grade (grade III intestinal type, and diffuse type adenocarcinomas). Formalin-fixed, paraffin-embedded 4µm sections were subjected to immunohistochemistry (streptavidin-biotin peroxidase) using monoclonal and polyclonal antibodies for p53, p21, p27, p14, p16, and Ki67. Results were expressed as % of positive cells. Mean follow-up time was 45.3 months (range 3.5-140 months).

Results: P53, p14, p16, p21 and p27 were detected in: 56%(65/116), 70%(81/116), 65%(75/116), 48%(56/116), and 82%(72/116) of the cases, respectively. Mean index for Ki67 was 25.34±7.3. High grade tumors exhibited higher indices for Ki67 (p=0.004), p53 (p=0.017) and p21 (p=0.03) compared to low grade tumors, whereas p14 and p16 were more frequently present in low grade tumors (p=0.001 and p=0.025 respectively). Ki67, p53 and p21 were more frequently expressed in advanced stage tumors (p=0.002, p=0.014 and 0.0012 respectively). Lower disease-free survival was correlated with **a)** high grade (p=0.0007), **b)** advanced stage (p=0.0008), **c)** higher Ki67 index (p=0.0075). p53 expression was associated with lower disease-free survival only in high grade p21(+) tumors (p=0.021). Spearman rank analysis revealed direct correlation between p21 and p27 (r=0.31562, p=0.00927) and p53 and p21 (r=0.5516, p=1.3079E-6). Finally, Cox regression analysis revealed that tumor grade, stage, Ki67 and p53 index were independent prognostic factors (CI: 0.032-0.502, p=0.03, CI:1.167-5.408, p=0.019, CI: 1.006-1.057, p=0.016, CI:1.000-1.0044, p=0.028).

Conclusions: The study shows that in cases of gastric carcinomas, tumor grade and stage and Ki67 and p53 indices are independent predictors of the outcome of the patients. p53 was associated with poor prognosis only in tumors overexpressing p21; that means high levels of p21 in tumor cells associated with aberrant p53 protein expression, may result in tumor recurrence.

Genitourinary

650 Hypermethylation of Tumor Suppressor Gene CpG Islands in Small Cell Carcinoma of the Urinary Bladder

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Background: Small cell carcinoma of the urinary bladder (SCBC) is a rare tumor which shows a common clonal origin with urothelial carcinoma. It bears a high metastatic potential, even when discovered in a localized state. Identifying the molecular underpinnings of this disease may elucidate useful clinical information regarding prevention, diagnosis, prognosis, treatment, and surveillance. DNA methylation is widely recognized as having a pivotal role in the process of carcinogenesis, but has not been explored in this pathology.

Design: We used quantitative methylation-specific PCR (qMSP) to analyze the DNA methylation status of four frequently hypermethylated tumor suppressors in small cell and transitional cell carcinoma (TCC) arising concomitantly in thirteen patients.

Results: We identify frequent methylation of *RASSF1* and *MGMT* and infrequent methylation of *MLH1* and *DAPK1* in cases of concomitant TCC and SCBC. Similar rates of methylation were found in pure and concomitant histopathologies with the exception of *MGMT*, which was much less frequently methylated in pure TCC.

Conclusions: These findings suggest that small cell bladder carcinoma and transitional cell carcinoma have common origins, establish DNA methylation of some tumor suppressors as frequent occurrences in both histopathologies, and suggest that *MGMT* methylation may be a SCBC-specific epimutation.

651 Germ Cell Origin of Testicular Carcinoid Tumors

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Background: Carcinoids are neuroendocrine tumors and most frequently occur within tissues derived from the embryonic gut. These tumors can occur in any organ site, but are rare in the testis. The cell type giving rise to testicular carcinoid is unknown. We hypothesized that testicular carcinoid may have a germ cell origin.

Design: We analyzed protein and genetic markers in four testicular carcinoid tumors using immunohistochemistry and fluorescence *in situ* hybridization methods.

Results: All four cases of testicular carcinoid tumor arose in a background of mature teratoma. Isochromosome 12p was identified in carcinoid tumor cells in all four samples. 12p overrepresentation was also observed in 3 cases. Isochromosome 12p and 12p overrepresentation were present in cells of co-existing mature teratoma in 3 cases. Carcinoid tumors showed strong immunoreactivity for synaptophysin and chromogranin; but no immunoreactivity for OCT4, CD30, c-kit, TTF-1, and CDX2. Membranous and cytoplasmic staining for beta-catenin was detected in 3 cases.

Conclusions: We found that the classic genetic alterations that characterize germ cell tumors, 12p isochromosomy and overrepresentation, are also demonstrable in testicular carcinoid tumors. These tumors showed uniform immunohistochemical expression of neuroendocrine markers, but lacked expression of CD30, OCT4, CDX2, TTF-1, and c-kit. Our findings suggest that testicular carcinoid is a phenotypic expression of teratoma and is of the same germ cell origin, rather than being derived from Leydig cells, as suggested by others.

652 Immunohistochemical Profile of Primary Urethral Carcinomas: Analysis of 60 Cases of Different Tumor Types

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Background: Primary carcinomas of the urethra are distinctly rare tumors and account for <1% of urinary tract malignancies. Because of the rarity of these tumors, their immunohistochemical profile has not been fully described. Goal of our study is to define the immunohistochemical profile of 60 cases of primary urethral carcinoma.

Design: 60 cases of primary urethral carcinoma (24 squamous cell carcinomas [SCC], 6 urothelial carcinomas [UC], 8 adenocarcinomas, 10 mixed carcinomas [MCa], 11 carcinoma not otherwise specified [CaNOS] and 1 lymphoepithelioma-like carcinoma) were evaluated. Secondary involvement of the urethra was clinically excluded. Formalin-fixed, paraffin-embedded tissue sections were used for immunohistochemistry. All

60 tumors were stained with the following antibodies: cytokeratin (CK) 7, CK 20, thrombomodulin, p63, CK 5/6 and CDX2.

Results: Results of the immunohistochemical stains are listed in table 1 below.

Table 1: Results of Immunohistochemical Stains

	CK7 (N positive/ N total)	CK20 (N positive/ N total)	CK5/6 (N positive/ N total)	p63 (N positive/ N total)	CDX2 (N positive/ N total)	Thrombomodulin (N positive/ N total)
Squamous carcinoma	14/24	0/24	22/24	22/24	0/24	21/24
Urothelial carcinoma	5/6	1/6	4/6	5/6	1/6	6/6
Adeno-carcinoma	8/8	6/8	0/8	0/8	5/8	2/8
Mixed carcinoma	7/10	2/10	9/10	8/10	1/10	8/10
Carcinoma NOS	8/11	1/11	10/11	7/11	1/11	8/11
LELC	0/1	0/1	1/1	1/1	0/1	1/1

The overall profiles of the different tumor types are listed in table 2.

Table 2: Immunohistochemical Profile

Squamous carcinoma	CK7, CK5/6, p63, Thrombomodulin positive. CK20, CDX2 negative
Urothelial carcinoma	CK7, CK5/6, p63, Thrombomodulin positive. CK20, CDX2 negative
Adeno-carcinoma	CK7, CK20, CDX2 positive. CK5/6, p63 negative. Thrombomodulin variable.
Mixed carcinoma	CK7, CK5/6, p63, Thrombomodulin positive. CK20, CDX2 negative
Carcinoma NOS	CK7, CK5/6, p63, Thrombomodulin positive. CK20, CDX2 negative

Conclusions: SCC, UC, MCa and CaNOS have a similar immunohistochemical profile (CK7, CK5/6, p63, Thrombomodulin positive. CK20, CDX2 negative), suggesting origin from a common progenitor cell. This profile makes it difficult to use immunohistochemical stains to reliably differentiate between these tumor types. Urethral adenocarcinomas have a distinctly different profile (CK7, CK20, CDX2 positive. CK5/6, p63 negative. Thrombomodulin variable), which is closer to that of tumors arising from the gastrointestinal tract.

653 Artifactual Mutations in FFPE Tissue Samples: The Case of TP53, EGFR and KLF6 in Prostate Cancer

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Background: Fresh tissue samples provide quality DNA or RNA for molecular studies in cancer. In some organs, such as the prostate, it is often difficult to freshly identify tumor foci, particularly in small, multifocal tumors, and tumor cells are often admixed with non-neoplastic epithelial cells and stromal tissues. Most studies on prostate cancer (PCa) are based on formalin-fixed, paraffin-embedded tissues (FFPE) and show a wide range of mutation rates for a given gene. Thus TP53 has been reported to harbor mutations in 3 to 42% of cases, and KLF6, in 0% to 55%. We assessed the mutation rate of TP53, KLF6, and EGFR with a double amplification strategy aimed to detect artifactual mutations, to investigate whether these discrepancies could be due to differences in the tumors included in each study or to methodological issues.

Design: 104 PCa samples were included (78 prostatectomies, 16 autopsies [latent PCa], plus 8 bone and 2 lymph node metastases). DNA was extracted from manually dissected areas in FFPE sections, using Dneasy Tissue Kit. Exons 1-4 from KLF6, 4-9 from TP53 and 18-21 from EGFR were amplified. Mutation analysis was performed by direct sequencing (ABIPRISM 377, Perkin-Elmer Applied Biosystems). Each PCR product was sequenced in both the forward and the reverse direction. When a change was detected in the first analysis, a second amplification and sequencing of the same DNA sample was performed. To check the quality of DNA in some tumors, particularly autopsy cases, amplification of 100 pb, to 600 pb fragments of the actin gene was performed.

Results: For KLF6, only one change of a total of 38 (3%) was confirmed by a second amplification, the remaining 37 were fixation-induced artifacts. For TP53, only 5 (19%) of 27 changes were confirmed as mutations. For EGFR, only 10 (18%) of 45 potential mutations were confirmed in a second amplification.

Conclusions: In FFPE tissues, most of the sequence changes are artifacts produced by Taq-polymerase, as this enzyme tends to insert nucleotides in the processing-induced DNA breaches. This explains many of the discrepancies found in the literature. It is mandatory to confirm all sequence changes with a second amplification, particularly when using FFPE tissue. Supported by FIS 06/1411, FIS 965190005 and Spanish Association Against Cancer.

654 Analysis of Microsatellite Instability in Urothelial Carcinomas of the Renal Pelvis and Ureter Based on Current Screening Practices: A Tissue Microarray Study of 106 Cases

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Background: Current screening practices for microsatellite instability (MSI) in colorectal carcinoma are based on the pattern of expression of four mismatch repair proteins: MSH6, MSH2, PMS2, and MLH1. While some urothelial carcinomas of the renal pelvis and ureter (UCRPU) have been associated with MSI, conflicting reports exist as to the proportion of cases in which this association is observed, ranging from 15 to 30%. We decided to study a large cohort of UCRPU to determine the proportion of cases with evidence of MSI.

Design: 106 UCRPU diagnosed over a period of 37 years were used to build a tissue microarray of 2.0 mm cores. Resulting sections were immunostained for MSH6, MSH2, PMS2 and MLH1. Cases were scored as negative (loss of expression) or positive (protein expressed) according to convincing absence or presence of nuclear staining in greater than or equal to 1% of tumor cells. A case was considered microsatellite unstable if both MLH1 and PMS2 showed absent nuclear staining, if both of MSH2 and MSH6 showed absent nuclear staining, or if either MSH6 or PMS2 showed absent nuclear staining. Controls stained appropriately and slides were read by 2 pathologists.

Results: Of 104 interpretable cases, 9 (8.6%) showed loss of expression of MSH6 and MSH2, 3 (2.8%) showed loss expression of MSH6 alone, and 1 (0.9%) showed loss of expression of MLH1 and PMS2, Thus, a total of 12.5% of cases showed evidence of MSI.

Conclusions: Based on current screening practices, at least 12.5% of cases of UCRPU are microsatellite unstable and can show alterations of the mismatch repair proteins studied. These data supports that UCRPU are part of the spectrum of neoplasms associated with Lynch syndrome. Additional studies are necessary to evaluate these cases for germline mutations.

655 B7-H3 and B7x Are Highly Expressed in Human Prostate Cancer and Associated with Disease Spread and Poor Outcome

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Background: The B7-CD28 family of costimulatory proteins encompasses critical ligands and receptors that regulate T lymphocyte activation and function. Several of these B7-CD28 molecules are expressed in human cancers functioning as coregulatory inhibitors that may induce T-cell anergy or apoptosis upon antigen recognition. B7-H3 and B7x (B7-H4, B7S1) were recently discovered and their precise physiologic roles in human malignancy are not yet elucidated. Observations suggest that malignancies expressing negative costimulatory molecules are more likely to exhibit aggressive behavior and are associated with poor clinical outcome. We studied the expression of B7-H3 and B7x on a large cohort of prostate cancer patients with long-term follow-up.

Design: Tissue microarrays (TMA) were constructed form 948 patients undergoing radical prostatectomy at our institution between 1985 and 2003. Immunohistochemistry was performed on TMA slides using anti-B7-H3 and anti-B7x. The percentage of positive tumor cells and staining intensity were blindly evaluated without knowledge of outcome and results were recorded semiquantitatively. The findings were then correlated with pathologic features and clinical outcome.

Results: Tissue was available from 823 patients. Both B7-H3 and B7x were highly expressed in the majority of cases with a median of 80% of tumor cells expressing these markers. Only 7% and 1% of tumors lacked expression of B7-H3 and B7x, respectively. Strong staining intensity for B7-H3 and B7x was noted in 212 (26%) and 120 (15%) patients, respectively. Strong intensity for B7-H3 and B7x was significantly associated with extraprostatic extension ($p<0.001$ and $p=0.018$) and seminal vesicle invasion ($p=0.019$ and $p<0.001$, respectively). Additionally, patients with strong intensity for B7-H3 and B7x were significantly more likely to develop metastatic or hormone-refractory disease (hazard ratio [HR] 2.79, $p<0.001$ and HR 2.22, $p=0.005$) and were at significant risk of subsequent cancer-specific death (HR 3.48, $p=0.004$ and HR 2.71, $p=0.040$, respectively).

Conclusions: This is the largest study on the expression of B7 family molecules in a human malignancy. B7-H3 and B7x are abundantly expressed in prostate cancer and are associated with locally advanced disease and poor outcome. Given the proposed immune-inhibitory mechanisms of B7-H3 and B7x, these molecules represent attractive targets for therapeutic manipulation in prostate cancer.

656 The Spectrum of Histopathological Findings in Vesical Diverticulum

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Background: Diverticula are saccular evaginations of urinary bladder mucosa that are occasionally encountered in all age groups. Intradiverticular neoplasms have a prevalence of 1%-10% and pose diagnostic and treatment challenges. The aim of this study was to document the common morphologic changes and neoplasms found in a large series of vesical diverticula.

Design: We evaluated the spectrum of neoplastic and non-neoplastic changes in 37 cases of urinary bladder diverticula diagnosed between the period of 2004-2007.

Results: A total of 37 cases were reviewed. 10 of the patients were children with a mean age of 7 years, and 27 patients were adults with a mean age of 65 years. 89% of patients were males. Non-neoplastic changes were noted in 27 cases, including chronic inflammation in 15 cases, acute inflammation in 2 cases, squamous metaplasia in 1 case, intestinal metaplasia in one case, and nephrogenic metaplasia in 1 case. Varying degrees of fibrosis and attenuation of the muscularis propria were present in all the non-neoplastic cases. Of the 10 neoplastic cases, 7 showed high-grade urothelial carcinoma, 2 showed carcinoma in situ and 1 case showed low grade non invasive urothelial carcinoma. One of the high grade urothelial carcinomas also had a component of small cell carcinoma. In 4 of 7 high grade urothelial carcinoma cases, infiltration of cancer into adjacent fat was noted. Coexisting intravesical urothelial carcinoma was seen in 8 of 10 neoplastic cases.

Conclusions: Diverticulum of the urinary bladder is a relatively common clinical disorder in pediatric and adult age groups. Fibrosis, inflammation, and urothelial metaplastic changes are frequently encountered in such specimens. In addition, specimens may harbor neoplasms, most commonly urothelial carcinoma. Attenuation of the muscle layer associated with diverticulum formation facilitates tumor invasion into peridiverticular soft tissues in many cases, particularly those cases in which high grade carcinoma is present.

657 Ureteral Endometriosis: A Clinicopathological and Immunohistochemical Study of 7 Cases

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Background: Ureteral endometriosis is a rare yet important entity, that can lead to renal failure due to silent obstruction of the ureter. Awareness of clinical and morphologic features can help in early detection and treatment.

Design: We analyzed the clinical, pathologic and immunohistochemical findings of 7 cases of ureteral endometriosis.

Results: Mean age of patients was 51 years. All patients presented with hydronephrosis and/or hydroureter with or without pyelonephritis. 57% of patients had a previous history of total abdominal hysterectomy with bilateral salpingo-oopherectomy. In 86% of cases, endometriosis involved the left ureter. The distal one third of the ureter was involved in 6 cases while the middle third was involved in one case. In 4 cases, endometriosis was located extrinsic to the ureter, while in 3 cases the ureter showed intrinsic involvement by endometriosis. In all cases, lesions were composed of endometrial stroma and glands. One case showed simple endometrial hyperplasia. Surgical management included nephrectomy in 2 cases, distal ureterectomy with reimplantation in 3 cases, and ureteral stent placement followed by ureteroureterostomy in one case. Immunostains for CK7 and PR were positive in all of the cases, whereas immunostains for ER were positive in 74% of cases and immunostains for CK-20 were negative in all cases. The stromal cells were positive for CD10 immunostaining in all cases, and CA125 immunostains were positive in 4 cases.

Conclusions: Ureteral endometriosis should be included in the differential diagnosis of obstructive ureteral lesions, even in postmenopausal patients. In keeping with the results of previous studies, our study shows that the left distal third of ureter is the most common site of involvement, and that endometriosis is more commonly extrinsic to the ureter rather than intrinsic in such cases. Ureteric endometriosis can show endometrial hyperplasia and/or malignant transformation. Immunostains for ER, PR, CK7, CA-125 and CD10 can be helpful in establishing a diagnosis. Treatment options include medical and /or surgical intervention.

658 The Morphology of Capsular Zone and Predicting Extracapsular Extension on Needle Biopsies of Prostate

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Background: The histologic features of the prostate capsular zone have not been described in needle biopsies and there is reluctance by pathologists to diagnose extracapsular extension (ECE) on needle biopsies. Predicting ECE on needle biopsy has therapeutic implications, regarding decision making for nerve-sparing surgery versus radiation therapy. Currently studies only correlate parameters such as Gleason score, number of cores involved and volume of cancer for prognostication and in the decision making process. The aim of this study was to describe the histologic features that suggest ECE on needle biopsy and to determine if involvement of this zone correlates with ECE on the final specimen.

Design: In this retrospective study, 28 patients with known stage pT3 cancer with available needle biopsy slides for review were selected. A control cohort of 20 pT2 cases was also similarly selected. Multiple detailed histologic parameters were studied in order to assess the presence or absence of capsule, and presence of capsular involvement by tumor along with other traditional tumor parameters.

Results: A total of 517 cores were studied in detail (302 for pT3 and 215 for pT2). A definite capsule was diagnosed by presence of fat and/or colon mucosa attached at the tip of a core, and was detected in 29% cores. Possibility of a capsule was entertained when the aglandular zone at the tip of a core had increased myxoid areas, nerves and ectatic thin walled vessels, which were detected in 24% cores. Tumor involved the tip in 53 cores of pT3 versus 14 of pT2. Definite ECE was identified in one core (pT3), and 17 cores with final stage pT3 had features suggesting tumor infiltration into the capsule versus 3 cases for pT2 tumors. There was a trend between number of cores positive, size of Ca, presence of Ca at the tip and level of capsular involvement with final pathologic stage.

Conclusions: Histologic features that define capsular zone in a needle core are presence of extraprostatic tissues (fat and colon mucosa), presence of loose myxoid stroma at tip of core, ectatic thin walled vessels and increased density of nerve bundles. Based on our histologic description, there was a strong correlation between involvement of the above-described space on needle core biopsy and ECE on final resection specimen. Diagnosing ECE on biopsy may provide clinicians additional helpful information in their decision for choice of therapy.

659 Vasculitis of Large Muscular Vessels in Lichen Sclerosus of Foreskin: Incidence and Clinicopathologic Significance

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Background: Lichen sclerosus (LS) is a chronic inflammatory skin disease. Vascular involvement in LS is mainly described as 'superficial phlebitis' of thin-walled vessels in and around the inflammatory infiltrate. We have encountered necrotizing vasculitis of large muscular vessels distant from the inflammation, a finding not well described in literature. Determining whether this represents localized versus systemic vasculitis has important therapeutic implications. Our aim was to systematically review LS of foreskin, to determine the incidence and clinicopathologic significance of large vessel vasculitis.

Design: We retrospectively reviewed 137 pathology specimens of foreskin (68 cases diagnosed as LS; 69 cases diagnosed as chronic balanitis, which served as controls). Demographic information of patients as well as presence of local versus systemic symptoms, history of autoimmune, collagen vascular disease or systemic vasculitis at time of diagnosis and during follow-up were recorded. Laboratory markers included ESR, blood count, and serologic tests (ANCA and antinuclear antibodies in prototype cases). Review of the pathology included determination of the presence of vasculitis, fibrinoid necrosis, or granulomas. Immunoperoxidase stains were performed in a subset of prototype cases (*n=10*) for CD3, CD4, CD8, CD35 and CD20 to characterize the inflammatory infiltrate.

Results: Vasculitis of large muscular vessels was identified in 12 (17%) patients with LS; four (5.8%) of which showed presence of fibrinoid necrosis within the vessel walls. None of the patients with chronic balanitis showed vasculitis. The vasculitis was characterized by transmural infiltrate of either lymphocytes or an admixture of lymphocytes and histiocytes. One case showed vasculitis with associated perivascular granulomas. Immunostains revealed an admixture of T and B-cells with few histiocytes and dendritic cells in the vascular infiltrate. No patient had symptoms suggestive of systemic vasculitis, or autoimmune disease at diagnosis or during the follow-up (range 3-94 months, mean: 39 months). Serologic studies performed for ANCA or antinuclear antibodies in the patients with fibrinoid necrosis were negative.

Conclusions: Vasculitis of large muscular vessels with or without fibrinoid necrosis can be seen in LS. The immunoprofile of infiltrate suggests a localized antigen mediated response, probably resulting in lymphocyte mediated vasculitis. There was no evidence of systemic involvement, and therefore does not necessitate systemic therapy.

660 Margin Assessment of Partial Nephrectomy Specimens: Analysis of 200 Cases

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Background: The utilization of partial nephrectomy (PN) is increasing, presenting pathologists with new challenges. Our study goal is to analyze clinicopathologic data from PNs, especially with regards to intra-operative evaluation (IOE).

Design: Data from 200 PN cases (one center, 2001 to 2003) were analyzed; including tumor characteristics, IOE of margin, and followup data.

Results: The 200 PNs were from 188 patients (pts); age range of 26-83 years (mean 57.6). 170 (85%) PNs had a single tumor, including: 131 (77%) renal cell carcinomas (RCC) (99 clear cell, 25 papillary, 6 chromophobe, 1 unclassified) and 39 (23%) benign tumors (21 oncocytoma, 14 angiomyolipoma, 3 renal cortical cyst, 1 metanephric adenoma). 30 (15%) PN (from 26 pts) had multifocal tumors (including clear RCC, papillary RCC, multilocular cystic clear RCC, oncocytoma, angiomyolipoma). 28 (14.8%) pts had bilateral tumors; of which 10 (5.3%) also had multifocal tumors. IOE was performed on 182 (91%) PNs. 2 PNs had only gross evaluation of resection margin status. In 18 PNs (13 malignant and 5 benign tumors) IOE was not requested (3 laparoscopic PN and 15 open PN). Total of 294 margins were submitted for frozen section (FS). In 118 PN only one FS was done (106 perpendicular margins, 12 en face margins). 62 PN had multiple FS (range 2-7); in 10 PN submitted only as en face margins, in 28 PN submitted only as perpendicular margins, and in 24 PN a combination. The mean distance of tumor to margin was 1.62 mm (median 0.9 mm, range 0.5 to 18 mm). 29 (14.5%) PN had less than 1 mm margin; none of these pts developed a local recurrence (follow-up range 11-70 months). Six PN had positive margins (3 did not have IOE), none of whom developed a local recurrence (follow-up range 0.3-146 months). 3 pts had a local recurrence despite negative margins, all had multifocal tumors. 5 pts had metastases at the time of PN (4 pts had prior radical nephrectomy of the other kidney, 1 had metastasis to pancreas). Nineteen patients died (8 from disease, 11 unrelated causes). One patient with multifocal tumor died of disease. Fifteen pts were alive with disease, 154 alive without disease and 8 alive with unknown disease status.

Conclusions: Margin status evaluation is an important component of the PN procedure. However, even with a margin distance of <1.0 mm patients may not develop a local recurrence. Multifocal tumors appear to be an important determinant of local recurrence.

661 The Likelihood Ratio of Prostatic Carcinoma and Its Relationship to PCA-3 Score

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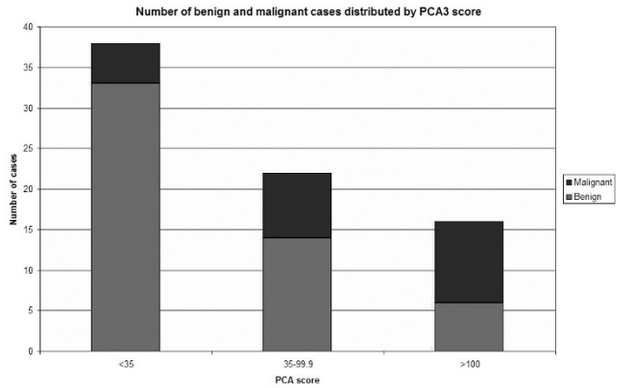
Background: Among the newer biomarkers for prostate cancer is prostate cancer gene 3 (PCA3). Its transcription produces a prostate specific non coding mRNA that has shown promise as a prostate cancer diagnostic tool. The likelihood ratio (LR) is the probability of a given test result among people with a disease divided by the probability of that test result among people without the disease. It has been suggested that patients with elevated PCA3 score be subjected to a repeat biopsy after an initial negative one. The objective of the current study is to evaluate the LR of prostatic carcinoma in biopsies at different levels of PCA3 score values.

Design: The current study included samples from 76 (53 benign and 23 malignant) referral cases submitted for PCA3 assay as well as prostate biopsies. The patients' age ranged between 46 and 90 years. The time interval between the PCA urine sample and the prostate biopsy ranged from 0-12 months. The PCA3 mRNA copies were quantitated using Gen-probe APTIMA technology and the results were expressed as PCA3/PSA ratio. The prostate biopsies were processed for H&E according to standard techniques. HMW CK and P63/ P504 IHC stains were performed as needed.

Results: The PCA3 scores were tabulated in three stratifications (<35, 35-99.9, and >100). The likelihood ratio was calculated and the results are shown in table (1) and figure (1).

Table (1)

PCA3 score	Benign	Malignant	Likelihood ratio
<35	33	5	0.35
35-99.9	14	8	1.4
100 or higher	6	10	3.9



Conclusions: The assay to determine the ratio of m RNA PCA3/PSA is a useful diagnostic test for evaluating the likelihood of prostate cancer in biopsies obtained within a year of the PCA3 urine test. The current study demonstrates the two fold utility of the test. A score below 35 carries a low likelihood of cancer and can safely weed out patients that do not need close follow up. The 89% negative predictive value at that score level confirms the low likelihood ratio's implication. On the other hand test scores of 100 and above portend a high likelihood of cancer and should warrant a more thorough follow up. This latter finding is confirmed by a specificity and accuracy rate of 91% and 76% respectively at 100 cut-off.

662 Papillary Renal Cell Carcinoma with Low Grade Spindle Cells Lining Angulated Tubules: A Mimic of Mucinous Tubular and Spindle Cell Carcinoma

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Background: The solid variant of papillary renal cell carcinoma (SV-PRCC) and mucinous tubular and spindle cell carcinoma (MTSC) overlap significantly at the morphologic and immunohistochemical (IHC) levels. PRCC may be definitively distinguished from MTSC by cytogenetics, since MTSC lacks PRCC's characteristic trisomies of chromosomes 7 and 17. Morphologically, low nuclear grade spindle cell foci are thought to distinguish MTSC from PRCC, since spindle cell areas in PRCC signify sarcomatoid transformation which is high grade.

Design: We identified 5 distinctive cases of SV-PRCC with low grade spindle cells lining angulated, elongated tubules, mimicking MTSC. The diagnosis of PRCC was confirmed by fluoresence in situ hybridization (FISH) demonstration of the characteristic trisomies of PRCC in formalin-fixed, paraffin embedded tissue.

Results: All patients were male, ranging in age from 17 to 68 years. The neoplasms ranged from 2.1 to 5.2cm and all were organ confined (pT1). All neoplasms were at least 80% solid, and 4 of 5 demonstrated papillary foci. Compact areas of bland spindled cells lining thin, angulated tubules (Figure 1) or forming solid sheets (Figure 2) comprised 10-70% of the neoplasms. Mucinous stroma was not seen in any case. By IHC, all neoplasms were diffusely immunoreactive for cytokeratin 7, and focally immunoreactive for CD10. By FISH, all 5 neoplasms demonstrated trisomy 7, and 4 of 5 demonstrated trisomy 17.

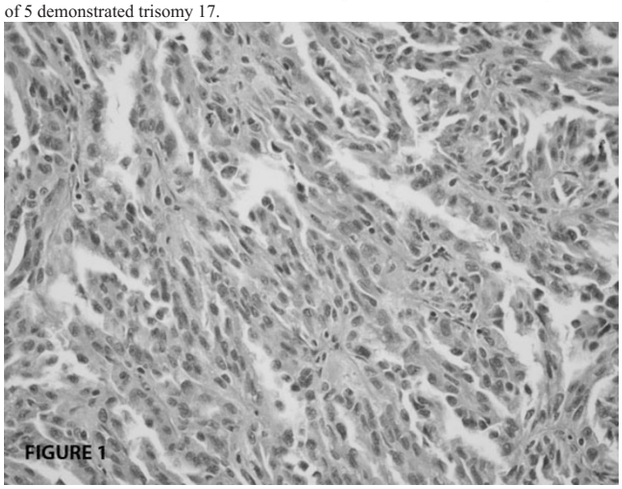
FIGURE 1

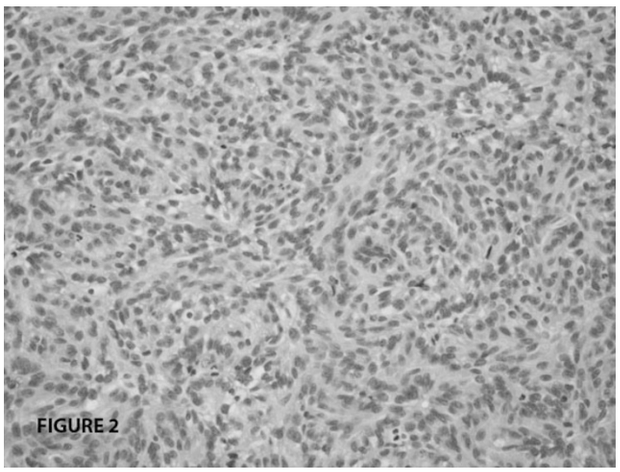
FIGURE 2

Conclusions: PRCC may demonstrate low grade spindle cells lining angulated tubules, mimicking MTSC. Demonstration of the characteristic trisomies of PRCC by FISH may confirm this diagnosis. Putative cases of MTSC with metastasis should be re-evaluated by FISH to exclude the possibility that they instead represent this unusual variant of PRCC.

663 p53 Mutational Analysis Supports Monoclonal Origin of Biphasic Sarcomatoid Urothelial Carcinoma (Carcinosarcoma) of the Urinary Bladder

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Background: Sarcomatoid urothelial carcinoma of the urinary bladder is an uncommon neoplasm with a biphasic morphology exhibiting both epithelial and mesenchymal components. Whether this tumor arises from a single stem cell with subsequent differentiation or represents collision of the progeny of two separate stem cells is still debated.

Design: We analyzed p53 mutational data from a series of 17 sarcomatoid urothelial carcinomas. Sarcomatous, carcinomatous and adjacent normal urothelium were separately microdissected. Following PCR amplification of p53 exons 5, 7 and 8, we used single-strand conformational polymorphism (SSCP) analysis to identify mobility shifts. P53 DNA in exons 5, 7 and 8 was additionally sequenced allowing mutational comparison between the different morphological components with adjacent normal urothelium as control.

Results: SSCP and DNA sequencing analysis of the tumors revealed that of the 17 sarcomatoid urothelial carcinomas studied five contained point mutations in the p53 exons examined. There was 100% concordance between the tumors with SSCP mobility shifts and those with point mutations identified by sequencing. In all five cases the mutations were identical in both the carcinomatous and sarcomatoid components. No p53 mutations were detected in any of the microdissected samples of surrounding normal tissue. Four of the tumors had point mutations in exon 8, all at different codons (261, 268, 272 and 275). Two tumors had point mutations in exon 5, both at codon 184 with identical G to T substitution. One of the tumors with a point mutation at codon 184 in exon 5 had a second mutation in codon 268 in exon 8. All of the point mutations led to amino acid alterations.

Conclusions: These findings support the monoclonal origin of carcinosarcoma of the urinary bladder with subsequent differentiation into its carcinomatous and sarcomatoid components.

664 Clinicopathologic and Immunohistochemical Characteristics of Clear Cell Renal Cell Carcinoma in Relationship to *von Hippel-Lindau* Gene Status

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Background: Dysregulation of von Hippel-Lindau (*VHL*) gene, by either gene mutation or promoter hypermethylation, plays critical role and is present in the majority of the sporadic clear cell renal cell carcinoma (CCRCC). The clinicopathologic and immunohistochemical characteristics of CCRCC in relationship to mutated, hypermethylated and wild type *VHL* gene status have not been documented.

Design: Sequencing of *VHL* gene was performed for 80 stage 4, sporadic CCRCC. Promoter hypermethylation was studied using methylation-specific PCR in those cases with wild type *VHL* gene. Immunohistochemical stains for CA IX, Glut-1 and Cyclin D1 (all regulated by *VHL* gene) were performed in all cases and were considered positive when staining was observed in ≥50% of tumor cells for CA IX and Glut-1, and in ≥25% of tumor cells for cyclin D1.

Results: *VHL* gene mutation (mutVHL) was detected in 35 (43.8%) cases, promoter hypermethylation (methVHL) in 11 (13.8%), and the remaining 34 (42.5%) had wild type *VHL* gene (wtVHL). *VHL* gene status correlated with Fuhrman grade with lower grade in mutVHL (grade 2, 3 and 4 in 19%, 53% and 26% mutVHL cases, vs 0%, 45% and 55% methVHL cases, and 6%, 50% and 44% wtVHL cases, Pearson correlation=0.222, p=0.029). In cases with *VHL* gene mutations, the exonal location of the mutations also

correlated with the dominant histological pattern with tubulocystic pattern more often and solid growth pattern less often in exon 1 mutants (solid, alveolar and tubulocystic pattern present in 7%, 57% and 36% of exon 1 mutVHL, 50%, 40% and 10% in exon 2 mutVHL, 33%, 50% and 17 % in exon 3 mutVHL, Pearson correlation=-0.322, p=0.041). CA9 and Glut-1 expression correlated with each other (Pearson correlation=0.239, p=0.025). *VHL* gene status correlated with the expression of CA9, Glut-1, and cyclin D1, with expression of all 3 proteins, 1 or 2 proteins, or none in 19%, 73% and 8% of mutVHL cases, 0%, 80% and 20% methVHL cases, and 15%, 56% and 30% wtVHL (Pearson correlation=-0.209, p=0.05).

Conclusions: Approximately 60% of sporadic CCRCC harbor *VHL* gene mutation or promoter methylation. Expression of CA9, Glut-1 and cyclin D1 correlates with VHL gene status. Further characterization of protein expression related to *VHL* status and response to therapy directed against the *VHL* pathway is ongoing.

665 Renal Angiomyolipomas: Clinicopathological Study of 202 Cases with Emphasis on the Epithelioid Variant

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Background: Classical renal angiomyolipoma (AML), composed of various proportions of adipose, vascular and myoid tissue, is a benign entity. The epithelioid AML is rare yet important to recognize for diagnostic and prognostic reasons. However, the clinicopathological features, including the clinical behavior, of epithelioid angiomyolipomas are not well defined.

Design: AMLs diagnosed between 1981 and 2007 were reviewed for histological features including presence of epithelioid component, microscopic foci of AML within renal parenchyma outside the main tumor and epithelial inclusion cysts within the tumor.Clinical information, including follow-up, was obtained by chart review and/or patient contact.

Results: Of 202 AMLs from 191 patients, 175 cases (86.6%) were triphasic with fat, vessels and smooth muscle, each present in significant proportion. 11 (5.4%) were monophasic with fat or smooth muscle accounting for >=95% of tumor. An epithelioid component, ranging from 10-100% (mean 39.1%), was present in 16 (7.9%) cases. Both epithelioid and triphasic AMLs predominantly affected female patients (male/female=1/3.3 in both). Patients with epithelioid histology were significantly younger than those with triphasic AMLs (36.5 vs 51.3 years, p<0.05), and more commonly had associated tuberous sclerosis (TS) (25% vs 7.2%, p<0.05). Epithelioid AMLs were significantly larger, and more often had epithelial inclusion cysts, microscopic foci of AML, necrosis, diffuse nuclear atypia and mitosis (Table 1). Epithelial inclusion cysts and microscopic AML foci were more common in patients with TS (37.5% and 68.8%, respectively). 189 patients (including all epithelioid AMLs) were followed for 3.4 years (range 0.1-23.7) and none had a recurrence.

Pathological features of epithelioid and triphasic AMLs

	Epitheloid AML (n=16)	Triphasic AML (n=167)
Size (cm, range)*	8.8 (1-30)	5.7 (0.2 - 1.9)
Epithelial inclusion cyst (%)	3 (18.9%)	16 (9.1%)
Microscopic AML foci*	6 (37.5%)	23 (13.1%)
Tumor necrosis*	3 (18.9%)	0
Marked/diffuse atypia*	10 (62.5%)	0
Mitosis*	7 (43.8%)	0

*Statistically significant

Conclusions: Significant % (7.9%) AMLs have epithelioid component. These epithelioid AMLs are more common in patients with TS history, and harbor rather worrisome histopathological features. However, they all behaved benign in our series. Presence of epithelial inclusion cysts and microscopic AML foci should raise the suspicion for TS.

666 Positive-Block Ratio vs Point-Count Method for Evaluating Tumor Volume in Radical Prostatectomies: A Comparative Study

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Background: One of the most controversial aspects of the pathologic assessment of radical prostatectomy specimens is the measurement of tumor volume. No accepted standard exists for reporting cancer volume or extent. Recently, a study showed that the ratio of tumor positive tissue blocks to the total number of blocks submitted is an independent predictor of biochemical progression following surgery. We compared the positive-block ratio to a semiquantitative point-count method for several clinicopathological variables.

Design: The study was based on 272 whole-mount consecutive surgical specimens. The data were analyzed using the Mann-Whitney test and the Fisher's exact test. Time to biochemical progression was compared using the Kaplan-Meier product-limit analysis.

Results: The mean, median and range for the positive-block ratio was 0.43, 0.43, and 0-1; using the point-count method was 36.15, 27, and 0-225 positive points. Comparing patients ≤ 0.43 with patients >0.43 positive-block ratio for several clinicopathological variables the findings were, respectively: mean age 63.6 yrs and 62.9 yrs (p=0.199); mean preoperative PSA 8.94ng/mL and 11.05ng/mL (p<0.001); mean Gleason score 6.39 and 6.95 (p<0.001); positive surgical margins 26.3% and 61.4% (p<0.01); extraprostatic extension (pT3a) 11.5% and 43.2% (p<0.001); and, seminal vesicle invasion 5.1% and 20% (p<0.01). Comparing patients ≤ 27 with patients >27 positive points the findings were, respectively: mean age 63.4 yrs and 63 yrs (p=0.414); preoperative PSA 9.09 ng/mL and 10.05ng/mL (p=0.008); Gleason score 6.40 and 6.96 (p<0.001); positive surgical margins 27.7% and 59.5% (p<0,01); extraprostatic extension (pT3a) 11.3% and 42.3% (p<0.001); and, seminal vesicle invasion (pT3b) 2.8% and 19.8% (p<0.001). At 5 years, the PSA progression-free survival rates were 60% and 51%, respectively, for patients with ≤0.43 and >0.43 positive-block ratio; and, 58% and 51%, respectively, for patients with ≤27 and >27 positive points.

Conclusions: The positive-block ratio and the point-count methods for measuring tumor extent are equivalent when evaluating several clinicopathological variables. The positive-block ratio is a practical and more simple method that can be used by pathologists in their routine practice.

667 Localized Prostate Cancers with Low AMACR Expression Are Associated with an Increased Risk of Prostate Cancer-Specific Death in the Physicians Health Study Cohort

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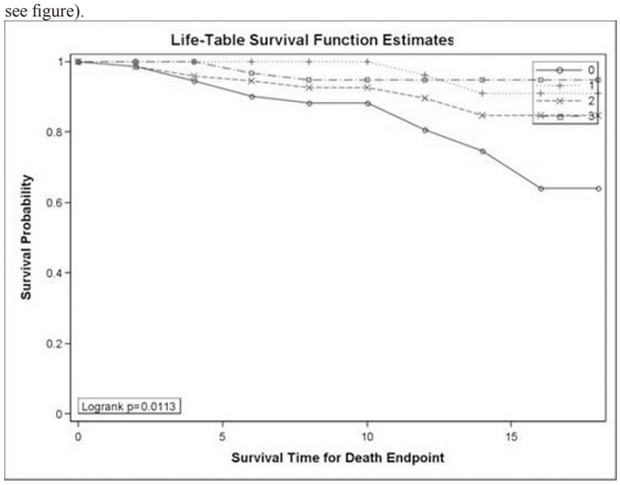
Background: Prostate cancer is a heterogeneous disease with wide variation in clinical outcome. The addition of validated tumor biomarkers to the classical clinical variables may better predict individual outcome. In a European cohort, decreased alpha-methylacyl CoA racemase (AMACR) expression was associated with prostate cancer-specific death. Here we sought to validate this finding in an American cohort: the Physicians Health Study (PHS) cohort, a randomized prevention trial of US male physicians followed prospectively for the development of cancer.

Design: We studied 298 men diagnosed with prostate cancer at a mean age of 66.8 years and followed for a mean of 10.4 years. Gleason scores were as follows: 4-5 (5.4%), 6 (27.5%), 7 (51.7%), 8-10 (15.4%). The cohort was divided into an indolent group (n=108), without prostate cancer-specific death at 10 years post diagnosis, and a lethal group (n=24), with prostate cancer-specific death. The remaining cases were censored. We analyzed AMACR expression by immunohistochemistry (p504S, Zeta Co.) on tumor tissue microarrays from the 298 cases and a quantitative image analysis system (ACIS II, Clarient Inc.). Tumor in each core was outlined electronically and scored for percent staining and staining intensity. The data was split into quartiles of AMACR percent staining and intensity respectively, from 0-3 (with 0 being the lowest). The endpoint for Kaplan-Meier survival analysis was death.

Results: There was no significant association between mean AMACR percent staining or intensity with Gleason score or age at diagnosis, though there was a trend for percent staining and intensity to be lower in patients older than 71 years. On survival analysis, cases in the lowest quartile of percent AMACR staining showed a significant association with the development of prostate cancer-specific death (logrank p=0.0113, see figure).



Conclusions: Low prostate cancer AMACR expression in a prospectively followed American cohort is associated with prostate-cancer specific death, further validating AMACR as a biomarker in prostate cancer.

668 Clinicopathologic Features of Hematolymphoid Neoplasms (HLN) Involving the Prostate in Surgical Pathology Specimens at The University of Pittsburgh

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Background: HLNs of the prostate are uncommon relative to prostatic adenocarcinoma (CaP). We have encountered HLNs in prostate needle bxs (PNBx), transurethral resection of prostate (TURP), and radical prostatectomy (RP) specimens. It is important to recognize prostatic HLNs since they may mimic chronic prostatitis when low-grade and high-grade CaP when high-grade. The aim of our study is to determine the incidence and clinicopathologic features of prostatic HLNs.

Design: We retrospectively searched the pathology information system database at The University of Pittsburgh medical system network for pts undergoing PNBx (n= >25,000), TURP (n= > 6,200), and RP (n=3343) (1/1/80 - 7/31/07) who were found to have HLN in prostate tissue. The HLNs were assessed by H&E and immunohistochemistry. We reviewed the clinical records and pathology for each pt.

Results: We identified 15 prostate specimens with HLNs: (PNbx (n= 8), TURP (n=6), RP (n=1). 13 pts originated from the UPMC medical system network, and 2 pts were from outside hospitals. Mean ± SD age was 71.1±5.8 yrs (62-81 yrs). Lymphoma subtypes were: B-cell SLL/CLL (n=7), diffuse large B-cell lymphoma (DLBCL; n=3), follicular center cell lymphoma (FCCL; n=3), mantle cell lymphoma (MCL; n=1), and small non-cleaved cell / Burkitt's lymphoma (BL; n=1). 6 /15 (40%) pts had documented extraprostatic HLNs: DLBCL (n=3; testis, thigh soft tissue, bladder, mesenteric mass, bone marrow, cervical / retroperitoneal LNs), MCL (n=1;GI tract, axillary / mesenteric LNs); BL (n=1; liver); B-cell CLL/SLL (n=1; pleural fluid). Two pts had concurrent

prostatic adenocarcinoma. 3 pts were ANED (FCCL (n=1), CLL/SLL (n=2); (median-72 mos)), 5 pts were AWD (CLL/SLL (n=3), DLBCL (n=1), FCCL (n=1); median- 20 mos)), 3 pts were DOD (DLBCL (n=1), MCL (n=1), FCCL (n=1); (median-58 mos)), 1 pt died of other causes (CLL/SLL), and 3 pts had no follow-up.
Conclusions: Prostatic involvement by HLN is rare, accounting for less than 0.05% of prostate tissue specimens in our pt population. Peak age range is similar to that of CaP. In our study, B-cell SLL/CLL was the most frequent cell type (seen most often in TURP specimens). This was followed by DLBCL and FCCL, occurring with equal frequency. DLBCL, MCL, and BL were more likely to have extraprostatic involvement and behaved more aggressively. It is important to consider the diagnosis of lymphoma in prostate specimens with diffuse cellular lymphoid infiltrates, and to distinguish from inflammatory processes and high-grade CaP.

669 MicroRNA Expression in Androgen Independent and Metastatic Prostate Cancer

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Background: MicroRNAs (miRNAs) are a newly discovered group of small noncoding RNAs that regulate target gene activity influencing cell proliferation and apoptosis. Their role in gene dysregulation in solid malignant tumors is evolving. In this study, we examined the expression of specific miRNA members (let7c, mi21, mi30c, mi27, 219, and 301) in late stage prostate cancer (androgen independent and metastatic cancer) based on their already implicated role in benign prostate hyperplasia and hormonally dependent prostate cancer.
Design: 1) Locked nucleic acid (LNA)-modified miRCURY probes (Exiqon, Inc) for the above miRNAs were used for tissue microassay based in situ hybridization. Hybridization and detection conditions for paraffin embedded tissue were used following the protocol provided by the company. 2) A tissue microarray (TMA)(n=60) including metastatic (n=20) and androgen-independent (n=18) prostate cancer and control tissues was analyzed. 3) Expression level was scored semiquantitatively; 0: negative, 1+: weak, 2+: moderate and 3+:strong, based on the intensity of color detection. 4) The expression of miRNA was analyzed for association with clinicopathologic parameters.
Results: The levels of five miRNAs, let-7c, miR21, miR27a, miR30c and miR219, were normalized according to the expression of U6. The expression of milet7c, mi219 and especially mi30c are significantly decreased in androgen independent prostate cancer with Gleason score > 8 as compared with lower grade androgen dependent prostate cancer. Further, mi219 levels showed a greater decrease in metastasis as compared with primary cancer. Additionally, we have observed a difference in miR21, miR30c and miR219 miRNA expression in prostate cancer between African-American and Caucasian populations.
Conclusions: miRNA dysregulation is associated with prostate growth and tumor metastasis. The relative levels in different groups show interesting trends and have implications for understanding growth control as well as for developing therapeutics. Our results are currently preliminary due to the limited statistical power of the small population cohort. Additional large population cohorts of androgen independent cancer are being collected from other sources to allow validation of findings. A validated role in androgen independent prostate cancer may offer potential alternative therapeutic options for this group with a generally poor prognosis.

670 Incidence of EDNRB Hypermethylation in Prostatic Adenocarcinoma

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Background: DNA hypermethylation of the gene promoter regions contributes to the carcinogenesis by silencing the expression of tumor suppressor and other regulatory genes. It refers to covalent bonding of a methyl group to the 5'-cytosine of the dinucleotide, CpG. Aberrant promoter methylations have been reported for several genes in prostate carcinoma. We have evaluated the methylation status of endothelin receptor B gene (EDNRB) and investigated its association with the disease prognosis.
Design: Radical prostatectomies from 68 clinically localized prostate cancer patients were included in the study. Preoperative and post-operative data were collected on all patients from the hospital charts. Slides were revieved and prognostically important pathological parameters were noted. Tumor DNA from each case was isolated using the paraffin blocks. ENDRB CpG island hypermethylation status was determined by real-time methylation specific polymerase chain reaction.
Results: Gleason score ranged from 6-9, and pathological stage ranged from T2N0Mx to T3BN1. EDNRB gene was found methylated in 16 cases. Unfavorable prognostic features were more frequent in radical prostatectomies with EDNRB hypermethylation (table1). The correlation between presence of hypermethylated gene and high Gleason score was statistically significant (p=0.015).

	Gleason score ≥7	EPE	SVI	SM (+)	LVI	pT ≥3a	PSA relapse
EDNRB Methylation (-)	69.2%	63.5%	23.1%	43.1%	15.4%	61.9%	31.3%
EDNRB Methylation (+)	100%	87.5%	37.5%	56.3%	18.8%	80.8%	37.5%

EPE: Extraprostatic extension, SVI: seminal vesicle invasion, SM (+): Surgical margin positivity, LVI: Lymphovascular invasion.
Conclusions: Hypermethylation of EDNRB is rare in prostate carcinoma, seen in 23.5% of cases. These patients are more likely to present with worse prognostic factors, which may imply the role of the genetic alteration in the disease progression.

671 KI-67 Is an Independent Predictor of Outcome in Conservatively Treated Clinically Localised Prostate Cancer

DM Berney, A Gopalan, S Kudahetti, L Ambroisine, G Fisher, M Kattan, H Moller, C Foster, V Reuter, J Cuzick, P Scardino, C Cooper, W Gerald. Barts and The London School of Medicine, London, United Kingdom; Memorial Sloan Kettering, New York; The Cleveland Clinic, Cleveland; The Royal Marsden Hospital, United Kingdom; King's College London.
Background: The Trans-Atlantic Prostate Group was set up to study men with clinically localised prostate cancer (PC), treated by conservative management. Gleason score and PSA are the most powerful clinico-pathologic predictors of death in this group, however,we sought to identify immunohistochemical (IHC) markers that might provide improved prediction accuracy. Preliminary analysis of multiple IHC markers in a subset of about 200 cases indicated that Ki-67 immunostaining index was a promising candidate for outcome prediction.
Design: Tissue microarrays of TURP specimens from 1756 men in the study were constructed. Immunochemistry was carried out using standard techniques. Analyses of Ki-67 was performed independently at two sites using either a quantitative (Q) or a semi quantitative (S) assessment. Results were analysed by dichotomous, grouped and continuous variables and compared with overall and prostate cancer specific survival. Multivariate comparisons in a model including serum PSA, age at diagnosis and Gleason score were also performed.
Results: 735 cases were assessed by Q and 680 by S methods. The agreement between methods based on dichotomized data was very good. Significant association between Ki-67 index and disease specific and overall survival were obtained by both methods. In univariate analysis of S, Ki-67 index (<5%) was a significant prognostic factor of PC specific death (HR=4.72, 95% CI=3.36-6.63, p<0.001) and remained so in multivariate analysis (HR=2.17, 95% CI=1.51-3.11, p<0.001). Q analysis demonstrated a continuous increase in HR with Ki-67 index in univariate analysis and was also significant in a model including age, PSA and grade. The continuous variable was the best predictor of survival in both scoring methods and both scoring methods showed better prediction at Gleason scores 7 or more.
Conclusions: Ki-67 is a potentially useful predictor of PC death, independent of Gleason score or other currently used clinical variables in this cohort. Multiple methods of Ki-67 assessment were informative. We suggest that, once standardised, measurement of proliferation will likely improve prediction and aid in therapeutic stratification.

672 Sporadic Clear Cell Renal Cell Carcinoma with Diffuse Cytokeratin-7 Immunoreactivity

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Background: Clear Cell Renal Carcinoma (CRCC) usually is not reactive for Cytokeratin-7 (CK7). The histology and immunohistochemistry of a new subtype of CRCC with diffuse CK7 immunoreactivity is described.
Design: All cases of CRCC over a period of 10 years, and measuring 20 mm or less in diameter, were examined. Areas of regenerative epithelial cell nests (REC) were also examined.
Results: Fifteen specimens containing 29 nodules were diagnosed as CRCC due to the characteristic clear cytoplasm. Of these 29 nodules, 21 showed diffuse CK7 positivity (ten patients) while 8 showed CK7 negativity. The CK7 positive CRCC measured less than 16mm and contained varying proportions of tumor cells with chromophil cytoplasm. Architecturally, CK7 positive CRCC consisted of cysts and solid cell nests with tubulo-acinar formations or papillary formations. Immunostaining for AMACR (Racemase), CD10 and RCC antigen showed negativity or focal reactivity in the CK7 positive CRCC, frequent positive reactivity in CK7 negative CRCC and negative reactivity in REC which also displayed strong CK7 reactivity. The ten patients, with CK7 positive CRCC developed no metastatic disease over a follow up time that ranged from 1 to 10 years (mean of 3 years).
Conclusions: Clear Cell Renal Carcinoma characterized by diffuse CK7 positivity and negativity for CD10, RCC, and Racemase represents a distinct type of CRCC with characteristic histopathological and immunohistochemical features. Further studies are indicated to investigate if it has a different biological behavior.

673 High Grade Prostatic Intraepithelial Neoplasia in the Prostatic Central Zone

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Background: The prostatic central zone (CZ) has a lower rate of prostatic adenocarcinoma (PCA) than the peripheral zone (PZ). Although the histology of the CZ has been documented, the HGPIN of the CZ has not been studied.
Design: We reviewed consecutive 300 prostatectomies for PAC to identify PCA and HGPIN in the CZ. The CZ was divided into two equal portions by a horizontal plane lower portion (lower 2/3of the height of the CZ) and basal portion (BP) (upper 1/3).
Results: There were 9 CZ PCA and 291 non-CZ PCA with HGPIN identified in 391 foci and 1662 foci in CZ (211 cases) and non-CZ (290 cases) respectively. The numbers of foci of HGPIN were 332 and 59 foci for LP and BP of the CZ respectively (p<0.01). In addition, the HGPIN were lager in size and displayed larger and more frequent nucleoli in the apical portion than in other portions of the CZ. Architecturally, the HGPIN were of papillary (40% of foci), cribriform (35%), tufting (10%) and flat types (15%). The papillae were usually slender. Cytologically, HGPIN displayed darker cytoplasm, more hyperchromatic and prominent nucleoli than those seen in the PZ. Cytoplasmic granules were rarely seen.
Conclusions: In comparison with the PZ, HGPIN of the CZ were rarer, smaller and often displayed slender papillae. There was a gradient of distribution of PCA and HGPIN in CZ with highest prevalence in the AP.

674 The Significance of Microscopic Bladder Neck Involvement in Radical Prostatectomies: pT4 Disease?

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Background: It is controversial whether microscopic invasion of the bladder neck has a high risk for biochemical progression following radical prostatectomy. The TNM classification for prostate cancer considers bladder neck involvement to be pT4 disease, equivalent to rectal or external sphincter invasion, however, it does not specifies whether the invasion is macroscopic or microscopic.

Design: The study was based on 290 whole-mount consecutive radical prostatectomies. The bladder neck and the apical margins were amputated and processed through perpendicular sections. Extraprostatic extension was diagnosed whenever cancer was seen in adipose tissue and, in case of desmoplastic response, whenever a protuberance corresponding to extension of tumor into the periprostatic tissue was seen. Tumor extent was evaluated with a point-count semiquantitative method. The data were analyzed using the Mann-Whitney test and the Fisher's exact test. Time to biochemical (PSA) progression was compared using the Kaplan-Meier product-limit analysis.

Results: Bladder neck invasion was present in 55/290 (18.96%) surgical specimens and absent in 235/290 (81.03%) patients. Comparing patients with and without bladder neck invasion for several clinicopathological variables, the findings were, respectively: mean age 64.2 yrs and 63.1 yrs (p=0.231); preoperative PSA 14.84ng/mL and 8.80ng/mL (p<0.001); mean Gleason score 6.98 and 6.59 (p<0.001); positive surgical margins 52.7% and 38% (p=0.049); extraprostatic extension (pT3a) 50.9% and 20.9% (p<0.001); and seminal vesicle invasion (pT3b) 25.5% and 8.9% (p=0.002). At 5 years, the PSA progression-free survival rates were 42% and 58% for patients with and without bladder neck invasion, respectively. In comparison, at 5 years, the PSA progression-free survival rates were 28% and 58% for patients with and without seminal vesicle invasion.

Conclusions: Patients with bladder neck invasion have significantly higher preoperative PSA, higher Gleason score, higher positive surgical margins, and more advanced pathological stage. However, PSA progression-free survival rate is higher comparing to seminal vesicle invasion (pT3b). This latter finding seems not to favor considering microscopic bladder neck invasion as stage pT4.

675 The Border between Low- and High-Grade Gleason Score for Prostate Carcinoma: 6 or 7a (3+4)?

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Background: Some authors consider that the prognostic value of Gleason scores 6 and 7a (3+4) may be similar. The border between low- and high-grade prostatic carcinoma may be probably Gleason score 7a (3+4) and 7b (4+3). This change could result in different therapeutic options for prostatic carcinomas. We compared Gleason score 6 vs 7a (3+4) vs 7b (4+3) in radical prostatectomies for several clinicopathological variables.

Design: The study was based on 300 whole-mount consecutive surgical specimens. The bladder neck and the apical margins were amputated and processed through perpendicular sections. Extraprostatic extension was diagnosed whenever cancer was seen in adipose tissue and, in case of desmoplastic response, whenever a protuberance corresponding to extension of tumor into the periprostatic tissue was seen. Tumor extent was evaluated with a point-count semiquantitative method. The data were analyzed using the Kruskal-Wallis Anova and the qui-square test. Time to biochemical (PSA) progression was compared using the Kaplan-Meier product-limit analysis.

Results: Gleason score 6, 7a (3+4), and 7b (4+3) were seen in 87/300 (29%), 140/300 (46.6%), and 37/300 (12.3%) specimens, respectively. Comparing patients with these Gleason scores for several clipathological variables, the findings were, respectively: mean age 61.6 yrs, 63.3 yrs, and 65.9 yrs (p=0.005); preoperative PSA 7.72ng/mL, 9.95ng/mL, and 12.74ng/mL (p<0.001); tumor extent 16.4, 39.8, and 57.6 positive points (p<0.001); positive surgical margins 26.4%, 51.4%, and 59.5% (p<0.001); extraprostatic extension (pT3a) 4.6%, 32.4%, and 59.5% (p<0.001); and, seminal vesical invasion 1.1%, 11.4%, and 27% (p<0.001). At 5 years, the PSA progression-free survival rates were 77%, 48% and 31% for patients with Gleason score 6, 7a (3+4) and 7b (4+3), respectively (log-rank, p<0.001).

Conclusions: In this series, Gleason score 6 was not statistically similar to Gleason score 7a for several clinicopathological variables and biochemical (PSA) progression following radical prostatectomy. It seems that the border between low- and high-grade Gleason score for prostate carcinoma should be 6 and 7a and not 7a and 7b.

676 MicroRNA Expression Profiles in Paraffin Embedded Seminoma and Benign Testicular Tissue

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Background: Seminoma is a primitive subtype of germ cell tumors. Mature microRNAs (miRNA) are small RNAs about 19-23 nucleotides in length. Studies show that miRNAs are key players in cell proliferation and differentiation. By screening a miRNA expression library, Voorhoeve et al (Cell 124, 1169-1181, 2006) found that miRNA-327 and miRNA-373 can neutralize p53-mediated CDK inhibition and permit tumorigenesis of primary human cells with both oncogenic RAS and wild type p53. Moreover, they identified high levels of expression of miRNA-372 and 373 in non-seminomatous germ cell tumor cell lines and miRNA-372 in both seminoma and non-seminotous germ cell tumors and miRNA-373 in non-seminomatous germ cell tumors, suggesting that these miRNA species may function as oncogenes in the development of human testicular germ cell tumors. In this study, we used miRNA array to identify aberrantly expressed miRNAs in seminoma.

Design: Formalin-fixed paraffin-embedded samples from 11 cases of normal testicular tissue resected due to non-neoplastic causes and 11 cases of testicular seminoma were race-paired and assessed for miRNA expression. The expression patterns of ~750 characterized and predicted human, mouse and rat miRNAs were evaluated. The relative differences in signal strength were assessed by applying a 1-sample t-test with a hypothesizing mean of 0 using a false discovery rate of 5%. Selected miRNAs were confirmed by reverse transcription and TaqMan® real time PCR.

Results: 112 miRNAs were found differentially expressed between seminoma and normal testicular tissue. 52 miRNAs were overexpressed while 60 miRNAs were down-regulated in seminoma. We did not observe significant difference between black and white populations in our race-paired study. Three microRNAs, hsa-mir-21, hsa-mir-372, hsa-mir-373 were dramatically up-regulated. This upregulation was validated by reverse transcription and real time PCR. Hsa-mir-372 was upregulated 1,268.7 fold (p=8.3e-9). Has-mir-373 was up-regulated 1,532.7 fold (p=8.3e-9). Hsa-mir-21 was up-regulated 12.2 fold (p=0.0006).

Conclusions: miRNA array can be successfully performed in formalin-fixed paraffin embedded tissue. We identified a unique miRNA profile in primary seminoma. miRNA-372 was dramatically upregulated, which concurred with the prior observation of Voorhoeve et al. Our identification of overexperssion of miRNA-373 is unique and is the first report of this finding in primary seminoma. The up-regulation of these miRNA species suggests a potential role of miRNAs in the tumorigenesis of seminoma.

677 Combining Interphase and Metaphase Analyses in the Differential Diagnosis between Chromophobe Renal Cell Carcinoma and Renal Oncocytoma

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Background: Cytogenetic analysis usually reveals low number of chromosomes 1, 2, 6, 10 and 17 in chromophobe renal cell carcinoma and a normal numerical complement of chromosomes in renal oncocytoma. However, different chromosomal patterns have been rarely reported in both renal cell neoplasms.

Design: We investigated 23 renal cell neoplasms (11 chromophobe renal cell carcinomas, 12 renal oncocytomas) by metaphase karyotyping and interphase FISH for chromosomes 1, 2, 6, 10 and 17 and flow cytometric analyses on tissue sections.

Results: FISH showed losses of two or more chromosomes in 10 chromophobe renal cell carcinomas (91%) and gains of multiple chromosomes in one (9%). Six (50%) renal oncocytomas were totally disomic, five (42%) showed one chromosomal loss (chromosome 1 in 3 cases), one case (8%) two losses. Among 9 chromophobe renal cell carcinomas with available istograms 6 (67%) showed aneuploid stemlines whereas the three remaining and 8/9 (89%) renal oncocytomas were diploid. Karyotypically, 3 chromophobe renal cell carcinomas (33%) were hypodiploid, 3 (33%) were polydiploid, one (11%) was diploid. Nine out of 12 (75%) renal oncocytomas were diploid, one showed -Y (8%), one 47XX (8%), one multiple different clones (9%). All chromophobe renal carcinomas which failed to grow and 2/3 (75%) showing gains by metaphase analyses displayed multiple chromosomal losses by FISH. Eight renal oncocytomas with normal DNA content and those three with additional chromosomal abnormalities (91%) by karyotyping showed normal complement of chromosomes by FISH.

Conclusions: 1) chromophobe renal carcinomas usually display multiple chromosomal losses by FISH analysis in spite of a different spectrum found by karyotyping and flow cytometric analyses; 2) chromophobe renal carcinomas that fail to grow in culture are characterized by chromosomal losses in FISH; 3) renal oncocytomas usually show a normal DNA content by both interphase and metaphase analyses.

678 Gene Expression Profiling Designs TFE/MiTF Renal Cell Translocation Carcinomas As a Distinct Group among Renal Cell Carcinomas

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Background: Translocation renal cell carcinomas (RCC) are uncommon, characterized by cytogenetic translocations involving transcription factors of the Microphtalmia transcription factors family MiTF/TFE: TFE3 or TFEB. They can be suspected in young adults of onset and/or atypical histologic patterns. Antibodies directed against TFE3 and TFEB are useful tools of good specificity and sensibility.

Design: To determine the genetic profile of these subtype of RCC, we realized DNA micro arrays of 5 TFE3 and 1 TFEB translocation RCC. Diagnostic was confirmed either by immunohistochemistry (all cases) or cytogenetic (one TFE3). RNA was extracted from frozen samples and quality was assessed on denaturing gel electrophoresis. Oligonucleotide expression profiling was performed using Affymetrix platform according manufacturer's recommendations. We compared transclocation RCC with 55 cases of renal tumors representative of their respective subtypes based on both histology and gene expression (11 clear cell, 7 chromophobe, 11 papillary type 1, 6 papillary type 2, 13 urothelial RCC and 7 oncocytomas). We included 13 samples of normal kidney. Statistical analyses were performed using the R statistical environment and Bioconductor project version 1.9. Genes differentially expressed between the samples of translocation RCC and our reference samples were identified using the Linear Models for Microarrays data (Limma) package from the Bioconductor project.

Results: From our clustering analysis against 6 other subtypes of kidney tumors and normal kidney, we demonstrated that the TFE3 and TFEB translocation carcinomas cluster closer to each other than to other renal tumors. Among the genes differentially expressed in TFE carcinomas, TRIM63, ITGB1BP3, SV2B, HABP2, CYP2B6 and CA XII represent potentially novel diagnostic or prognostic makers, although further validation studies are warranted.

Conclusions: Our results suggest that translocation RCC can be divided based on their molecular signatures. This is further evidence for considering TFE3 and TFEB renal translocation carcinomas as a distinct subgroup of RCC to be designed as MiTF/TFE carcinomas, as did already suggested.

679 Expression of 4EBP1 Is an Influential Prognostic Covariate in Renal Cell Carcinoma

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Background: The outcome of surgically treated localized renal cell carcinoma is unpredictable, up to 40% of patients apparently cured by surgery go on to develop metastasis and die from these. There is a need for better prognostic markers to determine the need for adjuvant therapy. Dysregulation of the MTOR pathway is recognized in renal cell carcinoma and some novel therapies inhibit this pathway; but to date there are no reports of the impact of the expression of phosphorolated 4E binding protein 1 (4EBP1), an important effector component of this pathway, on prognosis.

Design: A cohort of 174 clinically localized RCCs treated by radical nephrectomy and for whom good follow up was available were studied. Data derived from the original report and a chart review included tumor size, time to first recurrence and date and cause of death. Slides and blocks were retrieved and the tumors assessed for tumor type, nuclear grade, vascular invasion (VI) (IVC, renal vein or microvascular invasion) and invasion of perinephric tissue. A tissue microarray was constructed and stained by convention immunohistochemical technique for 4EPB1. Staining for these antigen was assessed blinded to the outcome and scored on a semiquantitative scale. This was converted into a binary value by thresholding according to the most informative cut off value on Kaplan Meier analysis. Impact on disease free survival (DFS) was assessed by Kaplan Meier method and Cox regression analysis.

Results: After thresholding 65 cases scored positively for 4EBP1. On Kaplan Meier analysis positive 4EBP1 had a adverse impact on DFS p=0.033 (Breslow test). On Cox regression analysis including grade, vascular invasion and invasion of perinephric tissue E4BP1 was found to have significant additional influence on the outcome: positive 4EBP1 vrs negative 4EBP1 HR: 3.2 ($p<0.001$).

Conclusions: This is the first report of the impact of the expression of 4EBP1 on disease free survival in localised RCC. and demonstrates it has powerful influence on survival in both univariate and multivariate analysis. This implies the pathway is important in the pathogenesis of the aggressiveness of the RCC and offers a novel prognostic marker. We postulate that expression of 4EBP1 may also be predictive in the response of tumors to inhibitors of the MTOR pathway.

680 MALDI-MSI (Mass Spectometry Imaging) Discriminates Malignant and Nonmalignant Disease of the Prostate and Identifies Specific Protein Markers

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Background: Malignant transformation involves alterations in protein expression. These alterations can be monitored at the protein level, *in situ b*oth qualitatively and quantitatively with a recently developed technique, known as matrix-assisted laser desorption/ionization mass spectrometric tissue imaging (MALDI-MSI). Prostatic adenocarcinoma (PCa) has traditionally been classified and staged using purely pathologic and clinical measures. Although the current system provides good prognostic information there is a need for molecular markers that may enhance early diagnosis, better predict tumor prognosis, and further stratifiy patients with PCa. We have used MALDI-MSI in order to identify specific protein markers of PCa.

Design: A total of 18 frozen prostate tissue samples (11 normal and 7 PCa), were collected from consenting patients undergoing radical prostatectomy. All tissue samples were sectioned at a thickness of 10 µm and mounted on specialized glass slides with a conductive coating. A mirror section was stained with H&E as a guide, and analyzed by a pathologist to determine tissue morphology and Gleason grading. A matrix solution of sinapinic acid (10 mg/ml) was sprayed uniformly over the tissue using the ImagePrep workstation (Bruker Daltonics). Spectra were acquired across the entire tissue area using the Ultraflex III MALDI-TOF/TOF instrument (Bruker Daltonics). Spectrum acquired at each spot, and the intensity of each signal specified was plotted as a function of the location on the tissue surface.

Results: One specific protein peak was found highly expressed primarily in tissue sections where prostate adenocarcinoma was present (7/7), whereas, only one of the normal prostate tissue samples displayed expression (1/11). Other protein peaks were also found to be differential expressed in the areas of normal prostatic tissue and prostatic stroma.

Conclusions: MALDI-MSI allows for the direct visualization of proteins and peptides in the context of the histology image. In the future, identification of the component proteins in different cell types from tumor sites should lead to the discovery of novel targets for new preventative and therapeutic strategies for PCa.

681 RNA Interference of HIF-1a Induces PC-3 Prostate Cancer Cell Death by Down-Regulation of the Inhibitor of Apoptosis Protein Family and the Bcl-2 Family Members

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Background: Hypoxia-inducible factor-1 alpha (HIF-1a) is a transcription factor, the level of which is upregulated in various tumors, including prostate cancer. Recent studies indicated that HIF-1a could directly upregulate survivin, a gene of the inhibitor of apoptosis protein (IAP) family, in addition to the more classical targets such as VEGF. We used RNA interference (RNAi) of HIF-1a to examine if other IAP family members, as well as the Bcl-2 family and the caspase family members, were under control of HIF-1a.

Design: Prostate cancer cell PC-3 was transfected with expression vector pRNAT-U6.1/Neo (GeneScript) containing an inserted sequence encoding siRNA designed for targeting HIF 1-alpha mRNA (according to the cDNA sequence NM_001530). A scrambled sequence was used as control. Stable transfectants were selected by using G418. The efficacy of RNAi was assessed by using real-time PCR and Western blotting of HIF-1a. Cell proliferation was measured by Ki67 immunocytochemistry. Apoptotic cell death was evaluated by TdT-mediated dUTP nick end-labeling (TUNEL) and caspase-3 activity assay. The mRNA and protein levels of the IAPs, the Bcl-2 family and the caspase family members were assessed by using conventional RT-PCR, real-time PCR and Western blotting.

Results: PC-3 cells with stable HIF-1a siRNA showed significantly decreased HIF-1a expression at both the mRNA and protein levels, while the control cells displayed very high expression of HIF-1a. Knocking down of HIF-1a was accompanied by decreased cell proliferation as well as increased cell death and caspase-3 activity, and higher sensitivity to flutamide treatment. However, no change was observed in expression levels of the caspases. Among the IAPs, cIAP2 and survivin displayed significant decrease in expression, but other IAPs did not. Interestingly, one of the Bcl-2 family genes, Bcl-xL, also showed significant decrease in expression.

Conclusions: Down-regulation of HIF-1a in PC-3 cells results in decreased expression of selected IAPs and Bcl-2 family members, which is correlated with increase in cell death. Studies aimed at exploring the underlying mechanisms of such down-regulation are underway.

682 Expression of Aurora Kinase B in Urothelial Carcinoma

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Background: Aurora kinase B (AURKB), a serine-threonine kinase (STK12), is a key controller of chromosome segregation during mitosis. Its activity and stability are regulated by other proteins involved in this process including survivin. Inhibitors targeting aurora kinases are in clinical trial for cancer therapy. We have previously showed survivin as a useful marker in separating low-grade (LG) vs. high-grade (HG) papillary urothelial carcinoma. To examine whether AURKB could potentially be a diagnostic marker and/or therapeutic target in urothelial carcinoma, the expression of AURKB in tissue and urine specimens was examined.

Design: Papillary urothelial carcinoma specimens were snap-frozen at the time of cystoscopic biopsy, and urine or bladder washing samples were collected from patients at the same visits. RNA expression of AURKB, survivin, and Ki67 was analyzed by quantitative RT-PCR, and their protein expression was evaluated by immunohistochemistry (IHC) using monoclonal antibodies and paraffin-embedded biopsy specimens.

Results: By IHC, normal urothelium was negative for AURKB, whereas urothelial carcinoma showed nuclear staining in from <1% to 26% of the tumor cells. Of 27 specimens (14 HG and 13 LG), HG lesions showed significantly higher percentage of AURKB positive cells ($p<0.001$), and paralleled to survivin expression in general. By qRT-PCR, expression of AURKB, survivin and Ki67 mRNA was successfully detected in all 27 tumor specimens and in all but one urine/washing specimens, with up to 100 fold differences of AURKB expression among the specimens. Strong correlations were found between all three markers, with the strongest correlation seen between AURKB and survivin mRNA, both in tumor samples ($r=0.926$) and in urine/washing samples ($r=0.904$). However, for all three markers, no significant correlation of the mRNA levels between tumor tissue and their corresponding urine samples was detected.

Conclusions: AURKB expression in urothelial carcinoma can be evaluated at both mRNA and protein levels. HG lesions showed higher AURKB expression than LG lesions by IHC, suggesting it as a new diagnostic/prognostic marker for urothelial carcinoma that can potentially be used on tissue sections in conjunction with survivin and/or Ki-67. Abundant expression of AURKB in HG also suggests it as a possible treatment target. However, no correlation of mRNA levels was found between tumors and corresponding urine samples, indicating that urine mRNA levels of these markers are not reliable diagnostic indicators at present.

683 Characterization of Renal Neoplasms Associated with Autosomal-Dominant Polycystic Kidney Disease

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Background: Autosomal-dominant polycystic kidney disease (ADPKD) is the most common renal cystic disease and accounts for 5-10% of dialysis patients. The mutations of PKD1 or PKD2 underlying this disease process have been implicated in the altered cell-cell/matrix interaction and cell proliferation that contribute to the development of cysts in kidneys. Despite early literature suggesting an increased risk of renal cell carcinoma (RCC) in ADPKD patients, it is questionable whether this disease is preneoplastic. The renal neoplasms developed in ADPKD have not been well characterized using the current standards for renal neoplasms.

Design: Archived materials of 31 kidneys from 22 ADPKD patients with unilateral or bilateral nephrectomy from 1997 to 2007 were retrieved. The pathological features of the kidneys and the coexisting solid neoplastic lesions were evaluated. Immunohistochemistry using a panel of renal tumor markers including CK7, AMACR, carbonic anhydrase IX (CA9), c-kit, vimentin and Ki-67 was performed on paraffin-embedded tissue sections.

Results: Microscopic papillary adenomas were found in 23/31(74%) kidneys with 12 (52%) demonstrating multifocality. Their sizes ranged from 0.1 to 0.5 cm. These papillary adenomas are most frequently characterized by intracystic papillae lined by a single layer of cuboidal cells with low nuclear grade and scant eosinophilc or partially clear cytoplasm, histologically slightly different from the usual type 1 papillary

adenoma. Immunohistochemically, the epithelial cells are strongly reactive for CK7, but non-reactive for AMACR, CA9, c-kit and vimentin, and showing low proliferation rate revealed by Ki-67 staining (<1%). These immunophenotypic findings also distinguished them from common subtypes of RCC as well as typical papillary adenomas which are often CK7 and AMACR positive. One case of papillary RCC was found associated with ADPKD in this series, which had homogenous papillary architectural pattern and was also CK7 (+) and AMACR (-), similar to the staining pattern of coexisting papillary adenoma in ADPKD, but in contrast to that of typical papillary RCC. On the other hand, this papillary RCC in ADPKD was still vimentin positive and had increased proliferation rate (Ki-67 5-10%).
Conclusions: There is a high incidence of small renal cell neoplasms arising in ADPKD, which are histologically and immunochemically different from renal cell carcinoma and typical papillary adenoma. Most of the ADPKD associated neoplasms are benign with a low incidence of renal cell carcinoma only found in one of 31 cases.

684 Absence of INI1 Expression in Renal Medullary Carcinoma: A Marker of Biologic Behavior Similar to Rhabdoid Tumors

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Background: Renal medullary carcinoma is a rare, highly aggressive tumor of varied histopathology which occurs in young patients with sickle cell trait or disease. The underlying molecular mechanism(s) for its aggressive behavior is unknown. Rhabdoid elements, occasionally seen in high grade renal tumors including renal medullary carcinoma, possibly represent a pathologic marker of aggressive behavior. INI1 (hSNF5/SMARCB1/BAF47) is a highly conserved factor in the ATP-dependent chromatin modifying complex; loss of this factor in mice results in aggressive rhabdoid tumors or lymphomas. Loss of INI1 expression has been reported in pediatric renal rhabdoid tumors, central nervous system atypical teratoid/rhabdoid tumors and epithelioid sarcomas. The aim of this study is to investigate INI1 expression in renal medullary carcinoma and other high grade renal tumors.
Design: 5 renal medullary carcinomas were compared with 10 high grade renal cell carcinomas and 2 urothelial carcinomas (5 with rhabdoid features). As a control, 2 renal rhabdoid tumors were included. An immunohistochemical panel, including antibody BAF47 (1:200, BD BioScience) was used.
Results: Similar to pediatric renal rhabdoid tumors, all renal medullary carcinomas, irrespective of histopathology, showed complete loss of INI1 expression. In contrast, all renal cell carcinomas or urothelial carcinomas, including those with histologic rhabdoid features, expressed this protein.
Conclusions: Rhabdoid histopathology may be a phenotypic expression of biologic aggressiveness in renal tumors only some of which lack the molecular mechanism of true rhabdoid tumors. Also, the absence of INI1 may occur in non-rhabdoid tumors such as some medullary carcinomas. In the present series, INI1 expression was absent in all renal medullary carcinomas with or without rhabdoid features, similar to its absence in true rhabdoid tumors. This study suggests that the absence of INI1 expression is independent of rhabdoid histopathology and is a marker of aggressive behavior of renal neoplasms.

685 Lymphatic Vessel Density in Radical Prostatectomy Specimens Is Not a Prognostic Factor

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Background: Formation of new lymphatic channels, or lymphangiogenesis, has been associated with poor prognosis in a number of human cancers. Its prognostic significance in prostate cancer is uncertain.
Design: We analyzed 122 radical prostatectomy specimens were analyzed. Immunohistochemistry for lymphatic vessels was performed using a mouse monoclonal antibody reactive with an O-linked sialoglycoprotein found on lymphatic endothelium (clone D2-40, Signet Laboratories, Dedham, MA, USA). The mean lymphatic vessel densities (LVD) of the three prostate compartments were compared. Lymphatic vessel densities were correlated with other clinical and pathologic characteristics.
Results: Mean values for intratumoral, peritumoral and normal prostate lymphatic vessel densities were 3.0, 5.2, and 4.8 lymphatic vessels per 200x field, respectively. The intratumoral lymphatic vessel density was significantly lower than the peritumoral or normal lymphatic vessel density (P<0.001), and the lymphatic vessel density of the latter two compartments was not significantly different (P=0.29). The intratumoral prostate lymphatic vessel density did not correlate with other clinical and pathological parameters.
Conclusions: Lymphatic vessel density is reduced in the intratumoral compartment compared to the peritumoral and normal prostate compartments, while the latter two have similar lymphatic vessel density. In contrast to other malignancies, quantitation of lymphangiogenesis in prostatic adenocarcinoma does not appear to offer useful prognostic information.

686 Gleason Percent Pattern 4/5 Predicts Cancer-Specific Survival after Radical Prostatectomy

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Background: Morphological and clinical heterogeneity within tumor grades is well recognized in prostate cancer. We sought to determine whether the combined percentage of Gleason patterns 4 and 5 in radical prostatectomy specimens is an independent predictor of cancer-specific survival in prostate cancer patients.
Design: We analyzed radical prostatectomy specimens from 504 consecutive prostate cancer patients who were treated at Indiana University Medical Center between 1990 and 1998. Various clinical and pathologic characteristics were analyzed.
Results: Higher combined percentage of Gleason patterns 4 and 5 was associated with older age, higher preoperative serum PSA level, higher pathologic stage, positive surgical margins, extraprostatic extension of tumor, higher Gleason score, perineural invasion and lymph node metastasis. In the multivariate Cox regression model, the combined percentage of Gleason patterns 4 and 5 was an independent predictor of cancer-specific survival ($P = 0.04$).
Conclusions: The combined percentage of Gleason patterns 4 and 5 is a powerful predictor of prostate cancer-specific survival. Assessment of high-grade cancer amount may allow for better stratification of patients into appropriate prognostic groups and treatment protocols.

687 H2A.X Phosphorylation in Low Grade Urothelial Carcinoma: Potential Role in Predicting Recurrence

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Background: Histone modifications have been linked to DNA replication, transcription and DNA repair. Phosphorylation of histone H2A.X at serine 139 (H2A.X-phos) is associated with DNA breaks and is involved in DNA repair. H2A.X phosphorylation has been suggested to be present in the majority of bladder urothelial carcinoma (URCa). We have previously demonstarted a lower rate of H2A.X phosphorylation in high grade (HG-URCa) compared to low grade (LG-URCa) bladder carcinomas. Here we evaluate the potential role of H2AX-phos in predicting recurrence in LG-URCa .
Design: A total of 73 cases of non invasive LG-URCa are analysed comprising two groups of cases. Group 1 included routine tranurethral biopsie sections from 31 archival LG-URCa retreived from our surgical pathology files (2004-2006). The second group consisted of tissue microarray sections constructed from transurethral biopsies from 42 LG-URCa (1975-1994) with more extended follow-up period (Mean F/U: 5 years compared to 1 year for group 1). Immunohistochemistry was performed using polyclonal antibody for H2A.X-phos (Upstate Biotech, NY). Immunoreactivity of H2A.X-phos was evaluated by two pathologists that were blinded to follow-up data. Tumor was considered to be positive if any nuclear reactivity was present.
Results: In the short term follow up group (Group 1), biopsy proven recurrence was encountered in 19/31 (61.3%) cases. Interestingly, recurrence was more likely to occur among H2A.X-phos negative cases (16/21; 76.2 %) compared to H2A.X-phos positive cases (3/10; 30%). The difference was statiscally significant ($p < 0.01$). In the long term follow-up group (Group 2), biopsy proven recurrence was seen in 24/42 (57%) cases. Again, recurrence was more likely to occur among H2A.X-phos negative cases (17/21; 81%) compared to H2A.X-phos positive cases (7/21; 33.3%). The difference was statistically significant ($p < 0.002$). Combining all LG-URCa cases from both groups (73 cases), H2A.X-phos negative cases had a statistically significant higher rate of recurrence (32/42; 76.1%) compared to H2A.X-phos positive cases (10/31; 32.3%), $p < 0.0002$.
Conclusions: H2A.X-phos positivity is associated with a significantly lower rate of tumor recurrence (32.3% vs. 76.1%, p<0.0002) in LG-URCa. H2A X-phos assessment could be of utility in predicting prognosis in low grade urothelial carcinoma.

688 MicroRNA Processing Proteins in Prostate Adenocarcinoma

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Background: MicroRNAs (miR) are small noncoding RNAs that are likely to control expression of about 30% of protein-encoding genes. Little is known about the mechanisms of microRNA regulation in normal and neoplastic tissues. It is our hypothesis that deregulation of miR-processing enzymes in prostate adenocarcinoma disrupts normal processing of a subset of miR.
Design: miR array was performed on benign (RWPE) and neoplastic (DU145) prostate cell lines. Western blot was performed on the same cell lines for PACT, SND1, and FMR1 miR-processing proteins. Cellular localization of PACT and p54 was studied by immunofluorescense in RWPE and DU145 cell lines. Finally, tissue microarray of 230 samples of normal and neopalstic prostate samples was studied for expression of PACT.
Results: In a miR array performed on RWPE1 and DU145 prostate cell lines we show 15 differentially expressed miRs. Western blot analysis showed higher PACT level in DU145 PCa cell line when compared with RWPE1 non-tumorigenic prostate cell line. Another miR–processing protein, SND1, was detected at a higher level in a DU145 cell line, also. Immunofluorescence analysis of PACT subcellular localization demonstrated the accumulation of PACT in aggregates that do not co-localize with Processing bodies (P-bodies; p54). An *in vitro* assay to study the processing of precursor miR into mature miR is presented. In benign prostate samples, PACT immunoreactivity was limited to basal cells. PACT is up-regulated in luminal neoplastic cells in a significant fraction of PCa. PACT up-regulation correlated with higher Gleason score and presence of metastatic disease.
Conclusions: The differential epxression of a subset of miR characterizes PCa. miR processing proteins are up-regulated in PCa and form cytoplasmic aggregates (PACT). PACT up-regulation correlates with a clinically agressive PCa. The knowledge of miR processing in PCa may help to diagnose PCa and predict the susceptibility of PCa to future RNA interference-based therapy.

689 Micropapillary Renal Cell Carcinoma, a Rare Aggressive Variant: Report of Five Cases

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Background: Micropapillary carcinoma is a well known aggressive histologic variant of carcinoma and has been reported predominantly from the bladder, breast, ovary, lung and recently from the colon. However, micropapillary RCC has never been described in the kidney.

Design: During review of 33 papillary renal cell carcinomas (RCC) of Asan Medical Center (AMC) and National Cancer Center (NCC) cases, we found 2 cases showing similar pictures with surface micropapillae and tight cell clusters in lacunar spaces seen in micropapillary carcinoma in the previously described organs. In addition, we found 3 more cases from the Methodist Hospital, Houston, TX. These five cases formed this study.

Results: A relative percentage of invasive micropapillary RCC was 6.0% (2 of 33 cases from AMC and NCC). The median age of those five cases was 59.0 years with age range from 32 to 78. There were 4 males and 1 female. Four patients presented with renal mass with gross hematuria and one with flank pain. Radical nephrectomy with lymph node dissection was performed in all patients. The tumor size ranged from 8 cm to 15 cm with a mean of 11.9 cm. All 5 patients had nodal metastases and 2 of 4 patients who had metastasis workup found distant metastasis. TNM stage grouping was Stage III in 1 patient and Stage IV in the remaining 4 patients. Fuhrman nuclear grade was 3 in 4 cases to 4 in 1 case and all cases were type 2 papillary RCC. Follow-up was done up to 12 months in 4 patients and revealed that 3 were alive with disease and 1 died of disease. Immunohistochemical results were similar to those of other papillary RCC on MUC1, CD10, CD117, CK7, CK20, E-cadherin, Ki-67, p53, racemase, and VEGF stainings and D240 stain revealed lymphatic invasion in all cases.

Conclusions: Micropapillary variant of papillary RCC has never been described in the kidney. This is a rare variant of papillary RCC (6.0%) and it appears to be an aggressive histologic variant with lymph node metastases in all cases. Although cases are rare with short term follow up, this variant has poor outcome with frequent distant metastasis. More detailed clinicopathologic characteristics and biologic behavior await further cases with long term follow up.

690 Histologic Features and Pitfalls of Positive Margins in Areas of Capsular Incision (CI) in Otherwise Organ-Confined Disease at Radical Prostatectomy (RP)

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Background: There is conflicting data on the prognosis of positive surgical margins due to CI in RP, in part due to differences in assessment of CI amongst pathologists.

Design: Between 1993 to 2004, we reviewed 186 RPs signed out as either: 1) CI into tumor, otherwise organ-confined (OC) (n=143); 2) Positive surgical margin (M+) in an area difficult to distinguish extra-prostatic extension (EPE) from CI into tumor, otherwise OC (n=36) or 3): Equivocal positive surgical margin (M?) in an area difficult to distinguish OC disease with tumor close to resection margins from CI into tumor, otherwise OC (n=7).

Results: The original diagnoses are listed in the column to the left with the changed diagnoses on review listed in the rows to the right.

Original diagnosis	CI M+	OC M-	EPE M+	Equivocal	Total
CI M+	119	11	10	3	143
M+ (CI v.s. EPE)	19	7	9	1	36
M? (CI v.s. OC)	4	2	0	1	7
Total	142	20	19	5	186

The 39 cases originally misdiagnosed as definitive or questionable CI with M+ were posterolateral (N=19, 48.7%), distal margin (N=12, 30.8%), posterior (N=6, 15.4%), and anterolateral (N=2, 5.1%). When tumor extended out of the prostate posterolaterally and posteriorly, there was often a fibrotic reaction, such that EPE was in fibrous not adipose tissue with the edge of the prostate noted by where condensed smooth muscle of prostatic stroma ended. Underdiagnosis of EPE resulted in overcalled CI when positive margins were present. Anteriorly, prostatic boundaries are vaguer with diagnosis of EPE often requiring seeing tumor in fat. Apically where prostatic boundries are also vague with benign glands in skeletal muscle, positive margins with EPE were diagnosed with tumor extending to the ink where benign glands were not cut across. It can be difficult to distinguish OC tumor close to the ink from CI into tumor, typically posterolaterally where urologists often try to preserve the neurovascular bundle leaving virtually no extraprostatic soft tissue on the gland in this region. The only clue to OC tumor is at low magnification the smooth rounded contour of the completely removed prostate.

Conclusions: Familiarity with different patterns of EPE in different anatomic locations and applying strict criteria for diagnosing CI into tumor can minimize overcalling CI and can provide accurate feedback to urologists to prevent iatrogenic positive margins.

691 Utility of a Combined Triple Immunohistochemical Stain Using CD44/CK20/p53 in the Diagnosis of Neoplastic and Non-Neoplastic Bladder Biopsies

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Background: The distinction between non-neoplastic and neoplastic bladder lesions is therapeutically and prognostically critical. Previous studies have demonstrated the diagnostic utility of CD44, CK20, and p53 immunohistochemistry (IHC) across the spectrum of urothelial neoplasia/dysplasia in bladder biopsies. We describe the use of triple IHC (TIHC) for CD44/CK20/P53 as a tool for detecting metasynchronous expression of all three markers in bladder biopsies.

Design: Sixty bladder biopsies were retrieved from the surgical pathology files at our institution, including 30 benign/reactive, 11 dysplasia and 19 urothelial carcinomas (9 carcinoma in situ (CIS) and 10 non-invasive papillary carcinomas). CD44/CK20/P53 TIHC (Biocare, Concord, CA) was performed and examined for each case (primary antibody dilution of CD44 1:600, CK20 1:200, P53 1:250). Membranous CD44 expression was evaluated for location in normal basal pattern or 2/3 to full-thickness urothelium staining including basal layer. CK20+ cells were assessed for location in upper third-umbrella cells, 2/3 urothelium sparing basal layer, or full-thickness stain including basal layer. P53 was scored as 0 (negative), 1+ (<15%, weak), 2+ (15-50%, moderate) and 3+ (>50%, strong).

Results: (1) Benign/reactive cases were CK20+ in umbrella cells and/or upper 1/3 urothelium in 26/30 cases (87%), and p53- in 22/30 cases (74%). Expression of CD44 was restricted to the basal cell layer in 22/30 cases (74%). (2) Among dysplastic cases, CK20 stained 2/3 to full-thickness in 7/11 cases (64%), with variable p53 staining (4/11, 36%) and loss of CD44 expression (10/11, 91%). (3) Among CIS cases, 7 of 9 (78%) had CK20+ involving 2/3 to full-thickness, and of those all (9/9) were p53+ and CD44-. (4) Among non-invasive papillary carcinomas, all (10/10) were CK20+ involving 2/3 to full-thickness and p53+, and 9/10 (90%) were CD44-.

Conclusions: (1) Benign/reactive lesions consistently show CD44+ basal cells, CK20+ umbrella cells and/or upper third urothelium, and p53 negativity. (2) Dysplastic lesions have CK20 staining involving 2/3 to full-thickness urothelium, with variable p53 staining and CD44-. (4) There is abnormal expression of CK20 and p53 with loss of CD44 in urothelial CIS and non-invasive papillary carcinomas, with 2/3 to full-thickness CK20+ and p53+ dual staining, and CD44-. (5) Our studies have demonstrated that CD44/CK20/P53 cocktail is a useful tool in the differential diagnosis of urothelial proliferative lesions.

692 Microsatellite Instability Analysis in Adenocarcinomas of the Urinary Tract

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Background: Approximately 20% of colorectal carcinomas are associated with microsatellite instability (MSI), and a proportion of these are due to Lynch syndrome. Significant morphologic overlap exists between colorectal adenocarcinoma and most adenocarcinomas of the urinary tract (AUT). It is unknown whether MSI plays any role in the pathogenesis of AUT.

Design: A tissue microarray containing samples of 34 primary AUT (15 enteric, 2 mucinous, 3 signet ring cell, 1 clear cell and 13 NOS; 27 bladder, 2 urachal, 5 urethral) was built and stained for the mismatch repair proteins MLH1, PMS2, MSH2, and MSH6. A case was considered microsatellite unstable if both MLH1 and PMS2 showed absent nuclear staining, if both MSH6 and MSH2 showed absent nuclear staining, or if either PMS2 or MSH6 showed absent nuclear staining.

Results: All cases showed presence of MSH2 and MSH6 nuclear expression. Five cases showed absent MLH1 staining, but only one of them (3%) had also absent PMS2 staining.

Conclusions: Contrary to colorectal neoplasia, MSI does not appear to play a significant role in the pathogenesis of AUT. Since absence of MLH1 and PMS2 expression can be related to methylation of the promoter of MLH1, the case presented herein does not necessarily represent a germline mutation. This limited data set would suggest that AUT may not be a neoplasm frequently associated with Lynch syndrome.

693 Prostatic Carcinoma in Young Patients: A Detailed Pathologic Study in the Era of Laparoscopic Surgery

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Background: In the United States, prostate cancer is the most common cancer affecting men, and the second leading cause of cancer-related deaths. With the advent of prostate-specific antigen (PSA) testing and increased screening, the rate of cancer detection in men younger than 50 years of age has increased from 1% to 3.7-4% since the 1970s. The recent use of laparoscopic techniques has dramatically changed the management of patients with prostate cancer. In this study, we set out to compare the pathologic findings in prostates from patients 50 years and younger versus patients older than 50 years in prostatectomies performed by laparoscopic surgery.

Design: Fifty consecutive radical prostatectomy specimens from patients 50 years and younger (Group 1) were reviewed for detailed pathologic analysis including Gleason score, surgical margin status (SM), extraprostatic extension (EPE), seminal vesicle involvement (SVI), perineural invasion (PNI), and PSA mean and median values. As a control group, 50 patients older than 50 years (Group 2) were also analyzed.

Results: Detailed results are listed in Table 1.

Table 1. Comparative Results of Detailed Pathologic Analysis Between Groups 1 and 2

	Group 1 (n=50)	Group 2 (n=50)
Gleason score		
3+3	10 (5)	18 (9)
3+4	58 (29)	48 (24)
≥4+3	32 (16)	34 (17)
tertiary	18 (9)	18 (9)
SM	24 (12)	6 (3)
EPE	18 (9)	22 (11)
SVI	4 (2)	2 (1)
PNI	60 (30)	74 (37)
PSA mean	5.4	6.6
PSA median	4.4	6

All values except PSA are %. ()=absolute numbers, SM=surgical margin, EPE=extra prostatic extension, SVI=seminal vesicle involvement, PNI=perineural involvement

No significant difference in Gleason score, EPE, SVI, PNI, and overall PSA values was observed between prostates of men in Group 1 versus Group 2. The rate of positive SM was greater in Group 1 than in Group 2 (24% versus 6%, $p<0.05$). PSA values were overall greater in Group 2 than in Group 1.

Conclusions: Unlike previous studies on radical retropubic prostatectomies that showed a lower rate of positive SM in men younger than 50 compared to older men, we found a significantly increased rate of positive SM in men younger than 50 verus older men in patients undergoing laparoscopic prostatectomies. This difference may reflect a more conservative, nerve-sparing laparoscopic approach to surgery in younger men. Despite having an overall slightly lower PSA values, PC in patients younger than 50 exhibits pathologic features and potentially biologic behavior similar to that of patients older than 50.

694 S100A1 Is a Reliable Marker in Distinguishing Nephrogenic Adenoma from Prostatic Adenocarcinoma

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Background: Nephrogenic adenoma (NA) is a benign lesion that may occur at any site of the urinary tract, usually in association with previous urothelial injuries. Although the pathogenesis of NA is still debated, recent studies seem to confirm its origin from renal tubular epithelium, rather than from a metaplastic process of urothelium. Due to its peculiar morphological features, NA could be misdiagnosed as prostatic adenocarcinoma, and immunohistochemical analyses are often needed to differentiate between these lesions. S100A1 is a calcium binding protein which has been recently reported to be expressed in renal tubules and in a subset of renal epithelial tumors.Aim of our study is to investigate the immunohistochemical expression of S100A1 in a series of NA and prostatic adenocarcinoma.

Design: A total of 15 cases of NA arising in the urinary bladder or the prostatic urethra, and 15 cases of prostatic adenocarcinoma from transurethral resection specimens or needle biopsies were retrieved from the archives of the Pathology Departments of the Universities of Sassari and Verona. Immunohistochemical analysis was performed on formalin-fixed, paraffin embedded tissue sections with a monoclonal antibody recognizing S100A1. Immunoreactions were developed using a non-biotin, highly sensitive system (Envision Peroxidase detection system, DAKO, Carpinteria, CA, USA). Cytoplasmic and or nuclear staining were considered positive. Staining intensity and percentages of positive tumor cells were scored for each case.

Results: The predominant architectural pattern was tubular or microcystic, whereas a pure papillary or mixed pattern was seen in a minority of cases. Fourteen out 15 cases (93%) of NA showed a strong, specific cytoplasmic or nucleocytoplasmic staining for S100A1; percentages of positive cells were scored as 40% (1 case), 60% (1 case), whereas the remaining 12 cases showed percentages ranging from 80 to 90%. Only one case of NA and all the prostatic adenocarcinomas included in the study were negative.

Conclusions: Our findings demonstrate that: 1) S100A1 could be considered as a reliable, specific and sensitive immunohistochemical marker to differentiate NA from prostatic adenocarcinoma; 2) since S100A1 has been reported to be expressed in renal tubules, our findings confirm the histogenetic relationship between NA and renal tubular epithelium.

695 Myofibroblasts vs. Smooth Muscle Cells and Muscularis Propria vs. Muscularis Mucosa: A Potential Role for Immunohistochemistry in Staging Bladder Carcinoma?

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Background: Although there are histological features that help distinguish bladder muscularis mucosa (MM) from muscularis propria (MP), determining the depth of invasion in bladder carcinoma can still be problematic in bladder biopsies and transurethral resection (TUR) specimens, upon which major therapeutic decisions are made. In addition, invasive carcinoma can be associated with a proliferative/desmoplastic myofibroblastic (MF) response that can resemble smooth muscle (SM) and potentially lead to over diagnosis of MP invasion. The aim of this study was to evaluate the role of immunohistochemistry (IHC) as an aid in distinguishing between MF and SM cells.

Design: The expression of vimentin (VM), smooth muscle actin (SMA), muscle specific actin (MSA), caldesmon (CAL), desmin (DES), and CD10 in MF and SM cells was evaluated by IHC in 15 cystectomy specimens with invasive urothelial or squamous carcinoma with an associated MF response. Expression was categorized as negative (0), weak (1+), moderate (2+), or strong (3+) and analyzed separately in SM cells of MM and MP. Selection of these markers was based on preliminary work evaluating 10 markers on a single case.

Results: Reactive MF cells were consistently VIM (3+) and SMA (2-3+) positive, CAL and DES negative, and with variable (1-3+) CD10 and weak MSA immunoreactivity in 11 (73%) and 8 (53%) cases, respectively. The SM cells of the MM and MP were consistently SMA (3+), MSA (3+), DES (3+) and CAL (1-3+) positive, and CD10 negative. Surprisingly, although strongly highlighting MP endothelial and endomysial cells, MP and SM cells were mostly VIM negative [only 1 case (7%) had weak staining]; this contrasted with MM and SM cells which were VIM positive (1-2+) in 9 of 11 cases (82%) ($P < 0.0002$). Details are in Table:

	VIM (%)	SMA (%)	MSA (%)	CAL (%)	DES (%)	CD10 (%)
MF (n = 15)						
0	0 (0)	0 (0)	7 (47)	15 (100)	15 (100)	4 (27)
1+	0 (0)	0 (0)	8 (53)	0 (0)	0 (0)	3 (20)
2+	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	2 (13)
3+	15 (100)	15 (100)	0 (0)	0 (0)	0 (0)	6 (40)
MP (n = 15)						
0	14 (93)	0 (0)	0 (0)	0 (0)	0 (0)	15 (100)
1+	1 (7)	0 (0)	0 (0)	1 (7)	0 (0)	0 (0)
2+	0 (0)	0 (0)	0 (0)	3 (20)	0 (0)	0 (0)
3+	0 (0)	15 (100)	15 (100)	11 (73)	15 (100)	0 (0)
MM (n = 11)						
0	2 (18)	0 (0)	0 (0)	0 (0)	0 (0)	11 (100)
1+	8 (73)	0 (0)	0 (0)	2 (18)	0 (0)	0 (0)
2+	1 (9)	0 (0)	0 (0)	2 (18)	0 (0)	0 (0)
3+	0 (0)	11 (100)	11 (100)	7 (64)	11 (100)	0 (0)

Conclusions: The findings suggest that (1) differential expression of muscle markers can help distinguish between reactive MF and SM cells in the bladder; (2) differential VIM expression may also help distinguish between SM cells of the MM and MP; and (3) that IHC is potentially useful for the staging of bladder carcinoma on biopsy and TUR specimens; additional studies are warranted.

696 Morfological Characterization and Distribution of Penile Precancerous Lesions Using a Simplified Nomenclature. A Study of 198 Lesions in 115 Patients

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Background: There is an heterogeneous spectrum of precancerous lesions affecting penile mucosal compartments with no uniformity in the nomenclature.

Design: The aim of this study was to characterize the morphological features and distribution of penile precancerous lesions using a simplified nomenclature. A total of 115 specimens with penile precancerous lesions were selected, the majority of which also had invasive squamous carcinoma (SCC; 105 cases). Penile intraepithelial neoplasia (PeIN) was classified as follows: squamous (differentiated), when predominantly composed of large keratinocytes with ample eosinophilic cytoplasm; warty, when the atypical cells showed koilocytic changes; basaloid, when predominantly composed of small cells with high nuclear/cytoplasmic ratio and round hyperchromatic nuclei. Warty-basaloid lesions had mixed features of both. Low grade lesions showed mild dysplasia affecting the lower third of the epithelium and high grade lesions included moderate and severe dysplasia affecting more than the lower third of the epithelium.

Results:

Penile Intraepithelial Neoplasia (PeIN), 198 lesions

Type of PeIN	# of lesions	%
Squmous (differentiated), low grade	89	45
Squamous differentiated, high grade	56	28
Basaloid, high grade	17	8.5
Warty, low grade	11	5.5
Warty high grade	14	7
Warty-basaloid, high grade	11	5.5

Basaloid and warty-basaloid PeIN were all high grade lesions.

Penile Intraepithelial Neoplasia (PeIN), 115 patients

Type of PeIN	# of patients	%
Squamous (differentiated)	75	65
Basaloid	12	10
Warty	3	3
Warty-basaloid	2	2
Mixed, squamous-warty/basaloid	23	20

Conclusions: Each type of PeIN revealed distinctive morphological features and combinations of various lesions in the same patient were frequent (55%). Differentiated PeIN was more prevalent than warty/basaloid variants (65 vs 35%). Considering that warty/basaloid PeIN represent precursors of HPV-related warty/basaloid invasive SCCs and differentiated PeIN represent the precursor lesion of non-HPV related keratinizing SCCs, these figures correlate with the reported incidence of HPV in penile invasive cancers of about 40%. It is important to be familiar with the sometimes subtle features of differentiated PeIN since it is considered to be a precursor of a well-characterized subset of penile SCCs.

697 PTOV-1 Expression Predicts Prostate Cancer in Men with Isolated High-Grade Prostatic Intraepithelial Neoplasia in Needle Biopsy

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Background: PTOV1 (Prostate Tumour Overexpressed One) was identified in our laboratory as a novel gene and protein during a differential display screening forgenes over-expressed in prostate cancer (CaP). PTOV1 was undetectable in normal prostate tissue and in benign prostate hyperplasia, whereas a strong immunoreactivity was showed in areas of carcinoma and high grade prostatic intraepithelial neoplasia (HGPIN). Because of the clinical management of HGPIN isolated in needle biopsy is still controversial, the present study analysed if PTOV1 expression could be a predictor factor of cancer in HGPIN diagnosed in needle biopsies.

Design: Inmunohistochemestry using a polyclonal Ab-anti PTOV1 in HGPIN areas of 139 patients was analyzed. A through positive control group of 79 radical prostatectomy specimens for CaP and a through negative one of 11 radical cistoprostatectomy specimens for bladder cancer without CaP were included. In a pilot study group of 50 patients with an isolated HGPIN at the first six core needle biopsy, CaP was detected in 11 patients (22%) with a mean of 2.5 repeated biopsies .PTOV1 expression was analysed

in all HGPIN areas in each group using a HistoScore between 0 and 300. Age, baseline PSA, PSA at the last biopsy and PSA velocity were also included as predictive variables in a statistical multivariated analysis with SPSS 12.0 program.
Results: The mean PTOV-1 expression in HGPIN lesions of positive controls was 162.6 and 67.0 in negative ones ($p< 0.001$). In patients with isolated HG-PIN the mean PTOV-1 expression was 151.4 when cancer was detected during the follow up and 94.6 when cancer could not be demonstrated ($p<0.001$). PTOV-1 expression was the only independent predictor of cancer in the multivariate analysis and the AUC was 0.803 (95%CI 0.728-0.878). A threshold of 100 for PTOV-1 expression provided 90.9% sensitivity, 51.3% specificity, 34.5% positive predictive value and 95.2% positive predictive value. Moreover, a 40% rate of non cancer biopsies had been avoided if PTOV-1 expression had been used to indicate an immediate repeat biopsy.
Conclusions: High PTOV1 expression in HGPIN is a predictive marker of cancer in needle-biopsies. In patients with an isolated HGPIN, PTOV1 expression could be used with high efficacy to warrant an immediate repeat biopsy.

698 Panel of Immunohistochemical Markers To Differentiate Metastatic Transitional Cell Carcinoma from Other Tumors with Micropapillary Histology

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Background: Micropapillary histology has been reported in TCC, ovarian serous carcinomas, and in carcinomas of breast, lung and pancreas. This knowledge is useful when observing micropapillary features in metastasis from unknown primary. Immunohistochemical staining can further help in the differentiation, and identification of the site of origin. We studied a panel of immunostains that would aid in the differential diagnosis of tumors with micropapillary histology in a metastatic setting.
Design: Cases were collected from three institutions. 25 primary tumors from breast (n=5), pancreas (n=5), ovary (n=5), lung (n=5) and bladder TCC (n=5) were included in this study. Additionally, we studied 7 cases of metastatic carcinoma with known or unknown primary, which showed a micropapillary pattern. The immunohistochemical panel used for evaluation included CK7, CK17, CK20, TTF1, WT1, p63 and estrogen receptor (ER).
Results: CK7 was positive in all the cases. All cases of TCC were positive for CK20 and 80% also showed focal positivity with p63. All ovarian serous carcinomas were positive for WT1 and 67% were also positive for ER. All cases of lung Ca were positive for TTF1. 80% of breast Ca are positive for ER. CK17 was focally positive in all ovarian serous Ca and pancreatic Ca, and in 80% of TCC. CK20 was positive in 60% of pancreatic Ca and 20% of breast Ca. In metastatic tumors, the panel was accurate in confirming the site of origin in all seven cases.
Conclusions: Micropapillary histology is a distinctive feature whose recognition can help in the identification of the site of primary. The staining patterns in all these different tumors showing micropapillary histology is quite distinctive [TCC (CK20+ and p63 focal+), serous Ca (WT-1+, ER+, CK17 focally+), breast Ca (ER+, WT1-), lung Ca (TTF1+) and pancreas (CK17 focally+, CK 20±, ER-, WT1-, TTF1-, p63-)]. The locations of metastases provide further clues, especially considering metastasis above the diaphragm (lung and breast) versus intra-abdominal (TCC, ovarian serous Ca and pancreas). A combination immunoprofile is of value in determining metastatic micropapillary TCC from other sites of primary.

699 Expression of hZip1 in Benign Prostatic Hyperplasia, Primary and Metastatic Prostatic Adeno-Carcinomas

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Background: Zinc uptake and intracellular accumulation occur as a result of activation of zinc transporters. We established that the *hZIP1*, *hZIP2* and *hZIP3* are major zinc transporters in human prostate cell lines. Alteration in these transporters becomes a major molecular target for the decrease in zinc accumulation seen in prostate cancer. The goal of the current work is to study the expression of hZIP1 in a large panel of malignant versus non-malignant prostate glands in addition to, for the first time, bone metastasis from prostate carcinomas.
Design: hZip1 protein expression in prostate tissue microarray sections (TMA) were determined by IHC using anti-hZip1 antibody. The TMA contains 26 prostate carcinomas with matched BPH, 4 tissue cores of bony prostate carcinomas metastases and 45 unmatched prostate carcinomas. hZip1 protein expression was also evaluated by IHC in sections prepared from paraffin blocks of HPR-1, BPH and PC3 prostatic cell lines. The appearance of membrane-associated immuno-positivity for hZip1 of the glandular epithelial cells were used for scoring as: negative, no; +, < 10%; ++, 10–50% and +++, > 50% positive cells.
Results: Analysis of the 26 matched cases results in a significant difference ($P<0.01$) between BPH glands (92% positive) and carcinomatous glands (54% positive) hZip1 expression. 51% (23/45) of unmatched prostate carcinoma cases are positive for hZIP1. Analysis for the presence of acini composed of >10% positive cells reveals that BPH glands exhibited this in 85% (22/26) compared to 20% (14/71) for the carcinomatous glands. The mean score of hZip1 immunoreactivity was lower (0.8) in carcinoma cases (n=71) compared to (2.4) in BPH (n=26) ($P<0.01$). No correlation was found between tumor stage and expression of hZip1; 64%, 37% and 63% of T2, T3 and T4 tumors were negative for hZip1, respectively. The 2 cases with lymph node metastases were negative for hZip1 in the primary tumor. Three out of 4 cores of bone metastasis are positive for hZip1. hZip1 is highly expressed in HPR-1, and BPH benign cell lines in contrast to low expression in PC3 prostate cancer cell line.
Conclusions: The present work consistently reveals that hZIP1 is always low expressed in malignant glandular epithelium compared to matched normsl and BPH glands. The work is in progress to further investigate the role of different zinc transporters in prostate gland carcinogenesis.

700 CAIX Expression Correlates with *VHL* Mutational Status in Sporadic Clear Cell Carcinoma of the Kidney

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Background: Clear cell renal cell carcinoma (cRCC) is the most common and most fatal kidney cancer subtype. It has been shown that Carbonic Anhydrase IX (CAIX) is expressed in RCC and that high expression is associated with improved survival and response to IL-2 based therapy. Inactivation of the von Hippel-Lindau gene (*VHL*) is known to stabilize hypoxia inducible factor (HIF) and induce the expression of hypoxia inducible genes, including CAIX. Somatic mutations in *VHL* occur in about 60% of sporadic cRCCs and novel treatments targeting the pVHL-HIF pathway show anti-tumor activity in the majority of patients with advanced RCC. To explore whether high CAIX levels are associated with *VHL* inactivation in human renal cancers, we investigated the relationship between CAIX expression, HIF1α expression and *VHL* mutational status in a series of cRCCs.
Design: Immunohistochemical analysis of CAIX and HIF1α was performed on a tissue microarray containing cores from thirty-six cRCC cases. Sequencing analysis of *VHL* was performed on the same cases. For both CAIX and HIF1α, a dichotomized variable was created that incorporated both expression intensity and percentage of positive cells. High staining was defined as a combined variable > 2. CAIX and HIF1α levels were correlated to each other and with the presence of *VHL* mutations.
Results: *VHL* mutations were detected in 20/36 tumors (55.6%). Of these mutations, 10 were frameshifts (50%), 6 missense (30%), 2 intronic (10%), 1 in-frame deletion (5%) and 1 in-frame insertion (5%). High CAIX expression correlated with presence of *VHL* mutations. 15/20 (75%) *VHL* mutant tumors expressed high CAIX compared to 5/16 (31%) *VHL* wild-type tumors ($p=0.0172$). No significant association was found between HIF1α and CAIX expression levels and between HIF1α expression levels and *VHL* mutational status.
Conclusions: CAIX expression might be utilized as a surrogate marker of *VHL* mutational status in cRCC tissues. Further studies will assess whether CAIX expression levels in cRCC can predict responses to therapies targeting the pVHL-HIF pathway.

701 Molecular Profiling of Transitional Cell Tumors of the Ovary and Bladder

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Background: Transitional cell tumors of the ovary (TCT-O) are surface epithelial tumors of the ovary with an associated benign or borderline Brenner tumor that morphologically resembles a transitional cell carcinoma of the bladder (TCC-B). Histologically, these tumors are similar, and some reported studies using immunohistochemistry to differentiate and attempts to prove their urothelial differentiation using urothelial markers have produced some conflicting results. The molecular pathology of ovarian carcinomas (OC) is heterogeneous and involves various putative precursor lesions and multiple pathways of development. The most common subtype, high-grade serous carcinoma, is characterized by p53 mutations, BRCA1 and/or BRCA2 dysfunction. Similarly; mucinous carcinomas have been shown to have KRAS mutations. WT1 is expressed at high frequency in patients with OC and has also been found to be expressed in ovarian transitional cell tumors; however the molecular changes in these tumors remains largely unknown. The development of reliable markers may be helpful in the distinction of TCT-O from a metastatic tumor TCC-B as well as from other primary ovarian tumors.
Design: 12 cases of TCT-O [1 case of malignant Brenner (MB), 10 cases of Benign Brenner's tumor (BT)] and **11** cases of TCC-B, were retrieved from our tumor registry. All tumors and corresponding normals were manually microdissected. DNA from formalin-fixed paraffin-embedded tissue (FFPE) was isolated using the DNeasy tissue extraction kit (Qiagen, USA). A Panel of 8 polymorphic microsatellite markers corresponding to p16 (CDKN2A), TP53, PTEN, and WT1 tumor suppressor genes were studied (2 more in process). Loss of heterozygosity (LOH) was determined using PCR amplification technique followed by capillary gel electrophoresis on ABI 3730 (Applied Biosystems, Foster City, CA).
Results:

	P16	PTEN	TP53	WT1
BT	LOH, 1/11(9%)	LOH, 5/11(45%)	LOH,1/11(9%)	No LOH,11/11(100%)
MB	No LOH, 1/1(100%)	LOH, 1/1(100%)	LOH, 1/1(100%)	No LOH,1/1(100%)
TCC-B	No LOH-11/11 (100%)	LOH, 7/11(64%)	NoLOH,11/11(100%)	LOH, 1/11(9%)

Conclusions: 1. There was a high frequency of LOH for the PTEN gene in all cases, and signifies this gene might have a role in carcinogenesis of these tumors. **2**. Loss of WT1 demonstrated in only one case of TCC-B, may be helpful to distinguish TCC-B from TCT-O. **3.** Loss of p16 was found only in one case of BT.

702 Detection of Cancer in Radical Prostatectomy Specimens with No Residual Carcinoma in the Initial Specimen

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Background: Radical prostatectomy (RP) specimens occasionally contain no carcinoma in the entirely submitted specimen.
Design: We evaluated 32 cases with no carcinoma in the initial slides and an additional 77 cases with minute cancer in 1 or 2 slides in the entirely submitted RP over a 2 year interval out of ~2,200 RPs. Our sequential protocol for cases with no initial tumor is:

1) review the biopsy; 2) do immunostains on suspicious foci; 3) perform levels on blocks with high grade PIN; 4) perform 3 levels on the sextant and adjacent sextant region where cancer was identified on biopsy; and 5) flip the blocks in these regions and perform 3 additional levels.
Results: The mean age was 58.6 years (41-73) with a mean PSA of 5.3 ng/ml (0.5-25.2). On review, all of the biopsies had carcinoma; 95% had a Gleason score of 3+3=6 with 3 cases Gleason score (GS)=7, 1 GS=8, and 1 GS=9. The number of positive cores was 1 [n=88 (81%)], 2 (n=18), 3 (n=2), and 3 (n=1). 54% of the biopsies had IHC for basal cells and/or AMACR. RPs on average weighed 67.7 gm (32-163). Of the 30 cases with no initial cancer, cancer was found in 22 (73%) and 8 (27%) had no residual carcinoma despite extensive leveling in all cases and IHC in 1 case. Of the 8 cases with no cancer, all biopsies had only 1 positive core with 6/8 with <10% of the core involved. IHC was done on 14/109 RP cases. Of 101 cases with cancer, 89% had cancer on only 1 slide, and 9% had cancer on 2 slides. 96% of cancers were GS=6, and the GS agreed with the corresponding biopsy in 94% of cases. In 5 cases, biopsy GS> RP GS and in 1 case biopsy GS<RP GS. In 81% of the 101 cases, RP carcinoma was ipsilateral to carcinoma in the biopsy. In 82% of the 101 cases, the location of the carcinoma in the radical was in the same or the adjacent inferior-superior sextant site. Of the 29 cases that required leveling, in 7 cases cancer was found only after flipping the blocks and doing additional levels.
Conclusions: In about 5% of RP cases, minute cancer will be found and in about 3% of RP cases, no tumor will be seen in the initially entirely submitted specimen. A methodical limited targeted approach to identifying cancer can identify cancer in 73% of the cases with no initial cancer, yet there will still be 0.4% of all RPs where cancer will not be identified. As cancer was seen in areas away from the biopsy site in some of our cases with minute tumor, leveling all the blocks may have identified cancer in some of the cases we found no tumor with our protocol.

703 Handling and Reporting of Radical Prostatectomy Specimens in Europe: A Web-Based Survey by the European Network of Uropathology (ENUP)

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Background: There has until now not been any easily accessible channel for distribution of professional information to urological pathologists in Europe or for data collection. Furthermore, most of the published data on radical prostatectomy handling and reporting is from North America and little information is available about European practices.
Design: A European Network of Uropathology (ENUP) was organized with the purpose of disseminating guidelines and consensus documents, carrying out survey studies and being a hub for research collaborations. Names and email addresses of uropathologists were collected from 321 pathology laboratories in 15 west European countries. The aim of the first ENUP survey was to collect information about handling and reporting of radical prostatectomy (RP) specimens.
Results: A total of 67.6% (217/321) of the ENUP members replied to a web-based questionnaire. Some routines were adopted by a large majority, e.g. inking of the specimen (95.6%), Gleason grading (99.5%), stratifying extra-prostatic extension (EPE) according to extent (88.2%), reporting TNM stage (88.6%) and reporting location of positive margins (98%). As many as 71.6% of respondents always embedded the entire RP specimen and only 10.8% always practiced partial embedment. Only 13.8% used special techniques for enhanced fixation and only 12.3% use special equipment (such as electric cutters or knife guides) for slicing of the prostate. The "Cone method" with sagittal sections was the most common method to cut the apex (practiced by 75.4%), somewhat less common at the base (64%). Whole mounts were used in all cases by 37.5% while standard blocks were used throughout the specimen by 55.5%. Among areas where the routines were much more variable were methods to define focal vs. extensive EPE and methods to quantify margin positivity, probably reflecting that we do not know yet which is the optimal method.
Conclusions: Some routines are almost universally adopted in Europe, while others still need to be standardized. The results of the study may be helpful when judging what recommendations are reasonable to issue.

704 Low-Grade Glial Tumors Following Chemotherapy for Primitive Neuroectodermal Tumor of Testicular Germ Cell Tumor Origin

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Background: The development of primitive neuroectodermal tumors (PNET) in metastatic sites following chemotherapy for advanced testicular germ cell tumors has been associated with a poor prognosis, with successful management in only a minority of cases by complete surgical excision. We recently observed metastatic tumors from two patients composed entirely of mature glial tissue that were excised following chemotherapy for metastatic PNET of germ cell tumor origin.
Design: A search was performed for a 17-year period for tumors of testicular germ cell tumor origin that were composed exclusively or predominantly of glial tissue.
Results: Aside from the two recent cases, no additional glial tumors following chemotherapy for PNET of testicular germ cell tumor origin were identified. Patient 1 presented at age 51 with a germ cell tumor having seminoma, teratoma, yolk sac tumor, and embryonal carcinoma components. He was treated with 2 cycles of adjuvant etoposide and cisplatin followed by excision of retroperitoneal teratoma. The patient had widely metastatic disease 28 months following orchiectomy, and PNET was excised from the perisplenic region. The patient was treated with 4 cycles of chemotherapy with alternating Cytoxan/Adriamycin/vincristine and ifosfamide/etoposide (CAV-IE), followed by 2 cycles of carboplatin and Taxol. A 5.5 cm residual retroperitoneal tumor was excised 44 months after orchiectomy and consisted solely of differentiated glial tissue. Patient 2 was 20 years old at presentation and had a germ cell tumor with yolk sac tumor, embryonal carcinoma, teratoma, and PNET components. The patient had widespread bone metastasis and 4 cycles of bleomycin/etoposide/cisplatin chemotherapy were given. A retroperitoneal recurrence at 40 months after orchiectomy was pure PNET, and he was treated with 6 cycles of chemotherapy with alternating CAV-IE. A pelvic recurrence at 71 months was treated with 4 cycles of alternating CAV-IE. A residual 1.7 cm pelvic mass was excised and was composed only of differentiated glial tissue. In both cases glial fibrillary acidic protein stains were positive.
Conclusions: Low-grade glial tumors may be seen following chemotherapy for PNET of testicular germ cell tumor origin, probably by destroying primitive cells and thereby permitting survival of differentiated elements. This finding may indicate an improved survival in these patients.

705 Incidental Prostatic Adenocarcinoma in Radical Cystoprostatectomy Specimens: The Impact of Embedding Protocols

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Background: Incidental prostatic adenocarcinoma (IPC) is found in radical cystoprostatectomies (RCP) for urothelial cancer (UC). Its reported incidence is variable and may reflect the thoroughness in the embedding of the prostate. The aim of our study has been to analyze the impact of different processing protocols on the identification of IPC in RCP for UC, and to characterize the clinico-pathological features of IPC in the different processing subgroups.
Design: This retrospective study is based on 120 RCP for UC in which the prostate had been embedded with different protocols along time: group 1, cases with extended processing (1a:Total embedding [12-28 blocs], n= 22; 1b: Extensive embedding, ≥8 blocs], n=11) and group 2, previous cases with limited prostate embedding (≤7 blocs, n= 87). Presence of IPC, tumor size, Gleason score, stage, margins, associated PIN, patient age, serum PSA, and UC stage were recorded. Statistical analysis was based on contingence tables and Fisher's test.
Results: There were 41 RCP with and 79 without IPC. The incidence of IPC was significantly higher in G1a (77.3%) and G1b (72.7%) than in G2 (18.8%) ($p<0.01$). Comparing cases with and without IPC, we found statistically significant differences in PIN (87.8% vs 27.8%; $p<0.001$) and serum PSA (3 vs 1.14ng/ml; $p<0.001$). There were no differences in age or prostate volume. 61% of IPC were clinically significant (tumor >0.5cc, Gleason score ≥7, extracapsular extension, seminal vesicle invasion, and/or positive margins). Comparing clinicopathologic features of IPC in G1 and G2 there were differences in tumor volume (G1: 0.24cc; G2: 0.08cc, p= 0.692), margin involvement (G1: 28%; G2: 18.8%; p=0.712), and associated PIN (G1: 99%; G2:75%; p=0.06).
Conclusions: IPC is found in around 70% of extensively or totally embedded RCP specimens. Interestingly, in this retrospective series, embedding ≥8 blocks yields a similar incidence than submitting the whole prostate, but this finding must be confirmed prospectively. Finding PIN in RCP should prompt an extensive embedding in search for IPC, as a substantial proportion of these cases will contain clinically significant prostatic tumors. Supported by Grants FIS 06/1411 (ISC III) and Asociacion Española Contra el Cancer.

706 Activation Status of the PI3K/AKT/mTOR Pathway in Metastatic Renal Cell Carcinomas and Their Matched Primary Tumors

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Background: Successful targeted therapy for metastatic renal cell carcinoma (RCC) has resulted in a paradigm shift in the management of this disease. Rational treatment strategy requires understanding the activation status of the putative signaling pathway being targeted at the desired stage of disease. We examined the activation state of the PI3K/AKT/mTOR/p70S6 pathway in metastatic RCC and compared it with matched primary RCC.
Design: Tissue microarray sections of 136 metastatic RCC and a subset of 25 cases with matched, primary RCC were immunostained with antibodies against PI3K, phosphorylated(p)-AKT, p-mTOR and p-70S6. The sites of metastases included adrenal, bone, brain, liver, lymph node, lung, GI tract, thyroid and skin. The histology of the primary and metastatic RCC was overwhelmingly conventional type(90%) with a median Fuhrman nuclear grade of 2.5. Immunohistochemistry on each core was scored as absent(0), weak(1+) or moderate/strong(2+). The mean score was considered positive if >1.
Results: Among metastatic RCC, immunopositivity was seen with PI3K (mean:1.7 with 78% 2+), p-mTOR (mean:1.1 with 41% 2+) and p-70S6 (mean:1.5 with 63% 2+). Primary matched RCC showed expression of PI3K (mean:1.3 with 44% 2+) and p-mTOR (mean: 1.1 with 40% 2+). Immunoexpression of pathway markers correlated with each other in metastatic RCC, ie. p-70S6 correlated with upstream p-mTOR, p-AKT and PI3K ($p<.01$). Comparison of marker expression between primary RCC and their paired metastasis, however, only showed a significant association with p-mTOR ($p=.002$). Expression of p-AKT, PI3K and p-70S6 in primary RCC *did not* correlate with their matched metastases.
Conclusions: The PI3K pathway is activated downstream to the level of the translational protein p-70S6 in metastatic RCC, thereby supporting a therapeutic role for PI3K pathway inhibitors. The differential staining of metastatic and paired primary RCC suggests that the metastatic process is associated with differential activation of this pathway that sampling of primary RCC may not accurately reflect.

707 Co-Overexpression of SmgGDS and COX-2 in Prostate Carcinoma

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Background: SmgGDS is a guanine nucleotide exchange factor with the unique ability to activate multiple small GTPases, implicating it in cancer development and progression. We previously demonstrated that SmgGDS is overexpressed in prostate carcinoma (Ca) and PIN using immunohistochemistry (IHC), indicating a potential role as a marker in the diagnosis of prostate cancer. We also showed that reduction of SmgGDS expression using siRNA in different cancer cells inhibits cell proliferation and reduces cell migration, further suggesting that SmgGDS may be a potential target in the treatment of prostate cancer. However, the molecular mechanisms of these findings are unclear. Recently, cyclooxygenase-2 (COX-2) has been believed to have an important role in the development and progression of prostate cancer, and represents an important target for treatment and prevention of prostate cancer. In this study, we tested the correlation of SmgGDS and COX-2 expression in different developmental stages of prostate cancer, and tested a causal relationship between SmgGDS and COX-2 expression in cultured prostate cancer cells.

Design: A total of 127 prostate carcinoma cases were selected, including 77 benign tissue, 47 PIN, 47 Gleason grade 3 (Ca-G3), 48 Ca-G4/5 and 11 metastatic carcinoma. Serial sections from each case were stained with monoclonal anti-SmgGDS and anti-COX-2, respectively. In functional studies, the LnCAP, PC-3 and Du-145 cell lines were transfected with SmgDGS siRNA to reduce SmgGDS expression. SmgGDS and COX-2 protein level was tested using western blotting.

Results: 1. SmgGDS and COX-2 are co-overexpressed in prostate tumors (see table 1). 2. The expression of SmgGDS and COX-2 is strongly correlated ($r = 0.65$; $p < 0.0001$). 3. Knockdown of SmgGDS inhibits the expression of COX-2.

Expression of SmgGDS and COX-2 in different types of prostate tissue by Immunohistochemistry

Tissues	N	SmgGDS positive (%)	COX-2 positive (%)
Benign	77	32 (41.6)	56 (72.7)
PIN	47	40 (85.1)	44 (93.6)
Ca-G3	47	40 (85.1)	47 (100)
Ca-G4/5	48	43 (89.6)	46 (91.7)
Metastatic	11	8 (72.7)	10 (90.9)
Total	230	163 (70.9)	203 (88.3)

Conclusions: 1. SmgGDS may participate in the development and progression of prostate cancer in part, through its ability to regulate COX-2 pathway. 2. SmgGDS may hold value as a marker for tumor diagnosis or a target for the prevention and treatment of prostate cancer.

708 Does TMPRSS2-ERG Gene Fusion Status in Prostate Cancer Correlate with Gleason Score?

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Background: Recent studies have suggested that TMPRSS2-ERG gene fusion-associated prostate cancers (PCA) may be associated with more aggressive phenotypes/poor outcomes. Conflicting data regarding Gleason score (GS) - TMPRSS2-ERG fusion correlation has been reported.

Design: We assigned GS in a blinded fashion to 512 PCA from men undergoing prostatectomy for localized disease, sampled in triplicate and arrayed in 8 tissue blocks (TMA). Fluorescence in situ hybridization (FISH) had previously been performed to delineate TMPRSS2-ERG aberrations. Fisher's exact test and logistic regression were performed for three PCA categories: TMPRSS2-ERG translocation/deletion (T/D), polyploidy (PP) ($\geq$3) of TMPRSS2-ERG, and T/D+PP. Maximum GS, based on evaluation of 3 cores, was used for analysis. Overall GS, based on review of all tissue sections, was also analyzed.

Results: 215 (41%) T/D, 30 (5.9%) PP, and 32 (6.3%) T/D+PP cases were detected by FISH. 237, 200, and 75 PCA had maximum GS of 6, 7, and 8-10, respectively. T/D: 108/237 (45.6%) GS 6, 89/200 (44.5%) GS 7, and 18/75 (24%) GS 8-10 cases had T/D ($p=0.002$). A similar result was seen when analyzing for overall GS with 71/145 (48.9%) GS 6 and 16/56 (28.5%) GS 8-10 cases showing T/D ($p=0.02$). Odds of having T/D among GS 8-10 relative to GS 6 PCA was statistically significant with an odds ratio (OR)=0.38 (95% CI 0.21-0.68; $p<0.01$). PP: For GS 6, 7, and 8-10 PCA, 6/237 (2.5%), 12/200 (6%), and 12/75 (16%) had PP ($p<0.001$). Odds of having PP among GS 8-10 relative to GS 6 PCA was also significant (OR=7.33; 95% CI: 2.65-20.31; $p<0.01$). T/D+PP: 9/237 (3.8%) GS 6, 15/200 (7.5%) GS 7, and 8/75 (10.7%) GS 8-10 PCA had T/D+PP, demonstrating a marginally significant trend toward higher GS ($p=0.052$). The odds of having T/D+PP in GS 8-10 PCA compared with GS 6 was statistically significant (OR=3.03; 95% CI: 1.12-8.15; $p=0.03$).

Conclusions: TMPRSS2-ERG gene fusion is associated with lower GS, both core-specific and overall. TMPRSS2-ERG polyploidy, with T/D or otherwise, is associated with higher GS. These findings suggest that T/D is not associated with histologic features of aggressive PCA in our patient population. Association of T/D+PP with high grade PCA may indicate that biological effects of increased TMPRSS2-ERG copy number contribute to aggressive disease or that more generalized copy number changes, including those on chromosome 21, are more frequent in PCA with aggressive histologic features.

709 Defining the Anterior Extraprostatic Space: Anatomical Considerations and Clinical Implications

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Background: Diagnosing extraprostatic extension (EPE) in prostate cancer is predicated upon finding tumor beyond condensed fibromuscular stroma at the gland periphery. In the anterior prostate, it has been observed that there is no clear demarcation separating intra- from extra-prostatic tissue. Given that one's criteria for anterior EPE will affect pathologic stage, we sought to define the composition of the anterior extraprostatic space (A-EPS).

Design: The tissue composition of the A-EPS and the proximity of anterior extraprostatic tissue to adjacent structures were detailed in 50 entirely-submitted, whole-mounted radical prostatectomies (RP) sectioned from apex to base in the transverse plane and 7 cystoprostatectomy (CYS-P) specimens in which the prostate / bladder neck were sagitally sectioned from left to right.

Results: RP: From apex through base, adipose tissue admixed with medium-sized blood vessels was visualized in the A-EPS, representing remnants of the dorsal vascular complex (DVC). From mid gland to base, well-formed medium- to large-sized smooth muscle bundles were also observed in the A-EPS, adjacent to and often interdigitating with adipose tissue. The anterior-most region of the gland did not exhibit a distinct 'capsule' in any case. Rather, it was composed of prostatic anterior fibromuscular stroma, intertwined with skeletal muscle fibers at the apex/mid gland and often merged into the smooth muscle bundles of the A-EPS at the base. CYS-P: sectioning in the sagital plane revealed that a variable number of medium- to large-sized smooth muscle bundles, anatomically extending from and morphologically identical to detrusor muscle of the bladder, cascade from the bladder neck over the anterior prostate from base to mid gland, halting abruptly at the mid gland. In all cases, this muscle was intermingled with the DVC-remnant tissue and was often situated immediately anterior to the anterior-most prostate.

Conclusions: - The A-EPS is composed of DVC-remnant adipose tissue and vessels as well as extensions of the detrusor muscle of the bladder (mid to base). - Anterior EPE should be defined as: tumor in adipose tissue and/or well-formed medium- to large-sized detrusor-like smooth muscle bundles adjacent to adipose tissue. - Akin to debate regarding clinical implications of bladder neck invasion, further study is necessary to determine whether anterior EPE of prostate cancer into detrusor muscle extensions characterized here, carries independent import for pathologic staging and clinical outcomes.

710 Leiomyosarcoma of the Urinary Bladder: A Clinicopathological Study of 25 Cases, with a Comparison of the FNCLCC, NCI, and Mayo Grading Systems

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Background: Leiomyosarcomas of the urinary bladder (LMS-UB) are rare, usually aggressive neoplasms. Owing to their rarity, only a limited number of cases with clinical follow-up information have been published. There is no current concensus on LMS-UB grading, and it is unknown whether the widely accepted FNCLCC and NCI grading systems for soft tissue sarcomas are applicable to LMS-UB. We studied 25 well-characterized LMS-UB and compared the prognostic power of the FNCLCC and NCI systems to that of one published grading scheme for LMS-UB (Mayo).

Design: All slides from 25 LMS-UB were retrieved and evaluated with regards to degree of differentiation, mitotic rate/ 10 HPF, and % necrosis. Cases were graded using published criteria for the FNCLCC, NCI and Mayo schemes. Follow-up information was obtained.

Results: The tumors occcurred in 12F and 13M, ranging from 40-91 years (median 67), and measured 2-12 cm in size. Twelve tumors were moderately differentiated and 13 poorly differentiated. Mitotic rates ranged from 1->25/10HPF (median 10/10HPF), and tumors showed 0-50% necrosis (median 20%). FNCLCC grades were 1 (2), 2 (7), and 3 (16). NCI grades were 1 (1), 2 (8), and 3 (16). Mayo grades were low (6) and high (19). FNCLCC and NCI grades were identical in 16/25 cases (64%). 4 cases were FNCLCC/NCI grade 2 or 3 and Mayo low-grade. Clinical follow-up (17 cases, range 1-120 months, mean 36.4 months, median 12 months) showed: mets (12/17, 71%), dead from disease (9/17, 53%), alive with disease (2/17, 12%), and disease free (6/17, 35%). The Table shows the relationship between grade and outcome.

LMS-UB: Grade and Outcome

Grade	Adverse Outcome	Non-adverse Outcome
FNCLCC 1	0	1
FNCLCC 2	3	2
FNCLCC 3	10	1
NCI 1	0	1
NCI 2	4	1
NCI 3	9	2
Mayo LG	2	2
Mayo HG	11	2

Conclusions: LMS-UB occurs in older adults of either sex, and is characterized by aggressive behavior, with death from disease in >50% of cases. Both the FNCLCC and NCI schemes may predict outcome, with adverse outcome noted in 13/16 (81%) of FNCLCC Gr 2+3 and NCI Gr 2+3 tumors, as compared with 0/1 FNCLCC or NCI Gr 1 tumors. However, these results are not statistically significant, because of the small number of Gr 1 tumors. Adverse outcome was seen in 11/13 (85%) of Mayo HG tumors as well as 2/4 (50%) of LG tumors, suggesting that this scheme may undergrade some potentially aggressive LMS-UB. Truly low-grade LMS-UB are extremely rare under any grading system, and great caution should be taken in making this diagnosis.

711 Mapping of *TMPRSS2-ERG* Fusions in the Context of Multi-Focal Prostate Cancer

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Background: *TMPRSS2-ERG* gene fusion leading to the androgenic induction of the *ERG* proto-oncogene expression is a highly prevalent oncogenic alteration in prostate tumor cells. Prostate cancer (PCa) is a multi-focal disease and the origins as well as biological contribution of multiple cancer foci remain unclear with respect to PCa onset or progression. To assess the role *TMPRSS2-ERG* alteration in PCa onset and/or

progression, we have evaluated the status of fusion transcript in benign glands, prostatic intraepithelial neoplasia (PIN) and multiple cancer foci of each malignant prostate.
Design: Quantitative expression of *TMPRSS2-ERG* fusion type A and C transcripts was analyzed in benign, tumor and PIN areas, selected from whole mounted radical prostatectomy slides. *TMPRSS2-ERG* expression was correlated with clinico-pathological features.
Results: Overall, 30 of 45 (66.7%) patients exhibited *TMPRSS2-ERG* fusion transcripts in at least one tumor foci. Of 80 tumor foci analyzed, 39 had *TMPRSS2-ERG* fusion (type A only: 30, type C only: 2, both type A and C: 7) with predominant detection of the *TMPRSS2-ERG* fusion type A (27/30, 90%) in the index tumors. Two of 14 (14.3%) PIN lesions were positive for type A fusion.
Conclusions: Frequent presence of the *TMPRSS2-ERG* in index tumors suggests critical roles of *ERG* alterations in the onset and progression of a large subset of PCa. However, heterogeneity of the *TMPRSS2-ERG* detection in the context of multiple cancer foci and its frequency in PIN also support the role of other genomic alterations in the origins of PCa.

712 Elevated Secreted Protein, Acidic, and Rich in Cysteine (SPARC) mRNA Expression in Neoplastic Prostate Epithelial Cells Correlates with PSA Recurrence after Radical Prostatectomy

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Background: Comparative gene expression signatures of well/moderately differentiated and poorly differentiated prostate cancer (CaP) cells along with knowledge based gene function and transcriptional regulation analyses highlighted alterations of SPARC, and genes linked to it, in poorly differentiated CaP. SPARC is a secreted glycoprotein that supports the migration of CaP cells to bone and demonstrates increased expression in CaP metastatic foci (mCaP) as well as mCaP cell lines. We hypothesized that quantitative determination of SPARC expression levels in prostate tumor cells may have potential to predict aggressive clinical behavior in newly diagnosed patients.
Design: One hundred twenty five hormone naïve post radical prostatectomy (RP) patients were studied. Patient-matched benign and neoplastic prostate epithelial cells (250 specimens) were collected with LCM from frozen tissue slides. Gene expression of SPARC (normalized to GAPDH) was measured in each specimen by Taqman quantitative RT-PCR and correlated with clinical-pathological features.
Results: Using Student t-test and ANOVA, higher SPARC mRNA expression was found in patients with overall Gleason sum of 8-9 (N=26, p=0.0061) and with poorly differentiated cells (N=23, p=0.0137). Kaplan-Meier unadjusted survival analysis revealed that patients with the highest SPARC expression across median split groups (p=0.0186) had increased risk of PSA recurrence (mean f/u 46.5 months).
Conclusions: High SPARC expression in malignant prostate cells is associated with an increased risk of PSA recurrence, with poorly differentiated cells, and with overall Gleason sum 8-9 in this patient cohort. Therefore, quantitative determination of SPARC gene expression levels in prostate tumor cells may have prognostic utility.

713 Synchronous Urinary Bladder and Prostate Cancer: A Retrospective Study

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Background: Recent studies have shown that patients with urinary bladder cancer are at increased risk of developing prostate cancer. Previous case-control studies reported that many patients with bladder cancer are also diagnosed with prostate cancer, suggesting that this comorbidity may be relatively common. The aim of the present study was to determine the incidence of synchronous prostate cancer in patients undergoing radical cystoprostatectomy for bladder cancer.
Design: Between 1992 and 2007 a total of 171 radical cystoprostatectomies were performed at our institutions, Rush University Medical Center, Chicago, IL and VA-Hospital, Houston TX. Age, tumor grade, and synchronous tumors were recorded for these cases.
Results: 59 (35%) out of 171 patients had synchronous adenocarcinoma of the prostate. The age of this group of patients ranged from 32 to 81 with a mean of 64 years. 18 (22%) patients were 60 years and younger. All 59 patients had a high grade transitional cell carcinoma. The Gleason pattern for the prostatic cancer was Gleason 6 in 39 (66%), Gleason 7 in 16 (27%) and Gleason 9 in 4 (7%) patients.
Conclusions: 1) In our study, the incidence of synchronous prostatic cancer in patients undergoing cystoprostatectomy for bladder cancer was 35%. 2) The mean age of patients with synchronous prostate cancer was 64 years with 18 patients (22%) 60 years old and younger. 3) Majority of prostatic cancers were well differentiated tumors with a Gleason pattern of 6. 4) Since about 22% of patients can be 60 years old or younger, we recommend that digital rectal examination and prostatic specific antigen (PSA) should be included as part of screening procedures for men with bladder cancer, irrespective of their age.

714 Heat Shock Proteins 27, 60 and 70 as Prognostic Markers of Prostate Cancer

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Background: Heat shock proteins (HSPs) are highly conserved, ubiquitous molecules, belonging to the family of chaperones. They also protect cells against injury associated with adverse stress. Major HSPs show an increased expression in a variety of malign tumors. Recent studies have suggested that HSPs correlate with outcome of prostate cancer. In the largest study on these proteins up to now, we aimed to investigate their value as prognostic markers.
Design: We analyzed the prognostic impact of the expression of HSP 27, 60 and 70 in primary prostate cancer samples. A tissue microarray (TMA) was constructed of radical prostatectomy (RP) specimens from 289 patients. The patients were followed 1-101 months (median 48.9). Intensity and extent of immunoreactivity (IR) and their product (IRp) were evaluated by two independent observers. The interobserver variability of the IR measures of HSP 27, 60 and 70 IR was investigated by kappa analysis.
Results: The IRp of HSP 27 and 60, but not 70 correlated with biochemical recurrence (p=0.014, 0.034 and 0.160, respectively). Recurrence-free survival in patients with strong HSP 27 and 60 IRp was shorter than in those with weak expression (p=0.019 and 0.001, respectively). HSP 27 and 60 IRp correlated with Gleason score ($p<0.01$), but not HSP 70 IRp. In multivariate analysis, HSP 60 IRp was an independent predictor for biochemical recurrence when extraprostatic extension, positive surgical margins, seminal vesicle invasion and Gleason score were included in the model as explanatory variables. Weighted kappa for interobserver agreement of HSP 27, 60 and 70 IR was 0.613 - 0.823 for intensity, 0.036 - 0.244 for extent and 0.584 - 0.719 for IRp.
Conclusions: HSP 60 is an independent predictor of biochemical recurrence-free survival after RP. For most HSP markers, the interobserver agreement was in the range of substantial to almost perfect for intensity and IRp while the reproducibility of the estimation of staining extent was poor. There is a need to further develop methods for measurement of IR in TMAs.

715 Renal Papillary Clear Cell Tumor Is a Distinct Entity in the Spectrum of Renal Cell Neoplasia: An Immunohistochemical and Cytogenetic Analysis

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Background: A group of renal tumors composed mainly of cells with clear cytoplasm arranged in papillary patterns and arising in end-stage kidneys has recently been identified. The aim of our study is to investigate the cytogenetic and immunohistochemical phenotypes of these unusual renal tumors, and of morphologically similar tumors arising in kidneys unaffected by end-stage renal disease.
Design: Seven tumors from 5 patients (age range: 53 to 64 years, mean: 60 years; 3 male and 2 female) were identified. Sections were obtained from paraffin blocks including the tumors and adjacent non-neoplastic renal parenchyma. Interphase fluorescence in situ hybridization was performed with centromeric probes for chromosomes 7, 17, Y, 3 and with a subtelomeric probe for 3p25. Immunohistochemistry was performed with antibodies against cytokeratin 7, carbonic anhydrase IX, alpha-methylacyl-CoA racemase (AMACR) and CD10.
Results: Three of the tumors arose in a kidney from a patient with end-stage renal disease. The other 4 tumors were from patients who did not have end-stage renal disease. All 7 tumors (ranging from 5 to 42 mm in diameter) were stage pT1. Six tumors lacked the gains of chromosomes 7 and 17 and losses of chromosome Y that are typical of papillary renal cell carcinoma. One tumor showed a gain of chromosome 17. Deletion of 3p, usually seen in clear cell renal cell carcinoma, was not detected. All tumors showed strongly positive immunohistochemical staining for cytokeratin 7 and carbonic anhydrase IX and negative immunostaining with antibodies against AMACR and CD10.
Conclusions: Renal papillary clear cell tumor arises in otherwise normal kidneys as well as kidneys with end-stage renal disease. Renal papillary clear cell tumor has immunophenotypic and genetic profiles distinct from those of either classic papillary or clear cell renal cell carcinoma, and should be considered a distinct entity in the spectrum of renal cell neoplasia.

716 Cytogenetic Findings of Hybrid Tumors from Renal Oncocytosis: A Comparison with Multiple Renal Oncocytomas

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Background: Renal oncocytosis is characterized by the presence of multiple tumors with oncocytic features associated with small clusters of tubule-like structures with oncocytic change. The morphologic features of the oncocytic nodules encompass a spectrum of appearances, with patterns typical of renal oncocytoma or classic chromophobe renal cell carcinoma, as well as "hybrid" tumors with features resembling both oncocytoma and chromophobe renal cell carcinoma. Based on these findings, some authors have proposed a close relationship between oncocytoma and chromophobe renal cell carcinoma, and suggested that some oncocytomas might progress to chromophobe carcinoma.
Design: This study was undertaken to elucidate the genetic pattern of 7 hybrid tumors from two cases of renal oncocytosis and to compare them with those found in 14 classical oncocytomas from 6 cases of multiple renal oncocytoma. Fluorescence in situ hybridization was performed to analyze chromosomes 1, 2, 6, 10, and 17 that are typically lost in chromophobe renal cell carcinoma. X-chromosome inactivation analyses were performed in 2 women.
Results: Twelve tumors from the patients with multiple oncocytoma showed no loss of any of the chromosomes 1, 2, 6, 10, or 17 and two tumors had loss of chromosome 1 only. Among renal oncocytosis, all three hybrid tumors from one case showed gains of all five chromosomes, one tumor from the other patient had gains of chromosomes 2 and 10. Four hybrid tumors showed strong and diffuse immunostainings for cytokeratin 7 and 2 showed positive and strong cytoplasmic reaction with Hale's colloidal iron stain. All oncocytomas showed rare or scattered cells immunoreactive for cytokeratin

7 and were negative for Hale's colloidal iron stain. A concordant pattern of nonrandom X-chromosome inactivation in the coexisting multiple lesions was seen in both cases of renal oncocytosis and multiple oncocytomas.
Conclusions: Although hybrid tumors seen in renal oncocytosis show overlapping morphologic features of oncytoma and chromophobe renal cell carcinoma, these tumors have distinct morphologic, immohistochemical and cytogenetic profiles. Our data do not support the hypothesis that these tumors represent the progression between oncocytoma and chromophobe renal cell carcinoma.

717 Clonality of Post-Radiation (RT) and Post-Prostatectomy Adenocarcinomas (adenoCAs): Some Local Recurrences Are Second Primary Neoplasms

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Background: It is generally assumed that post-therapy (RT and prostatectomy) local recurrences are due to the initial adenoCA. In the breast, about 33% of local recurrences are new second primary neoplasms. It is unknown if these relationships exist in the prostate.
Design: The clonality of 69 post-radiation therapy (postRT) prostate and 4 post-prostatectomy fossa adenoCAs were established using a 20-marker minimum allelic imbalance PCR assay using markers to genes commonly altered in prostate adenoCA. Clonality was classified as not-analyzed (no amplifiable DNA in PostRT bx due to RT-induced crosslinking), clonal, or distinct new (second) primary. postRT adenoCA morphology was classified as marked-RT effect (N=40), minimal-RT effect (N=3), or no-RT effect (N=26). Time interval between initial biopsy diagnosis and post-therapy adenoCA biopsy was recorded.
Results: PostRT AdenoCAs (N=69): 37 (53%) were clonally not-analyzed, 21 (30%) were clonal, 11 (16%) were clonally-distinct separate primary adenoCAs. Of the 40 postRT adenoCAs with marked-RT effects, 35 (87%) were clonally not-analyzed and 5 (12%) were clonal. Of the 26 postRT adenoCAs with no-RT effects, 2 (8%) were not-analyzed, 13 (50%) were clonal, and 11 (42%) were distinct separate primary AdenoCAs. All 3 minimal-RT effect postRT cases were clonal. The mean initial-postRT adenoCA time intervals were 2.35 (range, 0.81-5.02 yrs: SD=2.35 yrs) in clonal and 7.19 years (range, 4.27 – 9.92 yrs, SD=1.96 yrs) in distinct separate primary AdenoCAs ($p<0.001$). Post-prostatectomy fossa AdenoCAs (N=4): All 4 adenoCAs were clonally related to the primary adenoCA. The mean time interval of post-prostatectomy fossa adenoCAs was 2.43 years. The primary resection slides were reviewed. In all 4 cases, there was no extraprostatic extension, all margins were negative, and bilateral seminal vesicles were free of adenoCA.
Conclusions: Most postRT adenoCA with marked-RT effects and some with no-RT effects appear to be inert, evidenced by the absence of amplifiable DNA. Most post-RT adenoCAs with no or minimal RT effects detected within the first ~3 years after initial diagnosis are incompletely eradicated/persistent adenoCA (mean interval =2.4 years), whereas most late occurring (mean interval = 7.19 yrs) postRT adenoCAs are distinct second primary neoplasms. Post-prostatectomy fossa adenoCAs appear to clonally related to the primary adenoCA, despite the absence of unfavorable factors in the prostatectomy specimen.

718 Non-Bilharzial Squamous Cell Carcinoma of the Urinary Bladder: A Clinicopathological Study of 16 Cases

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Background: Urothelial carcinoma of the urinary bladder often demonstrates focal squamous differentiation. However, pure squamous cell carcinoma of the urinary bladder is rare in Western countries. We aim to study the pathological and clinical features of non-bilharzial squamous cell carcinoma in the urinary bladder.
Design: We searched our surgical pathology files from 1993 to 2007 and identified 16 patients with invasive squamous cell carcinoma in the urinary bladder. The specimens included 6 transurethral resections of the bladder tumor and 10 cystectomies. The H and E slides and clinicopathological data including follow-up were reviewed. Immunohistochemical stains for epidermal growth factor receptor (EGF-R), E-cadherin, and p53 were performed in all 16 cases.
Results: Of the sixteen patients, 8 were men and 8 were women. The average age of patients at diagnosis was 65.4 years old (range: 41-77). All tumors were pure squamous cell carcinomas. The 10 cystectomies showed muscularis propria invasion in 5 cases and invasion through the muscularis propria into perivesical soft tissue in the other 5 cases. The transurethral resections of bladder tumor also showed invasion into muscularis propria in all six cases. Five patients received radiation therapy and 4 patients received chemotherapy. The average follow-up was 29.8 months (range: 2-128). Eight of 16 patients died 3-26 months after diagnosis. Among the remaining eight patients who were alive, two developed distant metastases. Immunohistochemical studies showed positive membranous staining for EGF-R and decreased staining intensity for E-Cadherin in all 16 cases, when compared to normal urothelial cells. Positive signal for p53 in more than 20% of nuclei was observed in 11 cases.
Conclusions: Squamous cell carcinoma of the urinary bladder often presents at an advanced stage and is associated with poor prognosis. EGF-R and p53 likely play an important role in the oncogenesis and cancer progression of squamous cell carcinoma in the urinary bladder. As EGF-R is strongly expressed in these tumors, EGF-R based therapies may be of potential therapeutic benefit in patients with squamous cell carcinoma of the urinary bladder.

719 A Comparative Histopathologic Study of Prostate Cancer Treated by Conventional Radical Prostatectomy and Robotic-Assisted Laparoscopic Radical Prostatectomy: A Series of 1006 Cases

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Background: Conventional open radical prostatectomy (CRP) has been the main surgical treatment for prostate cancer. Robotic-assisted laparoscopic radical prostatectomy (RARP), a recently developed minimally invasive technique, has become a very attractive alternative. However, only a few studies have addressed the histopathologic features of prostate cancer treated by RARP and compared with that of CRP.
Design: We retrospectively reviewed the pathologic features of 1006 consecutive radical prostatectomy specimens done for prostate cancer treated by either CRP (n=603) or RARP (n=403). The histopathologic features examined were: prostate weight, tumor size, TNM stage, Gleason score, and status of surgical margins.
Results: The patient age, prostate weight and tumor size were well matched in both groups. The predominant pathological tumor stage for both RARP and CRP groups was T2 (89.3% and 76.3% respectively), and the predominant Gleason score for both groups was 7. However, the incidence of Gleason score ≥8 was 9.2% (37/401) in RARP and 21.6% (121/560) in CRP. Locally advanced tumor (≥pT3) was found in 10.7% (43/403) of RARP and 23.5% (142/600) of CRP ($P<0.0001$). Tumor was present at the inked surgical resection margin in 7.2% (29/403) of RARP cases and in 12.4% (75/603) of CRP cases ($P<0.0081$). The anterior margin was involved in 7% (2/29) of RARP and in 29% (27/75) of CRP ($P<0.018$). The apical margin was involved in 3.7% (15/403) of RARP cases and in 4% (24/603) of CRP cases. The bladder neck margin was involved in 1.7% (7/403) of RARP cases and in 1.6% (10/603) of CRP cases. The tumor extended beyond the prostatic capsule in 8.9% (36/403) of RARP cases and in 15.4% (93/603) of CRP cases.
Conclusions: The overall tumor stage and Gleason grading were similar in both RARP and CRP groups, but locally advanced disease (≥pT3) and high histologic grade (Gleason score ≥8) were more commonly found in the CRP group. RARP disclosed a decreased rate of positive margins, especially the anterior margin, when compared to CRP.

720 Increased Copy Number of TMPRSS2-ERG Fusion, but Not Translocation Alone, Is Associated with Aggressive Disease in Patients Treated by Prostatectomy

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Background: The relationship of TMPRSS2-ERG gene rearrangement in prostate cancer (PCA) to biologic behavior is uncertain. It has been variously reported that translocation (T) alone, T due to deletion (T/D) and T/D with polyploidy (PP) is associated with significantly worse survival. We analyzed TMPRSS2-ERG gene status in a large, well characterized retrospective cohort of PCA patients with long term follow-up (f/u) to evaluate associations with clinicopathologic variables and outcomes.
Design: Interphase fluorescence in situ hybridization (FISH) was performed on 8 tissue microarrays constructed from 538 patients with clinically localized PCA treated by radical prostatectomy. Outcome events included 144 patients with biochemical recurrence (BCR), 60 with metastases (M) and 55 deaths. Median f/u period was 95.5 months. FISH assay used a break apart probe with BAC DNA clones for 3' and 5' TMPRSS2 and 3' ERG. Assay was evaluable for 521 patients. Statistical correlations used Fisher's exact test.
Results: 217 (42%) of PCA had the gene fusion (T and T/D). Presence of fusion was significantly associated with low (≤6) Gleason score (GS) ($p=0.02$), but not with other pathologic variables, BCR, M or overall survival. Deletion alone was significantly associated with low GS ($p<0.01$) and absence of seminal vesicle invasion (SVI) ($p=0.02$). PP was found in 62 (12%) patients of whom 32 had concomitant fusion. Fusion with PP was significantly associated with SVI ($p<0.01$) and pathological stage ($p=0.02$). A subset of PCA with PP where deletion was seen in ≥2 copies of the gene (2D) constituted 7% of all deletion and 16% of all PP. 2D was significantly associated with SVI ($p=0.01$). Of 10 patients with 2D, 7(70%) had BCR, 4(40%) had M, and 3(30%) died, compared to 9(41%), 4 (18%) and 3(14%), respectively, of 22 patients showing fusion with PP.
Conclusions: 1. Fusion alone was associated with low Gleason score but not with other clinicopathologic variables or outcome. 2. Fusion with PP and 2D were significantly associated with higher stage. A greater proportion of patients with 2D had BCR, M and death compared to those showing fusion with PP (non-2D), although this did not reach statistical significance. 3. These findings suggest that increased copy number associated with fusion/deletion of TMPRSS2-ERG, and not fusion alone, is associated with aggressive clinical behavior of PCA.

721 A Comparison of Three Methods of Estimating Prostate Tumor Volume in Radical Prostatectomy Specimens

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Background: The volume of a prostate tumor measured following radical prostatectomy (RP) is an considered an important predictive indicator and is frequently calculated for inclusion routine RP reports. The best method of measuring tumor volume is controversial. The "gold standard": a formal morphometric method (MM) is time consuming and several alternative method have been proposed. Two methods, a "streamline" method (SM) calculating the volume from three perpendicular dimensions, and using the maximum dimension (MDM) are suggested to be a adequate proxies for the MM. To date there is only one validation each of these methods and there is no validation comparing the predictive power of any of the methods for other prognostic indicators. The aim of the study is to validate the both the SM and the MDM against the gold standard and to compare their predictive power in indicating extra-prostatic extension (EPE) and high Gleason score (>6).

Design: 129 RP specimens were fully embedded in 4mm slices and processed using conventional technology. All tumors identified by microscopy were outlined with a fine marker pen and the x and y perpendicular dimensions of the dominant tumor were measured on the glass slide. The depth (z dimension) was considered the number of blocks involved x 4mm; the streamline method volume being X x Y x Z x 0.4. Morphometric volume was calculated by scanning each slide, measuring the area of tumor in each using the software package ImagePro+, summing the areas and multiplying by 4mm. SM and MDM were compared with the MM by a scatter plot and the Spearman rank statistic. In addition the predictive power of each for extra-prostatic extension and Gleason score >6 was determined by receiver operating characteristic (ROC) curve.
Results: Both simple methods (SM and MDM) correlated well with the MM. However scatter plots showed significant outliers indicating irregularly shaped tumors. ROC curves showed all three methods significantly predicted both EPE and Gleason score >6, with each method having a similar area under the curve of approximately 0.75 (95% CIs of 0.65 to 0.85) for EPE and 0.70 (CIs 0.60 to 0.80) for Gleason score >6 indicating no significant difference between the methods.
Conclusions: Our findings suggest that both SM and MDM are adequate substitutes for the MM. The simplest method, i.e. measuring the maximum dimension, is recommended for routine surgical pathology practice.

722 Topographic Characteristics of Clinically Detected Prostate Cancer in Men under Fifty Years of Age Compared to Older Patients: Analysis of Radical Prostatectomy Specimens with Implication for Biopsy Strategies

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Background: The average age of men newly diagnosed with prostate cancer has been decreasing in recent years. The combination of wider use of PSA, increased number of biopsies from the six core sextant sampling to an average of 8-14 cores and the targeting the outer peripheral zone and the "apical/anterior horns of the prostate" has increased the efficiency of detecting smaller tumors in at the offset and improved the ability to confirm cancer diagnosis in patients who had repeat biopsies for a variety of reasons. Our objective was to investigate the potential differences in tumor volume and distribution between younger and older men with prostate cancer.
Design: The entirely submitted radical prostatectomy specimens (RPS) of patients under 50 years of age who had surgery at our institution between 1994-2005 were compared to RPS from a random subset of the older RP cohort (one of every 7 consecutive patients). Patients who had hormone and/or radiation treatment were not included. The two groups were compared with respect to pre operative PSA, tumor volume and distribution, Gleason score and pathologic stage.
Results: We identified 156 patients who had RP prior to age 50 (range 38-49 years), and 332 of the older cohort (range 50-78 years). Mean preoperative PSA for younger patients was 7.3 ng/ml (range 0.9 -39) compared to 8.9 ng/ml for the older cohort (range1.0-63). The proportion of tumors with GS =>7 was 45% in the younger group and 58% in the older patients while the proportion of pathologically organ confined disease was 59% and 48% respectively. Younger patients had significantly smaller tumors compared to older men with mean volumes of 1.9 cc (range 0.2-6.8) and 3.4 cc (range 0.2-13.7). Furthermore, a higher proportion of tumors in young patients were located exclusively within the 5 mm of the outer peripheral zone, 47% compared to 29% for the older cohort. Of interest also was the observation that while both groups often had an apical/distal one third tumor distribution, this was more evident in the younger than older men (55% vs 31% respectively). These differences were statistically significant.
Conclusions: Our data suggest that while patients under fifty years of age have a more favorable pathological parameters in their RPS, they also tend to have a more peripheral and distal/apical tumor distribution. These data may have implication for initial and particularly repeated biopsies in younger men.

723 Involvement of the Rectum by Prostatic Adenocarcinoma in Total Pelvic Exenteration Specimens

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Background: Advanced prostatic adenocarcinoma may involve the rectum and cause intractable perineal pain, requiring total pelvic exenteration for palliation. We report the clinical and pathological features of rectal involvement by prostatic adenocarcinoma in total pelvic exenteration specimens.
Design: We searched our pathology files from 1998 to 2007 and identified 18 patients who underwent total pelvic exenteration for prostatic adenocarcinoma. The original slides and clinical data including follow-up were reviewed.
Results: The average age of patients was 66.6 years (range: 56-73). The initial therapies included radiation therapy (11), radical prostatectomy (4) and hormonal therapy (3). All patients received additional hormonal therapy and chemotherapy prior to total pelvic exenteration. The specimens showed high grade prostatic adenocarcinoma (Gleason score 9 or 10) with no apparent therapy effect in 17 patients and angiosarcoma in 1 patient. The tumors diffusely involved the prostate and seminal vesicles in those patients (14) who had previously not undergone prostatectomy. The tumors invaded into bladder neck in all the patients, into rectal subserosa in 2, muscularis propria in 6, submucosa in 8 and mucosa in 2. The 2 tumors with invasion into the subserosa showed high grade prostatic adenocarcinoma without heterogeneous differentiation; Two of the 6 tumors with invasion into the muscularis propria showed neuroendocrine features. Of the 8 tumors with invasion into the submucosa, squamous differentiation was seen in 2, angiosarcoma in 1 and neuroendocrine features in 1. The 2 tumors with invasion into the mucosa showed extensive squamous differentiation. Metastatic carcinoma was found in 8 of 15 patients whose lymph nodes were dissected. After total pelvic exenteration, 9 of the 18 patients died at an average of 18.2 months (range 2 to 69). Nine patients were still alive with an average follow-up of 15.2 months (3-34) and 4 of these 9 surviving patients had developed distant metastases.
Conclusions: Rectal involvement by prostatic adenocarcinoma is associated with high-grade carcinomas showing frequent heterogeneous differentiation including squamous, neuroendocrine and sarcomatoid differentiation. Squamous differentiation is a distinctive feature in tumors invading into rectal mucosa. Prostatic carcinoma rarely involves the rectal mucosa, making its detection difficult using colonoscopy or rectal mucosal biopsies.

724 Nuclear C-MYC Protein Overexpression as an Early and Prevalent Marker of Human Prostate Carcinogenesis

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Background: C-MYC is an oncoprotein that regulates a number of cellular processes. Amplification of 8q24, encompassing the *MYC* gene, is a marker of aggressive disease in prostate cancer, and *MYC* mRNA has been previously shown to be over-expressed in prostate cancer as compared to normal prostate. Also, mice engineered to express C-MYC in their prostates develop PIN and carcinoma. Due to lack of suitable antibodies, previous studies of the cellular localisation of C-MYC protein in prostate tissue have not been consistent and the role of C-MYC during prostate carcinogenesis remains undefined.
Design: We used a new anti-C-MYC antibody for immunohistochemistry in tissue microarrays of normal, atrophy, prostatic intraepithelial neoplasia, primary adenocarcinoma, and metastatic adenocarcinoma. Genetically defined control experiments verified the antibody specificity. A total of 1896 tissue cores from 272 patients were evaluated with computerized image analysis to generate a nuclear C-MYC score.
Results: The median C-MYC score was 5.2 for normal and 5.8 for atrophy. Nearly 80% of PIN and carcinoma lesions showed elevated C-MYC protein compared to normal. The median C-MYC score for PIN was 17.1 ($p<0.0001$ vs. normal) and for primary prostatic carcinoma was 23.1 ($p<0.0001$ vs. normal). While the C-MYC score was somewhat lower, metastatic carcinoma was also elevated significantly compared to normal prostate (C-MYC score = 14.6, $p<0.0001$).
Conclusions: C-MYC protein overexpression occurred commonly in PIN, early clinically localized prostate cancers, and in hormone naive metastatic adenocarcinoma. Since C-MYC overexpression is the critical factor driving neoplastic transformation in a number of cancers, the current results suggest that deregulation of C-MYC expression is a critical, early and widely prevalent oncogenic event in prostate cancer and provide new molecular insights into prostate cancer initiation.

725 Impact of Monopolar and Bipolar Electrocautery Thermal Artifact on the Pathologic Diagnosis of Transurethral Bladder Resection Specimens

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Background: Transurethral resection of bladder (TURB) tissue by electrocautery (EC) is the usual basis for diagnosis and treatment of bladder cancer. EC causes thermal artifact (TA) that may hinder tumor grading and staging. The commonly used monopolar electrocautery (MEC) resecting loop requires hypotonic irrigation fluid, which may be associated with complications such as hemolysis, renal failure, and pulmonary/cerebral edema. The recently developed bipolar electrocautery (BEC) may have an advantage over MEC as it allows for the use of saline irrigation fluid, which is not known to be associated with these complications. Comparison of TA caused by MEC versus BEC and its impact on pathologic diagnosis has not been well studied.
Design: 81 TURB specimens were reviewed blindly. Each was assessed for usual pathologic parameters including presence of muscularis propria (MP), fragment size/uniformity, TA thickness, tissue most affected by TA, severity of tissue distortion, and diagnostic impact of TA.
Results: 37/81 (45.7%) of specimens were obtained by MEC and 44/81 (54.3%) by BEC. In both MEC and BEC cases, relatively equal numbers of tumor types, grades, stages, and fragment sizes were seen. The range in TA thickness was 0-1.0mm for MEC and 0-0.25mm for BEC. Connective tissue was the predominant TA-affected tissue in 22/37 (59.5%) of MEC and 32/44 (72.7%) of BEC cases. All 7 cases in which TA predominantly affected mucosa/tumor were MEC cases. Smooth muscle was generally preserved in both MEC and BEC cases, even in areas with TA-affected connective tissue. 8/37 (21.6%) of MEC and 11/44 (25.0%) of BEC cases showed no TA. The tissue most affected by TA was readable in 18/37 (48.6%) of MEC and 27/44 (61.4%) of BEC cases, and unreadable in 11/37 (29.7%) of MEC and 6/44 (13.6%) of BEC cases. TA caused moderate diagnostic difficulty (grading/staging difficult, but possible without immunohistochemistry) in 2/37 (5.4%) of MEC and 0/44 of BEC cases and severe diagnostic difficulty (grading/staging impossible) in 0/37 of MEC and 1/44 (2.3%) of BEC cases.
Conclusions: BEC causes less tissue distortion than MEC, which may facilitate grading and staging of TURB samples. This finding, as well as the fact that the irrigation fluid used with BEC is not known to be associated with complications, makes BEC a viable alternative to MEC. Smooth muscle, which has important staging implications, is minimally affected by TA in both MEC and BEC.

726 Transition Zone-Directed Needle Biopsies of the Prostate Uncommonly Sample Clinically Relevant Transition Zone Tumors

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Background: The utility of needle biopsies directed at prostatic transition zone (TZ-NB) has been debated. Correlation between prostate cancer (CA) detected in TZ-NB with that seen in radical prostatectomies (RP) had not been well studied.

Design: We reviewed all NB and RP slides from 61 patients in whom a CA diagnosis was made on TZ-NB. We assessed presence, extent, and location (mid +/- end) of CA on the core, Gleason score (GS), and presence/% TZ-LOOK (glands of variable size comprised of columnar cells with clear cytoplasm/basally-oriented nuclei) on NB and presence of TZ tumor, location of dominant tumor, presence/% TZ-LOOK, and NB-RP GS similarity on RP.
Results: Correlation of TZ-NB CA with Presence / Location of RP CA: - CA was detected in 25/61 (41%) left TZ-NB, 23/61 (38%) right TZ-NB, and 13/61 (21%) bilateral TZ-NB. In 8 cases the TZ-NB had the only detected CA and only 2 of these showed TZ-dominant CA on RP. - On RP, 24/61 (39.5%) cases each had either no TZ CA or non-dominant TZ CA, while 13/61 (21%) had TZ-dominant CA. - Of 38 cases with left TZ-NB CA, 18 (47%) showed either no TZ CA or right TZ CA only at RP. Similarly, 17/36 (47%) cases with right TZ-NB CA had no TZ CA or left TZ CA only. Correlation of TZ-NB CA Location on Core and TZ CA on RP:- 39, 13, and 22 cases were located in the middle, at the end(s) of, or at middle+end(s) of the core, respectively, with 23, 9, and 15 of these revealing CA in the TZ on RP. - Among the latter group, the % of dominant tumors progressively increased from 5/23 (22%) to 3/9 (33%) to 8/15 (53%). Correlation of GS on TZ-NB and RP: - 36/61 (59%) cases had matching GS between TZ-NB and dominant CA nodule on RP; for dominant TZ CA, only 4/13 (31%) had matching GS. Correlation of TZ-LOOK in TZ-NB and RP CA:- 53/61 (87%) RP cancers had either no / focal (≤ 25%) TZ-LOOK. - All 13 TZ-dominant tumors showed some degree of TZ-LOOK on RP: 8/13 >25%; 5/13 >50%. - 60/61 (98%) TZ-NB CA showed no / focal TZ-LOOK. - 6/49 (12%) NB with 0%, 6/11 (55%) NB with ≤ 25%, and 1/1 (100%) NB with > 50% TZ-LOOK were associated with TZ-dominant CA.
Conclusions: TZ-NB do not sample the TZ at all in ~ 40% of cases or a TZ-dominant CA in nearly 80% of cases. TZ-NB with CA at the middle+end(s) of a core are more commonly associated with TZ-dominant CA on RP. Greater than 25% TZ-LOOK is more commonly associated with TZ-dominant RP cancers. However, the presence of TZ-LOOK on NB is not predictive of TZ-dominant tumor on RP.

727 Identification of Histopathologic Features and Clinical Outcomes in 77 Cases of Bladder Diverticula

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Background: Bladder diverticula are frequently small and asymptomatic, and only a subset undergo histopathologic examination. Although many diverticula demonstrate benign histopathologic findings, prior reports have documented the presence of uncommon variants of bladder cancer arising within diverticula.
Design: We examined all cases of histologically sampled bladder diverticula at The Cleveland Clinic from 1981-2006 and examined patient outcomes.
Results: Demographics: 77 cases of bladder diverticula were identified. Patient age ranged from 1-81 yrs (mean 55 yrs), with 84% from adult patients. 74 diverticula were resected from males. The majority of patients presented with hematuria (n=22/51; 43%), urinary retention (n=10/51; 20%) and urinary tract infection (n=9/51; 18%). Histopathologic features: Diverticular size averaged 5.2 cm. Approximately half of all resected specimens demonstrated benign findings (n=41/77; 53%). The remaining cases (n=36/77; 47%) demonstrated neoplastic changes, including non-invasive low-grade papillary urothelial carcinoma (n=3/36; 8%), non-invasive high-grade papillary urothelial carcinoma (n=7/36; 19%), flat urothelial carcinoma in situ (n=6/36; 17%), and invasive urothelial carcinoma (n=12/36; 33%). In addition, less common variants of invasive bladder included small cell carcinoma (n=3/36; 8%), squamous cell carcinoma (n=2/36; 6%), adenocarcinoma (n=1/36; 3%) and sarcomatoid urothelial carcinoma (n=2/36; 6%). Approximately half of all carcinomas were non-invasive (n=16/36; 44%). The remainder invaded to the level of the lamina propria (n=9/36; 25%) or the perivesical fat (n=11/36; 31%). Clinical follow-up: Follow-up for benign diverticula demonstrated no subsequent neoplasm. Patient follow-up for the neoplastic group ranged from 1-126 mo (median 27 mo), with 4 cases of local bladder recurrence and 3 cases of subsequent metastases. In patients with pT1 disease, 11% (n=1/9) developed subsequent metastases. In contrast, patients with pT3 disease demonstrated a higher rate of both local recurrence (n=3/11; 27%) and subsequent metastases (n=2/11; 18%).
Conclusions: Of diverticula seen at surgical pathology, approximately half harbor neoplastic alterations, including an increased frequency of uncommon bladder cancer variants. Although patients with pT3 disease have worsened outcomes, further analysis on a larger population of patients is warranted.

728 *HER2* Amplification Affects a Subset of Metastatic Urothelial Carcinomas and Is Often Accompanied by *MYC* Amplification

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Background: Amplification and over-expression of the *HER2* proto-oncogene occurs in a variety of human cancers and often correlates with aggressive cancer growth and poor patient outcomes. In addition, *HER2* may be co-amplified with other oncogenes, such as *TOP2A* and *MYC*, which can influence long-term outcomes and response to therapy. We examined metastatic and non-metastatic urothelial carcinomas to identify the frequency of *HER2* amplification and over-expression, as well as the frequency of *TOP2A* and *MYC* co-amplification in these lesions.
Design: Paraffin-embedded material from flat urothelial carcinoma in situ (n=6; CIS), non-metastatic primary urothelial carcinomas (n=9), metastatic primary urothelial carcinomas (n=44), and paired pelvic lymph node metastases (n=42) were used for study. HER2 immunohistochemical (IHC) stains were scored according to a standardized Ventana PATHWAY HER-2/neu protocol. Fluorescent in situ hybridization (FISH) for *HER2/CEP17, TOP2A/CEP17* and *MYC/CEP8/LPL* (Abbott Molecular/Vysis; Des Plaines, IL) was scanned and scored using the Metasystems Metafer Metacyte v4.3.1.133 scanning system (Metasystems, Altlussheim, Germany).
Results: HER2 over-expression, assessed by IHC, occurred in 36% of urothelial carcinomas, including 3/9 non-metastatic and 16/44 metastatic primary lesions. Thirty percent of paired metastatic lesions showed HER2 over-expression (14/42), with an 88% concordance rate between primary and metastatic lesions. *HER2* amplification, as determined by florescent in situ hybridization (FISH), occurred in 10% of primary carcinomas and 11% of metastatic lesions, with a 100% concordance rate between primary and metastatic lesion. Normal urothelium did not demonstrate either *HER2* over-expression or amplification. Amplification of *TOP2A* was a rare event and was present in only 1 primary non-metastatic carcinoma. In contrast, *MYC* amplification occurred in 18% (7/40) of all urothelial carcinomas and in 12% of metastatic lesions, with a concordance rate of 50% between primary and metastatic lesions. Of 7 cases demonstrating *HER2* amplification, *MYC* co-amplification was present in 4 cases (56%; p=0.01), suggesting a possible cooperative effect between these oncogenes in urothelial carcinoma.
Conclusions: Amplification of *HER2* and *MYC* occurred more commonly in high-stage (pT4) carcinomas, but did not appear to be associated with either the presence of metastatic disease or long-term survival in this population.

729 Urethral Diverticula: A Clinicopathological Study of 50 Cases

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Background: Urethral diverticula are extremely uncommon and occur predominantly in females. Although the majority of surgically resected urethral diverticula are benign, reports of malignant transformation have been reported. We examined a large series of surgically resected urethral diverticula at our institution to determine associated histopathologic findings and subsequent clinical outcomes in this population.
Design: A total of 50 patients underwent urethral diverticulectomy at our institution between 1981 and 2006. The clinical findings and outcomes were determined by retrospective analysis of medical records. Pathologic slides were reviewed to determine associated histopathologic findings.
Results: Patient age ranged from 2 to 75 years (median 42 years), with peak incidence in the 3rd and 4th decades. Forty-seven patients were female and 3 patients were male. The majority of patients were Caucasian (33/50; 66%), with remainder of patients African-American (17/50; 33%). Clinical signs and symptoms were reported in 38 patients (76%). The most common clinical presentations included urinary incontinence (18/38; 47%), dysuria (14/38; 37%), recurrent urinary tract infection (9/38; 24%), pelvic and urethral pain (9/38; 24%), frequency (8/38; 21%), and dyspareunia (6/38; 16%). Diverticular size was reported in 32 cases (32/50; 64%) and ranged from 0.5 to 5.0 cm (mean 2.0 cm). Six cases circumferentially involved the urethra. On histopathologic examination, most cases demonstrated benign reactive findings, including acute inflammation (10/50; 20%), chronic inflammation (35/50; 70%), squamous metaplasia (15/50; 30%), and erosion with granulation tissue formation (16/50; 32%). Seven cases demonstrated the presence of nephrogenic adenoma (7/50; 14%). Although no carcinoma was identified in these specimens, 1 patient demonstrated extensive intestinal metaplasia (1/50; 2%) and 1 patient demonstrated a villous adenoma with low-grade dysplasia (1/50; 2%). No patient demonstrated subsequent recurrence or malignant transformation on follow-up, including the 2 patients with atypical findings on resection.
Conclusions: Urethral diverticula are uncommon findings that predominantly affect females. Most cases of surgically resected diverticula demonstrate reactive changes, including the relatively common occurrence of nephrogenic adenoma. Only 2 patients demonstrated atypical findings, including extensive intestinal metaplasia and villous adenoma. Long-term follow-up demonstrated a benign course in all patients examined.

730 Interobserver Variability (IV) between Uropathologists for Extraprostatic Extension (EPE) and Margins (M) in Radical Prostatectomy (RP) Specimens

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Background: Gleason score, EPE and M status are critical factors used to plan post-RP management of prostate cancer. Previous studies have evaluated IV between uropathologists for Gleason score, however similar data for EPE and M is sparse.
Design: A panel of 3 uropathologists selected slides from 41 RPs comprising 6 groups of 10 designated as positive, negative or equivocal for either EPE or M. Equivocal slides contained factors known to complicate EPE and M interpretation. The panel designations served as the gold standard (GS). Twelve of fifteen invited uropathologists (The GU Expert Group) assessed EPE and M on each slide by reviewing high-resolution 40x whole-slide scans via the internet. For slides with clear EPE/M diagnoses, sensitivity, specificity and % correct values with 95% confidence intervals (CI) were obtained. Multi-reader kappa (κ) values with 95% CI were calculated for all 60 slides as well as for clear GS diagnoses and EPE/M equivocal status subgroups.
Results: The data from the slide review are summarized in the table shown below.

	Specificity	Sensitivity	% Correct	κ All	κ GS Clear	κ GS Equivocal
EPE *(CI)*	87.5 *(63.6,96.6)*	95.0 *(88.0,97.8)*	91.2 *(79.2,96.6)*	0.63 *(0.60,0.88)*	0.81 *(0.63,0.98)*	0.29 *(0.00,0.57)*
M *(CI)*	97.5 *(93.7,99.0)*	83.3 *(68.9,91.9)*	90.4 *(81.6,95.3)*	0.73 *(0.60,0.88)*	0.73 *(0.53,0.93)*	0.62 *(0.31,0.93)*

Specificity, sensitivity, and % correct values were high for both EPE and M. Overall κ values were good, with κ for M being better than that for EPE. κ was higher for slides with clear GS EPE/M diagnoses than the EPE/M equivocal examples. This difference was significant for EPE and appeared to be a function of how individual pathologists define the boundary of the prostate on a given slide.
Conclusions: Uropathologists show good to excellent overall agreement when evaluating EPE and M. The lack of a clearly definable prostatic capsule can create appreciable IV concerning the interpretation of EPE.

731 End-Stage Kidney Disease: Gains of Chromosomes 7, 17 and Loss of Y Chromosome in Non-Neoplastic Tissue

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Background: Premalignant lesion in the kidney parenchyma has already been described, but there has been only little information about tubular changes in ESKD and specifically about their chromosomal alterations. The aim of this study was to determine the copy number changes of chromosomes 7, 17 and Y in non-neoplastic tubular epithelium in ESKD.

Design: Eleven patients with ESKD were detected in the files of the Departments of Pathology and Urology, Charles University Plzen. Seventeen kidneys were histologically examined. Histologic sections of formalin-fixed, paraffin embedded tissue were stained with hematoxylin and eosin. Non-neoplastic kidney tissue was examined only. Tissues from papillary adenomas or renal cell carcinomas were excluded from further molecular-biologic examination.

Results: Tubular changes in the kidney parenchyma were mapped. There were 3 major types of changes: 1) The vast majority of tubules were entirely atrophic. 2) Several tubules were hyperplastic: i.e. the tubules with undifferentiated large epithelial cells, in which it was impossible to establish specific type of renal tubulus. 3) Dysplastic tubules were dilated, sometimes wrinkled. Basal membranes were lined by large eosinophilic epithelial cells with polymorphic nuclei and pseudostratification. Nucleoli were clearly visible. These tubular changes were multifocal with a haphazard distribution to the atrophic parenchyma. Results of FISH analysis: Chromosomal abnormalities were found in the second and the third group of tubules, i.e. in the dysplastic and hyperplastic tubules. Trisomy of chromosome 7 was detected in 6, trisomy of chromosome 17 in 8 cases. A combination of both trisomies was found in 5 cases. Loss of chromosome Y was found in 2 cases.

Conclusions: It is possible to speculate that trisomies of chromosomes 7 and 17 are the early chromosomal changes in dysplastic tubules. The loss of chromosome Y could be the next step in cancerogenesis of renal papillary tumors, at least in the end-stage kidney background.

732 Renal Angiomyoadenomatous Tumor: Morphologic, Immunohistochemical and Genetic Study of 5 Cases

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Background: Kidney tumors with angiomyoadenomatous morphology have been described recently. We describe a series of the clinicopathologic, morphologic and genetic characteristics of this distinct tumorous entity.

Design: 5 cases were retrieved from consultation files of Charles University Hospital Plzen and 1 from file of Red Cross Hospital Kochi. Histologic and immunohistochemical features were evaluated. Sequencing analysis of coding region of the VHL gene was carried out.

Results: All but one patient were men with age ranging from 49 to 93 years (mean 64.6 years), the size from 1.5 to 8.5 cm (mean 3.76 cm). Tumors were greyish to tan on cut section. Tumors were composed of admixture of epithelial, clear cell component and prominent leiomyomatous stroma. Epithelial cells formed adenomatous tubular formations endowed with blister-like snouts. All tubular/glandular structures were lined by fine capillary network. Prominent leiomyomatous stroma was present in all cases. Epithelial component was positive for EMA, CK7, AE1-AE3, CAM5.2, vimentin in all cases. 3/5 cases were positive for E cadherin. Stromal component was positive only for vimentin. No or weak focal positivity was observed for CK20, racemase, CD10, CD117, HMB45, TFE3, S100. In all analyzed cases, no mutation of the VHL gene was found.

Conclusions: Renal angiomyoadenomatous tumor (RAT) is distinct morphologic entity. RAT shares clear cell component with clear renal cell carcinoma (CRCC) but the architecture is completely different. No mutation of VHL gene, common in CRCC, was found in 5/5 cases. RAT is different from mixed epithelial and stromal tumor of the kidney or cystic nephroma both in epithelial and stromal components.

733 The Incidence of TFE3 Translocation Renal Carcinoma among Adult Patients

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Background: Renal cell carcinomas (RCC) expressing the TFE3 gene fusion product, produced by the Xp11.2 translocation, are rare neoplasms, which morphologically, may mimic clear cell RCCs. These tumors are most often encountered in the pediatric population and only a few reports have been published of TFE3 (+) RCCs in adults. Thus, the incidence of this unique tumor has not yet been established by a large cohort of cases.

Design: This study included 122 adult patients with RCC, initially diagnosed as clear cell type, who were treated at Loyola University Medical Center during the period 2004-2007. All cases were positive for CD10 and vimentin. All H&E, and immunohistochemical (IHC) stained slides were reviewed and 1 paraffin section per case was stained for TFE3 IHC as previously reported [Am J Clin Path 2007;128:70]. The staining intensity was graded on a scale of 0 to 3, with 0 corresponding to no staining (negative) and grade 3 corresponding to strong positive nuclear staining (strong positive). For positive cases, at least 30% of nuclear positivity was required. Appropriate negative and positive controls were also examined.

Results: Of the 122 tumors, 18 were TFE3 positive (3+, >30% of nuclei), and 104 were considered negative (grades 0, 1, or 2 staining intensity or <30% of 3+ positivity). Only nuclear stains in well preserved areas of tumor were interpreted as positive. TFE3 (+) cases accounted for 14.75% of the total consecutively collected cases with clear cell morphology. The mean age at presentaion in TFE3 positive cases was 55 years (range 18-81 years), while the mean age of presentation for TFE3 negative cases was 59 years (range 34-81 yrs). Stratification of TFE3 positive cases by age showed 2 tumors in patients aged 18-35 years, 7 tumors from 36-55 years, and 9 tumors in patients aged >55 years. The male to female ratio for TFE3 positive cases was 2.6:1, while the ratio for TFE3 negative cases was 1.5:1.

Conclusions: This study demonstrates that a significant percentage (14.75%) of adult RCCs is positive for TFE3. While these tumors can be detected in all age groups, males were affected more frequently. The significance of a focal (<30%) and weaker stain (<3+), if any, requires further studies. Thus, in view of the higher than expected rate of positivity for TFE3, routine testing for TFE3 in tumors with clear cell morphology from adult patients may be advocated. This distinction may be clinically relevant in view of differences in patient management i.e. immunotherapy versus targeted therapies.

734 Clinicopathological Features of 22 Cases of TFE3 Renal Carcinoma from Adult Patients

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Background: Renal cell carcinomas (RCC) expressing the TFE3 gene fusion product, produced by the Xp11.2 translocation, are rare neoplasms most often encountered in the pediatric population. To date, the clinicopathologic features of these neoplasms have not been well characterized and data from adult patients is scanty.

Design: This study includes 23 RCC positive for the TFE3 gene fusion product, and collected at Loyola Univ Med Ctr, Chicago during 2001-2007. All cases showed strong nuclear staining for TFE3, in immunohistochemical (IHC) stain using the standard protocol, on paraffin sections. Appropriate (+) and (-) controls were also examined. Pathologic and clinical data were reviewed for each of the cases using archived specimen reports and electronic medical records.

Results: The patients ranged in age from 18 to 81 years, with a mean age of 52 years. The male to female ratio was 3.4:1. Twelve tumors were in the right kidney and 10 in the left kidney; there were no bilateral tumors. The average tumor size at presentation was 10 cm in greatest dimension. In addition to clear cell features, 6 tumors exhibited a sarcomatoid component, 5 had eosinophilic cytoplasm, 2 had focal papillary architecture, and 1 was cystic; 4 exhibited varying combinations of the above morphologies. The Fuhrman grade was: 4 (x10), 3 (x 10) and 2 (x 3). All tumors were positive for vimentin and CD10. CK7, EMA, and high molecular weight keratin were all negative when tested; stain for pankeratin, when tested, showed only focal positivity. At presentation, solid organ metastases were in 9 cases (in 3 patients combined with nodal metastases), isolated lymph nodes metastases were in 3 cases, rest negative or unknown. Clinically, presenting symptoms included flank/abdominal/back pain, hematuria, and weight loss; in 3 cases the renal tumors were incidental findings, and in 3 cases unknown. Only 5 patients were alive without recurrence and 2 were alive with metastases while 14 were discharged with extremely poor performance status; 1 additional patient died of cardiac etiology.

Conclusions: There was a male predominance, ratio > 3:1. Symptoms at presentation were not significantly different from the classic triad; 14% were incidental findings. There was a tendency for a large tumor size at presentation, as well as a high nuclear grade. Tumors exhibited multiple morphologies rendering this characteristic unreliable for identification. Overall, the tumors showed aggressive clinical nature, in particular in patients with solid organ metastases.

735 Frequency of Expression of Estrogen Receptor Subunit Alpha on TMPRSS2:ERG Positive Prostate Cancer and Its Association with Aggressive Disease

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Background: The fusion of the androgen-regulated TMPRSS2 promoter with ETS transcription factors is found in the majority of prostate cancer (PCa). Recent work suggests that the TMPRSS2-ERG fusion is associated with an aggressive phenotype and we were able to identify an association of TMPRSS2:ERG fusion and estrogen receptor (ER) signaling. More specifically, the subunit alpha of ER (ERa) appeared to modulate TMPRSS2:ERG status. In the current study we are analyzing the frequency of ERa expression in a high-risk PCa cohort, its impact on disease-free recurrence and its association with aggressive disease.

Design: We analyzed 111 patients for TMPRSS2:ERG status using fluorescent in-situ hybridization and for ERa expression using immunohistochemistry and an automated microscope system (Ariol). Mean age of our cohort was 63.3 years (48.6-76.1), 17/101 (17%) patients had a Gleason score (GS) of <7, 29/101 (29%) of 7, and 55/101 (55%) of >7 (with 10 patients with missing data). Lymph node metastases at time of radical prostatectomy were present in 59/108 (53%) of patients (with 3 cases with missing data). 54/111 (49%) of patients were fusion positive. Median follow up was 22.83 months (0.2-151 mo). ERa expression showed a bimodal distribution and we divided our cohort into ERa positive (>mean) and ERa negative (<mean) patients.

Results: ERa positivity was identified in 24/54 (44%) TMPRSS2:ERG2 patients. Its expression was significantly associated with higher Gleason scores (r=0.35, p=0.034) and development of lymph node metastases (r=0.39, p=0.021). Of 21 TMPRSS2:ERG positive and ERa positive patients, 8 (38%) developed PSA recurrence whereas only 5 of 22 (18.5%) TMPRSS2:ERG positive and ERa negative patients relapsed. Median survival was 4 months for TMPRSS2:ERG positive and ERa positive patients and 18.9 months for TMPRSS2:ERG positive and ERa negative patients.

Conclusions: Our study suggests that ERa overexpression is present in half of TMPRSS2:ERG positive patients in this high risk cohort. ERa expression appears to be associated with aggressive disease demonstrated by its association with higher Gleason scores and presence of lymph node metastases. We also observed a marked

difference in failure rates and time to recurrence between ERa positive and negative patients. These results warrant a further investigation of effect modulation of TMPRS2: ERG through steroid receptors.

736 Surgical Resection Adequacy in 500 Robotic Radical Prostatectomies

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Background: Robotic-assisted laparoscopic radical prostatectomy (RRP) is a minimally invasive alternative for the treatment of prostate cancer and is now becoming increasingly popular. Limited information exists regarding surgical margins and adequacy of surgical resection in RRP.

Design: To avoid selective bias 500 consecutive RRP performed between 7/2005 and 10/2006 at our institution were evaluated for tumor grade, extraprostatic extension, seminal vesicle involvement, frequency and site of capsular incision, resection margin status, and location and amount of attached periprostatic fatty tissue. Capsular incision was defined as that benign glands expose to the inked resection margin due to surgical cut of capsule.

Results: Gleason score ranged from 5 to 10 (10 with score 5, 203 with score 6, 236 with score 7, 23 with score 8, 24 with score 9, 1 with score 10, 3 with treatment and not graded). 87 cases had extraprostatic extension, and 42 seminal vesicle involvement. 68 cases showed evidence of capsular incision (64 definitive and 4 equivocal). Of these, the incision was seen at the apex in 44 cases, at the mid in 21, and at the base in 4 (one case with two sites). Of 64 cases with positive margins, 39 showed positivity at the apex, 20 at the mid and 7 at the base (some cases with more than one positive margin). In 33 cases the positive margins were seen at sites of capsular incision. The percentage of prostate circumference covered with attached periprostatic fibroadipose tissue ranged form 0% to 100% (mean 27%, median 20%). The mean circumference covered for the 64 cases with positive margins was 19.2%, compared to 28.5% for the 436 cases with negative margins (p=0.0002). The average circumference covered for the 68 cases with capsular incision was19.0%, compared to 28.5% for the 432 cases without capsular incision (p=0.0002).

Conclusions: In RRP the most common site of both capsular incision and margin positivity is at the apex. Attached periprostatic fatty tissue is more commonly present at the anterior, right posterior lateral and left posterior lateral aspects of the gland. The presence of fibroadipose tissue correlates with less incidence of capsular incision or margin positivity. This systematic analysis can provide useful information to improve surgical management of prostate cancer with RRP.

737 Prospective Characterization of the Diagnostic Utility of p63/AMACR Cocktail Immunohistochemistry in the Contemporary Evaluation of Prostate Needle Biopsies

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Background: Immunohistochemistry (IHC), utilizing basal cell marker/AMACR antibody cocktails, is increasingly being utilized as an aid in the diagnosis of prostatic adenocarcinoma (PCa); however, to our knowledge there are no prospectively designed studies that analyze its use in routine daily practice.

Design: A prospective study was designed to evaluate the frequency of using a p63/AMACR antibody cocktail in the evaluation of routine prostate needle biopsies (PNBs) as utilized by a group of surgical pathologists in a large academic medical center. In addition, the utility of p63/AMACR cocktail IHC for the diagnosis of PCa as performed on saved un-stained interval sections (IS) was compared to that performed on recut sections (RS) from the block; these 2 methods were performed together on a subset of cases and both sections were made available to the primary pathologist at the time of original diagnosis.

Results: Over a one year period, 682 consecutive PNB cases were evaluated. Of these, 376 (55.1%) were benign, and 232 (34%), 46 (6.7%) and 28 (4.1%) were diagnosed as PCa, focal glandular atypia (FGA), and high-grade prostatic intraepithelial neoplasia (HGPIN), respectively. IHC was performed on 73 (10.7%) of all specimens, including 3 (0.8%), 45 (19.4%), 17 (37%), and 8 (30%) of the cases diagnosed as benign, PCa, FGA, and HGPIN, respectively. Forty two (93%) of cases of PCa diagnosed with the aid of IHC had a Gleason grade of 3+3 = 6, the remainder had a grade of 3+4 = 7; 41 (91%) cases involved a single core and 38 (84%) were <1mm in length. The p63/AMACR cocktail, performed on IS, was utilized as an aid to the diagnosis of 31 cases of PCa, and in 2 (6%) cases was non-contributory due to loss of tissue from slides; 21 cases had cocktail IHC also performed on RS, of which 11 (52%) were non-contributory due to sectioning through and loss of the focus of concern ($P < 0.001$).

Conclusions: (1) The frequency of using IHC in the evaluation of PNBs in this study (~11%) is half of what has been reported in a prior prospective study; (2) IHC is most frequently used to aid in the diagnosis of minimal and non-high grade (< Gleason score 7) PCa; and (3) use of saved unstained interval sections for IHC is significantly superior to using recut sections from the block.

738 The Telomere Shortening in Prostatic Atrophy Lesions

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Background: Telomeres stabilize the ends of chromosomes, but progressively shorten with cell division. Telomere shortening and dysfunction have been implicated in human carcinogenesis. Using a fluorescence in situ hybridization for assay for telomere length (Tel-FISH) assessment, telomere lengths are reduced in most cases of prostate cancer and high grade prostatic intraepithelial neoplasia (PIN), as compared to matched normal appearing epithelium. Focal areas of epithelial atrophy exist as a number of different histological patterns, some of which have been implicated in prostatic carcinogenesis. The aim of this study was to evaluate the telomere lengths in prostatic atrophy lesions.

Design: Focal atrophy lesions were classified into simple atrophy (SA), simple atrophy with cyst formation (SACF), post atrophic hyperplasia (PAH) and partial atrophy (PA). We used a recently developed chromogenic in situ telomere length assay to evaluate telomere shortening by light microscopy on high density tissue microarrays containing matched atrophy (mostly simple atrophy) and normal tissues from radical prostatectomies. To enrich for cases with simple atrophy with cyst formation and simple atrophy merging with PIN and/or carcinoma, additional standard tissue sections were used. Any lesion considered short by the chromogenic assay was verified by the traditional FISH assay. Using TMAs 214 cores from 20 patients were analyzed. On standard sections 113 different areas from 20 patients were evaluated.

Results: Using the TMAs, 2 of 115 spots (1.7%) of SA had short telomeres and 3 of 13 spots (23.1%) of SACF were short. Using the standard sections, 9 out of 11 (81.8%) SA merging with PIN or cancer lesions and 18 out of 26 (69.2 %) SACF lesions showed short telomeres, respectively. 2 out of 10 (20 %) PAH lesions had short length in telomere. 6 out of 7 lesions of low grade PIN, all of the 20 lesions of high grade PIN and all of the 13 lesions of cancer had short telomeres.

Conclusions: This is the first report that telomere lengths of prostatic atrophy lesions were evaluated using in situ hybridization. Telomere shortening was seen commonly in SACF and much less frequently SA unless this latter lesion was directly merging with PIN or cancer. These findings are consistent with certain types of focal atrophy lesions as possible precursor lesions of PIN and prostate cancer.

739 EGFR v III Mutation and EGFR Expression in Urothelial Carcinoma of Urinary Bladder: High EGFR Expression Is Not Associated with EGFR v III Mutation

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Background: Epidermal growth factor receptor (EGFR) is a receptor tyrosine kinase member of the ErbB family. Anti-EGFR receptor tyrosine kinase inhibitors (TKI), Gefitinib and Erlotinib, are low-molecular-weight ATP-competitive inhibitors that have been used with some success in the treatment of non small cell lung carcinoma (NSCLC) and other solid tumors. The presence of somatic EGFR variant III mutations has been shown to be predictive of response to TKI in NSCLC. Clinical trials of TKI agents in patients with advanced urothelial carcinoma (UrCa) are underway. The current pilot study assesses EGFR mutation status in UrCa.

Design: 22 archival cystectomy specimens were analyzed. Representative paraffin sections from 18 invasive high grade UrCa, 3 papillary non invasive UrCa and one flat CIS and one metastatic UrCa were mapped for tumor areas and microdissected for DNA extraction using Zymo Pinpoint Isolation System. Two PCR reactions were performed on each sample for EGFR exon 19 deletion and exon 21 L858R mutation analyses using previously described primers and parametrs. PCR products were analyzed using ABI3100 genetic analyzer with Gene Scan 3.7 software (Applied Biosystems). For Exon 21 L858R mutation, PCR product was purified using QIAquick Columns and digested with 2U Sau96I enzyme prior to analysis on ABI3100. Parallel paraffin sections from all 22 cases were immunostained using monoclonal antibody for EGFR (Dako, CA). Intensity and distribution of EGFR expression were assessed. Tumors were then classified as negative, low (1+ in 5% cells) or high (any 2+ and or 3+ staining) EGFR expressors.

Results: Mutation analysis was successful in 20/22 paraffin samples. None of the 20 informative UrCa samples revealed any evidence of EGFR Exon 19 deletion or Exon 21 L858R mutation. EGFR expression was high in 11/22 (50%) tumors, low in 5 and absent in the remaining 6 tumors.

Conclusions: Mutation analysis of EGFR exons 19 and 21 is feasible in microdissected paraffin sections from UrCa archival tissue. We found no evidence of EGFR exon 19 deletion nor exon 21 L858R mutation in any of the 20 UrCa studied including 10 with high EGFR protein expression. Analysis of larger number of samples is needed. Although the possibility of other types of mutations exists, our results suggest a low rate of studied EGFR mutations in UrCa. Other mechanisms of EGFR activation such as gene amplification should also be investigated in this setting.

740 Expression of Mammalian Target of Rapamycin (mTOR) and Phosphorylated – mTOR (p-mTOR) in Prostatic Adenocarcinomas (PACs)

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Background: Mammalian target of rapamycin (mTOR) regulates several cell functions critical to tumorigenesis including proliferation, growth, mobility and survival. The mTOR signaling cascade is reportedly activated (p-mTOR) through translational and phosphorylation mechanisms. Expression of this protein and dysregulation of its signaling pathway has been reported in several malignancies including carcinomas of breast, head & neck, liver and kidney, making it a potential target for anti-cancer therapy. The expression of p-mTOR has not been studied in detail in PAC.

Design: Formalin-fixed paraffin-embedded tissue sections from 132 PACs were immunostained by an automated method (Ventana Medical Systems, Tucson, AZ) using monoclonal rabbit anti-human mTOR and p-mTOR antibodies (Cell Signaling, Danvers, MA). Immunohistochemical assessment included intensity and percentage of positive cells in both benign and carcinomatous elements. Immunoreactivity for mTOR was essentially cytoplasmic, while p-mTOR expression was cytoplasmic with membranous accentuation. DNA ploidy was determined on Feulgen stained tissue sections by static image analysis. Results were correlated with morphologic and prognostic variables.

Results: Variable immunoreactivity was noted for both the tumor and adjacent benign glands for both proteins in all cases. mTOR tumor immunoreactivity was either increased or similar to the adjacent benign tissues in all cases, while p-mTOR expression ranged from increased, similar to, and decreased compared to the benign. Increased expression of mTOR correlated with high tumor grade (25/55 (45%) HG vs 18/77 (23%) LG, p=0.008). 25/55 (46%) high grade PACs showed loss of p-mTOR relative to benign glands as compared to 10/77 (13%) low grade tumors (p<0.0001). No correlations were found between mTOR and p-mTOR expression with tumor stage, aneuploidy or disease recurrence.
Conclusions: Total mTOR protein is increased in a subset of PACs. Activated mTOR protein - defined by phosphorylation of mTOR at Serine-2448 (p-mTOR) – is decreased in PACs. These findings suggest a potential role for mTOR dysregulation in prostate tumorigenesis and support the currently ongoing clinical trials investigating the therapeutic relevance of rapamycin analogs in the treatment of high grade PACs.

741 Fatty Acid Binding Protein 7: A Novel Marker for Clear Cell Renal Cell Carcinoma

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Background: Clear cell renal cell carcinoma (RCC) is responsible for much mortality and morbidity in patients with renal cancer. Advances in gene expression microarray technology have led to progress in identifying specific markers for renal cancer. However, many of the markers reported are also expressed in normal kidney. Using computer analysis of a large number of gene expression microarray data, we identified fatty acid binding protein 7 (FABP7) as one of the genes over-expressed in clear cell RCC compared to normal kidneys and other renal neoplasms. In this study, we examined the expression of FABP7 at mRNA and protein levels using gene expression microarrays and immunohistochemistry.
Design: FABP7 mRNA levels from 317 renal neoplasms, including 174 clear cell RCCs, were obtained from gene expression microarrays. Also, immunohistochemistry using an antibody specific for FABP7 was performed on 68 renal neoplasms, including 30 clear cell RCCs. The staining was interpreted as positive or negative; for the positive sections, the staining was interpreted as focal or diffuse.
Results: From expression microarrays, the FABP7 mRNA level (signal intensity units) was measured for each case. Mean values were obtained for each group. The mean FABP7 mRNA values were standardized using 1.0 for normal kidneys (n=15). The results of tumor groups were as follows: 61.62 (folds) for clear cell RCC (n=174), 4.92 for papillary RCC (n=51), 2.74 for Wilms tumor (n=32), 1.38 for chromophobe RCC (n=24), 1.35 for urothelial carcinoma (n=14), 1.23 for collecting duct carcinoma (n=4), and 1.12 for oncocytoma (n=18). By immunohistochemistry, FABP7 reactivity was absent in normal kidney tissues and was found in 73.3% of clear cell RCCs (22/30) with 15 cases showing diffuse staining. In contrast, 7.1% of papillary RCCs (1/14) showed reactivity. None of the chromophobe RCCs (0/11) or oncocytomas (0/13) showed reactivity.
Conclusions: Using gene expression microarrays and immunohistochemistry, we demonstrated high levels of FABP7 expression in the majority of clear cell RCCs but not in other types of kidney tumors and normal kidneys (sensitivity 73% and specificity 98%). The presence of FABP7 in clear cell RCC may be related to its function in fatty acid uptake, transport, and metabolism during tumor development. Our findings also suggest that FABP7 is a promising diagnostic marker for clear cell RCC.

742 Expression of FXYD3 in Urothelial Carcinoma

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Background: FXYD3 is a membrane protein functioning as an ion channel regulator. However, overexpression of FXYD3 is found in several types of cancers including breast, colon, and pancreatic carcinomas, which suggests that it may be important for cell differentiation and proliferation. In this study, we examined the expression of FXYD3 using gene expression microarrays and immunohistochemistry in order to evaluate its value as a marker for urothelial carcinoma.
Design: FXYD3 mRNA levels were obtained from the gene expression microarray data in 14 urothelial carcinomas. Immunohistochemistry using a polyclonal antibody specific for FXYD3 was carried out on paraffin-embedded tissue sections of 70 urothelial carcinomas, including 33 low-grade urothelial carcinomas and 37 high-grade urothelial carcinomas of the bladder. The staining was interpreted as positive or negative, and for the positively-staining sections, the staining pattern was interpreted as focal or diffuse.
Results: FXYD3 was one of the top three genes over-expressed in 14 urothelial carcinomas in Affymetrix HGU133 2.0 arrays examined, with a fold change of 10.5 (p= 0.0006) relative to its expression in 15 normal control samples. By immunohistochemistry, FXYD3 reactivity was found in 62.9% of urothelial carcinomas (44/70) with 9 sections demonstrating focal staining. Of the low-grade urothelial carcinomas, 39.4% (13/33) showed reactivity, while 83.8% (31/37) of the high-grade urothelial carcinomas showed FXYD3 immunoreactivity.
Conclusions: We confirmed the overexpression of FXYD3 in urothelial carcinoma. Furthermore, by immunohistochemistry, FXYD3 reactivity was demonstrated in a greater percentage of high-grade urothelial carcinomas as compared to low-grade urothelial carcinomas. Our findings suggest that FXYD3 may be related to urothelial carcinoma development and progression. The diagnostic value of this marker needs further evaluation.

743 Prognostic Value of p16, PTEN, E-cadherin, and bcl-2 Expression in High Risk Prostate Cancer of Korean Patients

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Background: Gleason score and serum prostatic specific antigen (PSA) level have some restrictions for evaluation of the prognosis of prostate carcinoma. Potential markers involved in numerous biological processes in the tumor cell have been investigated intensively. At present, adequate prognostic markers for tumor progression are still lacking. In this study, we examined the prognostic significance of p16, PTEN, E-cadherin, bcl-2 and Ki-67 expression in prostate cancers.
Design: Prostatic tumors were obtained from needle biopsied samples and surgical specimens of patients at Yeungnam University Hospital from May 2006 to April 2007. A total 131 specimens (90 cases ≥ 7 Gleason score, 21 cases < 7 Gleason score and 20 cases of nodular hyperplasia) were used for immunohistochemical analysis for p16, PTEN, e-cadherin, bcl-2 and ki-67.
Results: The inactivation of PTEN and overexpression of e-cadherin were significantly different between high risk and low risk prostatic cancer (p=0.017, 0.021, respectively). Especially, loss of expression of PTEN was a commom event in prostatic cancer with high Gleason scores. In high risk group of prostatic cancer, Bcl-2 expression was related to bone (p=0.027) and lymph node metastasis (p=0.017). Ki-67 labeling index was associated with lymph node metastasis (0.029) and loss of p16 expression (p<0.001). However, these biomarkers were not associated with serum PSA level and stage.
Conclusions: This study suggests that a combination of loss of PTEN expression and overexpression of e-cadherin may be a potential markers discriminating high- and low-risk tumors in prostatic cancer. In addition, high expression of bcl-2 and Ki-67 was significantly associated with distant metastasis in high risk prostate cancer. However, neither loss of PTEN nor loss of p16 expression was associated with adverse clinical outcome.

744 Genetic Alterations Detected by FISH (UroVysion™ Probe Set) in Non-Urothelial Bladder Cancers

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Background: Fluorescence in situ hybridization (FISH) with the UroVysion probe set has been shown to significantly increase the sensitivity of detecting urothelial carcinoma (UC) bladder cancer over conventional urine cytology with a slightly lower specificity than cytology. However, few studies have evaluated the ability of FISH to detect non-UC bladder carcinomas, which comprise 5%-10% of all bladder cancers. The goal of this study was to assess paraffin embedded non-UC bladder cancers to determine if UroVysion could detect both UC and non-UC of the bladder.
Design: Thirty-five paraffin embedded biopsy specimens from 29 males and 6 females with a mean age of 72 years (range, 53-94) were analyzed by FISH using the UroVysion probe set (Abbott Molecular Inc, Des Plaines, IL). A technologist (blinded to clinical and pathologic findings) enumerated 50 consecutive cells in areas pre-determined by a pathologist to contain cells that best represented the diagnosis of: adenocarcinoma (ADCA, 4); urachal ADCA (UA, 5) small cell carcinoma (SmCC; 4); squamous cell carcinoma (SCC; 4); UC with SmCC features (2); UC with SCC differentiation (3); UC (9); and bladder specimens without cancer (negative; 4). Polysomy was defined as the gain of two or more of the four chromosomal targets (CEP 3, 7, 17, and LSI 9p21) within an individual cell while homozygous 9p21 loss was defined as loss of both 9p21 chromosomal targets.
Results:

FISH Results

Tissue Diagnosis	N of Cases	Homoz. 9p21 Loss Cells, Mean (Range)	Polysomic Cells, Mean (Range)
Negative	4	16 (8-24)	<1 (0-1)
ADCA	4	10 (2-15)	28 (19-43)
Urachal ADCA	5	19 (9-44)	23 (1-34)
SmCC	4	12 (7-16)	15 (6-28)
UC with SmCC	2	6 (2-10)	34 (28-39)
SCC	4	46 (41-50)	18 (9-24)
UC with SCC	3	22 (10-39)	17 (1-27)
UC	9	18 (2-49)	33 (10-46)

Thirty-three of 35 cancer specimens demonstrated 6 or more polysomic cells (mean 25 cells) compared to 0-1 cells for negative specimens. The remaining two cancer specimens that had only 1 polysomic cell had a large number of cells (44 and 39) demonstrating homozygous 9p21 deletion.
Conclusions: The data from this study suggest that FISH with the UroVysion probe set can detect chromosomal abnormalities in tissue specimens from patients with either UC or less common nonurothelial carcinomas.

745 A Systematic Evaluation of Histopathologic Criteria for Adenocarcinoma of Prostate Using Frozen Sections as Compared with Routine Permanent Sections

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Background: Evaluation of frozen section (FS) of prostate cancer (CaP) has not been widely recommended because of difficulty in morphologic evaluation. FS analysis for margin status of radical prostatectomy specimens is routinely performed introoperatively at our institution. Additionally, prostate tissue is harvested for research from the majority of radical prostatectomy cases and is later characterized by FS analysis. As we have noted that criteria for diagnosis of CaP on FS differ from what are relevant on routine permanent sections (PS), we retrospectively evaluated a large series of cases to systematically compare differences between morphology on FS versus PS.

Design: To evaluate morphologic features which are useful in frozen diagnosis of CaP as compared with non-frozen PS of CaP, we studied 100 consecutive cases where tissue was harvested and then characterized by frozen section enabling comparison of cancer in the FS with the routine PS of the same case. Standard diagnostic criteria for adenocarcinoma including architectural, cytologic (nuclear and cytoplasmic) and ancillary features, were evaluated on FS and corresponding non-frozen PS of radical prostatectomy cases.
Results: The most striking difference in features is the shrinkage of nucleoli on FS (macronucleoli observed in 29% cases on FS compared with 93% cases on PS, $p< 0.0001$), readily apparent when comparing FS with PS of the same case. Nuclear enlargement and hyperchromatism however is preserved on FS when compared with PS. Amphophilia and mucinous sections are not readily appreciated on FS compared with PS ($p=0.01$), while pink amorphous secretions are equally observed on both FS & PS. Sharp luminal border, crowded and haphazard growth are characteristics of CaP, which are as easily observed on FS as on PS.
Conclusions: Some features of CaP that are seen on PS yet are not visible on corresponding FS, likely related to nuclear distortion due to freezing artifact. Search for macronucleoli on frozen section is therefore an unreliable way to make a diagnosis of CaP (or high grade PIN) on FS. Features such as enlarged & hyperchromatic nuclei, sharp luminal border of glands, and crowded and haphazard growth pattern which are maintained on FS, are more reliable criteria to use for intraoperative FS diagnosis of CaP.

746 Should Multiple Cores with Prostate Cancer Submitted in the Same Container Be Assigned Individual Gleason Scores?

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Background: The 2005 ISUP Consensus Committee recommends assigning individual Gleason Scores (GS) to cores with prostate cancer (PCa) submitted in separate containers and/or multiple prostate cores (MPCs) in same container where the site of individual core is specified by clinician. However, scenarios where MPCs are put in the same container without any site designation or only left/right designation are relatively common. Objective of this study is to assess if MPCs with different GS submitted within same container should be assigned individual GS?
Design: Patients with PCa diagnosed on extended 12 core biopsies (EB) with MPCs with different GS who subsequently underwent radical prostatectomy (RP) were retrieved. EB containing only 1 core with higher pattern and other core(s) 3+3 were excluded from analysis. For analysis, positive cores from different sites of same lobe were artificially lumped into either "Right" or "Left" and assigned a "Global GS" (whereby all positive cores were averaged as if it was one long positive core), Worst GS and Largest tumor volume GS and compared with GS of RP. In all cases, cores were intact. Cases with tertiary higher Gleason pattern were assigned GS using primary grade and highest grade. For comparisons, following scenarios were considered clinically significant upgrading: biopsy GS of 3+4 to a RP GS of ≥ 4+3; biopsy GS of 7 to a RP GS ≥8; biopsy GS 7 to RP GS of 7 with tertiary Gleason pattern 5.
Results: Overall, 51 of 203 (25%) cases met inclusion criteria for the study. With respect to clinically significant differences in GS between biopsy and RP: Global GS, Worst GS and Largest volume GS on biopsy correlated with RP GS in 43%, 55% and 35% of cases respectively. None of the biopsy GS parameters correlated with GS on RP in 12/51(23%) cases. The most common reason for non-correlation in these cases was biopsy GS 4+ 3 and RP GS 3+4 (10/12, 83%). Correlation Kappa statistics for this data is currently underway.
Conclusions: Our results suggest for clinically significant correlation, Worst GS on biopsy correlated best with GS on RP, compared to Global GS or Largest tumor volume GS. When multiple intact cores are submitted in the same container without specific identifiers, individual GS of the cores with cancer should be attempted and/or the worst GS should be recorded.

747 Prognostic Value of Chromogranin-A in Prostate Cancer. A Clinicopathological, Immunohistochemical and Molecular Study of 112 Cases

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Background: Current opinion states that neuroendocrine differentiation in prostate cancer is a finding of bad prognosis. In fact, it has been related with biochemical progression, hormone refractory disease and tumor-associated death in some previous studies. The present work aims to evaluate the prognostic impact of chromogranin-A expression in prostate adenocarcinoma.
Design: A total of 112 prostate adenocarcinomas with a long-term follow-up treated with radical prostatectomy or transurethral resection have been investigated for neuroendocrine differentiation. Chromogranin-A (CRG) expression has been evaluated both by immunohistochemistry and RT-PCR techniques in paraffin-embedded tissue following routine methods. The patient follow-up included, in every case, the cause of death, the evaluation of biochemical and clinical progression, and the time until tumor-related death. SPSS (13.0) software has been used for statistical analysis.
Results: The average of tumour-related death was 68.06 years (CI 60.58-75.55, SD 3.77) Positive immunostaining with CRG was detected in 27 cases (23.5%), the median being 71 (SD 39.24). Overall, 5 and 10 year survival was 76.1% (SD 0.043) and 62% (SD0.084) respectively. While 5 and 10-year CRG-positive survival was 74.1% (SD 0.067) and 61.8% (SD 0.126) respectively, CRG-negative survival was 78.2% (SD 0.054) and 53.9% (SD 0.129) (log-rank, $p=0.06$). Real-time PCR detected CRG mRNA in 34 cases (38.1%). This expression was 9.110.3 times lesser in immunohistochemically CRG-negative cases ($p<0.05$), 81.90.32 times bigger in patients with hormone refractory disease at the time of biopsy ($p<0.05$), and 1.770.31 times bigger in patients dying of prostate cancer ($p<0.05$).
Conclusions: In spite of the fact that genetic expression of CRG may be used as a good marker for identifying hormone refractory disease and also as a reliable predictor for higher risk of cancer-related death, the usefulness of the immunohistochemical expression of this neuropeptide as a prognostic marker is questionable in clinical practice. Supported by the grant ISCIII PI020964.

748 Morphologic and Clinical Features in 41 Patients with Small Cell Carcinoma of the Bladder

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Background: Small cell carcinoma (SCC) is an uncommon form of bladder cancer, generally associated with a poor prognosis. SCC can arise de novo or occur concomitantly with other forms of bladder cancer. We reviewed 41 cases of SCC at our institution to determine histopathologic features and outcomes.
Design: Slides of SCC cases were reviewed and clinical outcomes were determined by retrospective review of patient records.
Results: Patients consisted of 32 men and 9 women (~4:1 M:F ratio) and age ranged from 39 to 87 yrs (median 65 yrs). Symptoms included hematuria (30/41; 73%), dysuria (5/41; 12%), abdominal pain (3/41; 7%), and chronic urinary tract infection (2/41; 5%). All patients underwent biopsy/TUR, with subsequent radical cystectomy (25/41; 61%), partial cystectomy (5/41; 12%), and 2 autopsies (5%). Pathologic stage at diagnosis was determined in 36 cases and included pT1 (1/36; 3%), pT2 (16/36; 44%), pT3 (17/36; 47%) and pT4 (3/36; 8%) disease. Pure SCC was present in 9 cases (22%) and mixed morphology tumors were present in 32 cases (78%), which included SCC admixed with urothelial carcinoma (22/32), urothelial with glandular differentiation (5/32), urothelial with squamous differentiation (2/32), sarcomatoid urothelial carcinoma (2/32), and undifferentiated carcinoma (1/32). Co-existent metastases were present in 16/25 (64%) patients who underwent cystectomy to pelvic lymph nodes (15/16) and the pelvic sidewall (1/16). Half of concurrent metastases demonstrated features of urothelial carcinoma (7/16; 44%), whereas the remainder demonstrated pure SCC (9/16; 56%). Clinical follow-up was available for 39 patients (95%) and ranged from 2 wks (dead with disease) to 7 yrs (median 16 mo). Twenty-three patients received chemotherapy (59%). Subsequent metastases occurred in 25 patients (64%) and most commonly affected bone (10/25; 40%), liver (9/25; 36%), and brain (8/25; 32%). Of 10 sampled metastases, 9 demonstrated pure SCC (90%) and only 1 demonstrated urothelial carcinoma (10%). Twenty-five patients died of disease (64%), with a mean of 17.5 months between time of diagnosis and death. Only 6 patients were alive without disease (15%) ranging from 13-35 mo (average 25 mo).
Conclusions: SCC of the bladder is an aggressive neoplasm that often occurs in conjunction with an admixed component of bladder carcinoma. Although early metastases may demonstrate either small cell or urothelial carcinoma morphology, late stage metastases are more commonly small cell in nature.

749 Polypoid Cystitis: A Series of 41 Cases Misdiagnosed as Papillary Urothelial Neoplasia

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Background: Polypoid cystitis occurs in the bladder with identical lesions affecting the entire urinary tract, where they are called polypoid urethritis, polypoid ureteritis, etc. For this study, all cases are called polypoid cystitis regardless of location.
Design: In our consultation files (1/00-7/07), we found 41/155 cases of polypoid cystitis diagnosed as papillary urothelial neoplasms by contributing pathologists. Cases were sent to us, typically at the request of the urologist after the case had be signed out. Original diagnoses included: non-invasive low grade papillary urothelial carcinoma (n=23), non-invasive high grade papillary urothelial carcinoma (n=6), PUNLMP (n=5), papilloma (n=3), urothelial neoplasia (n=2), CIS (n=1), and squamous carcinoma (n=1).
Results: Mean age at diagnosis was 63 years (19-93). Male to female ratio was 3.1 to 1. Most common symptoms were gross hematuria, bladder/urethral stones, h/o prostate cancer treated with radiation, f/u after bladder/ureter carcinoma treatment, long term urinary stents, and colovesicular fistulas. At cystoscopy, lesions were noted as polypoid, trabeculations, bullous polyps, and diffuse erythema and edema. Polypoid cystitis occurred in the bladder (n=34), ureteral orifice (n=2), urethra (n=2), renal pelvis (n=2), and undesignated (n=1). 31 cases had isolated papillary fronds with in 1 case branching. The base of the papillary stalks were both broad and narrow (n=24), only broad (n=9), and only narrow (n=3). Overlying urothelium was diffusely and focally thickened in 8 cases and 5 cases, respectively. Umbrella cells were identified in 32 cases. Mixed inflammation was present in 28 cases, moderate in 15 and mild in 13 cases. 11 cases showed chronic inflammation, mild in 10 and moderate in 1 case. Reactive urothelial atypia was noted in 26 cases with mitoses in 22 cases, frequent in 3 and rare in 19 cases. Stromal edema was seen in 32 cases with fibrosis within the polypoid stalks seen in 16 cases.
Conclusions: The key to diagnosing polypoid cystitis is to note at low power its reactive nature with an inflamed background that is edematous or densely fibrous with predominantly simple, non-branching, broad-based fronds of relatively normal thickness urothelium, and not focus at higher power on the exceptional frond that may more closely resemble a urothelial neoplasm either architecturally or cytologically. Because urologists can often recognize the inflammatory nature of the lesion, the pathologist should hesitate diagnosing urothelial neoplasia when the histology is equivocal and the cystoscopic impression looks inflammatory.

750 Immunohistochemistry of Adenocarcinoma of the Bladder

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Background: Adenocarcinomas of the bladder are rare. Pathologists need to rule out metastases or direct invasion from other sites, such as prostate and colorectum.

Design: We identified 24 primary adenocarcinomas of the bladder including 2 signet ring cell and 1 urachal adenocarcinoma. These were compared to 2 analogous adenocarcinomas arising from the prostatic urethra, 2 bladder villous adenomas, and 3 cases of intestinal metaplasia in the bladder. A tissue microarray was constructed from representative regions of each case's tissue samples. Only nuclear beta-catenin staining was considered positive.

Results: Of the 24 adenocarcinomas, all were negative for PSA, PSAP, and thrombomodulin. P501s (prostein) showed 4 cases with moderate staining. Prostate specific membrane antigen (PSMA) showed 5 with moderate and 1 with strong diffuse cytoplasmic staining. CDX2 was moderate in 6 and strong in 4 cases. 2 cases expressed beta-catenin nuclear staining. Prostatic urethral adenocarcinoma was negative for beta catenin and CDX2. Of the 2 villous adenomas, beta-catenin was negative and 1 case had strong CDX2 staining. All cases of intestinal metaplasia were negative for CDX2 and beta-catenin. 18 bladder adenocarcinomas were CK7+/CK20+ with 4 CK20+ only and 2 CK7+ only.

Conclusions: A minority of bladder adenocarcinomas label with the "prostate markers" P501S and PSMA, in contrast to negative staining for PSA and PSAP. Bladder adenocarcinomas have diffuse cytoplasmic staining for P501S in contrast to granular perinuclear staining in prostate adenocarcinoma. Similarly PSMA in bladder adenocarcinoma exhibit diffuse cytoplasmic positivity versus the apical membranous staining in prostate cancer. However, 1 case of villous adenoma of the bladder had positive apical membranous staining. In the differential diagnosis of colorectal adenocarcinoma, beta-catenin and CDX2 in bladder adenocarcinoma overlapped with reported findings in colorectal tumors. CK7+/CK20+ was more frequently seen as opposed to the typical colorectal CK7-/CK20+ profile. Although the novel markers P501s and PSMA are more sensitive and specific in the differential diagnosis of prostate and urothelial carcinoma, care must be taken when bladder and prostate adenocarcinoma are in the differential.

751 Diagnostic Utility of Antibody to Smoothelin in the Distinction of Muscularis Mucosa (MM) from Muscularis Propria (MP) of the Urinary Bladder

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Background: The cornerstone of pathologic tumor staging for urothelial carcinoma is the level of invasion in the bladder wall. Invasion limited to the lamina propria is staged as pT1 and involvement of MP muscle is staged as at least pT2. Recognition of invasion into the MP muscle (pT2) serves as the critical crossroad between aggressive and conservative management for the patient. In the lamina propria, an inconsistent layer of MM muscle exists which can be hyperplastic and mimic the MP muscle making staging extremely difficult in limited or unoriented specimens such as biopsies and TUR specimens. Smoothelin is a novel smooth muscle marker, which selectively stains only the fully or terminally differentiated smooth muscle and does not stain non-contractile smooth muscles. Herein, we evaluate the immunohistochemical (IHC) expression of smoothelin in bladder MM and MP muscles.

Design: Sections from urinary bladder cystectomy specimens (n=32) containing MM (including conventional [MM-C] and hypertrophic [MM-H] forms) and MP are immunostained with smoothelin (clone R4A, Chemicon). Two bladder sections along the ureteral insertion are also stained with smoothelin.

Results:

IHC staining of Urinary Bladder MP and MM with Smoothelin

Positivity	MP	MM-C	MM-H
0	0/32 (0%)	20/32 (62%)	14/25 (56%)
1+	0/32 (0%)	12/32 (38%)	9/25 (36%)
2+	3/32 (9%)	0/32 (0%)	2/25 (8%)
3+	29/32 (91%)	0/32 (0%)	0/25 (0%)

Grade: 0 or negative, <5%+; 1+, 5-10%+; 2+, 11-50%+; 3+, >50%+

MP typically had diffusely intense cytoplasmic staining highlighting dense compact aggregates of plump muscle fibers. MM showed typically absent to vaguely patchy cytoplasmic staining which was never diffusely intense. There was always a significant difference in staining intensity between the MM and MP in every section. The two sections with ureteral insertion highlighted a more superficial smoothelin-positive MP with similar intense immunoreactivity to the deeply situated bladder MP.

Conclusions: The relatively distinct immunohistochemical staining pattern of smoothelin between MM (including hypertrophic form) and MP makes it a robust and attractive marker to be incorporated in the contemporary diagnostic IHC armamentarium for the sometimes difficult area of staging bladder cancer which has important prognostic and diagnostic implications.

752 Extraparenchymal Testicular Leydig Cells: An Analysis of Prominent Foci in Orchiectomies for Tumor and Non-Tumoral Testicular Pathologies

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Background: The existence of Leydig cells outside their usual testicular interstitial location is well established. These eosinophilic steroid cells most commonly are seen intimately associated with testicular nerves within the tunica albuginea and/or the rete testes. In routine practice, we occasionally noticed relative prominence of ectopic Leydig cells in orchiectomy specimens. The significance of these prominent ectopic Leydig cells (PELC) and their correlation with any testicular pathology or with their interstitial Leydig cell counterpart is not known.

Design: We performed an analysis of PELC (herein, defined as at least one ectopic focus of distinct aggregate with >5 Leydig cells) in 74 consecutive orchiectomies including 32 (43%) resected for tumors (24 germ cell tumors, 6 Leydig cell tumors, and 2 other tumors) and 42 (57%) for non-tumoral testicular pathologies. There were 60 adult orchiectomies and 14 pediatric orchiectomies. Immunohistochemical (IHC) analysis with inhibin and calretinin of selected sections with coexisting PELC and hyperplastic interstitial Leydig cells (n=7) was performed.

Results: PELC were encountered in 42 of 74 (57%) orchiectomies usually intimately associated with nerves (27/42, 54%), blood vessels (12/42, 29%), or seen in the connective tissue stroma of the tunica albuginea (21/42, 50%). Ectopic Leydig cells were typically more florid when associated with nerves. PELC were seen in 24 (75%) orchiectomies for tumor and 18 (43%) non-tumoral testes. Among the non-tumoral testes, PELC were more commonly encountered in adult (39/60, 65%) compared to pediatric (3/14, 21%) testes. All testes with PELC were associated with hyperplasia of the interstitial Leydig cells (prominent [39/42, 93%] and focal [3/42, 7%]). Five of the six Leydig cell tumors showed associated PELC and hyperplasia of interstitial Leydig cells. Immunohistochemistry revealed analogous expression of inhibin and calretinin in coexisting PELC and hyperplastic interstitial Leydig cells.

Conclusions: PELC occur in both tumoral and non-tumoral testes. This change is more commonly encountered in adults than in pediatric testes particularly in non tumoral testes. PELC always occurs in the background of interstitial Leydig cell hyperplasia suggesting close biologic pathway for both cells. The analogous immunohistochemical profile of both ectopic and interstitial Leydig cells further provides strong evidence for the similarity of these two cell types.

753 Grading of Infiltrating Cribriform Carcinoma on Prostate Needle Biopsy

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Background: The distinction between infiltrating cribriform pattern 3 and 4 prostate cancer is controversial.

Design: Out of 3590 prostate cancers sent to one of the authors over 7 months, 30 needle biopsy cases were selected (36 images taken) that possibly represented cribriform Gleason pattern 3 cancer. Images were sent to 10 other experts in prostate pathology. Consensus was defined when at least 7/10 agreed on the grade.

Results: 67% (n=24) of images reached consensus (23 pattern 4; 1 pattern 3). Of the 12 non-consensus images, 7 were favor pattern 4 (6/10 experts agree), 1 was favor pattern 3 (6/10 experts agree), and 4 were equivocal (<6 experts agree). Between 23 consensus pattern 4 images and 10 experts, there were 190 pattern 4 diagnoses. Of these, the most common criteria used to call pattern 4 were: irregular contour (n=122), irregular distribution of lumens (n=92), slit-like lumens (n=56), large glands (n=51), number of glands (n=45), and small lumens (n=28). In the only consensus pattern 3 image, criteria were regular contour (n=6), small glands (n=4), regular distribution of lumens (n=3) and uniform round lumens (n=2). 3 pathologists more commonly diagnosed cribriform pattern 3. Discrepancy between experts was qualified as primarily objective (different criteria present) in 38%, subjective (different interpretation of the same criteria) in 12%, and mixed (both ojective and subjective) in 50%. The most frequent criteria with different interpretations of the same criteria were: regular vs irregular contour and small vs large glands, with the former more common.

Conclusions: Even in this highly selected set of images thought to be the best candidates for cribriform pattern 3 from a busy consult service, most experts interpreted the cribriform patterns as pattern 4. The only consensus pattern 3 image came from a case in which 2 other images were interpreted as consensus pattern 4 and favor pattern 4. The criteria and their application to diagnose cribriform pattern 3 prostate cancer are so stringent that the diagnosis is virtually never made on needle biopsy by most experts.

754 Claudins 7 and 8 Expression Separate Chromophobe Renal Cell Carcinoma from Oncocytoma

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Background: Morphologic features of chromophobe renal cell carcinoma (chRCC) often overlap with those of oncocytomas (OC), which are benign in nature. Claudins comprise a multigene family with a major role in tight junction formation. Here we determined the usefulness of claudin 7 and 8 expression in differentiating human renal neoplasms.

Design: Tissue microarrays were created from paraffin-embedded tissue samples from 141 patients with renal cell carcinoma (RCC) or OC. The array included 90 clear cell (cRCC), 22 papillary (pRCC), 17 chRCC, and 12 OC. Representative areas of non-neoplastic renal tissue were included for all cases. The microarrays were stained for claudins 7 and 8 with epitope specific rabbit antibodies. The immunoreactivity was assessed based on a combined score of the extent and intensity of staining.

Results: The staining pattern for claudins 7 and 8 staining was membranous and cytoplasmic in both non-neoplastic renal tissue and tumors. Both claudins were strongly expressed in the distal convoluted tubules and collecting ducts. Moderate to strong claudin 7 was present in 94% of the chRCC tumors and in 55% of the OCs ($p=0.02$), whereas fewer of the cRCC stained significantly with claudin 7 (30%; $p<0.0001$). 75% of the pRCC stained moderate/strongly for claudin 7. The majority of the OCs demonstrated moderate to strong claudin 8 staining (92%), which differentiated it from pRCC (14%; $p<0.0001$) and cRCC (12%; $p<0.0001$). Importantly, only negative to weak claudin 8 staining was detected in chRCC; while in the OC group there were no negative tumors and 8% demonstrated weak claudin 8 staining ($p<0.0001$).

Conclusions: Distinct expression patterns of claudins 7 and 8 were detected in normal renal parenchyma and in the renal cell carcinoma subgroups. Claudins 7 and 8 could thus serve as useful immunohistochemical markers for the separation of chRCC from OC.

755 Immunohistochemical (IHC) Panel for Subtype Classification of Renal Neoplasms

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Background: Subtypes of renal cell carcinoma (RCC) and oncocytoma can share overlapping morphologic features making classification challenging. Correct classification is important since these lesions have different prognostic and therapeutic outcomes. Conventional renal IHC panel results are frequently ambiguous. Our study investigates the pattern, sensitivity and specificity of staining of four IHC markers: CA-9, caveolin, PAX-2 and parvalbumin, using a multi-tumor renal paraffin tissue microarray (TMA).

Design: The TMA consists of 240 cases of RCC subtypes (102 conventional clear cell, 50 papillary, 30 chromophobe), oncocytoma (28) and benign kidney (30). It was stained with antibodies against CA-9, caveolin, PAX-2 and parvalbumin. The staining intensity for each TMA core was scored from 0 to 3.

Results: CA-9 and parvalbumin demonstrated the most specific staining of the group. CA-9 showed strong, diffuse membranous staining of all clear cell RCC subtypes, with an average staining score of 3; CA-9 also stained a few papillary RCC, but the overall staining average was low (0.3). Parvalbumin stained only those neoplasms of distal nephron origin (chromophobe and oncocytoma), with a similar intensity (2.6 and 2.4, respectively). PAX-2 stained clear cell and papillary RCC with similar intensity (average score 1 for each), and showed either absent or weak staining of chromophobe RCC and oncocytoma. Caveolin staining was found in all neoplasms (clear cell 1.6, papillary 1, chromophobe 1, oncocytoma 1.57), and showed a variety of staining patterns (membranous, fine and/or coarse granular).

Conclusions: CA-9 and parvalbumin are most useful in discerning renal neoplasms of proximal versus distal nephron origin. CA-9 is especially useful in almost exclusively identifying clear cell RCC subtype. Parvalbumin highlights oncocytoma and chromophobe RCC equally. It is of great utility in identifying distal tubular origin but does not distinguish between the two tumor types. PAX-2 highlights proximal nephron origin tumors, but staining intensity is low. Caveolin appears to have variable results, and does not appear to be a useful aid in differentiating these tumors.

Staining Intensity

Renal Tumor	CA-9	Parvalbumin	PAX-2	Caveolin
Clear Cell RCC	3	0	1	1.6
Papillary RCC	0.3	0	1	1
Chromophobe RCC	0	2.6	0	1
Oncocytoma	0	2.4	0.1	1.6

756 A Useful Panel of Immunohistochemical Markers in Differentiating Papillary Renal Cell Carcinoma from Papillary Urothelial Carcinoma

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Background: Cryoablation is a conservative procedure for treatment of small (<7.0cm) renal cell tumors and has gained its popularity in recent years because of its lower complication rates. Percutaneous thin needle core biopsy is usually performed prior to cryoablation. Because of the limited tissue available for diagnosis, the distinction between papillary renal cell carcinoma (PRCC) and papillary urothelial carcinoma (PUC) may be problematic. In this study, we search for a panel of immunostaining markers to differentiate between these two entities.

Design: We immunohistochemically evaluated the diagnostic value of kidney injury molecule-1 (KIM-1), von Hipple-Lindau gene product (pVHL), S100P, P504S, CK7 and CK20 on tissue microarray sections containing 44 cases of PRCC and 66 cases of PUC. Of the 66 urothelial carcinomas, 26 were non-invasive, and 40 were invasive. The distribution was recorded as negative (less than 5% of tumor cells stained), 1+ (5-25% of tumor cells stained), 2+ (26-50% of tumor cells stained), 3+ (51-75% of tumor cells stained), or 4+ (more than 75% of tumor cells stained).

Results: The results demonstrated a nuclear and cytoplasmic staining pattern for S100P and membranous and cytoplasmic staining pattern for the remaining antibodies. Diffuse and strong positivity (>2+) for all 6 antibodies was observed in the majority of positively stained cases. PRCC is usually positive for P504S, pVHL, KIM-1, and CK 7; in contrast, PUC is usually positive for CK7, CK20 and S100P. The detailed staining results are summarized in Table 1.

Table 1. Immunostaining results in 44 cases of PRCC and 66 cases of PUC

Marker	PRCC (N=44)	Non-invasive PUC (N=26)	Invasive PUC (N=40)
CK7	41/44 (93%)	26/26 (100%)	38/40 (95%)
CK20	0/44 (0)	21/26 (80.8%)	22/40 (55%)
S100P	0/44 (0)	25/26 (96%)	30/40 (75%)
P504S	41/44 (93.2%)	2/26 (7.7%)	10/40 (25%)
pVHL	40/44 (91%)	0/26 (0)	0/40 (0)
KIM-1	40/44 (91%)	4/26 (15.4%)	6/40 (15%)

Conclusions: The useful staining profile for papillary renal cell carcinoma is P504S+/pVHL+/KIM-1+/S100P-/CK20-; and the useful staining profile for papillary urothelial carcinoma is S100P+/pVHL-/CK20+/P504S-/KIM-1-. Caution should be taken in interpretation of P504S and KIM-1, since a small percentage of urothelial carcinomas may be positive for P504S and/or KIM-1.

757 Pathologic Findings in 5912 Prostate Biopsies

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Background: Modern PSA screening, routine digital rectal examination, availability of PCA3 testing, extended needle core biopsy and popularity of P63/P504S immunoperoxidase stains have dramatically changed the practice of prostate cancer detection and management in recent years. This study demonstrates pathologic findings over the past several years to reflect the trend of prostate biopsies.

Design: A total of 5912 consecutive prostate biopsies from 50 urologists at multiple community practices in Arizona, Utah, Nevada, and Wyoming between 2002 and August 2007 were included. The following parameters were tabulated year by year for observation: PSA, % positive rate for prostate cancer (PCa), mean Gleason Score (GS), % cases with GS of 7 over all cancer cases, % GS ≥8, mean number of positive cores per case, % high grade prostatic intraepithelial neoplasia (HPIN), and % atypical small acinar proliferation (ASAP).

Results: The mean age was slightly downward (younger) with an obvious downward trend of PSA levels. The overall cancer positive rate was 36.3% (2149/5912 cases) with a clear uptrend from 31.4% in year 2002 to 39.1% in 2007 even though the PSA was decreasing. There was a linear trend of increasing GS. Both % GS=7 and % GS≥8 in 2007 were significantly higher than those in 2002 (P<0.005, Chi-Square Test). The tumor extent (size) as measuring by number of positive cores per case and % positive core per case was slightly increased year by year as well. Finally the HPIN and ASAP rates were constant at around 5.2% and 2.3% respectively.

Conclusions: Despite a downward PSA trend (suggesting earlier biopsy), the overall cancer detection rate has been increasing with an obvious uptrend of Gleason Scores and significantly increased higher grade cancer from year 2002 to 2007. So far, there is no evidence of increasing detection of minimal cancer or low grade clinically insignificant cancer in the urological practices this laboratory serves.

Years	Mean Age	Mean PSA	# Cancer (%)	Mean GS	GS=7 (%)	GS8 (%)≥	# Pos. Cores
2002	67.2	13.82	214(31.4)	6.29	19.6	4.8	2.6
2003	66.6	25.70	295(36.1)	6.49	26.0	10.2	3.1
2004	65.4	7.06	355(34.6)	6.32	17.5	6.2	3.2
2005	65.6	8.81	625(37.8)	6.52	28.6	9.9	3.4
2006	66.6	9.75	1036(37.2)	6.60	28.6	13.0	3.4
2007	66.1	10.90	274(39.1)	6.68	36.9	13.1	3.5

758 Prostate Cancer at One End of a Needle Biopsy Core Is Associated with Advanced Pathology Stage

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Background: Advanced pathologic stage such as extra-capsular extension is an adverse factor for prostate cancer (PCa). It is crucial to identify patients with high risk of advanced pathologic stage preoperatively. The location of the PCa at a needle biopsy core in relation with advanced pathologic stage has not been investigated. The hypothesis was that during the transrectal prostate needle biopsy, the needle travels through the prostate capsule at the inferior aspect to the superior edge. Any PCa at either end of a needle core might have high likelihood to be associated with advanced pathologic stage.

Design: Pathology stage of pT3a or pT3b as seen in prostatectomies was used as end points. The location of PCa at a needle biopsy core was documented and correlated with tumor stage. The PCa within 1 mm from one end of a needle core was defined as PCa at core end. Any men with Gleason Score ≥ 8 or tumor involving more than six cores at needle biopsies, regardless of the location of the PCa, were excluded from this study due to the high likelihood of higher disease stage in these patients. Perineural invasion (PNI) if present was also documented. Chi-Square Test was used for statistics.

Results: From January 2002 to August 2007, 185 cases of protatectomies were available for tumor stage follow up. Fifty-five men had PCa at core end at initials biopsies with mean age of 61.4, PSA 8.3 and 3.3 positive cores on average. The mean age, PSA and number of positive cores for 130 men who did not have PCa at core end were 62.5, 6.6, and 2.1. Twenty eight out of 55 men (50.9%) with PCa at needle core end showed pT3a or pT3b disease, while there was only 16.2% men showing pT3a or pT3b disease when the PCa was not at core end. The difference was very significant (P<0.001). Furthermore, 13 of 21 (61.9%) men with PCa at needle core end plus perineural invasion showed advanced tumor stage.

Conclusions: PCa at one end of a needle biopsy core with or without perineural invasion is highly predictive for advanced pathology tumor stage. These findings may be useful in treatment planning such as patient selection for nerve-sparing prostatectomy procedure.

	PCa at core end	PCa not at core end	PCa at core end with PNI
# of cases	55	130	21
# pT3a or pT3b	28 (50.9%)	21 (16.2)	13 (61.9%)

759 Characterization of Nuclear Hormone Receptor Cofactor TBLR1 Expression in Adenocarcinoma of the Prostate

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Background: The role of androgen receptor in the tumorigenesis and progression of prostatic adenocarcinoma has long been recognized. TBLR1 is a newly identified nuclear hormone receptor cofactor with both corepressor and coactivator activities. It interacts with hormone receptor transcription regulatory complex in the nucleus. This study characterizes the expression pattern of TBLR1 in malignant glands and its surrounding benign prostatic glands. The correlation of the TBLR1 expression pattern with clinicopathological factors in prostate cancer is also investigated.

Design: Immunohistochemical study with polyclonal TBLR1 antibody was used to characterize its expression pattern. Two sets of prostate cancer cases are used. In the first set, the whole tissue sections from 30 cases were studied. TBLR1 nuclear and cytoplasmic expression was compared between malignant glands and the surrounding benign glands on the same slide. In the second set, 86 samples of prostate cancer, including 18 androgen independent cases, were studied on a tissue microarray (TMA). The levels of TBLR1 nuclear and cytoplasmic expression were scored semi-quantitatively; 0 as negative, 1 as weak, 2 as moderate and 3 as strong expression.

Results: Comparing malignant glands to the surrounding benign glands on the same slide, the expression of TBLR1 in nucleus is significantly lower in the malignant glands ($p<0.005$). Among the 30 cases studied, TBLR1 nuclear expression in malignant glands were decreased in 19 cases, stay the same in 10 cases and increased in 1 case. No significant difference between the level of TBLR1 cytoplasm expression in malignant glands and benign glands was observed. Stratifying TMA case with clinicopathological factors, the levels of TBLR1 nuclear expression have positive correlation with the Gleason scores ($R^2= 0.96$, $p<0.05$). Significantly, patients with hormone refractory cancer express higher levels of nuclear TBLR1 compared to patients without prior therapy ($p<0.01$).
Conclusions: The study demonstrates significant down-regulation of TBLR1 nuclear expression in the malignant glands compared to the surrounding benign prostatic glands. However, higher levels of nuclear TBLR1 expression are observed in patients with high Gleason score and hormone refractory disease. The findings suggest that down-regulation of nuclear TBLR1 expression plays a role in the tumorigenesis. Further, high levels of nuclear TBLR1 is associated with progression of prostate cancer.

760 B-RAF Mutation in Prostatic Carcinoma: A Study of 93 Cases by High-Resolution Melting Amplicon Analysis (HRMA)

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Background: The RAF-MEK-ERK signal pathway is a conserved RAS-activated protein kinase cascade which regulates cell growth, proliferation and differentiation in response to growth factor, cytokines and hormones. Approximately 15% of human cancers harbor B-RAF somatic mutations. The highest incidence of B-RAF mutations is observed in melanoma, papillary thyroid carcinoma and colorectal cancer. The common B-RAF activating mutation is at V600E. Recently it has been reported that approximately 10% of prostatic carcinomas showed B-RAF V600E activating mutations, especially in the tumors with higher histological grade and advanced stage. The current study was designed to screen for B-RAF gene mutations in prostatic carcinoma by using HRMAA.
Design: Ninety three radical prostatectomies performed at Huntsman Cancer Hospital at the University of Utah during 2005 to 2007 were reviewed. The pathologic findings including Gleason scores and AJCC staging were re-evaluated and confirmed by two additional pathologists independently. After micro-dissection of the tumor, DNA was isolated from paraffin-embedded tissue and B-RAF mutation was detected by HRMA in conjunction with selective gene sequencing.
Results: Of the ninety three patients in this study, fifty four percent (50/93) presented with a prostatic carcinoma with total Gleason score of 7. Twenty six percent (24/93) had a Gleason score of 6 or below. Nineteen percent (18/93) showed a higher grade carcinoma (Gleason Score 8 or above) with advance stage diseases. Remarkably, no B-RAF mutations at V600E were found in any of the patients.
Conclusions: Approximately 10% of patients with prostatic carcinoma have recently been reported to contain B-RAF gene point mutations at V600E. Surprisingly we were unable to detect the V600E mutation in any of the 93 Caucasians patients in our study. Because the previous study reporting BRAF mutations in prostate cancer was performed in an Asian population, our results suggest the intriguing possibility that the presence of the mutation may have an ethnic bias. To test this possibility, a study of B-RAF mutation in major ethnic genetic populations will be valuable.

Results:

Mean age (year)	Gleason Scores	AJCC staging	B-RAF Mutation
60.5	<6	T3/T3 (20/3)	negative
62.8	7	T2/T3 (37/23)	negative
64.7	>8	T2/T3 (3/15)	negative

761 Evaluation of S100P as a Marker for Urothelial Carcinoma

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Background: Placental S100 (S100P) was recently reported as a potential marker for urothelial carcinoma. However, its value in distinguishing urothelial carcinoma from other benign and malignant lesions in the genitourinary tract has not been well investigated.
Design: Immunohistochemistry was performed on formalin-fixed, paraffin-embedded tissue sections and pre-constructed tissue microarray (TMA) slides using a monoclonal antibody against S100P (BD Transduction Laboratories, San Jose, CA; 1:200). Selected cases included 91 urothelial carcinomas (35 non-invasive and 56 invasive), 78 renal cell neoplasms of various types, 10 other poorly-differentiated malignant neoplasms involving the genitourinary (GU) tract including high grade prostatic adenocarcinoma (Gleason score ≥ 8), and 32 cases of benign urothelial conditions including 7 nephrogenic adenomas.
Results: Benign urothelium in all cases (25/25, 100%) showed strong cytoplasmic S100P immunoreactivity. The majority of nephrogenic adenomas showed S100P positivity (5/7, 70%). In comparison, S100P was positive in 73/91 (80%) urothelial carcinoma overall, demonstrating strong cytoplasmic stain in 33/35 (94%) non-invasive low-grade or high-grade cases. But the sensitivity decreased to 71% (40/56) in high-grade invasive carcinomas where a need for urothelial marker is most desired. In addition, the immunoreactivity in these invasive cases was often weaker in intensity and focally distributed. All 78 cases of renal cell neoplasm examined were negative for S100P (0/78), which included clear cell, papillary, chromophobe renal cell carcinomas and oncocytomas. In contrast, three of eight (38%) high-grade prostatic adenocarcinoma cases showed areas of weak to strong S100P staining. One signet ring cell colorectal carcinoma involving bladder was also positive for S100P.
Conclusions: Based on this study, we confirmed the presence of S100P immunoreactivity in the majority of urothelial carcinoma of either low or high grade, and the absence of S100P reactivity in renal cell neoplasms. Thus it might be a urothlial marker especially suitable for separating upper GU tract neoplasms. However, benign urothelium, nephrogenic adenomas and other poorly differentiated carcinomas involving the GU tract such as prostate adenocarcinoma can also be S100P positive. Therefore, S100P can not be used to differentiate benign urothelial lesions from urothelial carcinoma. Furthermore, caution should be excised when using S100P in distinguishing poorly-differentiated neoplasms in the GU tract.

762 Urothelial Carcinoma of the Bladder, Lipoid Cell Variant (UCBLCV). Clinicopathologic Findings and LOH Analysis

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Background: UCBLCV is a rare neoplasm defined by the WHO (1999, 2004) as an urothelial carcinoma which exhibits transition to a cell type resembling signet-ring lipoblasts, but typical features of urothelial carcinoma are generally present. It is currently considered as an ill-defined tumor variant with less than 10 reported cases. We report on clinicopathologic, immunohistochemical and LOH analysis in 23 such cases.
Design: Twenty three neoplasms with morphologic features of UCBLCV were reviewed. Clinical findings such as age/sex, initial symptom, and therapy and survival status were obtained from patients chart. The immunohistochemical panel included cytokeratins (AE1, AE3, 7, 20, and high molecular weight), CEA, EMA, LEA135, thrombomodulin, vimentin, and S100 protein. LOH analysis was performed on 8 cases using 4 polymorphic microsatellite markers (D9S171, D9S177, IFNA and TP 53).
Results: All 23 patients were male aged 56-to-92 (mean 74) years. At histology, the lipoid cell pattern varied from 10%-to-50% of the tumor specimen, with associated micropapillary (n=1), plasmacytoid (n=1), squamous carcinoma (n=1) and grade 3/3 conventional urothelial carcinomas (n=20). Immunohistochemistry showed an epithelial phenotype of the lipoid cells characterized by diffuse cytokeratins AE1/AE3/7(+++), EMA(++), and LEA135(+++), or focal CEA(+), Cytokeratin 20(+), high molecular weight cytokeratin(+), and thrombomodulin(+) expression. Vimentin and S100 protein were negative. LOH was present in 6 out of 8 cases. Pathologic stage was Ta (n=1), T1 (=1), T2 (n=6), T3a (n=3), T3b (n=7), and T4 (n=5). Sixteen (69.5%) patients died of cancer on follow up (range 6-to-58 months); four were alive with disease and three patients died of other causes.
Conclusions: UCBLCV is rare and usually presents in old men with high tumor stage and poor survival. The finding of a concordant pattern of LOH suggests that lipoid cells are clonally related with the associated urothelial carcinoma. The immunohistochemical profile supports that the lipoid cell component present in urothelial bladder carcinoma is of epithelial origin suggesting that these tumors should be classified as a variant of urothelial carcinoma.

763 Gleason Grading of Prostatic Adenocarcinoma with Glomeruloid Features on Needle Biopsy

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Background: Glomerulations in prostatic adenocarcinoma are characterized by dilated glands containing intraluminal cribriform structures with a single point of attachment, resembling a renal glomerulus. On prostate biopsy, glomerulations are exclusively associated with carcinoma and not associated with benign mimickers. However, the Gleason grading of carcinoma with glomerulations on needle biopsy remains controversial.
Design: We prospectively collected 45 prostate needle biopsies containing carcinoma with glomeruloid features from our consult files from 12/06 to 08/07. For each case, the Gleason score of the carcinoma on the same core as the glomerulations was recorded, as was the overall highest Gleason score for the entire case. Gleason pattern 4 carcinomas associated with glomerulations were further characterized by predominant architectural pattern.
Results: Glomerulations were overwhelmingly associated with high grade cancer on the same core, composed of either Gleason pattern 4 (n=36, 80% of cases) or Gleason pattern 5 (n=2, 4% of cases). Only a minority of glomerulations were surrounded exclusively by pattern 3 cancer (n=7, 16% of cases) on the same core. The majority of the cases with surrounding pattern 4 cancer were scored as 3+4=7 (n=24, 66%), while a smaller fraction were scored as 4+3=7 (n=9, 26%) and only a minority were 4+4=8 (n=3, 9%). Pattern 4 cases were evenly divided into those showing predominantly fused, poorly formed glands and those showing large or irregular cribriform glands (49% and 51% respectively). In most cases, glomeruloid change was present on the same core as the highest Gleason score carcinoma of the case. None of the pattern 3 cases with glomeruloid features showed higher grade carcinoma on other cores, and only a minority of the pattern 4 cancers had higher Gleason score carcinoma on additional cores (n=5, 14%).
Conclusions: Glomeruloid structures are a rare but diagnostic feature of prostatic carcinoma on needle biopsy. Our data indicate that glomerulations are overwhelmingly associated with Gleason pattern 4 or higher grade carcinoma, both on the same core, as well as on additional cores in the same case. In several cases, transition could be seen between small glomerulations, large glomeruloid structures, and cribriform pattern 4 cancer. This data suggests that glomerulations represent an early stage of cribriform pattern 4 cancer and until follow-up data is available, are best graded as Gleason pattern 4.

764 Significance of a New Entity Termed Diffuse Adenosis of the Prostatic Peripheral Zone

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Background: We have observed a group of typically younger patients with multiple foci of small, nonlobular, crowded, but relatively bland acini on needle biopsy and in

prostatectomy specimens. It is unclear whether this architectural pattern, which we have termed Diffuse Adenosis of the Peripheral Zone (DAdPZ), is simply a cellular variant of normal prostate morphology or whether it represents a risk factor for the development of prostatic carcinoma. Informally, we have designated these prostates as "funny looking prostates (FLPs)".
Design: We studied 61 cases of DAdPZ on needle biopsy in our consult practice from 2001 to 2007. The percentage of cores involved by DAdPZ was calculated and the PSA level at the time of biopsy and clinical follow-up were obtained. Although the background DAdPZ had no cytologic atypia, rare glands in some cases had focal atypia which we rated as moderate or severe.
Results: Cases on average showed 72% of cores involved by DAdPZ. Average patient age was 49 years (range=34-73) and the average PSA level at the time of biopsy was 5.2 ng/mL (n=40). 39 cases (64%) had available clinical follow-up, while 17 (28%) were confirmed lost to follow-up, and in 5 cases (8%) clinical follow-up is currently being pursued. 31/39 cases (79%) with available clinical follow-up were rebiopsied and 8 (21%) were followed with serial PSA measurements. Patients rebiopsied after DAdPZ diagnosis had higher PSA levels than those who were followed by PSA levels alone (6.2 vs 3.1 ng/mL, p = 0.04). Of the rebiopsied cases, 20 (65%) were subsequently diagnosed with carcinoma, with an average of 15.3 months elapsed between initial biopsy and carcinoma diagnosis. Patients with a subsequent diagnosis of carcinoma were more likely to have had at least one small focus of DAdPZ with moderate-severe cytological atypia (p=0.04). We histologically confirmed the carcinoma diagnosis in 14 cases (all were Gleason score 3+3=6) and are in the process of confirming the remaining cases. 9/11 radical prostatectomies were reviewed, of which 8 showed peripheral zone pT2c disease in a background of DAdPZ. In one case of DAdPZ misdiagnosed as cancer on biopsy, no carcinoma was found at prostatectomy.
Conclusions: DAdPZ is a newly described and diagnostically challenging entity on prostate needle biopsies from typically younger patients. It should not be mistaken for carcinoma, as we have seen at least one negative prostatectomy in this context. On the other hand, the high incidence of carcinoma following a DAdPZ diagnosis suggests that patients with this finding should be followed closely and rebiopsied.

765 Frequent FGFR3 Mutations in Inverted Urothelial Papilloma

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Background: The fibroblast growth factor receptor 3 (FGFR3) is a member of a family of tyrosine kinase receptors. The FGFR3 gene, on chromosome 4p16 is involved in cell signaling pathways and angiogenesis as well as cell proliferation, development, and differentiation. Activating point mutations of FGFR3 have been associated with several skeletal abnormalities and multiple myeloma. Interestingly, urothelial papillomas and low grade urothelial carcinoms have shown a high incidence of FGFR3 mutations and are associated with a favorable prognosis. The association of FGFR3 mutations with inverted papillomas is unknown.
Design: 10 cases of inverted papilloma in the bladder were evaluated for the presence of one of four specific missense mutations in the FGFR3 gene (codons 248, 249, 372/375, and 652).
Results: Activating point mutations of the FGFR3 gene were identified in 60% (6/10) cases of inverted papilloma with three cases exhibiting mutations at multiple exons. Mutations occurring were 4 at codon 248, 3 at codon 249, 1 at codon 372/375, and 2 at codon 652.
Conclusions: FGFR3 gene mutations may be involved in tumorigenesis of inverted urothelial papilloma.

766 Insulin-Like Growth Factor-1 Receptor (IGF-1R) Is Up-Regulated in Prostatic Adenocarcinoma (PAC) and Correlates with Gleason Grade and Stage

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Background: The insulin-like growth factor (IGF) signaling pathway components, especially IGF-1R and its docking molecules are associated with regulation of cell growth, establishment of neoplastic phenotype and apoptosis. Expression of IGF components has been studied in a variety of tumors and implicated in the pathogenesis of melanoma, breast, colorectal and prostate cancer. There is, however no consensus regarding the expression of IGF-1R in PAC. To clarify this we studied its expression in PAC.
Design: Formalin-fixed paraffin-embedded whole-mount radical prostatectomy specimens from 203 patients with PAC were evaluated for IGF-1R expression by immunohistochemistry. Slides were stained by automated methods (Ventana Medical Systems, Tucson, AZ) using mouse anti-human IGF-1R antibody (sc-462; Santa Cruz Biotechnology, Santa Cruz, CA). Immunohistochemical assessment included intensity and percentage of positive cells in both benign and carcinomatous elements. Hematoxylin and Eosin stained slides were reviewed and tumors graded based on the Gleason grading system. Tumors were scored semi quantitatively based on staining intensity (weak, moderate, strong) and distribution (focal <25%, regional 25-50%, diffuse>50%). These results were correlated with Gleason grade and tumor stage.
Results: All 203 prostatectomies showed dim expression of IGF-1R in the benign glandular epithelium as compared with neoplastic glands. A statistically strong correlation was seen with tumor grade (p<0.0001)). IGF-1R expression increased with increasing Gleason score with 90% (87/97) of high grade tumors (Gleason score of 7 or more) showing high expression of IGF-1R while only 48% (51/106) of low grade tumors (Gleason score of 6 or less) showing increased expression. Similarly, a statistically significant correlation was seen with pathological stage (p=0.002). 86% of high stage tumors (T3/ T4) showed high expression of IGF1R as opposed to only 62% of organ confined cancers.
Conclusions: Up-regulation of IGF-1R in PAC and increased expression in high grade and high stage PAC suggest its role in prostate cancer pathogenesis. Given its correlation with Gleason grade and pathological stage, known predictors of disease outcome, IGF-1R should be further investigated as a prognostic factor. Finally, IGF-1R mediated intracellular pathway components could provide additional potential targets for therapeutic intervention.

767 Neural Precursor Cell-Expressed Developmentally Down-Regulared 8 (NEDD8) Is Up-Regulated in Prostatic Adenocarcinoma (PAC) and Correlates with Gleason Grade

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Background: NEDD8 is a ubiquitin-like protein essential for protein processing and cell cycle progression. NEDD8 gene expression may be involved in tumorigenesis by modifying tumor suppressor genes such as p53, p73, and pVHL. NEDD8 gene expression is up-regulated in renal cancer and oral squamous cell carcinoma, but has not been well-characterized in PAC.
Design: Formalin-fixed paraffin-embedded whole-mount radical prostatectomy specimens from 135 patients with PAC were evaluated for NEDD8 expression by immunohistochemistry. Patients ranged from 40 ~ 81 in age (mean = 62), and with no therapy before radical prostatectomy. Slides were stained by automated methods (Ventana Medical Systems, Tucson, AZ) using NEDD8 rabbit mAb (#2754; Cell Signaling Technology, Danvers, MA). Immunohistochemical assessment included intensity and percentage of positive cells in both benign and carcinomatous elements. Hematoxylin and Eosin stained slides were reviewed and tumors graded based on the Gleason grading system. Tumors were scored semi-quantitatively based on staining intensity (weak=1, moderate=2, strong=3) and distribution (focal, regional, diffuse). These results were compared with Gleason grade and tumor stage.
Results: All 135 prostatectomy specimens showed weak to moderate expression of NEDD8 in the benign glandular epithelium as compared with neoplastic glands. A statistically significant correlation was seen with tumor grade (p= 0.05). NEDD8 expression increased with increasing Gleason score with 60% (43/72) of high-grade tumors (Gleason score of 7 or more) showing high expression of NEDD8 while only 43% (27/63) of low-grade tumors (Gleason score of 6 or less) showing increased expression. NEDD8 expression did not correlate with pathological stage (p= 0.63).
Conclusions: Increased NEDD8 expression is identified in PAC and is associated with high tumor grade suggesting that this biomarker may play a role in prostate cancer pathogenesis. Further study of NEDD8 expression in prostate cancer both as a potential prognostic factor and target of therapy appears warranted.

768 Precursor of PSA (Pro-PSA) Expression in Prostatic Intraepithelial Neoplasia and Adenocarcinoma: A Study of 90 Cases

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Background: The precursor of PSA (pro-PSA), a distinct molecular form of free PSA in serum, includes native and several truncated forms. Its expression in benign epithelium, high-grade prostatic intraepithelial neoplasia (HGPIN), and prostatic adenocarcinoma has not been well characterized.
Design: The authors performed an immunohistochemical (IHC) study of tissue sections from 90 prostate needle biopsies at Bostwick Laboratories using monoclonal antibodies against [-2] pro-PSA, native [-5/-7] pro-PSA, prostate-specific membrane antigen, PSA, and racemase. Staining intensity was recorded using a scale of 0 to 3 (0 = no staining, 3 = highest). The percentage of immunoreactive cells in benign epithelium, HGPIN, and adenocarcinoma was estimated in increments of 10%.
Results: All cases exhibited [-5/-7] pro-PSA immunoreactivity. Weak focal perinuclear cytoplasmic immunoreactivity for [-5/-7] pro-PSA was observed in 62.3% of benign epithelial cells, while strong diffuse cytoplasmic staining was observed in 83.3% of HGPIN epithelium and 87.2% of adenocarcinoma epithelial cells. Almost all (98.9%) cases were immunoreactive to [-2] pro-PSA. The number of cells reactive to [-2] pro-PSA was lower in benign epithelium (16.9%) than in HGPIN (55.0%, P<0.0001) and adenocarcinoma (55.1%, P<0.0001). The intensity of immunoreactivity to both isoforms increased from benign to neoplastic (HGPIN and adenocarcinoma) epithelium. No difference was observed for [-5/-7] and [-2] pro-PSA staining between high-grade and low-grade adenocarcinoma. Cases exhibiting racemase immunoreactivity were greater in HGPIN (69.2%) and adenocarcinoma (88.9%) epithelium compared to benign epithelium (33.8%). Interestingly, 31% of PIN and 11% of cancer cases with negative racemase staining showed strong positive staining for [-5/-7] pro-PSA. The immunoreactivity of PSA and PSMA staining were greater than 90% in all cases.
Conclusions: The different staining patterns for [-5/-7] pro-PSA in benign and neoplastic cells can be used to distinguish benign epithelium from HGPIN and adenocarcinoma, particularly when racemase staining is negative. Both isoforms are sensitive markers for prostatic epithelium, making them possible candidates for investigating carcinomas of unknown primary, particularly when PSA staining is negative and the level of suspicion is high.

769 Prostate Cancer Detection in Men with Initial Biopsy Diagnosis of "Atypical Glands Suspicious for Cancer": Impact of Expert Opinion and Ancillary Immunohistochemistry

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Background: A diagnosis of "atypical glands suspicious for cancer (ATYP)" in prostate biopsy (PBx) is associated with a 40-50% risk of detecting prostate cancer (PCa) in subsequent biopsies. Expert consultation and ancillary immunohistochemistry (IHC) in

such cases help reduce the incidence of ATYP. However, it is not known whether such additional work-up may also lead to reduced cancer risk in subsequent biopsies.
Design: We studied patients with an initial diagnosis of ATYP on PBx and subsequent follow-up biopsies between 1992 and August 2005. Before 2003, all PBx were reviewed by general surgical pathologists at our institution; in and after 2003, all PBx were reviewed only by GU pathologists.
Results: The ATYP incidence is significantly lower in PBx diagnosed by GU pathologists (1.3 % from the period 2003-Aug 2005, vs 2.1% prior to 2003, p=0.01), but the subsequent cancer risk remained the same. For 75 ATYP cases diagnosed since IHC for basal cell markers and AMACR was implemented in mid 1997 and 2004, respectively, the cancer risk in subsequent biopsies was 19/24 (79.2%) for cases with no IHC, 21/42 (50%) for cases with only basal cell IHC, and 1/9 (11.1%) with IHC for both AMACR and basal cell markers (p=0.0015 by Chi-square test).
Conclusions: Expert consultation reduces the incidence of ATYP diagnosis on PBx; however, it does not reduce the cancer risk in subsequent biopsies. The use of ancillary IHC, especially of both basal cell markers and AMACR, significantly reduces the cancer risk associated with an initial ATYP diagnosis. PBx with an ATYP diagnosis should be reviewed by a GU pathologist and work-up should include IHC for basal cell markers and AMACR.

Repeat PBx Findings for Patients With An Initial ATYP Diagnosis

		1992-1997	1998-2002	2003-2005	Total
# of cases		6695	7663	3702	18396
# of ATYP		138	163	49	350
ATYP Incidence*		2.1%	2.1%	1.3%	1.9%
f/u available		43 (31.2%)	47 (28.8)	30 (61.2)	120 (34.3%)
Mean f/u time (range, month)		15.3 (0.5-73)	16.1 (0.5-74.5)	12.5 (2-42)	14.8 (0.5-74.5)
f/u diagnoses+	Binign	14 (32.6%)	14 (30%)	5 (16.7%)	33 (27.5)
	HGPIN	3 (7.0%)	4 (8.5%)	5 (16.7%)	12 (10.0%)
	ATYP	0 (0%)	3 (6.4%)	4 (13.3%)	7 (5.8%)
	PCa	26 (60.5%)	26 (55.3%)	16 (53.3%)	68 (56.7%)

*p=0.01 by chi square test +The follow-up diagnoses are not statistically different during 1992-1997, 1998-2002 and 2003-2005 (p=0.162 by chi-square test)

770 Malignant Profiles in Normal- or Hyperplastic-Appearing Prostate Acini and Ducts

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Background: The development of prostate cancer is believed to be a multi-step process, progressing sequentially from normal to hyperplasia, to prostatic intraepithelial neoplasia (PIN), and to invasion. Progression from PIN to invasion is believed to be triggered by overproduction of proteolytic enzymes that cause degradation of the basement membrane (BM). Our recent studies, however, showed that some normal or hyperplastic duct and acinar clusters had a high frequency of focal disruptions in the BM and basal cell layers (Man et al. Cancer Detect Prev 29:161-169, 2005; Man and Gardner. Medical Hypotheses. 2007 Jul 19; Epub ahead of print). As degradation of the BM and basal sell layers is a distinct sign of invasive lesions, this study intended to further elucidate the profiles of these clusters.
Design: H&E and immunostained sections from our previous studies were reviewed and 17 such cases were identified. Consecutive sections were prepared and subjected to molecular and immuno-histochemical assessment with a panel of basal cell-, BM-, aggressiveness-, and invasiveness-related biomarkers.
Results: These clusters varied markedly in size. At low magnification of H&E stained sections, they were indistinguishable from their clear-cut normal or hyperplastic counterparts. At high magnification, the epithelial cells showed nuclear enlargement with prominent nucleoli, and the loss of nuclear polarity. In immunostained sections, the BM and basal cell layers were fragmented with multiple small disruptions or were totally absent. The residual basal cells showed significantly reduced expression of tumor suppressor p63 and proliferating cell nuclear antigen, but elevated rate of apoptosis and leukocyte infiltration. In contrast, the epithelial cells showed distinct signs of aggressiveness and invasiveness: [1] significantly elevated proliferation rate; [2] significantly elevated androgen receptor expression; [3] associated with stromal tissues that show markedly elevated vascular density and tenascin expression; [4] direct physical continuity with invasive lesions.
Conclusions: These findings suggest that these acinar or duct clusters may represent previously unrecognized malignant lesions, or progression and invasion of prostate malignancies may not always follow the hypothesized steps. (Supported by grants PC051308, DAMD17-01-1-0129, DAMD17-01-1-0130 from Congressionally Directed Medical Research Programs, and BCTR0706983 from the Susan Komen Breast Cancer Foundation to Yan-gao Man).

771 Elevated Tenascin Expression in Stroma near Focally Disrupted Prostate Basal Cell Layers: Implications for Tumor Progression and Invasion

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Background: Our recent studies revealed that a subset of pre-invasive breast tumors contained focal disruptions in surrounding basement membrane and myoepithelial cell layers, and that stromal tissues adjacent to these focal disruptions consistently showed a significantly higher rate of tenascin expression and leukocyte infiltration (Man. Medical Hypotheses 2007 May 8; Epub ahead of print). Since elevated tenascin expression and leukocyte infiltration are believed to promote tumor invasion, this study attempted to assess whether similar alterations could be seen in human prostate tumor tissues.
Design: Consecutive sections were prepared from 50 formalin-fixed, paraffin-embedded human prostate tumors with co-existing normal, hyperplastic, pre-invasive, and invasive components. Sections were subjected to double immunohistochemistry with a panel of monoclonal antibodies against tenascin, basal cells, leukocytes, and cell proliferation-related proteins. The expression levels and frequencies of these molecules in ducts and acini with and without focal basal cell layer disruptions were statistically compared.
Results: Similar to those seen in human breast tumors, 34% of the ducts and acini with pre-invasive tumors were found to contain a high frequency of focal basal cell layer disruptions (FBCLD). In stromal tissues immediately surrounding these FBCLD, substantially elevated tenascin expression was detected in over 60%, and leukocyte infiltration was detected in over 90%, of the cases. Elevated tenascin and infiltrated leukocytes were predominantly located at the center of FBCLD. A vast majority of the proliferating tumor cells were also located at or near the center of FBCLD. In sharp contrast, all or nearly all the stromal tissues surrounding non-disrupted basal cell layers showed no distant tenascin expression or leukocyte infiltration.
Conclusions: These findings suggest that progression and invasion of prostate and breast cancer are likely to share a similar mechanism, promoted by correlated alterations in epithelial and stromal cells. Consequently, identification of these correlated events and interactive molecules may have significant value in early detection, treatment, and prevention of prostate cancer invasion. (Supported by grants PC051308, DAMD17-01-1-0129, DAMD17-01-1-0130 from Congressionally Directed Medical Research Programs, and BCTR0706983 from the Susan Komen Breast Cancer Foundation to Dr. Yan-gao Man.)

772 Histopathologic Outcomes of Robotic Assisted Laparoscopic and Open Prostatectomies

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Background: Robotic assisted laparoscopic radical prostatectomy (RALRP) has become an alternative to traditional open radical prostatectomy (ORP) for the treatment of prostate carcinoma. Studies in the clinical urology literature have shown that RALRP has similar pathologic outcomes to ORP based on review of pathology reports, but few studies have provided detailed investigation of histopathologic differences that may affect the manner in which pathologists examine such specimens.
Design: A retrospective comparison of 61 consecutive RALRP and 61 consecutive ORP was performed. All cases were grossed by a standard procedure for prostatectomies. The pathology reports and glass slides were re-examined and assessed for prostate weight, final Gleason sum (GS), pathologic stage, margins, location and extent of positive margins, number of step sections, effect of cautery on margin assessment, completeness of prostate gland excision and prostatic capsule integrity. Clinical data collected included pre-operative PSA and GS, clinical stage, and nerve-sparing status.
Results: The two groups were statistically similar with respect to pre-op PSA (p=0.589), pre-op GS, final GS, clinical stage, pathologic stage, and nerve-sparing status (p=1.000). There was no statistical difference in the prostate weight, number (no.) of blocks submitted, positive margin rate (16.1% RALRP, 27.9% ORP, p=0.190), positive margin location, no. of cases with multiple positive margins, no. of cases requiring step sections, extent of positive margin, or effect of cautery artifact. There was no significant difference between the two groups in the integrity of the capsule (p=0.273). The apex was more likely to have loss of the capsule in ORP (p=0.022), but this did not reach statistical significance in positive apical margin rate (ORP 19.7%, RALRP 6.56%, p=0.058). Positive surgical margins were more likely to be extensively involved at the apex in ORP (p=0.011).
Conclusions: No clinically significant histopathologic differences were identified between RALRP and ORP specimens. The RALRP group had a lower positive margin rate than ORP, but the difference was not statistically significant. The completeness of prostate excision was higher in the RARLP group for the anterior and apical areas, but this was not associated with statistically significant difference in margin positivity rates. These results suggest that standard/traditional techniques for the pathologic assessment of prostatectomies apply equally well to both types of procedures.

773 Epidermal Growth Factor Receptor (EGFR) Expression in Prostatic Adenocarcinoma after Hormonal Therapy

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Background: The progression of normal prostatic epithelium to androgen-dependent cancer and, eventually, hormone-refractory prostate cancer is a complex process involving many different growth regulatory signals. Activation of epidermal growth factor receptor (EGFR) has been implicated in prostate cancer cell growth. **Methods:** This study was undertaken to investigate both amplification of EGFR gene by fluorescence in situ hybridization and over-expression of EGFR by immunohistochemical staining in prostate tissue from 71 patients treated by hormonal therapy.
Design: This study was undertaken to investigate both amplification of EGFR gene by fluorescence in situ hybridization and over-expression of EGFR by immunohistochemical staining in prostate tissue from 71 patients treated by hormonal therapy.
Results: EGFR gene amplification was present in 1 of 71 tumors, and polysomy of chromosome 7 was present in 24 of 71 tumors. Immunohistochemically, EGFR expression was demonstrable in 57 of 71 tumors. Membranous immunostaining for EGFR was observed in >75% of tumor cells in 11% of cases, in 51-75% of tumor cells in 20% of cases, in 26-50% of tumor cells in 21% of cases, in 11-25% of tumor cells in 21% of cases, and in 1-10% of tumor cells in 7% of cases. No immunostaining for EGFR was seen in 20% of cases. There was no correlation between EGFR protein expression and gene amplification. There was also no correlation between EGFR expression and clinicopathological characteristics or clinical outcome.

Conclusions: We found that EGFR gene expression was detectable in 35% of this large series of hormone-treated prostate cancer, and that EGFR protein is frequently expressed in tissue from these patients. EGFR over-expression may serve as a reasonable target for therapeutic intervention in this otherwise difficult to treat subset of prostate cancer.

774 Heterogeneity of *TMPRSS2* Gene Rearrangements in Multifocal Prostate Adenocarcinoma: Molecular Evidence for an Independent Group of Diseases

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Background: Recurrent gene fusions between the androgen-regulated gene *TMPRSS2* and the *ETS* family transcription factors *ERG*, *ETV1* and *ETV4* have been identified in the majority of prostate adenocarcinomas (PCAs). PCA is often multifocal with histologic heterogeneity of different tumor foci.

Design: As *TMPRSS2* is a common 5' partner of *ETS* gene fusions, we monitored *TMPRSS2* rearrangement by fluorescence in situ hybridization (FISH) to study the origin and molecular basis of multifocal PCA heterogeneity. *TMPRSS2* rearrangement was evaluated by FISH on a tissue microarray representing 93 multifocal PCAs from 43 radical prostatectomy resections.

Results: Overall, 70% (30/43) of the cases demonstrated *TMPRSS2* rearrangement, including 63% through deletion (loss of the 3' *TMPRSS2* signal), 27% through translocation (split of 5' and 3' *TMPRSS2* signals) and 10% through both mechanisms in different tumor foci. Of the 30 *TMPRSS2* rearranged cases, 30% demonstrated concordance in all tumor foci while 70% were discordant in at least one focus. In *TMPRSS2* rearranged cases, the largest (index) tumor was rearranged 83% of the time. Pathologic stage, size, or Gleason grade of the multifocal PCA did not correlate with overall *TMPRSS2* rearrangement.

Conclusions: Our results suggest that multifocal PCA is a heterogeneous group of diseases arising from multiple, independent clonal expansions. Understanding this molecular heterogeneity is critical to the future development and utility of diagnostic and prognostic PCA biomarkers and development of tumor specific focal therapy.

775 *TMPRSS2: ETS* Gene Fusions in Androgen Independent Metastatic Prostate Cancers: An Association of *TMPRSS2: ERG* Fusions through Intronic Deletions and Molecular Evidence of Clonal Expansion

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Background: Recurrent gene fusions between the androgen-regulated gene *TMPRSS2* and the *ETS* family transcription factors *ERG*, *ETV1* and *ETV4* have been identified as a critical molecular event in prostate cancer (PCA) carcinogenesis. The objective of this study was to comprehensively characterize frequency, mechanism, and significance of these gene fusions in a cohort of androgen independent lethal metastatic PCAs.

Design: A tissue microarray (TMA) was constructed representing 12 primary and 84 non-osseous metastatic samples from 30 rapid autopsies of men died of androgen independent metastatic PCA. Gene fusions between *TMPRSS2* and three *ETS* family members were analyzed by fluorescence in situ hybridization (FISH) assay using 5' and 3' split probes flanking 4 genes. FISH signals were scored manually (100x oil immersion) in morphologically intact, non-overlapping nuclei and a minimum of 50 cancer cells from each site were recorded. Comparisons were made in between patients as well as within different metastatic sites of same patient.

Results: Rearrangement for *TMPRSS2*, *ERG*, *ETV1* and *ETV4* were seen in 48, 37, 11 and 4% of cases respectively. Overall, 37, 4, and 4% cases demonstrated *TMPRSS2: ERG*, *TMPRSS2:ETV1* and *TMPRSS2:ETV4* fusions respectively. As intronic loss of genomic DNA between *TMPRSS2* and *ERG* has been identified as a mechanism of *TMPRSS2: ERG* fusion, our assays allowed us to detect deletion between them in 100% of rearranged cases for this molecular subtype. For each case demonstrating gene rearrangement, all metastatic sites and primary site when present uniformly maintained similar gene rearrangement.

Conclusions: Our results indicate that high level of *TMPRSS2: ERG* gene fusions is maintained during progression to androgen independent state however this fusion is seen exclusively thorough intronic deletions between two genes, indicating that this molecular subtype is associated with aggressive lethal PCAs. Metastatic PCAs maintain homogeneity of the respective molecular subtype of gene fusion, indicating that they arise through clonal expansion of seeded primary malignant cells in different metastatic sites.

776 How Different Are the Clinicopathologic Features of Predominantly Transition Zone Tumors in Radical Prostatectomies?

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Background: Tumors arising in the transition zone of the prostate are generally considered well differentiated and clinically unimportant, however, some reports have questioned this assumption.

Design: The study was based on 214/272 (78.7%) whole-mount consecutive surgical specimens; 58/272 (21.3%) very limited (insignificant) tumors were excluded for this study. Tumor extent was evaluated with a point-count semiquantitative method previously described. The bladder neck and the apical margins were amputated. From each cone-shaped amputated margin, 8 fragments were processed through sections perpendicular to the margins. The rest of the prostate was serially cut at 3 to 5mm transverse sections intervals. The prostate slices were subdivided into quadrants and labeled to allow for reconstruction as whole-mount sections. Tumors were considered predominantly in the transition zone whenever $\geq$ 70% of the positive points using the semiquantitative method were in the anterolateral quadrants. Extraprostatic extension was diagnosed whenever cancer was seen in adipose tissue and, in case of desmoplastic response, whenever a protuberance corresponding to extension of tumor into the periprostatic tissue was seen. The data were analyzed using the Mann-Whitney test and the Fisher's exact test. Time to biochemical (PSA) progression was compared using the Kaplan-Meier product-limit analysis.

Results: We found 40/214 (18.7%) surgical specimens with predominantly transition zone tumors (group 1). Comparing group 1 with the remaining cases (group 2) for several clinicopathological variables, the findings were, respectively: mean age 64.3yrs and 63.1yrs (p=0.14); clinical stage T1c 34.2% and 43.5% (p=0.37); preoperative PSA 9.2ng/mL and 9.6ng/mL (p=0.96), Gleason score 6.6 and 6.9 (p=0.02); positive surgical margins 52.5% and 50.6% (p=0.86); extraprostatic extension (pT3a) 30% and 33.5% (p=0.70); and, seminal vesical invasion (pT3b) 15% and 14.4% (p=1.00). At 5 years, the PSA progression-free survival rates were 75% and 48%, respectively, for patients of group 1 and group 2 (log-rank, p=0.12).

Conclusions: Predominantly transition zone tumors showed lower Gleason score in radical prostatectomies. This was the only statistically significant clinicopathologic finding in this series.

777 Premalignant Lesions of the Kidney

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Background: It has been estimated that there will be 35,710 new cases and 12,480 deaths due to RCC in the United States in 2007. There are no markers for early detection, and better forms of therapy are still under investigation. Kidney cancer, comprises several histologic subtypes with specific molecular hallmarks which support their classification as distinct entities. While the vast majority of renal tumors occur sporadically, 6% or more are hereditary. Premalignant lesions have been well characterized in organs such as colon, and cervix. We investigated the presence of premalignant lesions in patients with sporadic and hereditary kidney cancer, and compared their morphologic and genetic alterations with those of the primary tumor.

Design: Forty five cases of renal cell carcinoma were studied. Fifteen were sporadic cancers including 6 CDC, 2 Medullary and 7 clear cell. Thirty five cases were hereditary (10 VHL, 10 Papillary type I, 10 BHD and 5 HLRCC). Adjacent normal parenchyma was evaluated in all cases for Renal Intratubular Neoplasia as well as presence of lesions other than the primary tumor regardless of size. Genetic alterations were investigated for LOH in Ch 3p,17q and 1q in the early lesions as well as the tumors by tissue microdissection. CISH/FISH was performed to detect trisomies in chr 7 and 17. IHC for p53, MiB1, CK7 and CD10 was also done.

Results: Sporadic cases showed a higher incidence of RIN near the distal tubules specially in cases of CDC (4/6) and Medullary (1/2) cancer. Clear cell demonstrated atypical changes predominantly in the cortical tubules. Hereditary tumors had a spectrum of early lesions according to the tumor type; cases of VHL had cystic lesions with variable degrees of cellular atypia, and nests of tubules with prominent clear cytoplasm. Cases of Pap type I had microscopic lesions predominantly in the cortex, that progressed from individual tubules showing atypia to papillary growths that resembled the large tumor masses. Oncocitosis, or clusters of clear and eosinophilic cells scattered throughout the adjacent parenchyma was seen in most cases of BHD. RIN was seen in some of the HLRCC cases. Similar genetic changes were seen in the premalignant and tumoral tissues.

Conclusions: Our results suggest that early lesions identified in hereditary kidney cancers, represent neoplasms, and that genetic alterations occur early in their development. RIN may be more frequent in sporadic and other cancers that arise in the distal nephron. Identification of premalignant lesions may be important as new surgical modalities of treatment are developed.

778 Is High Grade Intraepithelial Neoplasia (HPIN) an Independent Risk Factor for Prostatic Adenocarcinoma (PCA)?

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Background: HPIN is pathogenically related to PCa but there is debate in the literature regarding its current role as a risk factor for subsequent detection of PCa in prostatic needle biopsy (PNB). Many previous studies are limited by small sample sizes, lack of controls and other statistical issues. Using a large data set we evaluated whether HPIN on initial PNB is an independent risk factor for PCa.

Design: We reviewed the pathologic findings from 12304 men who underwent initial PNB during an 8 year period (May 1999- June 2007). PNBs were obtained from 28 community-based urologists in a setting without population based screening, typically for abnormalities in serum PSA or digital rectal examination (DRE). The PNBs were read by 3 urologic pathologists. Patients were included in the study if their initial diagnosis was HPIN alone or a benign diagnosis and if at least one follow-up PNB was performed.

Results: Of the 12304 patients, 4938 (40.13%) and 1283 (10.43%) had initial benign and HPIN diagnoses, respectively. A total of 1409 patients were included on the study, with 845 and 564 in the benign and HPIN groups. The mean follow-up time for the benign and HPIN groups was 2.36 and 1.37 years, respectively, with each group having an average of 2.3 total biopsies. 27.48% of patients with HPIN and 22.01% with benign diagnoses were subsequently diagnosed with PCa (p=0.019). A complete data set was available for 1120 patients (451 HPIN,669 benign). When age, PSA, extent of sampling and sign-out pathologist were adjusted for, the risk of PCa after an initial diagnosis of HPIN remained significant with an odds ratio of 1.38 (p=0.033). The risk of PCa was related to the extent of HPIN in the initial sampling, with odds ratios of 1.52, 1.95, 2.61 and 2.32 if 2,3,4 or 5 sites were involved, respectively (p=0.041).

Conclusions: Patients with an initial diagnosis of HPIN are at greater risk for subsequent PCa than those with benign diagnoses. The risk is independent of other variables (age, PSA, sampling and sign-out pathologist) and varies with number of sites involved with HPIN but may be related in part to more frequent PNB in the HPIN group.

779 *TMPRSS2-ERG* Fusion Prostate Cancer Is a Molecularly Distinct Estrogen-Sensitive Subclass of Aggressive Prostate Cancer

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Background: *TMPRSS2-ERG* fusion prostate cancer (PCA) is associated with a more aggressive clinical phenotype. We performed expression array profiling to better understand the molecular correlates of *TMPRSS2-ERG* PCA.

Design: DNA-mediated Annealing, Selection, Ligation and Extension (DASL) was used for the analysis of 455 formalin-fixed paraffin embedded tumor samples derived from a Swedish Watchful Waiting cohort (1987-1999) and the Physicians Health Study cohort (1983-2003). A custom array was designed for the study to interrogate ~6000K genes.

Results: An 87-gene signature distinguished *TMPRSS2-ERG* fusion PCA as a discrete molecular entity. This signature was associated with estrogen receptor signaling. Functional studies demonstrated regulation of the *TMPRSS2-ERG* fusion transcript by estrogenic compounds via estrogen receptor (ER) action. Stimulation of ERa resulted in *TMPRSS2-ERG* up-regulation, thereby explaining *TMPRSS2-ERG* expression in the androgen receptor (AR) negative NCI-H660 cell line. ERb agonism led to *TMPRSS2-ERG* down-regulation resulting in growth suppression of ERb positive NCI-H660 cells. Chromatin immunoprecipitation experiments showed that ERb localized to the *TMPRSS2* promoter in NCI-H660 cells. This result suggests that ERb regulation of *TMPRSS2-ERG* expression occurs through direct transcriptional regulation of the gene fusion.

Conclusions: We showed that *TMPRSS2-ERG* fusion PCA is a distinct molecular subclass and present a previously unrecognized mechanism for regulation of *TMPRSS2-ERG* expression. Our data suggest that inhibition of *TMPRSS2-ERG* expression using drugs that antagonize *ERa* activity and function may have promise as a new therapeutic approach for PCA.

780 Direct Detection of TMPRSS2:ETS^{family} Gene Fusion mRNAs in Prostatectomy Tissue: Prevalence and Correlation with Clinicopathologic Data

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Background: Fusion between TMPRSS2 (T2) and the ETS family of transcription factors is the most common specific gene arrangement identified to date among solid human malignancies (50 to 70 % of prostate cancer). In this research study, we characterized the T2:ETS^{family} mRNAs in tumor foci from formalin-fixed paraffin embedded (FFPE) radical prostatectomy (RP) tissue, and correlated these results with clinicopathologic data.

Design: One hundred consecutive RP patients (mean age 68 years) from 1999 to 2001 were included in the study: 26 cases ≥ pT3 cancer and 14 had seminal vesicle involvement (SVI). Ten-micron sections were cut from 524 zinc FFPE tissue blocks, then processed for histopathology or detection of T2:ETS^{family} mRNAs by using transcription-mediated amplification (TMA). Prototype TMA assays targeted the three most prevalent T2:ERG splice variants: T2exon1:ERGexon4 (T2:ERGa), T2exon1:ERGexon2 (T2:ERGb) or T2exon2:ERGexon4 (T2:ERGc), or T2:ETV1a,b. PSA mRNA levels were quantified to verify the integrity of the RNA.

Results: Based on PSA mRNA levels, 520/524 (99.2%) of the samples contained sufficient RNA for analysis. T2:ERG mRNAs were identified in 66% of tumor foci and 86% of patients. In fusion positive foci, T2:ERGa fusion was most frequently identified (83%) followed by T2:ERGc (61%). Among T2:ERG positive patients, 38/85 (45%) showed fusions in all tumor foci, while the remaining 47 patients showed fusion heterogeneity (i.e., T2:ERG ± foci). By patient, T2:ERGc was significantly associated with SVI (P = 0.01, PPV = 93%). There was no significant correlation between presence of T2:ERG and Gleason Score (6 vs. ≥ 7) or pathological stage (pT2 vs. pT3).

Conclusions: The high informative specimen rate (>99%) demonstrated the robustness of the specimen processing and TMA methods for detecting mRNA in FFPE tissue. The T2:ERG positive rate in tumor foci (66%) was similar to previous studies that utilized FISH; 86% of patients had at least one tumor focus containing T2:ERG mRNA. The association between T2:ERGc and SVI confirms that the presence of this splice variant is an adverse prognostic indicator after RP. Taken together, these data suggest that a T2:ERG tissue or urine test could be used to identify "significant" PCa, and might assist in selecting patients for active surveillance and/or adjuvant therapy.

781 An Oncofetal Protein, IMP3: A Novel Biomarker To Predict of Tumor Progression in the Low Grade Ta Urothelial Carcinoma of the Bladder

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Background: Tumor progression (deeper invasion and metastasis) is main cause of death from urothelial carcinomas (UC) of the bladder. Only a small portion of low-grade non invasive papillary (Ta) UC shows tumor progression. However, clinically it is important to identify these high-risk patients in order to treat and follow-up differently. In this study, we aimed to evaluate IMP3, an oncofetal protein, can serve as a molecular marker to predict tumor progression in low grade Ta UC of the bladder.

Design: The expression of IMP3 was evaluated by immunohistochemistry on 171 patients' initial bladder tissue biopsy with Ta UCs. Progression survival analyses were performed on 151 patients with available clinical follow-up data. Median follow-up was 44 months (range =2 –146 months). Tumor progression was measured as the date when tumors were demonstrated on subsequent biopsy to have evolved to a higher stage (deeper invasion) or when metastases were proven by clinical or pathologic diagnosis. The WHO/ISUP grading system was used [Papillary Urothelial Neoplasms of Low Malignant Potential (PUNLMP), n = 33; low grade, n = 118; and high grade, n = 20].

Results: The expression of IMP3 protein was observed in the cytoplasm of tumor cells, but not in benign tissue adjacent to the cancer. Twenty seven out of 171 (16%) primary Ta UCs expressed IMP3. IMP3 expression was strongly associate tumor grade (P=0.003). IMP3 was expressed in 9 (45%) of high grade tumors and 18 (15%) of low grade tumors. None of the PUNLMP cases expressed IMP3. Kaplan-Meier plots showed that patients with IMP3 positive low grade UC showed significantly higher progression rate as compared to patients with IMP3 negative low grade cases (p = 0.01). Thirty five percent of patients with IMP3 positive low grade UC showed progression within 5 years, whereas only 8 % of progression rate was found in IMP3 negative patients. No significant difference in progression free survival was found between IMP3 positive and IMP3 negative patients with high-grade Ta disease (P=0.45).

Conclusions: IMP3 is a useful prognostic marker that can identify a subgroup of patients with low grade non invasive UC who have a high potential to develop tumor invasion or metastasis and who may benefit from early and aggressive treatment.

782 Improving Diagnosis of Renal Cell Carcinoma through Chromosome Copy Number Analysis with SNP Arrays

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Background: Although the majority of renal tumors are classifiable based on morphologic characteristics, a subset of these tumors are difficult due to unusual features or mixed morphology. Single nucleotide polymorphism (SNP) arrays allow high resolution, whole-genome interrogation of chromosome copy number changes (CCNC) and loss-of-heterozygosity in paraffin-embedded tissues. We propose that this technique can be used for incorporating the existing knowledge of genetic alterations in renal cell carcinoma for its accurate classification in the clinical setting.

Design: We identified 28 renal epithelial neoplasms with challenging morphologic characteristics on routine signout. All tumors were reviewed by at least 2 pathologists who agreed that a definitive diagnosis could not be rendered. DNA from paraffin-embedded samples from these tumors were analyzed for CCNC using the Affymetrix GeneChip 10K 2.0 Mapping arrays. CCNC and loss-of-heterozygosity data were assessed using Copy Number Analyzer for Affymetrix GeneChip Mapping (CNAG 3.0). Patterns of CCNC were compared with patterns previously derived from known renal epithelial tumors (conventional RCC, papillary RCC, chromophobe RCC, and oncocytoma). Results were compared to IHC and FISH performance (when available).

Results: Patterns of CCNC derived from the morphologically challenging cases matched known patterns in 78% of cases (22/28). Only 1 case with good array performance did not match a known pattern. 5 cases resulted in bad quality data thus not available for analysis. FISH was diagnostic in only 8% of cases (3/39) and IHC in 16% of cases (6/38). In this cohort, 11 cases where the pathologist could not confidently categorize the tumor, were easily classified by the CCNC pattern. In 9 cases SNP confirmed the pathologist's favored diagnosis. In 4 cases with a final diagnosis of carcinoma, the CCNC pattern matched more closely that of a benign oncocytoma.

Conclusions: SNP arrays can detect characteristic chromosomal aberrations in paraffin-embedded renal tumors. This technique offers a high-resolution, genome-wide study of CCNC to the ancillary studies currently used for the classification/prognostic stratification of these tumors.This analysis can be particularly useful to confirm or establish the diagnosis in renal tumors with challenging morphology.

783 Chromosomal Abnormalities Associated with Intestinal Metaplasia in the Urinary Bladder

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Background: We recently demonstrated significant telomere shortening in intestinal metaplasia of the urinary bladder, supporting its potential to progress to adenocarcinoma. Cellular replication with dysfunctional telomeres has been shown to generate chromosomal instability, indicating a mechanism for epithelial carcinogenesis. We used UroVysion FISH to detect chromosomal abnormalities in bladder specimens with intestinal metaplasia and documented telomere shortening to learn more about the nature of this lesion.

Design: UroVysion FISH was performed on tissue blocks from 34 patients with intestinal metaplasia of the bladder. Centromeric probes (CEP) for chromosomes 3, 7, and 17 and a locus specific probe (LSI 9p21) were hybridized to tissue sections and analyzed using multicolor FISH. 50 nuclei were examined in each target lesion.

Results: Chromosomal gains were detected in 3 of the 34 cases of intestinal metaplasia: CEP 3 gain, CEP 3/7 gain, and CEP 3/7/9 gain. No deletion for the LSI 9p21 was identified. All 3 cases with chromosomal gains were previously found to have shortened telomeres.

Conclusions: The finding of cytogenetic abnormalities with associated telomere shortening further supports the role of intestinal metaplasia as a precursor to adenocarcinoma in the urinary bladder. As the UroVysion probes were developed for

the detection of urothelial carcinomas, additional studies with probes directed toward chromosomal abnormalities implicated in the development of adenocarcinoma may demonstrate additional evidence of chromosomal instability.

784 Characterization of *TMPRSS2-ERG* Fusion High-Grade Prostatic Intraepithelial Neoplasia (HGPIN) and Potential Clinical Implications

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Background: HGPIN has a low predictive value for identifying prostate cancer on subsequent needle biopsies. More than 1,300,000 prostate needle biopsies are performed annually in the United States with an approximate 15% incidence of isolated high-grade prostatic intraepithelial neoplasia. *TMPRSS2-ERG* fusion has been demonstrated to occur in a subset of HGPIN, and in prostate cancer is associated with higher tumor stage and tumor-specific death or metastasis. We studied HGPIN lesions with paired prostate cancer and correlated *TMPRSS2-ERG* fusion status.

Design: We assessed 143 HGPIN cases for *TMPRSS2-ERG* fusion status using FISH. 87% had paired prostate cancer and the remaining 19 cases demonstrated isolated HGPIN without evidence of concurrent cancer. These included two cases of HGPIN with adjacent atypical small acinar proliferation.

Results: Of the 143 HGPIN cases, 16% demonstrated *TMPRSS2-ERG* gene fusion. All cases shared the same fusion status with the paired prostate cancer. Of 120 *TMPRSS2-ERG* fusion negative HGPIN cases, 85% had matching adenocarcinoma, and in 32% of these the paired prostate cancer demonstrated *TMPRSS2-ERG* fusion. Two cases of HGPIN also demonstrated adjacent small atypical glands: one was fusion positive in both areas, whereas the other one showed fusion negative HGPIN with adjacent fusion positive atypical glands. We also identified two cases that showed presence of *TMPRSS2-ERG* fusion HGPIN and adjacent normal epithelium (with no fusion), within the same gland.

Conclusions: Our results suggest that the detection of isolated *TMPRSS2-ERG* fusion HGPIN would improve the positive predictive value of finding prostate cancer in subsequent biopsies. Further, *TMPRSS2-ERG* fusion HGPIN may represent a discrete molecular subtype with clinical implication in management, given the greater risk of predicting aggressive prostate cancer. These findings would also be helpful in clinical trials for chemoprevention of prostate cancer where one of the inclusion criteria is the diagnosis of isolated HGPIN.

785 Validation of a *TFE3* Break-Apart FISH Assay in Xp11.2 Translocation Renal Cell Carcinomas

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Background: RCCs with an Xp11.2 translocation (TR-RCC) share morphologic features and a gene fusion product (PRCC-TFE3) with alveolar soft part sarcoma (ASPS). Their distinction from clear cell and papillary RCC can be difficult secondary to overlapping histology. In addition to conventional cytogenetics, use of a commercial antibody to TFE3 has traditionally been used to confirm TR-RCCs, but in our experience newer clones of this antibody demonstrate less than optimal staining patterns in both TR-RCCs and ASPS, leading to a potential false-negative result. The goal of this study was to demonstrate that FISH analysis can be used to diagnose TR-RCCs in formalin fixed paraffin embedded (FFPE) tissue.

Design: Two TMAs containing 158 consecutive renal tumors (108 clear cell RCCs, 20 papillary RCCs, 3 RCCs with mixed clear cell and papillary features, 8 chromophobe RCCs, 10 oncocytomas, 7 AMLs, and 2 TR-RCCs) were blindly evaluated for t(X;1) using a break-apart FISH assay at Xp11.2. ~65% of these tumors had diagnostic karyotypes, while the remaining harbored either normal or non-diagnostic karyotypes. 13 samples of non-neoplastic renal parenchyma were also examined. The findings were retrospectively correlated with cytogenetics and TFE3 immunohistochemistry (IHC). Further, whole mount 5 µm-thick FFPE sections were assessed by FISH in 4 TR-RCCs, 4 (non-TR) RCCs with mixed clear cell and papillary features, and 5 ASPSs.

Results: FISH analysis was technically successful, as well as highly sensitive and specific for TR-RCCs. The 2 TMA TR-RCCs, the 4 whole mount TR-RCCs, and the ASPSs were all positive for break-apart signals homogeneously throughout tumor cells; all remaining TMA and whole mount RCCs were negative for a translocation involving Xp11.2. The current TFE3 clone did not immunoreact with any of the tumors, including the 2 TR-RCCs, on the TMAs.

Conclusions: This is the first study to screen a large number of renal tumors for an Xp11.2 aberration using break-apart FISH analysis in FFPE tissue. Correlation with cytogenetics confirms that t(X;1) is not randomly found in other renal tumors, including those with mixed clear cell and papillary morphology. FISH analysis is an alternative adjunctive tool that can be used for difficult cases and/or when optimal TFE3 IHC and cytogenetics and are not possible. Appropriate diagnosis of TR-RCC is important as new therapies are being investigated for this rare entity.

786 Prevalence of *TMPRSS2-ERG* Fusion Prostate Cancer in Men Undergoing Prostate Biopsy in the United States

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Background: Since its initial discovery, the prevalence of *TMPRSS2-ERG* prostate cancer was reported to vary from 15% in a watchful-waiting Swedish cohort to close to 50% in German and Portuguese cohorts where prostatectomies were studied. In recent publications from Canada and the United States, the prevalence in prostatectomy specimens ranges between 36% and 54%. A question has been raised as to whether the high prevalence in prostatectomies is due to selection bias. The main purpose of this study was to assess the *TMPRSS2-ERG* fusion status in men undergoing prostate biopsy in the United States.

Design: The analysis involved biopsies from 155 unselected men undergoing PSA screening as part of a prospective Early Detection Research Network clinical trial. The *TMPRSS2-ERG* fusion status was assessed by FISH. Morphological features of prostate cancer were evaluated blinded to fusion status. The study was conducted at two institutions and 30 cases were exchanged for cross-validation.

Results: 147 biopsies were assessable by FISH, 110 were positive for cancer and 46% demonstrated *TMPRSS2-ERG* fusion. All 37 benign cases were fusion negative. Morphological features associated with gene fusion were cribriform growth, blue mucin, macronucleoli, and collagenous micronodules ($p \leq 0.01$). No association with Gleason score was found. There was complete agreement in gene fusion status evaluation by FISH between the two institutions. There were minor discrepancies in Gleason grading, and presence or absence of morphologic features.

Conclusions: This is the first study of *TMPRSS2-ERG* prevalence in a North American cohort of patients undergoing needle biopsy. Our results show that this gene rearrangement is common among prostate biopsies having cancer, absent in benign prostate biopsy, and is associated with specific morphological features.

787 PIK3CA Gene Mutational Analysis in Urothelial Carcinoma of Urinary Bladder

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Background: PIK3CA is a member of the Phosphatidylinositol 3-Kinase (PI3K) family. PI3K/akt/mTOR pathway regulates cell proliferation, adhesion, survival and mobility. Genetic alterations in PIK3CA and other members of the pathway such as PTEN have been frequently found in a variety of epithelial neoplasms including colorectal and gastric carcinoma. PIK3CA gene mutations are clustered in well defined domains making it an excellent potential marker for cancer detection and monitoring. Our study assesses PIK3CA mutation in urothelial carcinoma (UrCa).

Design: A total of 22 archival cystectomy specimens were analyzed. Representative 5 micron paraffin sections from18 invasive high grade UrCa, 2 papillary non invasive UrCa and one flat CIS and one metastatic UrCa were mapped for tumor areas and microdissected for DNA extraction using Zymo Pinpoint Isolation System. Six PCR reactions were performed on each sample to span the target regions covering exons 1, 9 and 20. Amplification products were cycle sequenced using automated capillary electrophoresis (ABI3700) sequencer and analyzed with Sequencher 4.6 software.

Results: Sequencing failed in two cases. Sequencing of exons 1, 9 and 20 was successful in 16 samples and was limited to two of the three exons in remaining four cases. None of the successfully sequenced PIK3CA genes showed any evidence of a mutation.

Conclusions: Mutation analysis of PIK3CA exons 1,9,and 20 is feasible in microdissected paraffin sections from archival tisue. We found no PIK3CA gene mutations in any of the 20 UrCa included in our pilot study. Although the possibility of mutations outside the hot spots analyzed in our study cannot be excluded, our results suggest a low rate of PIK3CA mutations in UrCa. Additional studies including assessment of PIK3CA amplification would help further investigate any role of PIK3CA oncogene activation in this setting.

788 Lymphatic Invasion in Pathologic Stage T3 Radical Prostatectomy Specimens as a Predictor of Biochemical Progression

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Background: Podoplanin antibody (D2-40) is a new marker used to identify lymphatic endothelial cells which express podoplanin, as opposed to other vascular endothelium. The purpose of this study is to determine if lymphatic invasion (LI) is an independent predictor of biochemical recurrence.

Design: All the patients in the AFIP database diagnosed with pT3 disease in their radical prostatectomy specimens from January 1, 1996 to December 31, 2001 were identified. There were a total of 221 patients, with pathology slides available for 203. The sections were incubated in D2-40 Antibody (Biocare Medical Concord, CA) at a dilution of 1:20 for 1 hour, followed by 30 minutes in biotinylated horse antimouse (Vector Burlingham, CA) at a dilution of 1:400, and ABC (Vector, Burlingham, CA) Vector VIP was used as chromogen. The slides were then stained in hematoxylin, dehydrated and mounted. Two definitions of biochemical recurrence were used. Definition 1: a PSA value of ≥0.2ng/ml at any time 2 months or later post surgery. Definition 2: a PSA value of ≥0.4ng/ml and one additional PSA rise 1 month or later post surgery (181 patients had sufficient data for this calculation).

Results: 191 of the 203 patients had sufficient prostate specific antigen (PSA) data, with a median follow up of 68.2 months (range 2.3 – 151.5). LI was demonstrated in

38.7% (74/191). LI was present in 10% (4/40) with a Gleason score (GS) of 3 + 3. 43.5% (40/92) with a GS of 3 + 4, and 50.8% (30/59) with a GS of 4 + 3 or higher. LI correlated strongly with GS (p<0.0001), but not with age at diagnosis, PSA at diagnosis, or race. Using definition 1, LI correlates with biochemical recurrence on univariate analysis (p=0.0294) but not on multivariate analysis (p=0.1348) in all the GS. Using definition 2 there is a marginal increase in biochemical recurrence with LI on univariate analysis (p=0.0511), but not on multivariate analysis (p=0.2749). Stratifying by GS, there is a statistically significant increase in definition 1 recurrence with LI for GS 4+3 or higher on univariate (p = 0.0301), and multivariate (p = 0.0111) analyses. There is a trend towards increased recurrence with LI in GS 2 – 6, though not in GS 3+4.

Conclusions: The presence of LI directly correlates with GS and indicates an increased risk of biochemical recurrence in high grade disease.

789 Expression of MDM2 and CDK4 in Angiomyolipomas: A Potential Pitfall in Differential Diagnosis

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Background: Angiomyolipoma (AML) is a benign mesenchymal tumor composed of an admixture of thick-walled blood vessels, spindled to epithelioid smooth muscle-like cells, and adipocytes. AMLs characteristically coexpress melanocytic and smooth muscle markers, but staining may be very focal. Some cases are dominated by adipose tissue or smooth muscle, which can pose problems in diagnosis, particularly in limited biopsy material when renal origin is uncertain. In such instances, the differential diagnosis can include well-differentiated liposarcoma (WDLPS) and leiomyosarcoma. Recent studies have demonstrated the utility of immunohistochemical detection of MDM2 and CDK4 to confirm the diagnosis of WDLPS. However, expression of these oncogenes has previously been examined in only very few AML cases. The aim of this study was to evaluate the expression of MDM2 and CDK4 in AML.

Design: Thirty-five cases of classical AML were retrieved from surgical pathology files. All H&E stained slides were available for review, and immunostains for MDM2 and CDK4 were performed in all cases. The intensity of nuclear staining was graded as weak, moderate, or strong. Fluorescence in situ hybridization (FISH) analysis using probes directed against HMGA2 and MDM2 loci (12q13-15) was performed on isolated nuclei from 50 micron paraffin sections.

Results: Twenty-nine tumors were located in the kidney, 4 retroperitoneum, 1 mesentery, and 1 liver. Histologically, 16 tumors were composed of conventional mixed components, 13 were predominantly smooth muscle, 5 predominantly adipocytic, and 1 predominantly epithelioid (the hepatic case). By immunohistochemistry, 18 (51%) tumors were positive for MDM2, and 17 (49%) were positive for CDK4; 16 (46%) were positive for both MDM2 and CDK4. Of the MDM2-positive tumors, 2 showed strong, 13 moderate, and 3 weak staining. Of the CDK4-positive tumors, 3 showed strong, 7 moderate, and 7 weak staining. Most positive cases showed staining in a subset of lesional cells (10-30%). By FISH analysis, no amplification of MDM2 was detected, although amplification of HMGA2 was detected in a minority of tumor cells.

Conclusions: Since MDM2 and CDK4 are expressed in a subset of AML, these markers are of limited utility in the differential diagnosis with WDLPS, especially when dealing with small biopsy samples. However, in contrast to WDLPS, there is no evidence for MDM2 gene amplification in AML, although other chromosome 12q abnormalities may be present and this merits further study.

790 Oncocytic Tumors of Undetermine Malignant Potential

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Background: Oncocytomas account for approximately 6 to 9% of all renal tumors. In general, these are indolent tumors with a benign clinical course. Oncocytomas with atypical features that include prominent nuclear pleomorphism, intravascular tumor emboli, and extension into adjacent perirenal fat have been reported. The significance of these atypical features as well as the existence of malignant oncocytomas remains controversial. We report five cases of oncocytic tumors with atypical features including immunohistochemical evaluation and clinical follow-up. We propose that oncocytomas with atypical features be included as a borderline/uncertain malignant potential neoplasms.

Design: Five cases of oncocytoma with atypical features were identified. Atypical features included vascular invasion, perirenal fat infiltration, foci of necrosis, marked nuclear pleomorphism and small foci/nests of cytoplasm clearing. Proliferative markers (MiB1), p53, CD10, CK7 and cKit were performed in all cases.

Results: Patients ranged in age from 48 to 74 years. In two cases, the renal mass was an incidental finding when the patients were evaluated for other conditions. Three patients complained of back pain. The tumors involved the right kidney in 4 cases and the left in one. The tumor size varied between 3 and 10 cm and exhibited the characteristic brown to mahogany color with a central fibrous scar. Histologically, marked nuclear pleomorphism, infiltration into perirenal fat, small foci of necrosis and tumor cells in vessels were seen in all cases. In one tumor, occasional nests of cells with marked clear cytoplasm were noted. Only in one instance rare mitotic figures were noted. The proliferative index (MiB1) was low; p53 and CK7 were negative. CKIT was positive in all cases. Follow up: two patients developed liver metastasis; one with intraparenchymal nodules one year after diagnosis (patient is alive with disease 10 years after diagnosis) and one intravascular 15 years after diagnosis (patient dead of disease). The remaining three patients are free of disease.

Conclusions: Renal oncocytomas are tumors that predominantly follow a benign course. However, when atypical features such as those described are present, the potential for the development of metastasis involving predominantly liver and death exist. Therefore, long term follow up should be recommended for these patients. We propose to classify these lesions in the Undetermine malignant potential category to assure proper follow up.

791 Interleukin-6 and -10 Ratio (IL-6/IL-10) as a Prognostic Marker of Recurrence in Intermediate-Risk Urothelial Bladder Carcinoma Patients

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Background: Several potential markers have been investigated so as to improve non-invasive diagnosis of superficial bladder carcinoma (SBC). The aim of this study is to evaluate the interleukin-6 and interleukin-10 ratio (IL-6/IL-10) as a prognostic marker of recurrence in patients with intermediate-risk SBC.

Design: Sixty-five consecutive urologic out-patients (41 affected with intermediate-risk SBC and 24 controls) were selected for this prospective study. A total of five urine samples for urinary cytology, urine dipstick test and interleukin analysis were collected from each subject before transurethral resection of the bladder tumor (TUR-BT) and, after TUR-BT, at 3, 6, 9 and 12 months. IL-6 and IL-10 concentrations in urine were determined by solid phase ELISA Quantikine IL-6 and IL-10 immunoassay, respectively. Sensitivity, specificity and positive and negative predictive values of the method were calculated.

Results: At pre-TURBT sample collection, IL-6/IL-10 was not statistically different in patients and controls (p=0.58). IL-6/IL-10 was statistically different in patients with recurrence and in those without recurrence at 3 (0.009 *vs.* 0.408), 6 (0.011 *vs.* 0.268), 9 (0.012 *vs.* 0.288) and 12 (0.009 *vs.* 0.302) months after TURBT (each p<0.001). Multivariate analysis indicated that IL-6/IL-10 was an independent prognostic factor for recurrence (HR= 3.62, 95% CI 2.80-4.92, p<0.001). Test sensitivity and specificity was 0.83% (95% CI 0.57-0.95) and 0.76% (95% CI 0.45-0.93), respectively.

Conclusions: The current study highlights the feasible role of the IL-6/IL-10 value in predicting intermediate-risk SBC recurrence. This method will be instrumental in improving urinary cytology accuracy in the monitoring of SBC patients.

792 Prognostic Role of Cell Apoptotic Rate in Prostate Cancer Patients after Radical Prostatectomy

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Background: The tumour apoptotic pattern is considered a good predictor of outcome in patients with prostate cancer (PCa). Until now no authors have evaluated the apoptotic characteristics of clinically localised PCa treated with radical prostatectomy alone. The aim of the present study is to ascertain the prognostic role of the apoptotic index (AI) in a group of patients with prostatic adenocarcinoma subjected to prostatectomy with no adjuvant therapy.

Design: The study population consisted of 50 consecutive patients who had undergone retropubic radical prostatectomy. In order to evaluate the AI and correlate these results with pathological findings and follow-up data, we used a standardised apoptotic regulatory terminal deoxynucleotidyl transferase (TdT) mediated biotinylated deoxyuridine-triphosphate (dUTP)-biotin nick-end labelling (TUNEL) technique (Becton Dickinson Immunocytometry Systems, San Jose, CA, USA). The mean follow-up period was 66 months.

Results: Significant correlations were found between the AI and pathological features such as tumour stage (p<0.001) and grade (p<0.001). Out of 50 patients, 13 (26%) had biochemical recurrence and clinical disease progression, with an AI of 1.93 (range: 0.76-5.22), while 37 patients (74%) without disease progression had an AI of 0.58 (range: 0.1-3.12). Furthermore, the AI was significantly correlated with vital status at the end of follow-up (r=0.75, p=0.002), these data being confirmed by Kaplan-Meier curves (p<0.001). On multivariate analysis, the AI proved to be an independent prognostic factor of progression-free probability (p<0.001).

Conclusions: Our results demonstrated the utility of AI analysis in assessing the probability risk of clinical progression in PCa patients. In addition, the AI seems to be a feasible marker in everyday urological practice in the planning of a more appropriate follow-up schedule and treatment for patients undergoing radical prostatectomy.

793 Contrasting Global Methylation Status between Seminomatous and Non-Seminomatous Male Germ Cell Tumors

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Background: Alterations in methylation of cytosine residues at the 5' position in CpG dinucleotides (5'mC) are a hallmark of cancer. While specific genes can be silenced by acquiring somatic 5'mC in their promoter regions, global hypomethylation of the remainder of the genome has been observed in many types of somatic malignancies including lung, and gastrointestinal carcinomas. Studies on global 5'mC status in germ cell tumors (GCT) are lacking. We evaluated global DNA methylation status and DNA methyl transferase 1 expression (DNMT1) in seminomatous (Sm), non-semnomatous (NSm) and intratubular germ cell neoplasm (IGCN).

Design: A total of 54 GCT (31 Sm and 23 NSm) from 48 pts were retrieved from our archives (1995-2005). six tumors were mixed Sm/NSm. Associated IGCN was evaluated in 20 cases. Paraffin sections were immunostained using monoclonal antibodies for 5'mC and DNMT1. Distribution (percentage of cells staining) and intensity of nuclear staining (0,1,2,3+ categories) were assessed for each marker. 5'mC and DMNT1 scores (intensity x percentage) were then calculated for each tumor.

Results: 5MC: 5'mC content was strikingly lower in IGCN and Sm (mean scores 21 and 85 respectively) compared to NSm (mean 289); p 0.0001. 14/31 SM-GCT had no evidence of 5'mC staining compared to 0/23 NSm. Markedly reduced 5'mC status was also observed in Sm component of 5/6 mixed GCT compared to associated NSm component in the same tumor (p 0.003). 5'mC staining was absent in 17/20 IGCN. 5'mC status in IGCN was strongly correlated with that of associated seminoma (CC= 0.73; p 0.0006) but not associated NSm. **DNMT1:** Overall, no significant difference in DNMT1 expression was detected between IGCN, Sm and NSm groups (mean scores: 204, 223 and 212 rspectively). DNMT1 scores were significantly higher in emryonal carcinoma (mean score: 284) compared to seminoma and IGCN (p 0.03). Furthermore, in NSm

group, DNMT1 staining correlated with 5'mC status (CC= 0.46; p 0.03). No correlation between 5'mC and DNMT1 status was present in the Sm or IGCN groups.
Conclusions: Markedly reduced global 5'mC staining was frequently present in IGCN and Seminomas but not in non-seminomatous tumors. Our findings suggest that higher 5'mC content in a subset of NSm, eg. embryonal ca., could be related to associated higher DMNT1 expression. Alternative mechanism(s) must also be at play to account for the observed low 5'mC status in seminomas. Additional studies are needed to confirm our findings and further explore their biologic significance in GCT.

794 Promoter Hypermethylation of Multiple Genes Detected in Germ Cells Tumors

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Background: CG dinucleotide-rich (CpG islands) gene promoter regions are frequent targets of DNA methylation leading to transcriptional silencing. Aberrant promoter hypermethylation of tumor suppressor genes (TSG) occurs frequently during carcinogenesis and has been used as a molecular marker in cancer. CpG methylation is increasingly recognized as a potential mechanism of TSG inactivation in germ cell tumor (GCT) progression.
Design: Methylation status of 7 genes [3 known (*GSTP1, MGMT, RASSF1A*) and 4 novel (*SSBP2, NISCH, KIF1A, PAK3*)] is assessed by quantitative fluorogenic real-time methylation specific PCR (QMSP) in 9 GCTs. Tumors included 4 seminomas, 3 non-seminomas and 2 mixed seminoma/non-seminomatous GCT. Microdissection of unstained paraffin embedded sections was performed to enrich tumor DNA content. Bisulfite-modified DNA was used as a template. Primers and DNA probes were designed to specifically amplify promoters of the 7 genes of interest and a reference gene, β-*Actin*. In each sample, the relative level of methylated DNA for each gene was determined as a ratio of methylation specific PCR-amplified gene to β-*Actin*.
Results: With the exception of GSTP1 gene, all six remaining tested genes revealed frequent evidence of methylation. Overall, the rate of promoter methylation ranged from 22% to 88% with three of the six genes (*RASSF1A, SSBP2, NISCH*) being methylated in over half of tested tumors. Methylation of any of the seven tested genes in a given sample resulted in GCT detection sensitivity of 100% by binary dichotomization. Promoter hypermethylation of at least two of the genes was detected in all 9/9 (100%) GCT. Methylation frequency of *NISCH, SSBP2, RASSF1, MGMT, PAK3, KIF1A,* and *GSTP1* were 8/9(88%), 8/9 (88%), 7/9(77%), 4/9(44%), 3/9(33%), 2/9(22%) and 0/9(0%) respectively. Details of methylation frequencies is given in Table 1.

GCT Type	GSTP1	MGMT	RASSF1A	SSBP2	NISCH	KIF1A	PAK3
Seminoma	0/4	2/4	2/4	4/4	4/4	1/4	0/4
Non Seminoma	0/3	1/3	3/3	2/3	2/3	1/3	2/3
Mixed GCT	0/2	1/2	2/2	2/2	2/2	0/2	1/2
Total	0/9 (0%)	4/9 (44%)	7/9 (77%)	8/9 (88%)	8/9 (88%)	2/9 (22%)	3/9 (33%)

Conclusions: Promotor hypermethylation is detected in 6 of 7 examined genes with a high rate of methylation seen in 3 candidate TSGs (*RASSF1, NISCH, SSBP2)*. Further analysis, in a larger GCT cohort is needed to elucidate the role of these epigenetic alterations in GCT development. If confirmed, our findings may offer new potential targets of therapy, early detection, and disease monitoring in GCT.

795 Papillary Renal Cell Carcinoma of the Kidney: A Clinicopathologic Study of 76 Cases

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Background: Papillary renal cell carcinoma (pRCC), the second most common type of renal cell carcinoma, accounts for approximately 10% of all renal carcinomas. Histologically these tumors have traditionally been divided into type I and type II mainly based on amount of cytoplasm. However, oncocytic and acquired cystic disease-associated pRCC with clear cells have recently been described. Prognosis is related to tumor grade, stage and presence of sarcomatoid differentiation. Furthermore, type I pRCC has ben reported to have a better prognosis than type II as they are associated with lower stage and tumor grade. The goal of our study was to evaluate the morphologic features and clinical behavior in a large series of pRCC.
Design: Seventy six patients with pRCCs diagnosed from 1984 to 2005 were reviewed. Tumors less than 1 cm in greatest diameter were excluded. Morphologic features were evaluated in adittion to gender, age, tumor stage and follow-up.
Results: There were 56 males and 20 females who ranged in age from 27 to 86 (mean 64) years. The tumors ranged in size from 1.4 to 16 (mean 4.6) cm. Although most tumors appeared well circunscribed and encapsulated, infiltration into and through the capsule was not infrequent. Forty-nine tumors were classified as type I, 21 as type II, and 2 were mixed. Four tumors showed an unusual morphology; 3 were composed exclusely of clear cells (one of them arising in an end-stage kidney disease) and one had a prominent spindle cell morphology although obvious areas of type I pRCC were present. Two tumors had oncocytic features (1 diffuse and one focal and classified as type II) and one tumor had a metanephric-like morphology (classified as type I). No sarcomatoid differentiation was identified. Fifty six tumors were Fuhrman grade 2/4, 17 grade 3/4 and 3 were grade 4/4. Thirty five tumors were stage Ia, 18 stage Ib, 8 stage II and 15 stage III. Follow-up was availbale in 69 patients and range from 6 months to 158 (average 64) months.Only 5 patients died of disease, 4 had type II pRCC (2 grade 2 and 2 grade 3) and they were all stage III. One patient had a type I pRCC, grade 2, stage II tumor with a focus highly suspicious for lymphovascular invasion.
Conclusions: pRCCs more frequently affect male patients (2.8 to1) and are usually type I. Most pRCCs are stage I at the time of diagnosis and they are associated with a good prognosis irrespective of tumor type. In this study, only higher stage tumors especially type II were associated with an adverse outcome.

796 Reproducibility of Reporting Seminal Pathologic Parameters in Robotic Radical Prostatectomies (RP) Specimens: Comparison of Central Versus Institutional Review Data in 173 Cases

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Background: Following robotic radical prostatectomy (RP), the Gleason's score (GS), pathologic stage (pT) and resection margin (RM) status are key determinants of prognosis and subsequent management. Interobserver variability in reporting these parameters has not been well documented, except Gleason's score, which primarily has been evaluated in needle biopsies.
Design: 173 RP specimens from 3 academic institutions were entirely sectioned using a standard protocol, and findings for each case were reported by a dedicated urologic pathologist (Institutional review-IR). All cases were subsequently reviewed by a central reviewer (CR) and results on pT, GS and RM between IR and CR were analyzed; kappa statistics were used to document measure of agreement.
Results: See table.

Results

	Concordant %	Upgraded %	Downgraded	Kappa value
Pathologic stage, EPE*	82	8	10	0.55
Pathologic stage, seminal vesicle invasion	98	0.5	1.5	0.71
Pathologic stage, Overall**	82	10.5	7.5	0.77
Gleason's score	79	6	15	0.59
Resection margins	92	6	2	0.75

*EPE: Extraprostatic extension; **Includes seminal vesicle involvement

Conclusions: 1) Interobserver reproducibility was the highest for resection margin status (k=0.75) and was lower for pathologic stage (k=0.55) and Gleason's score assignment (k=0.59). 2) The GS was more frequently downgraded by CR; issues involved varying thresholds for assigning Gleason pattern 4 and factoring in multifocality and dominant nodule score into the final GS. 3) Evaluation of EPE was largely responsible for pT stage discrepancies; most of these cases had microscopic and focal involvement and reproducibility issues revolved around the definition of extraprostatic space.

797 Morphologic Correlates of Individual Splice Variants of TMPRSS2-ETS Prostate Cancer (PCa) Detected by Transcription-Mediated Amplification (TMA) in Radical Prostatectomy (RP) Specimens

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Background: The PCa TMPRSS2:ETS^{family} gene fusions with its splice variants TMPRSS2:ERGa (T2:ERGa), TMPRSS2:ERGb (T2:ERGb) and TMPRSS2:ERGc (T2:ERGc) and more rarely, TMPRSS2: ETV1a,b (T2:ETV1) are the most prevalent genetic defects observed in any solid tumor. A study using an ERG break-apart FISH assay has shown correlation of certain histopathologic characteristics with gene fusion status, but correlation of the splice variants with morphology has not been investigated.
Design: 520 blocks from 100 RP were analyzed utilizing a semiquantitative scale from 0-4 for the presence of perineural invasion (PNI), blue mucin (MU), macronucleoli (MA), cribriform architecture (CB), intraductal spread (IDS), signet ring cell features (SR) & associated desmoplasia (DM). RNA amplification was performed on nucleic acid from 10 micron formalin fixed sections by using a research prototype Gen-Probe TMA® test that includes end-point luminescence detection. Associations were identified with Chi-square analysis and Fisher's Exact Test.
Results: PNI, MU and IDS were present in the same block in 19% of cases. When all three parameters were present, the presence of the gene fusion was accurately predicted in 67% of cases.
Conclusions: Perineural invasion, intracytoplasmic mucin and intraductal spread were associated with all splice variants especially T2:ERGc. Since TMPRSS2:ETS^{family} gene fusions status in PCa may have prognostic and therapeutic implications, knowledge of morphologic characteristics associated with these molecular events may help identify cases for further testing.

	T2:ERGa	T2:ERGb	T2:ERGc	T2:ETV1	fusion
PNI 0 vs ≥1	0	0.006	0.001	0.105	0
MA 0 vs ≥1	0.545	0.007	0.702	0.282	0.481
CB 0 vs ≥1	0.587	0.005	0.060	0.046	0.236
MU ≤1 vs ≥ 2	0	0.133	0	0.031	0
IDS 0 vs ≥ 1	0.142	0.922	0.002	0.152	0.030
SR 0 vs ≥1	0.224	0.114	0.710	0.656	0.447
DM 0 vs ≥1	0.266	0.885	0.741	0.656	0.447

798 Aberrant Diffuse Expression of p63 in Adenocarcinoma of the Prostate on Needle Biopsy and Radical Prostatectomy: Report of 21 Cases

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Background: Pathologists often use the absence of the basal cell markers p63 & high molecular weight cytokeratin (HMWCK) to aid in the diagnosis of prostate cancer. Conversely, positive basal cell markers typically rules out invasive cancer. Aberrant diffuse expression of p63 in prostate carcinoma cells is a rare and poorly recognized phenomenon.
Design: We studied 19 cases of prostate cancer with aberrant diffuse expression of p63 on needle biopsy and reviewed the subsequent radical prostatectomies in 6 cases. Two additional radical prostatectomies with aberrant p63 staining with no needle biopsies available for review were also analyzed.

Results: On the H&E stained slides, 19/21 cases showed a distinctive morphology composed predominantly of glands, nests, and cords with atrophic cytoplasm, hyperchromatic nuclei, and visible nucleoli. Needle biopsy cases ranged from Gleason patterns 3 to 5 with tumor identified on one or more cores, ranging from a minute focus to 80% of the core. In 2/8 cases the radical prostatectomy was characterized by both p63 positive cancer and usual p63 negative acinar prostate cancer. In all 8 cases the tumors were organ confined with negative margins and there was no seminal vesicle involvement or lymph node metastasis. The 20 cases that had stains for HMWCK performed were all negative in the foci of p63 positive cancer. All13 cases that had stains for alpha-methylacyl-CoA-racemace (AMACR) performed showed variable degrees of positivity in the foci of p63 positive cancer. 2/19 cases with needle biopsies had a separate focus of high grade prostatic intraepithelial neoplasia.
Conclusions: The presence of p63 positive atypical glands with an infiltrative pattern and perineural invasion on radical prostatectomy confirmed the needle biopsy diagnosis of carcinoma. Rarely prostate cancer can aberrantly express diffuse p63 staining in a nonbasal cell distribution leading to the erroneous diagnosis of atrophy or atypical basal cell proliferation. The diagnosis of prostate cancer is based on morphology and confirmed by the absence of HMWCK staining and positivity for AMACR in the atypical glands. Pathologists need to be aware of this rare and unusual phenomenon which is a potential pitfall in prostate cancer diagnosis. The use of both p63 and HMWCK is recommended to prevent a misdiagnosis.

799 Prognosis of Mucinous Adenocarcinoma of the Prostate Treated by Radical Prostatectomy: A Study of 47 Cases

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Background: Mucinous adenocarcinoma of the prostate is one of the least common variants of prostate cancer. The prognosis of this variant of prostate cancer remains controversial. We present herein the largest series to date of mucinous carcinomas treated by radical prostatectomy.
Design: 47 cases of mucinous adenocarcinoma of the prostate removed by radical prostatectomy with follow-up were retrieved from the surgical pathology files of The Johns Hopkins Hospital from 1991-2006. Mucinous adenocarcinoma was diagnosed only when extracellular mucin resulted in pools of mucin involving more than 25% of the tumor volume.
Results: Mean patient age was 56 years (range 44-69 years). The mean preoperative PSA level was 9.0 ng/ml (range 1.9-34.3ng/ml). Clinical stages were T1c (34 cases), T2a (7 cases) and T2b (6 cases). The mean percentage of tumor composed of the mucinous component was 52% (range 25%-90%). The mean Gleason score was 7 with scores of 6 in 6 cases, 7 in 37 cases and 8 in 4 cases. Margins were positive in 4 cases of mucinous adenocarcinoma of the prostate, one of which also had simultaneous nonmucinous acinar cancer positivity at another site. Only 1 case had isolated margin positivity of the nonmucinous acinar component of cancer. In 11 cases, mucinous adenocarcinoma had established extraprostatic extension (EEPE), one of which also had simultaneous nonmucinous cancer EEPE at another site. Only 6 cases had isolated EEPE of nonmucinous cancer. The 1 lymph node metastasis contained nonmucinous cancer. The mean follow up for those without progression was 5.6 years (median 6 years, range 1-15 years). One patient progressed 3 years following his radical prostatectomy. The 5 year actuarial PSA progression free risk was 97.2%. Using the Kattan nomogram, the predicted mean 5 year PSA progression free risk for nonmucinous prostate cancer with the same PSA and postoperative findings as in the current study was 85.4%.
Conclusions: The grading of mucinous adenocarcinoma of the prostate is controversial. Given the excellent prognosis of mucinous carcinomas in our study, our data supports grading mucinous prostate carcinomas based on the underlying architectural pattern, rather than assuming that all of these tumors are aggressive and assigning Gleason pattern 4 to the mucinous component. This study confirms that mucinous adenocarcinoma of the prostate treated by radical prostatectomy is not more aggressive, and possibly even less aggressive than nonmucinous prostatic adenocarcinoma.

800 Do Men with Insignificant Cancer on Radical Prostatectomy Have Predictably Favorable Preoperative Clinical and Biopsy Findings?

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Background: Due to increased number of serum PSA tests and biopsies performed in the United States, earlier and smaller prostate cancers are being detected and removed by radical prostatectomy (RP). Information about the preoperative clinical and pathological findings in these cases might provide useful data for other men to predict who has a high likelihood of "insignificant" prostate cancer and could be candidates for active surveillance.
Design: A search was performed through the surgical pathology files of the Johns Hopkins Hospital from July 2004 to July 2006 for RP cases with only 1 to 3 slides involved by small foci of 3+3=6 adenocarcinoma of the prostate.
Results: Post-Op: A total of 151 RP cases were obtained. The extent of cancer was: 1 slide 69 cases (45.7%); 2 slides 61 cases (40.4%); and 3 slides 21 cases (13.9%). The mean weight of the prostate gland was 66 gms (range 28-159 gms). The largest tumor nodule on radical prostatectomy was $<2mm^3$. Pre-Op: The mean patient age was 57.1 yrs (range 41-73 yrs). 22 patients (14.6%) had positive DRE. The mean serum PSA and percentage free PSA were 5.2 ng/dl (range 0.3-16.7 ng/dl) and 15.5% (range 8-36%) respectively. 19 patients had a PSA density (PSAD) of >0.15. The mean number of cores obtained was 12 (range 4-27 cores). All cases were Gleason 3+3=6 cancers on biopsy. 114 cases (75.5%) had 1 core positive, 28 cases (18.5%) 2 cores and 9 cases (6%) had ≥3 cores positive. Only three cases had >50% of cancer involving one core with 2 of these cases having discontinuous foci.
Conclusions: Based on multiple prior studies, we have used the following criteria for deciding who are potential candidates for active surveillance: 1) Nonpalpable prostate cancer; 2) PSA density (PSA/gland weight) <0.15; and 3) biopsies with only 1 or 2 positive cores & no core with >50% involvement by cancer & no Gleason pattern 4 or 5. "Working backward" from men with minute prostate cancer at RP, there were only a few men whose preoperative criteria exceeded our definition of potentially insignificant cancer. Clinically, 12.6% had a PSAD of >0.15 and 14.6% had an abnormal DRE, demonstrating that these tests may uncommonly give a false indication of substantial cancer. In uncommon cases there may also appear to be more extensive cancer on biopsy (≥3 cores in 6%; >50% cancer per core in 2%) yet still insignificant cancer at RP. Acknowledging these exceptions, minute cancers at RP are typically associated with predictably favorable preoperative findings.

801 Rhabdomyosarcoma (RMS) of the Urinary Bladder in Adults: Predilection for Alveolar Morphology with Anaplasia and Significant Morphologic Overlap with Small Cell Carcinoma

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Background: RMS represents the most common malignant soft tissue tumor in children and adolescents with the urinary bladder representing a frequent site. Most of these urinary bladder tumors are embryonal RMS, predominantly the botryoid subtype. RMSs of the urinary bladder in adults are distinctively rare and the subject of only case reports.
Design: We report 5 bladder neoplasms with rhabdomyosarcomatous differentiation in adults and emphasize the differential diagnosis in the adult setting.
Results: The patients, 4 male and 1 female, ranged in age from 23 to 85 years (mean 65.4 years). Gross hematuria was the most common initial symptom, although 2 patients had metastatic disease at presentation. Four cases were pure primary RMSs of the bladder and 1 case was a sarcomatoid urothelial carcinoma with RMS representing the extensive heterologous component. All 5 cases demonstrated a diffuse growth pattern (i.e., non-nested) and 4 cases with nuclear anaplasia (Wilms criteria without the atypical mitotic figure requirement); only 1 case (the sarcomatoid carcinoma) showed obvious rhabdomyoblastic differentiation (i.e. strap cells). Three cases were of the alveolar subtype (1 admixed with embryonal histology) and 2 were RMS, not further classified. Microscopically, all tumors had a primitive undifferentiated morphology with cells containing scant cytoplasm, varying round to fusiform nuclei with even chromatin distribution, and frequent mitoses. The degree of morphologic overlap with small cell carcinoma of the bladder, a relatively more common round cell tumor in adults, was striking. All tumors showed immunohistochemical expression for desmin, myogenin, and/or MyoD1. Synaptophysin was performed in 4 cases, and 3 showed weak cytoplasmic reactivity. Two patients received chemotherapy, 2 underwent cystectomy, and 1 had TUR alone. Outcome data was available in 4 cases, and all 4 died of disease (1, 4, 8, and 8 months).
Conclusions: 1) RMS of the urinary bladder in adults more commonly presents as a primitive round blue cell neoplasm that has significant morphologic and immunohistochemical overlap with small cell carcinoma of the bladder. 2) While RMS in children generally have a botryoid embryonal histology with favorable outcome, bladder RMS in adults frequently demonstrates alveolar or unclassified histology, commonly with anaplasia, and have a uniformly aggressive clinical course.

802 Papillary Renal Cell Carcinomas with Inverted and Micropapillary Patterns: A Clinicopathologic Study of 33 Cases

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Background: Papillary renal cell carcinoma (PRCC) is the second most common RCC. The current histologic subclassification based on the cytoplasm and nuclear grade has variable prognostic significance. In this study, we describe clinicopathologic and immunohistochemical features of two distinct histologic patterns of PRCC (inverted and micropapillary) which have not been described before.
Design: Thirty three cases of pure PRCC were reviewed. The inverted pattern was defined as polarization of nuclei toward the surface of papillae as a single layer with abundant eosinophilic cytoplasm, exhibiting features of both type 1 and 2 PRCC. The micropapillary (MP) pattern were divided into surface or invasive forms. The surface form showed filiform proliferation of tumor cells from the surface of papillae or floating cell nests in the free spaces between papillae. The invasive MP was defined as small clusters of tumor cells surrounded by lacunar spaces. The expressions of MUC1, CD10, CD117, CK7, CK20, E-cadherin, Ki-67, p53, racemase, and VEGF were studied using a tissue microarray of all cases. Immunoreactivity was recorded semiquantitatively according to the percentage of positive cells.
Results: The patients' mean age was 55 years with a male to female ratio of 1.2: 1. The mean tumor size was 7.0 cm. Eight cases (24.2%) exhibited the inverted pattern and none of them developed recurrence or metastasis except for one case. The surface MP pattern was noted in 15 cases (45.5%) and 2 of them with invasive MP pattern (6.0%). The remaining 10 cases were usual PRCC. Extrarenal fat invasion by tumor was noted more frequently in PRCC with MP pattern than the usual PRCC. Both tumors with invasive MP pattern developed nodal and distant organ metastases. None of the PRCC with inverted pattern revealed the MP pattern. The immunohistochemical study showed that the inverted pattern had higher expressions of MUC1, E-cadherin, CD117 and lower expression of CD10 than usual PRCC, and was negative for Ki-67 and CK20. In contrast, the immunoprofile of the MP pattern was similar to those of the usual PRCC.
Conclusions: Our results suggest that PRCC with inverted pattern is a distinct type of PRCC with unique immunohistochemical profile with relatively favorable prognosis and that PRCC with MP pattern, especially the invasive form, may represent an aggressive variant of PRCC.

803 Aurora Kinase A as a Biomarker for the Detection of Bladder Cancer

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Background: Chromosomal missegregation resulting in aneuploidy is a common change in neoplasia, and genes regulating segregation of chromosomes may be potential cancer detection markers. Amplification/overexpression of aurora kinase A, a key regulator of mitosis, has been frequently detected in cancer and the degree of amplification correlates with the degree of aneuploidy.

Design: We measured the level of aurora kinase A expression in bladder cancer cells and correlated it with the copy number of three selected chromosomes as well as total nuclear DNA content. The effect of aurora kinase A on centrosome multiplication and chromosome copy number was measured *in vitro* using an adenoviral expression construct. To assess the applicability of this gene as a biomarker for bladder cancer, we tested the aurora kinase A gene copy number using fluorescence *in situ* hybridization (FISH) on exfoliated cells from urine sediments of bladder cancer patients.

Results: Overexpression of aurora kinase A in urothelial cells induced amplification of centrosomes, chromosome missegregation, and aneuploidy. Moreover, overexpression of this kinase could be detected in *in situ* lesions as well as in urine of patients with bladder cancer. A FISH test for aurora kinase A gene copy number performed on a blinded validation test consisting of 51 voided urine samples from patients with bladder cancer and 30 controls detected bladder cancer with specificity 1.0 and sensitivity 0.86.

Conclusions: Our findings indicate that overexpressed aurora kinase A can cause aneuploidy in urothelial cells and is a promising biomarker for detection of bladder cancer.

804 Margin Assessment of the Radical Prostatectomy (RP) Utilizing a Sectioning Method That Optimizes Margin Evaluation and Permits Tissue Banking

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Background: Positive margins (PM) in RPs are due to both surgical technique and biologic aggressiveness of the prostatic carcinoma (CaP). RP margin assessment aids in predicting local CaP recurrence, influencing adjuvant therapy. We utilize a method of RP sectioning that permits complete margin assessment with retention of fresh tissue for banking. The aim of our study is to determine the incidence, extent and location of RP margin positivity in our pt population.

Design: At UPMC, RP capsular margins are completely stripped and serially sectioned fresh. We retrospectively searched the CoPath RP database (1/1/80 - 7/31/07), identifying all cases with PMs: microscopic (group 1), focal (group 2) and extensive/multifocal (group 3). We assessed the size, number of PM sections and PM locations in each RP, correlating with Gleason score (GS), presence and location of the extraprostatic extension (ECE), and tumor stage.

Results: Of 3,343 RPs, 297 (9%) had PMs: group 1 (n=42; 14.1%), group 2 (n=180; 60.6%), group 3 (n=75; 25.3%). 192/297 (65%) RPs had PMs limited to a single slide.

Table 1

Location	Apex/DUM	Base/PUM	Left mid	Right mid	Left base	Right base	SV
Group 1	18	16	3	3	5	4	8
Group 2	76	62	19	23	11	8	23
Group 3	40	29	18	14	12	9	26

Mean (range) GS for the 3 groups were: 7.2 (6-9), 7.6 (6-9), and 7.7 (6-10). ECE was present in 205 (69%) cases in the respective 3 groups: 25/42 (60%), 126/180 (70%), 54/75 (72%).

Table 2.

Group	1	2	3
Focal ECE	16	88	25
Multifocal / established ECE	9	38	29

The location of the PM corresponded to the location of ECE in 70 (24%) cases distributed in the 3 groups: 3 (4.3%), 34 (48.6%) and 33 (47.1%). Metastatic CaP per group was as follows: 0/42 (0%), 5/180 (3%), 11/75 (15%).

Conclusions: Apical CaPs are more likely to result in PMs because the surgeon is more limited in the amount of tissue that can be resected if continence is to be preserved. Microscopic PM is more likely to be associated with organ-confined CaP, suggesting that the PM is often due to close dissection in an attempt to preserve the neurovascular bundles. CaPs with more extensive PMs have higher stage and grade, suggesting that PMs in these pts are more frequently due to the increased biologic aggressiveness of these tumors. Complete margin sampling is optimal, since 65% of RPs had PMs limited to a single slide. We are in the process of correlating local recurrence risk and our urologists' thresholds for recommending adjuvant therapy in relation to the extent and location of the RP PMs.

805 The Incidence of Lymphoma in Pelvic Lymph Nodes (PLNs) in Patients Undergoing Radical Prostatectomy (RP)

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Background: PLNs are removed during RP to assess for metastatic carcinoma of the prostate (CaP), but may harbor other disease processes. Coexisting hematolymphoid neoplasia (HLN) at the time of RP for CaP may occur due to the similarities in age group at risk for these diseases. We have identified incidental HLN in PLNs in some pts undergoing RP. The aim of this study is to determine the incidence and histologic subtypes of HLN in PLNs in pts undergoing RP.

Design: We retrospectively searched the CoPath database at UPMC for pts undergoing RP (n=3343) and cystoprostatectomy (CYSP) (n=800) with PLND (1/1/80 - 7/31/07) who were found to have HLN in PLNs. We reviewed the clinical records and pathology for each pt.

Results: Of the 3343 pts undergoing RP and PLND, 16 (0.5%) were found to have HLNs in PLNs. All pts were male, ranging from 53 to 71 yrs (mean 64 years). The HLNs included: B-cell small lymphocytic lymphoma/chronic lymphocytic leukemia (SLL/CLL; n= 11), B-cell follicular lymphoma (n=1), mantle cell lymphoma (n=1) and B-cell marginal zone / MALT lymphoma (n=3). No high-grade lymphomas were identified. No pt had an antecedent dx of a HLN. The HLN concurrently involved the prostate in two pts (0.06%), with the remaining pts having involvement limited to PLNs. 3 pts had LN enlargement with effaced architecture but immunohistochemistry revealed reactive lymphoid proliferations. CaP stages were pT2N0 (n=7) and pT3N0 (n=8), with Gleason score $\leq$ 6 (n = 3) and >6 (n=12). The single CYSP contained a pT1+N0 high-grade urothelial carcinoma and an incidental CaP. Follow-up in 10 pts revealed systemic HLN involvement in 6 pts at the following sites: bone marrow (n=3), pleura (n=1), hard palate (n=1) and appendix (n=1).

Conclusions: HLN in PLNs in pts undergoing RP and CYSP is rare (0.5%), with none of the pts having an antecedent dx of HLN. Since a large number of RPs are performed, it is important to closely scrutinize the LN morphology to avoid missing a HLN. The majority of pts have B-cell SLL/CLL (69%), which may be missed because the lymphocytes are cytologically normal-appearing and the lymph node sinuses are frequently preserved. One clue to lymphoma is the absence of fibrosis and calcifications that are usually present in benign PLNs. Concurrent prostatic involvement by HLN is unusual. On follow-up, a subset of pts will have systemic disease. Hematopathologic workup is required in all cases with atypical lymphoid proliferation in PLNs during evaluation of RP specimens to exclude HLN.

806 Empiric Evaluation of the AJCC TNM Staging System for T1a,b Prostate Cancers

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Background: The AJCC TNM staging for prostate cancer (PCa) differentiates T1a and T1b as tumor incidental histological finding in 5% or less vs >5% of tissue resected. Here, we report on the emperic data derived from a large population-based incidental PCa cohort with long-term outcome and disease specific death as endpoint.

Design: In a population-based cohort of men (n=1,016) with localized PCa diagnosed through transurethral resection of the prostate (TURP), managed by Watchful Waiting, we reviewed all histologic sections for evaluation of tumor percentage. Standardized criteria were used to classify disease progression and cause of death. We evaluated the association between tumor extent and time to lethal PCa. Receiver operator characteristic (ROC) curves were calculated at 15 years after diagnosis to assess the accuracy of tumor percentage as predictors of PCa death.

Results: Of the 1016 patients, 185 died of PCa, 584 died of other causes, and 242 were still alive at the study endpoint. The median follow-up time was 84 month. A cut-off point of 5% tumor volume had a negative predictive value (NPV) of 0.91 (sensitivity = 0.81, specificity = 0.40), a cut-off point of 25% tumor volume had a NPV of 0.86 (sens. = 0.42, spec. = 0.79), and a cut-off point of 50% of tumor volume had a NPV of 0.85 (sens. = 0.28, spec. = 0.89) for predicting PCa death.

Conclusions: Although not very specific, the cut-off point of 5% tumor volume has the best NPV for predicting PCa death in our study. 25% and 50% tumor volume as cut-off points do only slightly worse in the NPV of PCa death, but are much more specific. This is the first study with long-term PCa specific outcome as endpoint providing a rational for using 5% tumor volume as cut-off point to differentiate T1a and T1b PCa.

807 Expression of p53 and p16 in Differentiated and Warty/Basaloid Penile Intraepithelial Neoplasia (PeIN)

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Background: There is an heterogeneous spectrum of penile epithelial alterations and precancerous lesions. Two groups of lesions appear to be morphologically distinctive and precursors of specific tumors leading to different pathways of cancer progression, the squamous differentiated and the HPV-related warty and basaloid PeIN.

Design: The study was design to evaluate expression of p16 and p53 (presumptive surrogate markers of HPV-related and differentiated PeIN respectively) in penile precancerous lesions. There were 22 lesions classified as follow: squamous (differentiated) low grade PeIN, 8 cases; squamous (differentiated) high grade, 5 cases; warty PeIN, high grade, 3 cases; basaloid PeIN, high grade, 3 cases and warty PeIN, low grade (condylomas), 3 cases. Differentiated PeINs showed parakeratosis, epithelial thickening, elongation and thinning of the rete ridges, enlargement of keratinocytes, prominence of bridges, cytoplasmic eosinophilia, occasional pearls. Atypical cells were at the base in low grade and suprabasal in high grade lesions. High grade warty PeIN had an spiky appearance, hyper and parakeratosis, plemorphic koilocytosis and atypias throughout the epithelium. In high grade basaloid PeIN the cells were anaplastic, small and uniform affecting the full thickness of the epithelium. p16 was considered positive when showing intense staining of nuclei and cytoplasm of the entire (full thickness) lesion. Positivity for p53 was defined as the suprabasal extension of nuclear staining.

Results:

	Differentiated PeIN n=13	%	Warty/basaloid PeIN n=6	%	Condylomas n=3	%
p16 positive	1	8	6	100	0	0
p53 positive	10	77	1	17	3	100

Conclusions: The pattern of p53 staining was similar in low and high grade differentiated PeINs indicating their close relationship. There was an almost mutually

exclusive staining for p16 and p53 in HPV high grade and differentitated PeIN lesions corroborating the distinctness of both groups and suggesting a different pathway of cancer progression. Condylomas tend to be associated with non high-risk HPV, hence it is not surprising the lack of expression of p16 in these lesions. p16 may be a useful marker to identify HPV related high grade lesions specially in small biopsies.

808 Hilar Lymph Nodes in Radical Nephrectomy Specimens: Should They Be Expected?

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Background: Lymph node (LN) status is essential in staging renal cell carcinoma (RCC). The rate of regional LN involvement in RCC depends on pT stage, extent of the surgical resection, and accuracy of pathological examination, but published rates range from 3.3-24%. It is commonly quoted in the surgical pathology literature that lymph nodes should be grossly sought in the hilum of radical nephrectomy (RN) specimens, but that their presence should not be expected. To our knowledge, no study has assessed the presence of hilar nodes in RN specimens by entirely submitting the hilar adipose tissue for histologic examination.

Design: Consecutive RN specimens performed from July 2006 to September 2007 at a single academic institution were evaluated by submitting the renal hilar adipose tissue in its entirety for complete histologic evaluation in addition to standard sections. One H&E section was prepared from each block and reviewed by 2 investigators (NG, DP) to determine the number of LNs present and to correlate with subtype, grade, and stage of the primary tumor.

Results: 29 consecutive RN specimens were studied, and lymph nodes were identified in 3 (10%). The number of additional/non-standard hilar blocks ranged from 3 to 25 (mean: 11). Of the 29 cases, 1/20 (5%) with RCC had a single 2.0 cm lymph node that was identifiable at gross evaluation and was involved by metastatic RCC. 2 of 5 cases (40%) with renal pelvic urothelial carcinoma (UC) had LNs present by histology that were not identified grossly (two nodes in each case, the largest measuring 3.0 mm and 0.4 mm in greatest dimension).

Conclusions: In this study, the only RN specimen with LN involvement by carcinoma was identifiable by gross palpation (1/29; 3%). Two additional RNs with UC had small microscopic nodes identifiable by histology alone, measuring 3.0 and 0.4 mm. The presence of microscopic LNs in 2/5 RN with renal pelvic UC, but not in RCC cases suggests that regional inflammatory reactions may be involved. This initial study supports the current standard of renal hilar palpation with sampling of grossly identified LNs in the staging of RCC.

809 Molecular Signature of Prostatic Atrophy

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Background: Adenocarcinoma of the prostate is the most commonly newly diagnosed malignancy among men in the United States. Despite its prevalence in the population, however, the precise pathogenesis of the disease is still poorly understood. The prevailing notion today is that prostatic intraepithelial neoplasia (PIN) is a precursor to some adenocarcinomas. It has been hypothesized that atrophy, especially proliferative forms of focal atrophy with associated inflammation is another potential precursor to prostate cancer. Little is known about the genomic profile of prostatic atrophy compared to non-atrophic benign prostatic tissue and prostatic neoplasia.

Design: Twenty five snap frozen tumor tissues were selected from 20 patients through the Siteman Cancer Center Tissue Procurement Core facility. Laser capture microdissection was utilized to isolate 13 foci of PIN, 7 foci of PIA, 8 foci of invasive carcinoma, and 5 foci of normal glands (33 samples total) and RNA from these was analyzed on Affymetrix U133 gene expression microarrays.

Results: Using a variety of statistical approaches (ANOVA, DCHIP, SAM), we identified a set of 172 probe sets whose expression repeatedly distinguished between the microdissected cell types. Nonetheless, some of these genes showed overlapping patterns between PIA and PIN lesions, and PIN and invasive carcinoma, suggesting a gradation of molecular phenotypes, even within these histologically distinct lesions. Among the genes most highly expressed in the PIA lesions were an oncogene whose expression is critical for short-term tumor cell survival, and a developmentally regulated homeobox gene. We are currently using conventional immunohistochemical approaches to confirm the expression pattern of these genes in independent cases.

Conclusions: Our data show that prostatic tissue can be stratified at a molecular level into normal, atrophic, PIN, and invasive subcategories. However, even within these histologically defined categories, molecular variability still exists, particularly within PIA and PIN. We have identified a number of biomarkers that may be specific for these individual cell phenotypes, may provide insight into abnormal growth processes leading to prostate adenocarcinoma, and may ultimately be useful for classifying these lesions when traditional histology is ambiguous.

810 Increased Survivin Expression in Urothelial Carcinoma of the Bladder

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Background: The survivin protein shuttles between the nucleus and cytoplasm and can perform diverse functions such as inhibition of apoptosis and regulation of microtubule dynamics. Increased expression of survivin has been reported in multiple cancer types and has been suggested to correlate with pathologic grade and stage in bladder carcinoma. We examined survivin protein expression in normal and neoplastic urothelium to determine a putative role for this molecule in bladder carcinoma.

Design: Tissue microarrays consisting of normal urothelium (n=21), urothelial carcinoma in situ (n=8), non-metastatic urothelial carcinoma (n=9), metastatic urothelial carcinoma (n=46) and paired lymph node metastases (n = 46) were immunostained for survivin (1:100, Dako, Carpinteria, CA). Nuclear immunoreactivity was scored as the % positive cells/total cell number and tissues that demonstrated equal to or greater than 10% immunoreactivity were considered strongly positive. Clinical outcomes were determined from a retrospective review of patient records.

Results: Normal urothelial tissue did not demonstrate any noticeable survivin immunoreactivity (0/21; 0%). Half of all urothelial carcinoma in situ specimens (4/8; 50%) demonstrated a marked increase in nuclear survivin reactivity, ranging from 10 to 40% of cells. In addition, approximately half of all invasive urothelial carcinomas (20/32; 41%) demonstrated increased nuclear survivin, ranging from 10 to 30%. Paired lymph node metastases showed similar staining of survivin relative to the matching primary carcinoma and nuclear survivin expression was present in 13 metastatic lesions (28%). No correlation was evident between survivin expression and clinicopathologic characteristics, including pathologic stage.

Conclusions: Although survivin expression does not appear to correlate with metastatic behavior, the marked increase in nuclear survivin in both in situ and invasive urothelial carcinoma suggests a role for this molecule in urothelial carcinogenesis.

811 Somatic Transformation to Sarcoma in Testicular Germ Cell Tumors: A Clinicopathologic Analysis of 33 Cases

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Background: Secondary somatic transformation to sarcoma in germ cell tumors (GCT) is a rare occurrence. In these cases the mesenchymal component of teratoma demonstrates malignant morphology, and usually has an aggressive clinical course. We report the clinical and pathological features of testicular GCT with somatic transformation to sarcoma.

Design: We searched our pathology files from 1990 to 2007 and identified 33 patients who presented with somatic transformation to sarcoma in testicular germ cell tumors. All available pathology material and clinical data, including follow up were reviewed.

Results: The average age of patients was 31.5 years old (range: 16 to 55). The sarcoma types included: rhabdomyosarcoma (n=29), angiosarcoma (n=1) and high grade unclassified sarcoma (n=3). The average percentage of sarcomatous component in the tumor was 33.4% (2-99%). All patients underwent an orchiectomy. Four patients who presented with a metastatic tumor received neoadjuvant chemotherapy before orchiectomy; 3 did not have any residual tumor in the testis, and 1 only had residual mature teratoma. Of the 29 patients who did not receive neoadjuvant chemotherapy, all had teratoma in the testis (29), which was mixed with other GCT types, including: embryonal carcinoma (21), yolk sac tumor (20), seminoma (15) and choriocarcinoma (3). 20 patients had sarcoma in the primary testicular tumor, which included rhabdomyosarcoma (n=18), angiosarcoma (n=1) and high grade unclassified sarcoma (n=3). The other 13 patients only had sarcoma (all rhabdomyosarcomas) at the metastatic site. 12 patients presented with retroperitoneal lymph node metastasis, 5 patients presented with distant metastases (lungs, liver, mediastinum and neck) and 2 patients with retroperitoneal lymph node metastases and distant metastases (lungs and liver). Clinical follow-up was available for 25 patients (mean: 70.8 months; median: 68; range: 5-240). Ten patients were alive with no evidence of disease and under follow-up (mean: 88.6 months; median: 74; range: 5-240) and 9 patients died of disease (mean: 85.8 months; median: 70; range: 20-146). Follow-up period was short (mean 27.2 months; median: 18; range: 7-81) in the 6 patients who had no evidence of disease.

Conclusions: Rhabdomyosarcoma is the most common sarcoma observed in somatic transformation of testicular GCT. Sarcomatous transformation in germ cell tumors are associated with frequent tumor metastasis and poor prognosis.

812 Oncocytic Papillary Renal Cell Carcinoma with Solid Architecture: A Renal Cell Carcinoma That Mimics Renal Oncocytoma

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Background: Distinguishing between the different variants of renal cell carcinoma (RCC) poses a diagnostic challenge to the pathologist. Subclassification of papillary renal cell carcinoma (PRCC) includes the presence or absence of solid features, and the non-solid type of PRCC includes a recently described oncocytic variant. In this study we describe a novel oncocytic variant of solid PRCC.

Design: Eleven renal cell neoplasms possessing oncocytic features and solid architecture were examined retrospectively. The histologic differential diagnosis generated from these tumors included renal oncocytoma (RO), oncocytic PRCC, eosinophilic (granular) clear cell RCC, and oncocytic chromophobe RCC. Immunohistochemical analysis was performed and included CD117, PR, racemase (AMACR), RCC, Vimentin, CD10, and CK7.

Results: The eleven neoplasms consisted of circumscribed tumours exhibiting solid and diffuse growth patterns. Tubular structures were identified in six of the specimens, non-tubular structures in five, and occasional papillae in four. Results of immunohistochemical analysis demonstrated six tumours with CD117+/PR+ (features consistent with RO) and five with CD117-/PR- (features consistent with PRCC). The five CD117-/PR- neoplasms also displayed AMACR+, RCC+, VIM+, CD10+, and mainly focal reactivity for CK7. None of the oncocytic renal cell neoplasms had features of eosinophilic CRCC. The five neoplasms exhibiting the immunohistochemical profile of PRCC had sizes ranging from 1 to 6 cm, patient ages ranging from 52 to 67 years, and a male to female ratio of 4:1. Three of the patients in this group developed progression of their disease: one had tumour extension up to the right atrium; two of the patients had metastases, one of them extensive and resulting in death.

Conclusions: In this study we describe five solid oncocytic neoplasms that share the morphological characteristics of RO but possess the immunohistochemical profile and behaviour of classic PRCC. We propose that these tumours represent a previously undescribed oncocytic variant of solid PRCC. When confronted with an oncocytic renal cell neoplasm, awareness of this solid oncocytic variant of PRCC may prove useful in making the correct diagnosis.

813 Clinical Significance of Atypical Small Acinar Proliferation with and without High Grade Prostatic Intraepithelial Neoplasia. A Large Single Institution's Contemporary Experience of 3200 Consecutive Prostate Biopsies Reviewed by a Urologic Pathology Team

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Background: It is well established that 45-60% of patients with a diagnosis of atypical small acinar proliferation (ASAP) will have prostate cancer (PCa) on repeat biopsy. This number has been relatively stable over 15 years of reporting. In contrast, initial studies (1993-2000) showed that approximately 50% of patients with a diagnosis of high grade prostatic intraepithelial neoplasia (HGPIN) had subsequent PCa compared to recent studies which show only 27-33% with PCa on follow-up. Data on the significance of concurrent ASAP and HGPIN diagnoses is very limited.

Design: This study reviews the contemporary experience of a urologic pathology subspecialty service at a large, single institution. 3200 consecutive prostate biopsies from a 4-year period (2003-2006) were reviewed to address the detection rate of PCa after an initial diagnosis of ASAP with or without HGPIN. All cases of ASAP reviewed required a minimum of 1 year of follow-up.

Results: The 3200 biopsies had initial diagnoses of benign (n=1602; 50.1%); PCa (n=1232; 38.5%); ASAP with or without HGPIN (n=119; 3.7%) and HGPIN alone (n=267; 8.3%). 115 of 119 ASAP cases were studied by using antibodies (high molecular weight cytokeratin, p63 and racemase). Follow-up biopsy was performed in 37% of ASAP ± HGPIN cases (n=44). On re-biopsy, 70% of patients with ASAP had an abnormal biopsy, which included PCa (45%), ASAP (9.1%), HGPIN (11.4%) and ASAP with HGPIN (4.5%). Of the 46 cases with ASAP and HGPIN, 57% had PCa on repeat biopsy, compared to 40% of cases with ASAP alone. PCa was ipsilateral to ASAP ± HGPIN in 80% of repeat biopsies.

Conclusions: Our large experience of ASAP cases signed out by a subspecialty urologic pathology team using reliable current immunohistochemical markers reinforces the high positive predictive value of an ASAP diagnosis in the subsequent detection of PCa. Patients with both ASAP and HGPIN diagnoses are more likely to have PCa on a subsequent biopsy (57%). While the clinical significance of isolated HGPIN in needle biopsy in current times is being debated, its presence in patients with ASAP compounds the likelihood of subsequent detection of PCa. Patients with ASAP with or without HGPIN diagnoses should continue to be candidates for re-biopsy, including potentially more aggressive sampling from the ipsilateral side of ASAP diagnosis.

814 Role of Urovysion™ Fluorescence In-Situ Hybridization (FISH) in the Diagnosis and Surveillance of Squamous Cell Carcinoma of Bladder

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Background: Multitarget Urovysion™ (UV) fluorescent in-situ hybridization (FISH) is an established tool for detection of urothelial carcinoma (UC). Specific chromosome (chr) abnormalities are identified using probes which highlight the centromeres on chr 3, 7, 17 and 9, detecting polysomies of chr 3, 7 and 17 and deletions of chr 9p21. Non-bilharzial squamous cell carcinoma (SCC) is a rare form of vesical cancer seen predominantly in patients with prolonged indwelling urethral catheters. These patients often undergo routine surveillance with UV FISH. While UV FISH has proven useful in the diagnosis and monitoring of UC, its usefulness in SCC of bladder has not been established.

Design: UV FISH probes were applied to representative paraffin blocks of formalin-fixed specimens from 6 pure UCs and 17 SCCs. Fifteen of 17 (88%) SCCs were pure and 2 were UC with extensive squamous differentiation (SD) (12%). Tumors were evaluated for polysomy 3, 7 and 17 and loss of both 9p21 alleles in > or =10% of cells.

Results: The results of hybridization are shown in table 1 and 2.

Distribution of UV FISH Results

Diagnosis	Positive FISH	Negative FISH	Failed FISH	Total
SCC	1	8	6	15 (65%)
UC with SD	2	0	0	2 (9%)
UC	3	3	0	6 (26%)
Total	6 (26%)	11 (48%)	6 (26%)	23 (100%)

Polysomy Distribution in Tumor Types

Diagnosis	Polysomy 3, 17	Polysomy 7, 17	Polysomy 3	Polysomy 3, 7, 17
SCC	1			
UC with SD	1	1		
UC		1	1	1

Of the 23 cases hybridization failed in 6 (26%), 4 of which had blocks that were over 5 years old.

Conclusions: The characteristic UC-associated chromosomal abnormalities tested for by UV FISH were not identified in the majority of cases of pure SCC. UV FISH may therefore not be useful for the diagnosis and surveillance of pure bladder SCC. UV FISH may help to distinguish cases of pure SCC from conventional UC with extensive squamous differentiation. It will be necessary to conduct larger studies to confirm the clinical utility of UV FISH in resolving this differential diagnosis.

815 Hypoxia Inducible Factor (HIF) Pathway in Papillary Renal Cell Carcinoma: A Tissue Microarray Based Immunohistochemical (IHC) Study

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Background: Targeted therapies against HIF pathway molecules for high stage renal cell carcinoma (RCC) have recently shown promise in these refractory tumors. Most RCC's are of the clear cell type (CRCC) and often show genetic abnormalities in the *VHL* pathway. A non-functional VHL complex leads to over-expression of HIF1-α and its downstream factors including vascular endothelial growth factor (VEGF), glucose transporter1 (GLUT1), and carbonic anhydrase IX (CA9). Previously, we reported on the expression of these markers in CRCC. Among the metastases from RCC, papillary RCC (PRCC) is the second most common type. Recent data suggests that some of the HIF pathway markers may be expressed in PRCC. We investigated the expression patterns of several of these markers in PRCC.

Design: 85 cases of PRCC were examined using a tissue microarray (TMA), and were sub-classified as type 1 (n = 46), type 2A (n = 21) and type 2B (n = 18) [Yang et al. Cancer Res. 2005;65:5628]. IHC was performed using antibodies against HIF1-α, CA9, VEGF-receptor 2 (VEGF-R2), and GLUT1. The staining was graded from 0 to 3+ (0, no staining; 1+, 1-25% cells positive; 2+; 26-50%; and 3+, >50%). Cytoplasmic/membranous expression of CA9, GLUT1 and VEGF-R2 and only nuclear expression of HIF1-α were considered positive. Since only tumoral vessel expression with no tumor cell positivity was observed with VEGF-R2, vascular expression was graded as low or high in the vessels alone.

Results:

Staining grades for the IHC markers in tumor cells

Antibody	Staining Grade (%)			
	0	1+	2+	3+
HIF-1α (n= 85)	54 (64)	25 (28)	3 (4)	3 (4)
CA9 (n= 83)	62 (74)	14 (17)	3 (4)	4 (5)
GLUT1 (n= 85)	78 (91)	3 (4)	3 (4)	1 (1)
VEGF R2 (n= 85)*	0	0	0	0

* Low expression in tumoral vessels in 84 (99%), and high in 1 (1%).

The majority of cases (84%) showed either no (0) or weak (1+) expression with one or more marker (see table). The most frequently highly expressed (2 or 3+) marker was CA9, seen in 7 cases (9%). Necrosis was present in 10 cases; of these 3 and 1 showed 2 or 3+ expression of CA9 and HIF1-α, respectively. No correlation between PRCC type and expression of any of the markers was observed.

Conclusions: The majority of PRCC do not express CA9, HIF1-α, GLUT-1 or VEGF-R2. Among the small proportion of PRCC that overexpress CA9, such expression is often related to tumor necrosis. These results suggest that targeted therapies against HIF pathway molecules may not be effective in PRCC.

816 Testing a Multigene Model To Predict Lethal Prostate Cancer

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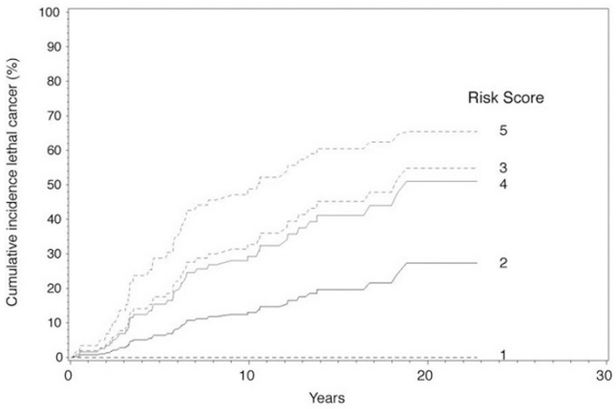
Background: While prostate cancer is a leading cause of cancer death, most men die with and not from their disease, underscoring the urgency to distinguish potentially lethal from indolent prostate cancer. We tested the prognostic value of a previously identified multigene signature of prostate cancer progression to predict cancer-specific death.

Design: The Örebro Watchful Waiting Cohort included 172 men with localized prostate cancer of whom 40 developed lethal disease. We quantified protein expression of the markers in tumor tissue by immunohistochemistry, and stratified the cohort by quintiles according to risk classification (Low Risk=1; High Risk=5). We accounted for clinical parameters (age, Gleason, nuclear grade, tumor volume) using Cox regression, and calculated Receiver Operator Curves to compare discriminatory ability.

Results: The hazard ratio of prostate-specific death increased with increasing risk classification by the multigene model, with a 16-fold greater risk comparing highest versus lowest risk strata, and predicted outcome independent of clinical factors (p=0.002) (Figure). The best discrimination came from combining information from the multigene markers and clinical data, which perfectly classified the lowest risk stratum where no one died of cancer; using the two lowest risk groups as referent, the hazard ratio (95% confidence interval) was 11.3 (4.0-32.8) for the highest risk group and the cumulative incidence difference at 15 years was 60% (50-70%). The combined model provided greater discriminatory ability (AUC 0.78) than the clinical model alone (AUC 0.71), p=0.04.

Conclusions: Molecular tumor markers can add to clinical parameters to help distinguish lethal and indolent prostate cancer, and hold promise to guide treatment decisions.



817 Secondary Intraurothelial Carcinoma and Muscle-Invasive Urothelial Carcinoma of the Bladder Shows Stem Cell-Like Features and Somatic Down-Regulation of DNA Mismatch Repair

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Background: The clonal relationship between low-grade urothelial dysplasia (LGUD), carcinomas in situ (CIS) and muscle-invasive urothelial carcinoma (UCC) remains unknown, and the role of stem cells and mismatch repair in bladder carcinogenesis has not been analyzed by topographic compartments.

Design: LGUD (18) and CIS (12) found in 25 patients (coexistent in 5) with muscle-invasive UCC were microdissected, and RNA and DNA extracted from intraepithelial and invasive tumor cells (superficial and deep to muscularis mucosa). The same areas were analyzed for: methylation pattern of androgen receptor alleles, microsatellite analysis of TP53, RB1, WT1, and NF1; the expression of Ki-67, TP53, nuclear DNA content, in situ end labeling; MLH1 and MSH2 sequencing and immunostaining; OCT-4 and CD44v6 expression (quantitative RT-PCR, immunostaining). Appropriate controls were run in each case.

Results: Clonal patterns were revealed monoclonal in CIS (6, 100%), invasive UCC (13, 100%), and LGUD (2, 20%), and polyclonal in LGUD only (8, 80%). CIS showed stem cell-like phenotype (OCT-4/CD44v6 positive), aneuploid DNA content and more abnormal microsatellite loci than the corresponding invasive compartments (always involving TP53 loci). LGUD revealed diploid DNA content, no stem cell phenotype (OCT-4/CD44v6 negative), and no microsatellite abnormalities except in 2 cases, one monoclonal and one polyclonal. Opposite proliferation/apoptosis patterns were observed for CIS and LGUD, advantageous for the former. Absent mlh1 protein expression with no gene mutations were identified in CIS and nodular-trabecular UCC with high microsatellite abnormalities.

Conclusions: The stem cell-like features and the kinetic patterns would contribute to the accumulation of genetic abnormalities in CIS by somatic down-regulation of mismatch repair system. These genetic findings (higher incidence of microsatellite abnormalities in CIS than in invasive components) suggest an independent evolution of CIS from muscle-invasive UCC, whereas LGUD should not be closely connected with this molecular progression.

818 Anterior Dominant Prostate Cancer Compared with Posterior Cancers in a PSA Non-Screened Population

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Background: Anterior prostate cancers can be difficult to detect with transrectal ultrasound (TRUS) guided biopsies with a consequent delay in diagnosis compared with posterior cancers. The clinical significance of anterior dominant prostate cancer (APC) is uncertain.

Design: We reviewed 280 consecutive radical prostatectomy specimens from the PSA non-screened population and identified cases with APC with the largest tumors anterior to the urethra. There were pure anterior cancers and anterior > posterior cancers. Clinicopathological features of these were analysed and compared with those of pure or dominant posterior cancers (PPC). Gleason score 8-10 or 7 with a third component of pattern 5 were considered high-grade.

Results: There were 40 patients (14.3%) with APC. The mean age was 58 years, not significantly different from that of PPC. There was a trend for higher mean serum PSA in APC of 8.40 ng/ml compared with 7.16 ng/ml in PPC. However, this difference was not statistically significant ($p = 0.08$). The APC involved the anterior peripheral zone (PZ) in all cases with 35 and 28 cases also displaying transitional zone (TZ) and anterior fibromuscular stromal involvement. The GS in APC was 7 in 32 cases with high-grade carcinoma found in the other 8 cases. High grade tumors were more common in APC compared with PPC ($p = 0.01$). The location of dominant cancer did not have any effect on stage with 15 of the 40 patients with APC (37.5%) in comparison to 90 of 140 (37%) with PPC displaying extraprostatic extension (pT3). All stage pT3 APC had extraprostatic extension into anterior fat. In APC, the mean tumor volume was 2.95 cc (range 0.6-6.1 cc) and surgical margin positivity was seen in 10 cases (25%) which were not significantly different from that seen in PPC ($p = 0$ .17 and 0.5 respectively).

Conclusions: In populations without PSA screening, APC is seen in a significant proportion of prostate cancer cases. These are pathologically significant tumors with a higher risk of high-grade cancer than PPC and a similar risk of high-volume, high stage disease and surgical margin positivity. With a high or rising PSA and negative posterior biopsies, it is important to perform appropriate biopsies to sample the anterior part of the prostate.

819 Any Proportion of Ductal Adenocarcinoma of the Prostate Predicts Higher Stage Disease

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Background: Ductal adenocarcinoma of the prostate (DAP) is recognized to be an aggressive high-grade malignancy that often presents at an advanced stage. In mixed DAP and acinar cancer, it is unknown whether the proportion of the ductal component influences the pathological stage. It is also unknown whether aggressive behaviour is due to the high-grade of these tumors.

Design: We reviewed 268 consecutive radical prostatectomy specimens and identified cases with a DAP component. Clinicopathological features of these were analysed and compared with those of pure acinar adenocarcinoma.

Results: 34 patients (12.7%) had a DAP component, varying from 5% to 100% of the tumor. The mean age, serum PSA and surgical margin status in the group with DAP did not differ significantly from the pure acinar group. All patients with DAP had peripheral zone involvement with 16 cases (46%) also involving the periurethral region. 25 of 34 patients with DAP (73%) had extraprostatic extension with or without seminal vesicle involvement (pT3) in comparison to 33 of 234 (14%) patients with acinar adenocarcinoma. Presence of any DAP component significantly predicted pT3 ($p < 0.0001$) and high tumor volume ($p = 0.001$). Presence of any DAP component remained a significant predictor for pT3, after adjusting for tumor volume. In cases with DAP, the proportion of DAP did not significantly modify the strength of the association with pathological stage or tumor volume. 22 of 34 patients with DAP (64%) in comparison with 24 of 234 (10%) patients with acinar adenocarcinoma had a Gleason score (GS) of > 7. Presence of any DAP in comparison with GS> 7 pure acinar adenocarcinoma significantly predicted pT3 ($p= 0.04$).

Conclusions: In a population of patients selected for radical prostatectomy, DAP is not uncommon. These involve the peripheral zone with less common involvement of the periurethral region. Any ductal component in a prostate cancer is associated with a high risk of extraprostatic extension, and therefore a worse prognosis. Since we observed an association with stage at all proportions of ductal adenocarcinoma involvement, the presence of any amount of ductal adenocarcinoma needs to be reported. The aggressive nature appears to be independent of the high-grade of these tumors.

820 Gene Expression Profiles of Ductal Versus Acinar Adenocarcinoma of the Prostate

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Background: Ductal adenocarcinoma (DC) is an uncommon variant of prostatic adenocarcinoma (PC) with a generally more aggressive clinical course and worse outcome than acinar adenocarcinoma (AC). Limited data exist on comparative protein expression in ductal vs. acinar adenocarcinoma and global gene expression profiling has not been performed. The aim of this investigation was to evaluate the relatedness of ductal vs. acinar PC by comparative gene expression profiling.

Design: Archived, de-identified, snap frozen tumor tissue from 5 DC, 3 mixed DC/AC, and 11 matched AC cases were obtained from the Siteman Cancer Center Tissue Procurement Core Facility. All cases of AC and DC were matched by Gleason grade. RNA from whole sections of 5 DC and 11 AC cases were subjected to gene expression profiling on Affymetrix U133Plus2 microarrays. Laser-capture microdissection (LCM) was performed on 3 mixed DC/AC (DC and AC components collected separately) and 5 matched AC cases. RNA was extracted and seven high quality LCM RNA samples (3 DC and 4 AC) were similarly analyzed on U133Plus2 arrays.

Results: Analysis of data from whole sections of DC and AC using both traditional ANOVA and SAM analyses showed few transcripts with a greater than 2 fold significant difference in expression between DC and AC. On the other hand, a similar analysis of microdissected cell populations identified several transcripts with significant differences in expression of 5-27 fold between DC and AC. The identity of several of these transcripts suggests interesting differences between the biology of AC and DC.

Conclusions: Although the molecular profiles of ductal and acinar adenocarcinoma are highly related based upon this pilot comparative gene expression analysis, we have identified several genes whose expression is characteristic of each tumor type. Such differences were only apparent by microdissection of independent tumor cell populations, reinforcing the concept that PC is a heterogeneous disease composed of cell populations with differing histological and molecular phenotypes, even in the context of the same tumor.

821 BRAF Mutation Status and Growth Factor Receptor Expression Do Not Predict Baseline Factors or Outcome in Hormone Refractory Prostate Carcinoma

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Background: BRAF, a gene whose protein product is an intracellular serine/threonine kinase involved in the mitogen-activated protein kinase growth factor receptor pathway, is frequently mutated in melanomas and smaller proportions of other neoplasms. Screening studies have not previously identified BRAF mutations in prostate carcinoma.

We investigated BRAF mutation status and growth factor receptor and signaling intermediary expression in a highly selected group of prostate carcinomas, in conjunction with a clinical trial of a BRAF inhibitor.

Design: Patients (n=26) with a diagnosis of prostate adenocarcinoma, metastatic or recurrent disease with PSA≥10µg/L, evidence of PSA progression while receiving androgen ablative therapy, and with archived paraffin-embedded tumour tissue were included. DNA was extracted and subjected to polymerase chain reaction amplification of BRAF exons 11 and 15, followed by denaturing high-performance liquid chromatography (DHPLC) mutation screening. Immunohistochemical (IHC) staining of tumour sections for epidermal growth factor receptor (EGFR), insulin-like growth factor 1 receptor (IGF1-R), ERK, phospho-ERK (pERK), and vascular endothelial growth factor receptor 2 (VEGFR-2) was completed. Slides were scored by two pathologists, using a three-point scale. Gleason scores were assigned by consensus.

Results: DHLPC revealed one variant profile for BRAF exon 15. By direct sequencing, two novel synonymous changes, c.1794C>T p.A598A and c.1742 p.I582I, were observed. While neither variant affects the BRAF protein structure, ESEfinder analysis revealed that the A598A variant was predicted to create a new splice enhancer and potentially cause altered processing of BRAF mRNA. The patient had an increasing PSA as best response to experimental treatment. IHC staining was noted in 76% of cases for VEGFR-2, 61% for ERK, 45% for pERK, 36% for IGF1R, and 5% for EGFR. Using Fisher's exact test, there were no statistically significant correlations between IHC scores and baseline factors or PSA changes during or after protocol therapy.

Conclusions: Prostate carcinomas may harbour BRAF mutations, a finding not previously demonstrated. A high proportion of hormone-refractory prostate carcinomas express VEGFR-2, though this does not correlate with measurable clinical characteristics or outcomes at the time of hormone refractory disease.

822 Cross-Study Microarray Evaluation of Aggressiveness Factors in Prostate Cancer

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Background: Men diagnosed with prostate cancer experience variable outcomes after prostatectomy. Most men have tumors with low biological aggressiveness, whereas some men have aggressive tumors leading to metastasis and death. Currently standard prognostic methods do not adequately predict whether an individual patient's tumor is likely to behave aggressively.

Design: Affymetrix U95A microarray data are publicly available for two expression profiling studies on prostate cancers with known clinical outcome[1,2]. We performed a cross-study comparison to identify genes related to outcome in both data sets. We rescaled each datasets by thresholded the data using maximal and minimal values of 97.5th and 2.5th percentile, respectively. We normalized the the data by dividing each array's values by 1/slope of a least-squares linear fit of data in a reference sample, and then divided each array value by the maximal/upper threshold value. This method rescaled data on all arrays from the lower threshold to 1. For aggressive and non-aggressive cancers in each dataset (based on PSA recurrence), we calculated a signal-to-noise metric: $Sx = (\mu_{class0} - \mu_{class1}) / (\sigma_{class0} + \sigma_{class1})$, where μ_{class0} represents the mean and σ_{class0} the standard deviation for each gene in all samples of class 0[3]. Genes identified as having high *Sx* values across both datasets were used in n-genes weighted-voting classification algorithms to predict prostate cancer non-aggressiviness.

Results: We found that a 20 gene algorithm best distinguished between aggressive and non-aggressive prostate cancers. Listed in decreasing order of *Sx* values, 10 genes were associated with aggressiveness: *APOE, AP3B2, HOXC4, CDC20, ALOX12P2, IL23A, HAS1, ATP12A, PVALB, CCNF*. 10 genes were associated with non-aggressiveness: *SFTPB, MAGEB1, RARB, OLIG2, CCDC88C, CDC27, PSCA, PLA2G6, UCHL1, TGM4*. A weighted-voting algorith demonstrated that the the specificity of predicting non-aggressiveness was 100% and 76%, in the two data sets (refs. 1 and 2, respectively). Seven (35%) of these 20 genes have previously published roles in prostate cancer biology.

Conclusions: Cross-study evaluation of datasets can lead to identification of useful prostate cancer aggressiveness markers. Availability of additional datasets will importantly contribute to the effort to identify and validate aggressiveness markers in prostate cancer. 1. D Singh, et al. 2002. Cancer Cell 1:203-9. 2. YP Yu, et al. 2004. J. Clin. Oncol. 15: 2790-99. 3. S Ramaswamy, et al. 2003. Nature Genetics. 33:49-54.

823 Pathologic Findings in Men Undergoing Post-Treatment Biopsy "For Cause" after Primary Cryoablation for Prostate Cancer

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Background: Prostate cryosurgery is a promising technique for the treatment of clinically localized prostate carcinoma (PCA), although its effectiveness is still unknown. We assessed the efficacy of cryoablation as primary treatment for PCA with emphasis on post-treatment pathologic finding in a selective group of patients undergoing biopsy "for cause".

Design: From 2004 to 2005, 34 men had primary cryosurgery for clinically localized PCA at one institution. All patients had pre-treatment (pre-PBx) biopsy-confirmed PCA and underwent a dual freeze-thaw cycle using third-generation cryotechnology via percutaneous transperineal approach. Thirteen men (38.2%) underwent post-treatment prostate needle biopsies (post-PBx) either for rising prostate-specific antigen (PSA) or to confirm efficacy of subtotal cryotherapy (4 cases). We reviewed the post-PBx pathologic findings.

Results: The mean time interval between cryotherapy and pt-PBx was 13.2 months (range 4 to 31). Eight of 13 patients (61.5%) had no evidence of PCA in post-PBx ("non-failures"). Five (38.5%) had persistent PCA confirmed by post-PBx ("failures"). Four of 5 "failures" had total cryoablation, 1 had focal treatment. Their immediate post-cryotherapy PSA was ≤0.6 ng/ml, although their PSA ≥6 months post-treatment was >0.5 ng/ml. Pre-PBx Gleason score (GS) and post-PBx diagnosis for "failures" are reported in table 1.

Patients with PCA in post-PBx

Pre-treatment GS	Post-PBx diagnosis
GS6 (R)*	GS6 (L)
GS6 (L)	benign (R); GS7 (L)
GS7 (R, L)	GS8 (R, L)
GS6 (R); GS8 (L)	benign (R); GS8 (L)
GS9 (R, L)	GS9 (R, L)

*subtotal treatment

Bilateral PCA was present at diagnosis in 60% of "failures" and 37.5% of "non-failures". The "non-failures" post-PBx showed stromal fibrosis, elastosis, hemosiderin deposition, focal inflammation and occasional coagulative necrosis. Post-treatment PCA showed no specific histologic changes. Although hemosiderin deposition was detected in the stroma of the post-PBx cores with PCA, the intervening stroma between malignant glands did not display any post-cryosurgical change, raising the possibility that these areas were inadequately frozen.

Conclusions: Primary cryoablation of the prostate is a viable treatment option in patients with localized PCA. Five of 34 (14.7%) men had persistent PCA confirmed by PBx. Additional studies with larger cohorts and longer follow-up are necessary to assess the sustained efficacy of this procedure.

824 Renal Tubulocystic Carcinoma Is Related to Papillary Renal Cell Carcinoma: Cytogenetic and Histological Evidence

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Background: Specific cytogenetic changes have been used to classify renal cell carcinomas. For example, papillary renal cell carcinoma (PRCC) is characterized by chromosome 7 and 17 gains. Renal tubulocystic carcinoma is a rare subtype and the clinicopathological features have only recently been described (Yang et al, Am J Surg Pathol, in press). In this report, we examined its relationship to PRCC in terms of cytogenetics and histology.

Design: Formalin-fixed, paraffin-embedded sections from 8 tubulocystic RCCs and 20 PRCCs were subjected to a multicolor FISH assay (UroVysion, Abbott/Vysis) containing probes for the pericentromeric regions of chromosomes 7, 17 and 9p21. 100 nuclei were examined to enumerate the copy numbers of chromosomes in each tumor and its corresponding normal kidney tissue. Tumors with % cells harboring a chromosomal change ≥ mean + 3 standard deviation (S.D.) of the normal tissue were considered to harbor that chromosomal change. 17 tubulocystic RCCs (including 14 cases from the original AJSP article) were studied for the presence of associated PRCC or papillary component within the tumor.

Results: All 20 PRCCs had chromosome 7 and 17 gains (Table 1). All 8 tubulocystic RCCs also had chromosome 7 and 17 gains. However, the mean % of chromosome 7 and 17 gains were lower (p=0.000 and 0.000), and only 3 cases had % of chromosome 7 and 17 gains within the range observed in PRCC. Loss of chr 9p21 was present in all 8 tubulocystic RCCs, but in none of PRCCs. Of the 17 tubulocystic RCCs, 4 (23.5%) had concomitant PRCC in the same kidney, and 1 had minor papillary component in the tumor. In 2 other cases, the majority of the tumors were papillary with a minor tubulocystic component.

Chromosomal changes in tubulocystic and papillary RCC

		Tumor: % cells with:			Normal tissue: % +3 S.D.		
		+7	+17	-9p21	+7	+17	-9p21
Tubulocystic RCC	1	11	5	67	2	0	49.3
	2	56	68	75	0	0	63
	3	13	3	94	4	0	56.8
	4	32	25	76	0	4	52.6
	5	12	3	71	6.2	0	40.3
	6	63	72	82	0	0	47.1
	7	22	22	89	2	2	57.2
	8	69	55	77	0	0	51.0
	Mean	35	32	77	0.9	0.5	50.8
PRCC	Mean of 20 cases	82 (45-100)	69 (42.5-90)	24 (10-52.5)	2.25 (0-15)	3.7 (0-12.5)	54.3 (31.9 - 76.2)

Conclusions: Tubulocystic RCC harbors chromosome 7 and 17 gains. In addition, PRCC is frequently present together with, or as a component of, tubulocystic RCC (41% cases). These findings suggest tubulocystic RCC and PRCC are related lesions.

825 Percentage of Fuhrman Nuclear Grades 3 and 4 as a Prognostic Factor in Renal Cell Carcinoma

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Background: The prognostic value of nuclear grade in the outcome of patients with renal cell carcinoma (RCC) has been well established. However, the impact of the amount of high grade RCC on clinical outcome has not been assessed.

Design: Using a computer database search for partial and radical nephrectomies performed at Barnes-Jewish and St. Louis Children's Hospitals during the years 1989 to 2003, we identified a total of 926 samples from 900 patients. All tumors were typed according to the 2004 WHO classification. The nuclear grade was evaluated using the 4-tier system. The percentage of high nuclear Fuhrman grade 3 and 4 within each tumor was recorded.

Results: The study group included 569 men and 331 women. 693 (74%) tumors were clear cell type, 138 (14.9%) papillary, 29 (3.1%) chromophobe, 13 (1.4%) multilocular cystic, and 48 (5.1%) unclassified. The remaining cases were as follows: 3 collecting duct carcinomas, 1 renal medullary carcinoma and 1 translocation carcinoma. 125 cases (13.4%) had nuclear grade 1, 505 (54.5%) grade 2, 232 (25%) grade 3 and 64 (6.9%) grade 4. 69% of cases had no high grade component, 14% had 1-50% nuclear grade 3 or 4, and 17% had 51-100% high nuclear grade. At a mean follow-up of 80 months,

58 of 630 (9.4%) patients with 0% high-grade RCC died of disease, 26 of 125 (20%) patients with 1-50% high-grade RCC died of disease, and 68 of 161 (42%) patients with 51-100% high-grade RCC died of disease ($p < 0.0001$).
Conclusions: The outcome for patients with high-grade RCC appears more favorable when less than 50% of the tumor is high grade.

826 Prostate Carcinoma within the Invaginated Extra-Prostatic Space Is Frequently Associated with Higher Stage Grouping: A Study of 73 Cases of Radical Prostatectomy Specimens with pN1 and/or pT3b

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Background: A comprehensive understanding of the zonal anatomy and the likely sites of carcinoma within the prostate gland may provide insights into the way in which prostate carcinoma (PC) can spread. The invaginated extra-prostatic space (IES) is the space around the ejaculatory ducts as well as the verumontanum, composed of loose connective tissue with numerous thin-walled lymphatic and vascular channels. It is considered a true extra-prostatic space and presence of PC in IES may lead to direct or contribute to involvement of the seminal vesicles (SV) and increased incidence of lymph node (LN) metastases. In order to further understand the significance of IES, we retrospectively reviewed the radical prostatectomy and lymph node dissection of patients with carcinomatous involvement of SV and/ or LN metastases.
Design: Seventy-three cases of radical prostatectomy specimens with pathologic staging of pN1 or pT3b or both were reviewed. Whole mount sections of prostate and pelvic LNs were examined for histopathologic features including Gleason scores, location of carcinoma, perineural invasion, capsular involvement, LN metastasis, SV and IES carcinoma involvement.
Results: Of the 73 cases, 44 (60%) were found to have IES PC involvement. Of those 44 cases, 21 (48%) had positive LN metastases and 37 (84%) had SV involvement. Fifteen (36%) cases had both LN metastases and SV involvement. Tumor size ranged from 0.8 to 5.8 cm in greatest dimension. Sixty-two (85%) of the 73 cases had capsular invasion or focal extra-prostatic invasion. Twenty-six (36%) cases had a Gleason score of 7, 14 (19%) cases had score of 8, 30 (41%) cases had score of 9 and 2 (3%) cases had a score of 10. Only 1 case (1.3%) had a score of 6. Perineural invasion was identified in 68 (93%) cases.
Conclusions: Almost half (48%) of LN metastases and 84% of SV involvement were associated with IES involvement by PC, indicating that IES is an important pathway for carcinoma spread. Our results suggest that finding of IES involvement warrants careful examination of pelvic LNs and SV that may eventually lead to a higher staging for PC.

827 Comparison of Selective and Whole Mount Section for Detection of Prostatic Urothelial Carcinoma in Patients with Bladder Cancer: A Meta-Analysis of 2653 Cases

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Background: Accurate assessment of prostatic involvement by urothelial carcinoma (UC) in patients with bladder cancer provides critical information regarding tumor staging, prognosis, choice of urinary diversion, the risk assessment of urethral recurrence, and overall treatment strategies. However, the reported incidence varied widely in the literature. In this study, we perform a meta-analysis to compare selective and whole mount sections of radical cystoprostatectomy specimens.
Design: We performed a meta-analysis of 10 studies published from 1977 to 2007 with primary or secondary objective to evaluate the incidence, as well as in-situ and stromal invasion of prostatic UC in radical cystprostatectomy specimens. The studies were divided into two groups based on the manner of examination: routine selective section of prostate versus whole-mount sections of entire prostate. Information regarding urothelial carcinoma in-situ of prostatic urethra or ducts and prostatic stroma invasion are collected and analyzed.
Results: A total of 2653 patients underwent radical cystoprostatectomy for bladder cancers were included in this analysis. Among them, 1884 cases of prostate were examined by routine selective sections and 769 were examined by whole-mount sections. By selective section, the incidence of prostatic UC ranged from 12.0% to 35% with average of 19.1%. In-situ and prostatic stroma invasion of UC were identified in 8.4% and 10.7% respectively. In contrast, the incidence of prostatic UC was 35.5% when the prostate was examined by whole-mount sections, ranging from 29% to 48%, which is significantly higher than that of selective sections ($p<0.001$). In-situ and prostatic stroma invasion of UC were identified in 20.0% and 17.5% respectively, both were significant higher than selective sections ($p<0.001$).
Conclusions: The incidence of prostatic involvement by UC (both in-situ and stromal invasive UC) in patients with bladder cancer is significantly underestimated in routine selective sections for radical cystprostatectomy specimen. More extensive and selective sections are warranted for accurate assessment of prostatic UC.

828 Papillary Renal Cell Carcinoma with Clear Cell Features: A Clinicopathologic Study of 55 Cases

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Background: Papillary renal cell carcinoma (PRCC) is the second most common RCC that has a unique morphologic growth pattern and cytogenetic abnormalities. Subclassification of PRCC based primarily on the cytoplasmic features and nuclear grade has been proposed and appear to have some prognostic significance. Clear cell features in PRCC are not an infrequent finding and their prognostic significance has not been thoroughly studied. We attempt to investigate the clinicopathologic features of PRCC with clear cell features and compare them with that of PRCC without clear cell changes.
Design: 55 cases of PRCC with clear cell features (defined by PRCC with lining neoplastic cells with abundant clear cytoplasm or intimate admixture of PRCC with clear cell RCC features) were identified from a retrospective review of 174 PRCC from a single institution from 1990 to 2006. The tumors were graded as low (Fuhrman grades 1 and 2) or high grade (grade 3 and 4) and were staged based on AJCC 2002. Demographic data as well as clinicopathologic information including tumor stage, tumor size, and follow up information were obtained.
Results: Overall, 55 (31.6%) of total 174 PRCCs were identified to have clear cell features. Compared to PRCCs without clear cell features, PRCCs with clear cell features had more high grade (69% vs. 45%, $p<0.01$), larger tumor sizes (6.7±4.4 vs. 4.7±3.8 cm, $p<0.01$), and more tumors with advanced (≥T3) stages (31% vs. 16.8%, $p<0.05$). No differences in age or gender distribution were seen. Among 153 patients with follow-up (mean 39 months and range 0.1-177 months), the 5-year and 10-year overall survival for PRCC with clear cell were 42% and 25% respectively, comparing with 69% and 44% for PRCC without clear cell features ($P<0.01$). Multivariate analysis showed that PRCC with clear cell features was an independent prognostic factor in addition to tumor stage and gender.
Conclusions: The presence of clear cell features in PRCC is a relatively frequent finding (31.6%) and is associated with high grade tumor and high stage presentation. Furthermore, clear cell features in PRCC imparts a poor prognosis. Our results indicate that papillary RCC with clear cell features may actually be a specific clincopathologic entity that requires further cytogenetic or molecular studies.

829 Is Their a Grade Progression of Prostate Cancer with Advancing Age? An Analysis of Needle Biopsy, Radical Prostatectomy and Autopsy Databases

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Background: It continues to be debated as to whether lower grade prostate cancers (PCa) can give rise to higher grade tumors as time passes or whether the latter arise de novo. It is well established that while high grade tumors can be present adjacent to or within the same tumor "nodule" containing lower grade cancer, small volume, higher grad PCa can be identified in isolation of a lower grade tumor. As longitudinal studies sampling PCa from the same individual over time are practically impossible to conduct, our objective was to tabulate results from three large databases documenting the Gleason score of cancers diagnosed in needle biopsies (NB), radical prostatectomy specimens (RPS) and prostate gland obtained from autopsies men.
Design: Gleason score (GS) of all patients who had NB with a diagnosis of PCa and all patients treated by radical prostatectomy between 1990-2003 as well as specimen GS of entirely submitted prostate glands obtained from autopsied men between 1991-1998 were stratified according to age of diagnosis, surgical treatment or death by decade.
Results: A total of 3120 patients, with PCa on NB were identified with an age distribution of 4%, 21%, 42% and 37% for the 5th, 6th, 7th and 8th decade respectively. The proportion of GS =>7 tumors for this group increased from 31% to 37%, 46% and 55% by decade ($p=0.001$). The corresponding increase for the 1902 RPS cohort by the same decade distribution was 51% to 57%, 68% and 71% respectively,($p=0.01$). The age distribution for the 1027 men from the autopsy cohort was different as this group represents higher proportion of younger men starting at age 20. The overall subclinical cancer prevalence in this group was 395 (38%) that reflected a rate ranging from 6% in the third decade to 74% in the eighth decade. The proportion of Gleason score>= 7 tumors in autopsied men with PCa was 0%, 1.5%, 3.2%, 10%, 15% and 23% for the 3rd, 4th, 5th, 6th and 7th decade respectively, ($p< 0.01$).
Conclusions: For the three cohorts of diagnostic needle biopsies, surgically resected glands and entirely examined prostates from a wide age range of autopsied men, the Gleason score of cancers in these specimens increases significantly with age. These data may have implications for the natural history of PCa and for screening and watchful waiting as a management choice for some patients.

830 ProEx C Is a Better Immunohistochemical Marker in Differentiating between Non-Invasive Low-Grade and High-Grade Papillary Urothelial Carcinomas

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Background: Even though MIB-1 (Ki-67) and p53 are popular biomarkers in identifying urothelial dysplasia and differentiating between low-grade and high-grade papillary urothelial carcinomas, a significant overlapping of the staining results exists. Our recent study demonstrated that ProEx C (a cocktail of minichromosome maintenance protein2 and DNA topoisomerase II alpha) has some value in identifying cervical squamous intraepithelial lesions (Human Pathology 2007;38:1335-1344). This study evaluates the utility of ProEx C in identifying and differentiating urothelial lesions when compared to Ki-67 and p53.
Design: We immunohistochemically evaluated the expression of ProEx C (TriPath Imaging Inc), MIB-1 and p53 on conventional tissue sections in benign urothelium and urothelial lesions. A total of 121 cases was included in the study: Group #0 (G0) – 12 cases of normal urothelium; group #1 (G1) –25 cases of low-grade papillary urothelial carcinoma (LPUC); group #2 (G2) – 27 cases of high-grade papillary urothelial carcinoma (HPUC); group #3 (G#3) –15 cases of urothelial carcinoma in situ; and group #4 (G4) – 42 cases of invasive urothelial carcinoma. The staining intensity was graded as weak (1+), intermediate (2+) or strong (3+). The percentage of the staining was recorded as negative (no staining), 1+ (<25% of tumor cells stained), 2+ (26-50%), 3+ (51-75%), or 4+ (>75%). The distribution of staining thickness in tissues was recorded as negative (no staining), 1+ (basal 1-2 layers), 2+ (lower 1/3), 3+ (lower 2/3), or 4+

(full thickness). For the purpose of statistical analysis, the 1+, 2+, 3+ and 4+ were converted into the score of 1, 2, 3, and 4, respectively.

Results: The results demonstrated a nuclear staining pattern for ProEx C, Ki-67 and p53. The results are summarized in the Table 1.

Table 1. Comparison of Positive Immunoreactivity for ProEx C, Ki-67 and p53 in Urothelial Lesions

Group	ProExC	Ki-67	p53
G1	6.3±1.6 (n=25)	4.7±2.5 (n=25)	1.7±2.3 (n=25)
G2	8.6±1.8 (n=27)*	6.3±2.4 (n=27)*	2.6±2.9 (n=27)
G3	9.3±1.3 (n=15)*	6.9±0.9 (n=15)*	3.8±2.5 (n=15)
G4	9.5±0.9 (n=42)*	8.4±1.0 (n=42)*Δ	5.3±3.6 (n=42)*#

*Compare to G1, $p < 0.01$;ΔCompare to G2 and G3, $p< 0.01$; #Compare to G2, $p<0.01$

Conclusions: The results demonstrate that 1) ProEx C is a better maker in differentiating non-invasive LGPUC from HGPUC when compared to Ki-67 and p53; and 2) overexpression of ProEx C is associated with the progression of non-invasive urothelial carcinoma to invasive urothelial carcinoma.

831 CD44 Is a Useful Marker for Prostatic Small Cell Carcinoma

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Background: CD44 and its isoforms mediate epithelial cell adhesion and are implicated in cell proliferation and migration. Their levels of expression have prognostic value in certain malignancies. CD44 has attracted significant attention because of its potential as a stem cell marker in many tumors including prostate cancer. It has been reported that CD44 is expressed in basal cells of benign prostate but not in the majority of the prostate adenocarcinoma cells. Here we report the value of studying CD44 expression in the diagnosis of prostatic small cell carcinoma (SCC).

Design: Immunohistochemical staining was performed on 59 cases of SCCs using a monoclonocal anti-CD44 antibody (Santa Cruz, sc-7297, 1:1000) which recognizes CD44s and all isoforms. Origins of SCCs included prostate (12), lung (11), female genital organs (cervix/lower uterine segment/vagina/ovary, 14), bladder (10), head and neck (6), stomach (3), and pancreas (3). Staining was evaluated for the percentage of positive tumor cells and intensity. A case was considered positive only when there was strong membrane staining in greater than 50% of tumor cells. Statistical analysis was performed using Fisher Exact Test.

Results: As reported in the literature, CD44 staining was strong and diffuse in basal cells of the benign prostate but focal (<5%) in prostatic adenocarcinoma. SCCs of prostate were positive for CD44 in 92% (11/12) of the cases with strong and diffuse membrane staining. In contrast, only occasional cases were positive in SCCs of the lung (3/11, 27%), head and neck (1/6, 17%), and female genital organs (2/14, 14%). CD44 staining was negative in all other SCCs (bladder, 0/10; stomach, 0/3; pancreas, 0/3). The difference between prostatic SCC and bladder SCC was statistically significant ($p < 0.001$).

Conclusions: 1. Strong and diffuse membrane staining for CD44 is a feature characteristic of prostatic SCCs which distinguishes them from prostatic adenocarcinoma with a solid growth pattern. 2. CD44 expression is rare in SCCs of non-prostatic origin. 3. CD44 expression reliably distinguishes prostatic SCC from bladder SCC, which has important diagnostic value.

832 PTEN Gene Dosage Affects Downstream AKT Pathway in Hormone Refractory Prostate Cancer

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Background: Hormone refractory prostate cancer (HRPC) represents the terminal stage of prostate cancer (CaP) with limited treatment options. The PTEN gene negatively regulates signaling through the PI3K/AKT/mTOR pathway that contributes to tumor cell growth and survival. PTEN haploinsufficiency is common in hormone sensitive CaP though its copy number and downstream effects have not been elucidated in HRPC. We evaluated the copy number of PTEN as well as the activation status of downstream effectors of this pathway in a cohort of HRPC patients.

Design: Transurethral resectates of 59 HRPC patients (median, 77.5 years; range 57-95 years; PSA median 29.2; range 0.1-4537) were arrayed and immunostained with antibodies against phosporylated (p)-AKT, p-mTOR, and p-70S6. Immunohistochemistry on each core was scored as absent(0), weak(1+) or moderate/strong(2+). The mean score was considered positive if >1. Four-color FISH was performed on the TMA using 6 BAC clones spanning both flanking PTEN genomic region and PTEN gene locus, and centromeric DNA probe (CEP10) for region 10p11.1-q11.1. PTEN copy number was evaluated for each probe in 100 non-overlapped, intact interphase nuclei. Hemizygous deletion of PTEN was defined as >30% of tumor nuclei containing one PTEN locus signal with control CEP 10 signals. Homozygous deletion exhibited simultaneous lack of the both PTEN locus signals with control signals in >20% of cells.

Results: The PTEN gene was deleted in 80% of cases with 20% showing homozygous deletions, 25% homozygous and hemizygous deletions and 35% hemizygous deletions. No deletions were seen in 20% of cases. Allelic loss of PTEN was correlated to expression of downstream p-AKT ($p<.001$) and p-70S6 ($p<.03$) but not to p-mTOR ($p=.53$). Immunoexpression of p-AKT correlated with p-mTOR ($p=.016$) while p-mTOR correlated with p-70S6 ($p<.029$).

Conclusions: The PTEN gene shows a greater incidence of bi-allelic loss in HRPC compared to hormone sensitive CaP. This coupled with net activation of downstream effectors appears to implicate PTEN gene dosage as a driver of the PI3K/AKT pathway. The foregoing supports ongoing and emerging clinical trials of PI3K/AKT pathway inhibitors in HRPC.

833 MUC2 Expression in Primary Mucinous and Nonmucinous Adenocarcinoma of the Prostate: An Analysis of 50 Cases on Radical Prostatectomy

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Background: The expression of mucin stains (MUC) in prostate cancer has not been well studied previously and may be of prognostic and pathobiologic significance. In exocrine organs including breast, skin and pancreas, the expression of MUC2 (mucin associated with gel formation) is limited to mucinous colloid type carcinomas, not seen in conventional adenocarcinomas of these organs, or their usual type pre-invasive precursors. It is however well known that MUC2 expression in mucinous pancreatic and breast cancer represents an indolent pathway since these tumors have a significantly better outcome than their conventional counterparts.

Design: Twenty five cases each of Gleason pattern 3 and 4 mucinous adenocarcinoma of the prostate (MAC) defined by greater than 25% mucinous component and nonmucinous adenocarcinoma of the prostate where obtained from the surgical pathology files of the Johns Hopkins Hospital and Emory University Hospital. Immunohistochemical stains were performed for MUC2 on all 50 cases.

Results: The mean age of patients was 60 yrs (range 44-72 yrs). MUC2 was expressed in all 25 cases (100%) of MAC irrespective of the Gleason pattern. The nonmucinous component of these cases was negative for MUC2. In contrast, MUC2 expression was significantly lower in nonmucinous adenocarcinoma of the prostate, detected in only 6/25 cases as a focal finding, while 19/25 (76%) of non-mucinous adenocarcinoma of the prostate were completely negative for MUC2 ($p < 0.01$). The six cases that showed focal patchy positivity for MUC2 were in areas with Gleason pattern 3 cancer with extensive mucinous fibroplasia (1 case) and prominent intraluminal mucin (5 cases). Other areas of these tumors were negative for MUC2.

Conclusions: MAC shows diffuse expression of MUC2, a known tumor suppressor which is not present in either normal prostate or majority of conventional adenocarcinomas of this organ. This indicates that MAC is indeed of the "colloid-type" akin to those in other exocrine organs. It is highly conceivable that this de-novo expression of MUC2 has a role not only in the mucinous differentiation of these tumors and their colloid pattern, but also in their relatively indolent behavior that has been recently elucidated.

834 EGFR 1 but Not HER-2 Is Expressed in Penile Cancers: Potential Chemotherapy Targets

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Background: The molecular biology of squamous cell carcinoma of the penis (PC) is not well understood, and the treatment of metastatic disease is not defined as the number of cases is not sufficient for prospective phase 2 clinical drug trials. For this reason the investigation of the molecular biology of the disease, and comparison with its most likely equivalent, squamous cell carcinoma of the cervix (as it is an HPV driven disease), may guide future therapeutic options. We wished to investigate the expression of epidermal growth factor receptor (EGFR 1) and HER-2 in PC to determine whether they are potential chemotherapeutic targets for metastatic disease.

Design: We constructed a tissue micro array of PC samples from 68 patients, with 3 samples from each tumor to account for tumor heterogeneity. All cases were derived from a single surgeon's practice. Tumors were all typed graded and staged. Immunochemistry for EGFR 1 (Novacastra clone 113) and three different antibodies for HER-2 was performed (Novacastra clones cerB-2-316, CBE-356 and CB 11). Staining was assessed in a semi-quantitaive manner on a scale from 0 to 3+ as a hercep score using the intensity and nature of membrane staining. The results compared with tumor type, grade and stage. 2+ and 3+ were considered positive results.

Results: Forty-nine out of 68 (72%) of the tumors showed positive expression of EGFR 1. In contrast none of the tumors showed positive expression of HER-2 with any of the clones used. The EGFR 1 positivity showed no correlation with tumor grade, type or stage.

Conclusions: There is considerable over-expression of EGFR 1 but none for HER-2 in PC. These results are comparable with those in carcinoma of the cervix, where the expression of HER-2 is present in adenocarcinomas and not squamous cell carcinomas, whereas EGFR 1 is expressed in squamous cell carcinomas. Therefore, we suggest that EGFR 1 and not HER-2 should be the subject of further investigation in PC. The EGFR 1 overexpression has been correlated specifically with HPV infection in the cervix and may be a consequnce of viral infection, driven by E6/7 protein overexpression. We plan to investigate this in PC to further define the disease pathogenesis and possibly suggest potential chemotherapeutic targets.

835 Cyclin D1 Overexpression and *CCND1* Rearrangement in Renal Oncocytoma: Frequency, Clinicopathologic Features and Utility in Differentiation from Chromophobe Renal Cell Carcinoma

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Background: Renal oncocytoma (RO) is a benign neoplasm with histologic features that on occasion overlap with those of chromophobe renal cell carcinoma (ChRCC). As ChRCC is malignant and can be aggressive, differentiating these tumors is clinically important. Recent studies have shown cyclin D1 overexpression by immunohistochemistry (IHC) in a subgroup of RO. Fluorescence in situ hybridization (FISH) studies have also shown that RO may have rearrangements of the *CCND1* locus. However, the frequency and clinical implications of cyclin D1 overexpression and *CCD1* rearrangements in RO and ChRCC has not been studied.

Design: Formalin-fixed, paraffin-embedded tissue from 61 RO and 11 ChRCC was evaluated for morphologic features, for cyclin D1 overexpression by IHC and for rearrangement of the *CCND1* locus by FISH using a commercially available break-apart

probe for the *CCND1* locus (Vysis). Fresh tissue was also obtained for conventional cytogenetic analysis in two of these RO.

Results: All 11 ChRCC did not exhibit cyclin D1 overexpression or rearrangement of the *CCND1* locus. Findings for RO are described below:

Cyclin D1 overexpression	Total	CCND1 FISH split	Multiple &/or bilateral lesions	Male	Female	Recurrence
Absent	40/61 (66%)	1/40 (2%)	15/40 (38%)	31/40 (78%)	9/40 (23%)	6/25 (24%)
Present	21/61 (34%)	13/21 (62%)	1/21 (5%)	10/21 (48%)	11/21 (52%)	1/15 (7%)
p-value		<0.0001	<0.0001			0.1052

Histologically, RO with and without cyclin D1 overexpression were indistinguishable. Two ROs showed karyotypes with alterations of the 11q region: der(11)ins(11;3)(q13 ;q13.2q25) and add(11)(q13). Both cases showed cyclin D1 overexpression by IHC, however, the RO with der(11)ins(11;3)(q13;q13.2q25) showed *CCND1* rearrangement by FISH while the RO with add(11)(q13) did not.

Conclusions: Cyclin D1 overexpression appears to be present in about 34% of RO and absent in ChRCC suggesting that cyclin D1 may be useful to distinguish these lesions. Overexpression of cyclin D1 in RO is associated with *CCND1* rearrangement in 62% of cases suggesting that overexpression of cyclin D1 in RO may be due to more than one genetic mechanism. In this series of 61 RO, the lack of cyclin D1 overexpression is more frequently associated with bilateral and/or multiple tumors and male sex while RO exhibiting cyclin D1 overexpression are more likely solitary and not associated with gender.

836 Histogenesis of Clear Cell Adenocarcinoma in the Urinary Tract: Evidence of Urothelial Origin by Histology, Immunohistochemistry and Fluorescence In Situ Hybridization Analysis

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Background: Clear cell adenocarcinoma in the urinary tract is a rare entity with an appearance resembling its counterpart in the female genital tract. Although a number of theories have been proposed regarding the origin of this tumor, its exact histogenesis has remained uncertain.

Design: In the current study, we integrated molecular genetic evaluation by fluorescence in situ hybridization with conventional morphological and immunohistochemical analyses to elucidate tumor histogenesis in 12 clear cell adenocarcinomas in the urinary tract.

Results: Concurrent urothelial carcinoma or urothelial carcinoma in situ were present in 6 cases (50%) and foci of cystitis glandularis were observed in 4 cases (33%). Neither intestinal metaplasia nor Müllerian component was identified in any case. Cytoplasmic expression of α-methylacyl-CoA racemase was demonstrable in 10 of 12 tumors (83%). Moderate to diffuse immunostaining for CK7 was identified in all 12 tumors (100%), whereas only 3 of 12 (25%) tumors showed positive immunostaining for CK20. In 5 cases (42%), focal to moderate CD10 immunoreactivity was observed. Immunostains for OCT4 and CDX-2 were completely negative in all tumors. In UroVysion FISH assays, all tumors displayed chromosomal alterations similar to those commonly found in urothelial carcinoma.

Conclusions: Our study confirms the frequent coexistence of clear cell adenocarcinoma with conventional urothelial neoplasia, and demonstrates that these two entities have overlapping protein expression profiles and identical genetic aberrations as indicated by FISH analysis. Our findings strongly support a urothelial origin for most clear cell adenocarcinomas of the urinary tract, despite their morphologic resemblance to certain Müllerian-derived tumors of the female genital tract.

837 Vascular Tumors & Tumor-Like Lesions of the Urinary Bladder

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Background: Vascular tumors of the bladder are rare and a subject of small series and case reports.

Design: We retrospectively identified vascular tumors and tumor-like lesions of the urinary bladder from the consultation files from one of the authors.

Results: We identified 15 lesions that included 4 hemangiomas, 3 intravascular papillary endothelial hyperplasias, 2 arteriovenous malformations (AVM), 1 epithelioid hemangioendothelioma (EHE), 1 malignant hemangiopericytoma (HPC) and 4 angiosarcomas. One angiosarcoma was associated with high grade urothelial carcinoma (sarcomatoid carcinoma). Ages ranged from 18 to 85 years old (mean 63.3). There was no statistical difference among the various lesions in terms of age, although angiosarcomas tended to arise in older patients. Hematuria was the most common presentation with both benign and malignant lesions. Histologically, benign and malignant lesions were similar to the counterparts in other organ systems. 3 hemangiomas were capillary and 4th was cavernous subtype. They measured 1.1, 2.4, 3.2 and 5.1 cm. Both AVMs were clinically large broad-based masses measuring 5.5 and 5.8 cm in greatest diameter. One of the AVMs was associated with pseudoepitheliomatous hyperplasia of the urothelium mimicking invasive urothelial carcinoma. All 3 patients with Masson's hemangioma had history of radiation therapy for other causes. These presented as raised lesions and were all below 1.0 cm in size. Patients with hemangiomas, papillary endothelial hyperplasias and AVM had an invariably benign prognosis and needed no further therapy. These benign lesions had consistent involvement of the submucosa and spared the muscularis propria of the organ. All cases of angiosarcoma, EHE, and HPC involved the muscularis propria. Two of 4 patients with angiosarcoma had a history of prior radiation therapy and all 4 were dead of disease at 6 months. Angiosarcomas measure 3, 4.5, 5, and 5.8 cm in greatest diameter at cystoscopy. The patient with EHE had a single nodule treated by TURP and no evidence of disease at 4 years. The patient with HPC had distant metastasis and was lost to follow-up after diagnosis. None of the patients experienced marked gross hematuria that resulted in morbidity or mortality.

Conclusions: A wide spectrum of benign, intermediate malignant, and malignant vascular lesions primarily involve the bladder. Despite the potential of marked hemorrhage, none of the tumors resulted in marked hematuria. Papillary endothelial hyperplasia occurs in the bladder and must be differentiated from angiosarcoma, which has a rapidly fatal outcome.

838 Small Endoscopic Biopsies of the Ureter and Renal Pelvis: Diagnostic Pitfalls

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Background: Advances in endoscopic equipment have led to increased ureteroscopic biopsies of the upper urinary tract, resulting in limited biopsy material which can pose unique diagnostic challenges to pathologists.

Design: We reviewed 25 consecutive ureter and 42 renal pelvis biopsies submitted for consultation (1/04-8/07). The mean age was 71 years with 62% males. Major discrepancies (expert consultation modifying a benign to a malignant diagnosis or vice versa) and minor discrepancies (change inside the benign or malignant categories) were determined.

Results: In both anatomic sites the most common presenting symptom was a mass (37%), followed by hematuria (23%). Others included ureteral stricture (3%), calculus (3%) and hydronephrosis (3%). On review, 24% of lesions in both sites were diagnosed as malignant, 53% as benign, and in 21% a definitive diagnosis could not be reached due the small biopsy size. Major discrepancies were found in 9 cases (13%) and minor discrepancies in 23 cases (34%). In 88% of the major discrepancies, the diagnosis was changed from urothelial carcinoma to benign. These included cases diagnosed on review as: PUNLMP (n=2); papillary urothelial hyperplasia (n=1); polypoid ureteritis (n=1), proliferation of von Brunn nests (n=1); and detached strips of benign urothelium (n=4). In 1 case, a diagnosis of urothelial papilloma was changed to low grade urothelial carcinoma. The most common minor discrepancy was on review a change from atypical to benign/reactive urothelium in 13 cases (56% of minor discrepancies). Other minor discrepancies included: carcinoma changed to an uncertain diagnosis (n=4) and a change in the grade of urothelial carcinoma (n=3). There was a highly significant association between the histological diagnosis of tumor on review and the presence of a clinically suspected tumor (p=0.0001). In many of the cases with an outside diagnosis of neoplasm or atypia, yet on review a benign diagnosis was made, no tumor was present clinically. We are in the process of obtaining follow-up information on all cases.

Conclusions: Pathologists need to recognize that in 1/5th of renal pelvic/ureteral biopsies a definitive diagnosis can not be made due to inadequate tissue. These biopsies also have a tendency to be overdiagnosed as malignant or atypical when they often represent detached strips of benign or reactive urothelium mimicking a papillary neoplasm. Caution must be exercised in the evaluation of these limited specimens, especially in the absence of a clinical tumor, as overdiagnosis can result in radical nephroureterectomy.

839 High Grade Prostatic Intraepithelial Neoplasia-Like Ductal Adenocarcinoma of the Prostate: A Clinicopathologic Study of 28 Cases

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Background: The vast majority of prostatic ductal adenocarcinomas have cribriform and/or papillary architecture lined by columnar pseudostratified malignant epithelium.

Design: We report 28 cases of ductal adenocarcinomas on needle biopsy closely resembling high grade prostatic intraepithelial neoplasia (HGPIN) composed of simple glands with flat, tufting or micropapillary architecture.

Results: The mean age of the patients was 68 years (range 50-91). PSA serum level at diagnosis ranged from 1.2 to 12.1 ng/mL. Treatment included: radical prostatectomy (RP) (n=9), hormone therapy (n=7), radiotherapy (n=5), and cryotherapy (n=1). 3 patients had recent biopsies without information on treatment and 3 patients were lost to follow-up after diagnosis. The number of cores involved by tumor in each case ranged from 1 to 18, with >1 core involved in 13 cases. Flat was the most common pattern (45.2%), followed by tufted (39.4%), and micropapillary (13.1%). In 14 cases, segments of dilated glands were on the edge of the biopsies, suggesting a large gland component. In RPs, tumor was primarily composed of small (25%), medium (17%), or cystically dilated (58%) cancer glands, with all cases demonstrating a mixture of different gland sizes. Cytologically, tumors had tall columnar atypical cells, basally located nuclei and amphophilic cytoplasm. In 24/28 cases, there were less prominent nucleoli than typical HGPIN despite striking resemblance to extensive HGPIN at low power. AMACR staining was seen in 93% of cases, with the majority showing strong and diffuse staining. No basal cells were present on p63 and/or high molecular weight cytokeratin staining. In the RPs, tumor volumes ranged from a small focus (<0.01cm^3) to 1.2 cm^3. Concurrent conventional acinar Gleason score 6 adenocarcinomas were seen in 6 of the 9 RPs. Only 1 of the PIN-like ductal adenocarcinomas at RP had extra-prostatic extension, which was focal.

Conclusions: PIN-like ductal adenocarcinoma differs from HGPIN by cystically dilated glands, a greater predominance of flat architecture, and less frequent prominent nucleoli. Verification often requires the immunohistochemical documentation of the absence of basal cells in numerous atypical glands. Although usual ductal adenocarcinoma is considered comparable to Gleason score 8, PIN-like ductal adenocarcinoma was accompanied by Gleason score 6 acinar carcinoma and behaved similar to Gleason score 6 acinar cancer. Recognition of this entity is critical to differentiate it from both HGPIN and conventional ductal adenocarcinoma.

840 Alterations in Immunohistochemical and Clinicopathologic Features in Signet Ring Adenocarcinoma of the Bladder

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Background: Primary bladder adenocarcinomas (PBA) encompass both urachal carcinomas and adenocarcinomas arising at other bladder sites. PBA has been associated with a poor prognosis, and the presence of signet ring features has been suggested to imply worsened outcomes. We examined a large series of PBAs with and without signet ring features to determine whether alterations in immunohistochemical (IHC) features and/or clinical outcomes distinguish signet-ring from non-signet ring PBAs.

Design: Pure PBAs were identified; patients with any history of adenocarcinoma primary to other sites were excluded from further study. IHC stains included CK20 (1:20, DAKO, Carpinteria, CA), CK7 (1:40, DAKO), CDX2 (1:10, Biogenex, San Ramon, CA), beta-catenin (1:250, BD Biosciences, San Jose, CA), E-cadherin (1:500, Zymed, San Francisco, CA), racemase (1:100, Zeta Corp, Sierra Madre, CA), and villin-1 (1:50, Immunotech, Westbrook, ME).

Results: Twenty-five cases of PBA were identified, including 19 radical cystectomy specimens and 6 biospy/TUR specimens from patients who were unresectable at diagnosis. Patient age ranged from 28-78 yrs (mean 57 yrs) with a M:F ratio of 18:7. Seven cases were urachal in origin. The most common symptom was hematuria (12/25; 48%). In radical cystectomy specimens, pathologic stage included pT1 (1/19), pT3 (14/19) and pT4 (3/19) disease. Six cases demonstrated a positive soft tissue resection margin. Patient follow-up ranged from 1 to 69 mo (median 12 mo). Patients were subdivided into those with colonic-type adenocarcinoma (CTA; 5/25), signet-ring predominant adenocarcinoma (>80% signet ring; SR; 14/25) and mixed CTA/SR morphology (MIXED; 6/25). All patients with unresectable disease at diagnosis demonstrated SR morphology (6/6) with 5 patients dead of disease in <1 yr from diagnosis. Concurrent metastases were identified in 40%, 17%, and 63% of CTA, MIXED and SR patients. Of patients with resectable disease, the CTA, MIXED and SR groups demontrated comparable dead of disease rates of 20%, 17% and 13%, respectively. IHC studies demonstrated a decrease in several markers in the SR subgroup as compared to the CTA and MIXED groups, including CK20, CDX-2, beta-catenin and E-cadherin.

Conclusions: Patients with SR type PBA frequently demonstrate unresectable disease at diagnosis and an increased rate of metastatic disease. In addition, protein expression of a subset IHC markers are lost in SR adenocarcinoma relative to CTA and MIXED type adenocarcinomas.

841 How Much Additional Sampling Is Required When Minimal Cancer Is Identified in the Initial Sample of the Transurethral Resection of Prostate (TURP)?

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Background: When incidental prostate cancer is detected in ≤5% of the initial TURP specimen, additional tissue is typically submitted to rule out more extensive cancer and to ascertain the Gleason score. Controversy exists whether additional partial or complete sampling is required to accurately evaluate the remaining tissue.

Design: We prospectively examined all TURPs in our institution during one year (06/06 to 05/07). All specimens were initially sampled in 6 random cassettes. When minimal cancer was found in the initial sample, additional partial and complete tissue sampling was performed. Partial sampling included 1 block/5g of remaining specimen, followed by complete sampling that included all remaining tissue. Additional partial and complete samples were evaluated separately to identify changes in Gleason score and tumor volume. Cost analysis was performed for the cost of additional sampling.

Results: Of 747 TURPs evaluated by initial 6-block submission, 125 (16.7%) had prostate cancer. Minimal cancer was found in 26 (3.5%) patients with median age of 68 years (range 56–85). All patients with minimal cancer were clinically unsuspected to have cancer. Median weight of TURP samples with minimal cancer was 24.7g (range 10.2–125g); median weight/block was 1.7g (range 1–3.3g). After the first 6 blocks, 3.5 blocks/case (median, range 1-23) were submitted for partial examination of remaining tissue. Complete sampling included an additional 5.5 blocks/case (median, range 2-25). Gleason Scores of 5, 6, and 7 were reported in the initial samples in 3 (12%), 22 (84%) and 1 (4%) patient, respectively. The Gleason scores and the minimal cancers found in the initial samples were not altered in any of the cases after evaluating the additional partial and complete samples. Median costs per case for additional partial and complete samples were $52 and $79, respectively. In our laboratory, we calculated a total cost of $4336 per year for additional sampling of TURPs with minimal cancer ($1681 for additional partial and $2655 for additional complete sampling).

Conclusions: When minimal cancer was found in the first 6 blocks of TURP, additional partial and complete sampling did not change the initial Gleason score and tumor volume. For quality assurance, an additional partial sampling of 1 block/5g of remaining tissue should be considered sufficient. Additional partial sampling compared to complete sampling of TURPs with minimal cancer results in cost saving of $79/case.

842 Histopathological Findings in Renal Cell Carcinoma (RCC): A Contemporary Series (2000-2007) Based on WHO 2004 Classification

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Background: In the last decade a new classification of RCC emerged, which was endorsed in 2004 by the World Health Organization (WHO). Although there are larger retrospective studies that have documented the findings in large RCC series, histopathological findings of RCC have been rarely reported in large studies after year 2000.

Design: We examined the RCC histopathological findings in our institution over a seven-year period (07/2000 to 06/2007). The tumors were classified using the WHO 2004 classification. We investigated the incidence of the common and recently recognized RCC entities, patient demographics, and the tumor histopathological parameters (size, nuclear grade, stage, necrosis, sarcomatoid change).

Results: Of 665 RCC, 481 (72.3%) were clear cell type, 87 (13.1%) were papillary, 38 (5.7%) were chromophobe, 24 (3.6%) were multilocular cystic, 23 (3.5%) were unclassified and mixed, and 6 (1%) were tubulocystic RCC. The remaining RCC were as follows: 3 (0.5%) translocation type, 1 (0.1%) adult nephroblastoma, 1 (0.1%) low-grade myxoid/spindle cell, and 1 (0.1%) mixed epithelial-stromal tumor. The overall Male:Female ratio was 1.6:1. Median patient age was 61 years (range 26-94). Median tumor size was 4 cm (range 1-25 cm). Multifocality was seen in 5.9% of RCC, most commonly in papillary type (18%). In the clear cell type, 87 (18%) were nuclear grade 1, 178 (37%) were grade 2, 145 (30%) were grade 3, and 57 (12%) were grade 4 (not specified in 14 (3%) tumors). In the three most common types (clear cell, papillary and chromophobe), stage distribution was: 375 tumors (62%) stage pT1, 67 (11%) stage pT2, 130 (21.5%) stage pT3, and 3 (0.5%) stage pT4 (not specified in 31 (5%) tumors). Renal vein invasion was most commonly found in clear cell RCC (12.5%). Regional node metastases were found in only 11 (1.8%) of the three common types. Necrosis was identified in 201 (30%) RCC. Sarcomatoid change was found in 23 (3.5%) tumors; 16 (70%) were stage pT3 and 4. Sarcomatoid change was most common in chromophobe RCC (5) 13%, followed by unclassified (3) 11%, clear cell (14) 2.9%, and papillary (1) 1%.

Conclusions: This large single-institution RCC series, based on the WHO 2004 classification, documents the incidence rates of the common and recently defined tumor types and the histopathological findings in the current practice. More than 70% of the common RCC types (clear cell, papillary, chromophobe) were organ-confined. Sarcomatoid change in RCC was most often associated with high-stage tumors.

843 Hypoxia-Inducible Factor (HIF) and Mammalian Target of Rapamycin (mTOR) Pathways in Invasive Urothelial Carcinoma of the Bladder: An Immunohistochemical (IHC) Study with Potential Therapeutic Implications

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Background: Recently, targeted therapies against HIF and mTOR pathway molecules have shown promising results in advanced renal cell carcinoma. Some of the downstream molecules of these pathways, including EGFR, are known to be overexpressed in some urothelial carcinomas, suggesting potential utility of similar targeted therapies in them. We performed an IHC study to assess whether HIF and mTOR pathway markers are expressed in high stage bladder urothelial carcinoma.

Design: IHC was performed on a tissue microarray of 92 cases of invasive urothelial carcinoma of bladder (≥ pT2), using antibodies against phospho-S6 and phospho-4E BP1 (activated mTOR pathway markers), and HIF-1α and VEGFR2 (HIF pathway markers). Positivity was graded as 0 to 3+ (0=0-5%; 1+=6-25%; 2+=26-50%; and 3+=>50% tumor cells positive) for phospho-S6 (cytoplasmic), phospho-4E BP1 (cytoplasmic and/or nuclear), HIF-1α (nuclear) and VEGF R2 (cytoplasmic). Since VEGFR2 positivity was also observed in tumor vessels, vascular expression was also independently graded for staining intensity as low (absent/weak) or high (intense staining) grade.

Results: The results of IHC in the tumor cells are tabulated.

Antibody (No.)	0 (%)	1+ (%)	2+ (%)	3+ (%)
HIF-1 (85)	41 (48)	14 (17)	8 (9)	22 (26)
VEGF R2 (86)	66 (77)	6 (7)	6 (7)	8 (9)
p-S6 (85)	45 (53)	11 (13)	9 (11)	20 (23)
p-4E BP1 (84)	25 (30)	10 (12)	17 (20)	32 (38)

Analyses using Spearman's correlation coefficient indicated significant relationship between expression of phospho-S6 and 4E BP1 (rho= 0.411, p= <0.001), and HIF-1α and VEGFR2 (in tumor cells) (rho= 0.265, p=0.015). The relationship was not significant for HIF-1α, and phospho-S6 (rho= .117) or phospho-4E BP1 (rho= -.149). There was no significant relationship between VEGFR2 tumor cell and vasculature expressions, or between VEGFR2 and p-S6 or p-4E BP1.

Conclusions: The overexpression of HIF and mTOR pathway markers suggests that targeted therapies against these pathways should be further investigated in urothelial carcinoma. There is a significant relationship between marker expression of molecules within same pathways (i.e. HIF-1 with VEGF R2, and p-S6 with p-4E BP1), but not among markers of separate pathways. The differential expression of these markers indicates that antigen evaluation of tumors may be required to select a specific targeted therapy in urothelial carcinoma.

844 Mammalian Target of Rapamycin (mTOR) and Hypoxia-Inducible Factor (HIF) Pathways in Clear Cell Renal Cell Carcinoma: An Immunohistochemical Study with Possible Therapeutic Implications

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Background: Targeted therapies against HIF pathway molecules have recently shown promising results in metastatic clear cell renal cell carcinoma (CRCC). However, not all cases have had a positive or uniform response. Therefore, alternative pathway targets are being sought. We have previously reported on expression of HIF pathway markers, including HIF-1α, carbonic anhydrase IX, VEGF and GLUT-1, in CRCC. We have now investigated the expression of activated mTOR pathway molecules that act as translation regulators of multiple downstream proteins including HIF-1 in CRCC.

Design: Immunohistochemistry was performed on a tissue microarray consisting of 104 cases of CRCC, using antibodies against HIF-1α, VEGF-receptor (R)2 (markers of HIF pathway), and phospho-S6 and phospho-4E BP1 (markers of activated mTOR pathway). Positivity was graded as 0 to 3+ (0=0-5%; 1+=6-25%; 2+=26-50%; and 3+=>50% tumor cells positive) for phospho-S6 (cytoplasmic), phospho-4E BP1 (cytoplasmic and/or nuclear), HIF-1α (nuclear) and VEGF R2 (cytoplasmic). Since

VEGF R2 positivity was only observed in the tumoral vessels and not in tumor cells, vascular expression was graded by intensity of staining as low (absent/0 or weak/1+) or high (intense staining/2+) grade.

Results: The results on staining grades are tabulated.

Antibody (# cases)	Grade 0 (%)	Grade 1+ (%)	Grade 2+ (%)	Grade 3+ (%)
HIF-1α (98)	23 (24)	15 (15)	11 (11)	49 (50)
VEGF R2 (102)*	12 (12)	37 (36)	53 (52)	-
p-S6 (98)	37 (38)	15 (15)	13 (13)	33 (34)
p-4E BP1 (99)	27 (27)	13 (13)	18 (18)	41 (42)

*Only in tumor vessels

Analyses using Spearman's correlation coefficient indicated a significant relationship between the expression of HIF-1α and VEGF R2 (rho= .475, p= <0.001), HIF-1α and phospho-4E BP1 (rho= .397, p= <0.001), VEGFR2 and phospho-4E BP1 (rho= .305, p=0.002), and phospho-S6 and phospho-4E BP1 (rho= .393, p= <0.001). The relationship was not significant for positivity for phospho-S6 and HIF-1 (rho= -.046), or VEGFR2 (rho= .058).

Conclusions: Expression of HIF pathway markers show significant relationship in clear cell RCC, as do the markers of mTOR pathway. HIF pathway markers show significant relationship with phospho-4E BP1. However, there was no significant relationship with phospho-S6, the other marker of mTOR pathway. These findings suggest that systemic therapies in clear cell RCC may be more effective if more than one pathways are tested and targeted.

845 Radical Prostatectomy Findings in Patients Considered Potential Candidates for Active Surveillance

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Background: Active surveillance (AS) or "watchful waiting" is a management option offered to patients with "low risk" prostate cancer. Its therapeutic safety and efficacy is currently being evaluated in clinical trials. However, there is no inter-institutional consensus regarding low-risk enrollment criteria or parameters of tumor progression. We report here the pathologic findings of extirpated specimens from patients undergoing radical prostatectomy who would be considered potential candidates for AS.

Design: Between years 2000-2007, thirty one patients undergoing radical prostatectomy (group A) matched our AS protocol eligibility criteria: clinical stage T1c/T2, PSA< 4.0 ng/ml, tumor in only one core of a minimum of 10 and Gleason score (GS) 6 (<3.0 mm) / GS7 (3+4) (<1.0 mm). Twenty six additional patients (group B) fulfilled these entry criteria except for having a serum PSA level between 4.0-9.9 ng/ml. All pts had a minimum of 10 biopsy cores; however, the biopsy scheme was not uniform.

Results: There were no significant differences between the two groups (A/B) for median age (58.0/58.5), clinical stage (T1c:81%/92%), median number of biopsy cores obtained (12/12), median size of tumor (1.0 mm/1.0 mm) or biopsy GS (GS6: 93.5%/92.3%). The median PSA levels and median prostate weights were statistically different between the groups (2.5/6.1 ng/ml, p < 0.001 and 36.5/46 grams, p=0.01), respectively. The majority of the prostates in both groups contained multiple tumor foci (71%/81%), with tumors located in both the peripheral and transition zones (45%/58%). Prostate tumors were organ confined in 100% of group A and 81% of group B specimens. Two (19%) of the specimens in group B had a positive margin without extraprostatic extension. The rate of upgrading for patients with a biopsy GS 6 was similar in both groups (26%/35%). None of the cases with GS7 in the biopsy was upgraded. The median total tumor volume (TTV) was 0.12 cc (range: 0.008-2.24 cc) (A) and 0.40 cc (range: 0.0008-5.06) (B) (p=0.12). Insignificant cancer (TTV < 0.5 cc, GS 6) was similar in both groups (61%/46%, p=0.29). Overall 13 (23%) of the specimens (4 A and 9 B) had dominant tumor foci exceeding 0.5 cc.

Conclusions: Although the currently available selection criteria are useful for AS of newly diagnosed prostate cancer patients, the results of this limited study underscore their limitations. Further refinement of the criteria used to select patients and to define tumor progression by incorporating molecular markers of biological aggressiveness are needed.

846 Neoadjuvant Total Androgen Deprivation Produces a Characteristic Prostate Phenotype

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Background: Recent studies report that conventional androgen deprivation fails to decrease serum and tissue androgens to a level where androgen activated pathways are inactivated. We initiated a neoadjuvant trial attempting to maximally suppress androgen levels using combinations of 4 androgen suppressing drugs in patients at intermediate risk for progression. We hypothesized that this neoadjuvant regimen would inactivate androgen mediated pathways and produce a distinct phenotype.

Design: Patients who meet study criteria (serum PSA < 20ng/ml, Gleason Score >6, and clinical stage < T2c) are given a combination of drugs - goserelin, bicalutamide, dutasteride, ketoconazole – prior to prostatectomy. Benign and cancer tissue in the prostatectomies of the 8 patients completing this protocol were evaluated histologically and immunohistochemically (androgen receptor, KI67, PSA, P27).

Results: The 8 treated cancers in this study exhibited distinct histological patterns – single, inconspicuous cells (all 8); focal cytoplasmic vacuolization (6), and a focal (4) intraductal growth pattern. In all cases cancer was histologically subtle. The Gleason score of the pre-treatment biopsies (ranging from 6 to 9) did not predict the histology of the androgen deprived cancer. Non-cancer glands in the prostatectomies had a distinctive cytoplasmic clearing. Immunohistochemically, the cancer glands exhibited markedly more intense PSA immunoreactivity than adjacent benign glands. Androgen receptor stains showed nuclear localization with a slight cytoplasmic staining in the androgen deprived cancer cells. Only rare cells in benign and malignant prostate expressed KI67. Although all benign epithelial cells expressed p27, virtually no cancer cells did.

Conclusions: Although similar to prostate cancers treated with a single androgen deprivation agent, the histologic features of these cancers are more marked, to such an extent that cancer might be easily overlooked. Of diagnostic help is the intense PSA immunoreactivity of cancer cells. That PSA expression is retained in the cancer is evidence that total androgen suppression may be insufficient to inactivate androgen receptor activated pathways. Furthermore, absent p27 staining is evidence that proliferation is not suppressed. Finally, the distinctive cytoplasmic clearing of benign secretory cells, in addition to atrophy, is a histologic clue that the prostate has been subjected to total androgen deprivation.

847 Cancer That "Abuts" or Is "Close to" the Surgical Margin of a Radical Prostatectomy Is a Marker of Adverse Outcome

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Background: Although the presence of carcinoma at the lateral surgical margin of a radical prostatectomy specimen is an adverse prognostic indicator, cancer that is "very close" to the margin has not been associated with a bad outcome in any published series. In cases where a nerve sparing procedure is a component of the surgery, prostate cancer is often no farther than a few cells from the lateral surgical margin. Given the possibility that in such cases cancer might actually be at the surgical margin were the block of tissue to be completely sectioned, we undertook an analysis of whether cancer virtually at, or, "cancer abutting" the margin predicted post-prostatectomy PSA failure.

Design: This study consisted of 904 patients who underwent radical prostatectomy for prostate cancer beginning in the year 2000 (when we began recording margin subcategories). All patients had stage pT2 tumor and were followed for a median of 2 years. Clinical (serum PSA, patient age, nature of surgical procedure) and pathologic features of the prostatectomy (Gleason score, pathologic stage, tumor volume, margin status) were recorded. We categorized tumors as "margin-negative", "margin close" (between 2 and approximately 6 cells from the margin), "margin abuts" (tumor no farther than two cells from the lateral surgical margin) and "margin-positive". Cases where tumor abutted the margin in one section, but was actually at the margin in another section were categorized as margin-positive. Time to PSA failure was recorded and correlated with margin status.

Results: Margin status correlated with likelihood of biochemical failure (defined as post-op rising serum PSA).

PSA Failure vs. Margin Status

	Negative	**Close**	**Abuts**	**Positive**
# of patients	621	94	35	154
% of patients who had PSA failure	7 %	9 %	12 %	18 %
Median time to failure (days)	244	422	295	278

Conclusions: We have identified histopathological features that are associated with an increased risk of biochemical failure following radical prostatectomy – tumor "close to margin" and tumor "abuts margin". Using explicit histological criteria, subcategories of tumor relationship to margin should be recorded since subcategory of margin status contributes to predicting patient outcome. Margin status provides value additive to Gleason score in predicting likelihood of biochemical failure following radical prostatectomy in this series of patients.

848 Diagnostic Utility of Commercially Available Prostate Marker for the Detection of Metastatic Prostatic Adenocarcinoma after Androgen Deprivation Therapy: Comparison with Prostate Specific Antigen (PSA), Prostate Specific Acid Phosphatase (PSAP), Prostate Specific Membrane Antigen (PSMA), and P501s (Prostain)

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Background: Many prostate specific markers are commercially available nowadays. They are widely used for diagnosis of prostatic adenocarcinoma (Pca), especially metastatic Pca (MPca). However, some of markers are reported that their expressions are downregulated after androgen deprivation therapy (ADT).

Design: We studied 46 Pca radical prostatectomies (RPca) after ADT, 14 RPca without ADT, 13 metastatic Pca after ADT, and 23 MPca without ADT. Representative sections were selected and stained with prostate specific antigen (PSA), prostate specific acid phosphatase (PSAP), prostate specific membrane antigen (PSMA), and p501s (Prostain). The extent of stain was evaluated semi-quantitatively.

Results: Most of RPca and MPca cases without ADT showed strong and diffuse immunoreactivity for PSA, PSAP, PSMA, and p501s. PSMA still showed strong and diffuse immunoreactivity in most of RPca and MPca cases with ADT. In contrast, the expression of p501s, PSA, and PSAP were down-regulated in both RPca and MPca cases with ADT.

Conclusions: PSMA is the most reliable marker regardless RPca or MPca, or ADT. Although p501s, PSA, and PSAP are also sensitive markers for RPca and MPca cases without ADT, their interpretation should be cautious in ADT cases.

P501s, PSA, PSAP, and PSMA expression in Pca.

		RPca, ADT(-)	RPca, ADT(+)	MPca, ADT(-)	MPca, ADT(+)
p501s	negative	0	25	1	6
	focal	0	8	1	4
	diffuse	14	13	21	3
PSA	negative	0	4	4	9
	focal	0	10	4	3
	diffuse	14	32	15	1
PSAP	negative	0	2	1	2
	focal	0	5	3	6
	diffuse	14	39	19	5
PSMA	negative	0	0	0	2
	focal	0	4	2	2
	diffuse	14	42	21	9

focal: less than 50% expression, diffuse: more than 50% expression

849 KAI-1/CD82 Is a Useful Biomarker To Distinguish Chromophobe Renal Cell Carcinoma from Oncocytoma

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Background: Chromophobe renal cell carcinoma (ChRCC) and oncocytoma (ONC) show overlapping morphological features. Although some immunohistochemical markers showed differential expression, none of them is diagnostically reliable as a single marker, and the use of a panel of antibodies has been the common practice. KAI-1/CD82 is a member of a new class of genes known as metastasis suppressor genes, which demonstrate *in vivo* capability to suppress metastasis. The loss of KAI1 expression has been observed in a wide range of high-grade cancer types and their metastasis, but has not been characterized in kidney cancer.

Design: Immunohistochemistry was performed with a KAI-1 monoclonal antibody (Santa Cruz) using a tissue microarray containing clear cell (CRCC) (N=47), papillary (PapRCC) (N =33), ChRCC (N=32), ONC (N=29), or normal kidney (N=10). KAI-1 expression was scored as - (none), +/- (minimal ≤10% cells with faint membrane staining), + (>10% cells with membrane staining). The positive staining category also included 1+ (weak), 2+ (moderate) or 3+ (strong).

Results: In normal kidney, KAI-1 showed weak to moderate membranous staining of the distal tubule cells but not cells in proximal tubules or glomeruli. In CRCC, KAI-1 expression was positive only in 4 of 47 cases (8.5%). Complete loss of KAI-1 expression was also observed in 90% (26/29) of ONC and 91% (30/33) of PapRCC. In contrast, ChRCC showed positive KAI-1 staining in most (28/32; 88%) specimens, with variable intensity (7 cases 1+, 11 of 2+ and 10 of 3+). The sensitivity of KAI-1 as a marker for ChRCC vs. ONC or CRCC or PapRCC is 87.5% for all tumors, and the specificity is 96.6%, 93.6%, and 97.0%, respectively. KAI-1 expression in PapRCC was scored base on the area without macrophages which showed positive staining.

Conclusions: The positive staining of the renal distal tubules suggests KAI-1 as a differentiation marker of the distal nephron, in addition to its proposed role as a metastasis suppressor gene. The expression of KAI-1 in ChRCC and not in CRCC or PapRCC likely reflects this lineage specificity, as ChRCC is known to be derived from distal nephron. Intriguingly, ONC, also considered distal nephron in origin, has lost KAI-1 expression in most cases, allowing KAI-1 to serve as a useful marker in distinguishing ChRCC from ONC.

KAI-1 expression in renal tumors

KAI-I staining	ChRCC (n=32)	ONC (n=29)	CRCC (n=47)	PapRCC (n=33)
+	28 (87.5%)	1 (3.4%)	3 (6.3%)	1 (3.0%)
+/-	2 (6.3%)	2 (6.9%)	1 (2.1%)	2 (6.1%)
-	2 (6.3%)	26 (89.7%)	43 (91.5%)	30 (90.9%)

850 A Distinct Clinicopathological Entity – Autoimmune Pancreatitis-Associated Prostatitis

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Background: Autoimmune pancreatitis (AIP) is a recently proposed disease entity, and an elevated serum IgG4 level is its characteristic finding. This disease is sometimes associated with other inflammatory diseases, such as retroperitoneal fibrosis or sclerosing cholangitis.

Design: To elucidate the clinicopathological characteristics of the AIP-associated prostatitis (AIP-P), we evaluated the clinicopathological findings and the immunohistochemical expressions of the IgG subclasses (IgG1, IgG2, IgG3, and IgG4) in AIP-P (6 patients) in comparison with patients of control group (10 patients) who were clinically diagnosed as suspicious for carcinoma based on the elevated serum PSA levels b but exhibited focal inflammation without adenocarcinoma on histological examination of the prostate.

Results: All patients with AIP-P had the characteristic findings of AIP, and their lower urinary tract symptoms (LUTS) improved after steroid therapy. In four of five patients with AIP-P, the digital rectal examination revealed prostate enlargement. Histologically, AIP-P showed lymphoplasmacytic infiltration, scattered eosinophilic infiltration, and obliterative phlebitis accompanying gland atrophy with dense fibrosis. Immunohistochemically, the IgG4-positive plasma cell / mononuclear cell ratio was significantly higher in AIP-P than in the control group (P=0.0011).

Conclusions: AIP-P is a distinct clinicopathological entity and a mechanism similar to that implicated in AIP may be involved in AIP-P.

851 Metastatic Carcinoma to the Testis: A Study of 26 Cases

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Background: Metastatic carcinomas to the testis may simulate primary testicular neoplasms, even in patients with known extra-testicular primaries. We reviewed our experience with such cases, about which information is limited.

Design: 26 metastatic carcinomas to the testis were retrieved from consultation (N=23) or hospital (N=3) files. The case histories, gross descriptions and microscopic sections were evaluated.

Results:

Features of 26 cases of metastatic carcinoma to the testis

	History of prior ca	Bilateral	Distinct mass	Conspicuous intertub. growth	Conspicuous intrarete/ intratub. growth	Conspicuous cyto. vacuoles	Lymph./ Paratestis inv.
Prostate ca (n=11)	6	1	8	6	3	2	8/4
Renal cell ca* (n=4)	0	0	4	1	1	1	2/1
Colonic ca (n=4)	0	0	4	0	0	0	1/1
Trans. cell ca (n=3)	2	0	2	3	1	1	3/0
Lung ca (n=2)†	1	0	2	0	0	0	2/0
Esophag. ca# (n=1)	1	0	1	1	0	0	1/0
Carcinoid (n=1)	0	1	1	0	0	0	1/0

*3 clear cell type, 1 unclassified; †1 micropapillary, 1 squamous; #small cell ca

Noteworthy findings (see table) are: the uncommon presence of a history of carcinoma (38%), the rarity of bilaterality (8%), the occasional absence of a grossly distinct mass (15%), the prominence of intertubular growth (42%), conspicuous intrarete or intratubular growth in some cases (19%), occasional prominent cytoplasmic vacuoles (15%) and the common occurrence of lymphatic invasion (69%). Four tumors with prominent intrarete and/or intratubular growth, which was the sole involvement in 2 prostate cases, had submitting diagnoses of rete adenocarcinoma or seminoma. Four tumors had diffuse patterns of pale cells with cytoplasmic vacuoles that simulated Sertoli cell tumor. Three renal cases had submitting diagnoses in the sex cord-stromal group. The transitional cell carcinomas had nested and anastomosing intertubular patterns.

Conclusions: Testicular metastases are usually unilateral and may simulate primary neoplasms.The prostate is the most common source. Prostatic carcinomas, and less commonly others, may show prominent growth in the rete and mimic rete adenocarcinoma or have diffuse arrangements of vacuolated cells simulating malignant Sertoli cell tumor. The clear cytoplasm of renal cell carcinoma frequently causes confusion with Sertoli cell tumor or other sex cord-stromal tumors. The bladder and renal pelvis should also not be overlooked as possible sources in cases of testicular metastasis.

852 Distribution of FOXP3, CD4 and CD8 Positive Lymphocytic Cells in Benign and Malignant Prostatic Tissues

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Background: Foxp3 is a transcription factor that is thought to be critical for the development and function of regulatory T cells (Treg), i.e. a subpopulation of CD4 positive lymphocytes with a CD4+CD25+/high phenotype. It has been suggested that Tregs inhibit the antitumor immune response, thereby promoting carcinogenesis. High levels of Tregs have been reported in several human cancers. Here, we have investigated the distribution of cells positive for Foxp3, CD4 and CD8 in benign tissue and carcinoma of the prostate.

Design: Tissue microarrays were constructed from 36 patients who underwent radical prostatectomy at the Karolinska University Hospital in Stockholm, Sweden. From each patient 6 cores were taken: 2 cores from cancer (primary and secondary Gleason patterns), 1 from benign tissue of the peripheral (PZ), transition (TZ) and central zones (CZ) and 1 from atrophic glands of the PZ. Sections were immunostained for Foxp3, CD4 and CD8. Number of cells per core were counted and corrected for tissue loss.

Results: The mean cell count of Foxp3 in PZ, TZ, CZ, atrophy and cancer was 2.4, 3.8, 0.5 (mean for all zones 2.2), 10.5 and 10.2, respectively. All 3 cell types were more common in cancer than in non-atrophic benign tissue ($p < 0.01$) and more common in atrophy than in non-atrophic PZ, but did not differ significantly between cancer and atrophy. Cells positive for Foxp3 and CD4 were less prevalent in CZ than in PZ and TZ. No correlation with Gleason score was found.

Conclusions: T lymphocytes positive for Foxp3, CD4 and CD8 are more common in prostate cancer than in non-atrophic benign prostatic tissue but almost equally common in areas of atrophy as in cancer. The CZ is less inflamed than the other anatomical zones. Whether this has pathogenetic importance for the low cancer rate in CZ prostate remains to be investigated. Likewise, these findings may have relevance for the hypothesis that atrophy is a potential precursor lesion of prostate cancer.

853 Proteomic Analysis Identifies Key Metabolic and Stress Proteins Differentially Expressed in Renal Cell Carcinomas

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Background: Renal cell carcinoma (RCC) comprises a heterogeneous group of tumors with various molecular and cytogenetic abnormalities. However, it is unclear whether the genetic abnormalities are followed by specific changes in the protein expression profile for each histological type. In this study, we analyzed the protein expression patterns in different histotypes of RCC to identify potential biomarkers that may help in the classification and prognosis of patients with kidney cancer.

Design: Twenty-three cases representing the spectrum of renal tumors were studied. Microdissected cell populations from frozen tissue sections were analyzed by standard 2-D polyacrylamide gel electrophoresis (2-D PAGE). Gels were digitally scanned, compared, and significant, differentially expressed spots were excised and analyzed by MALDI-TOF MS. Differential expression was further validated in a series of tumor samples by immunohistochemistry and western blot.

Results: Sixty-six protein spots were found statistically ($p<0.05$) up or down regulated between cancer and normal tissues, with changes ranging from 2 up to 13.5-fold. From these spots, eight different polypeptides were successfully identified, mainly representing

enzymes involved in metabolic pathways. Protein expression validation demonstrated that among the identified polypeptides, Triosephosphate Isomerase 1 (TPI-1) and Heat-shock protein 27 (HSP27) were the most related to clinicopathological features such as tumor type and grade.

Conclusions: Proteomic profiling of RCC by 2-D PAGE identified a series of differentially expressed proteins between neoplastic and normal tissue. Most of these proteins represent key metabolic, regulatory enzymes. Protein expression was consistent among histotypes, allowing classification of tumors based on spot pattern. This protein profile may prove to be useful in identifying biomarkers for disease progression and therapy.

854 Assessing the Clinical Significance of Limited Cancer in Prostate Needle Biopsies: Correlation with Gland Size May Be the Key

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Background: Smaller prostates are relatively better sampled by extended needle biopsy protocols as compared to larger glands. Hence, it is likely that limited cancer in prostate biopsies would be more predictive of clinically insignificant cancer in smaller prostate glands. However, no data is available to support or refute this hypothesis.

Design: 48 patients were identified who had undergone radical prostatectomy (RP) after detection of limited prostate cancer (≤3mm in a single core) in diagnostic needle biopsies following an 8 or 10 core extended biopsy protocol. The biopsy cores had been submitted in separate cassettes and examined through at least 3 levels. The RP specimens were submitted in total for histological assessment. Tumour volume in the RP specimen was measured using digital morphometry: the RP slides were scanned and the tumour volume calculated using the software package ImagePro+. Clinically insignificant cancer in RP was defined as one that was <0.5cc, Gleason score <7 and pT2. The tumour volume and the incidence of clinically insignificant cancer in small prostates (≤40gms) were compared with those in larger glands (>40gms).

Results: In 14 (29.2%) of the 48 cases with limited cancer in prostate needle biopsy, the weight of the prostate gland was ≤40gms. The tumour volume was smaller and the incidence of clinically insignificant cancer higher in these small prostate glands as compared to larger glands:

Wt of prostate (gms)	Number of cases	Mean tumour volume (cc)	Median tumour volume (cc)	Number of cases with clinically insignificant cancer
≤40	14	0.6	0.4	9 (64.3%)
>40	34	1.7	0.7	14 (41.2%)

Conclusions: Limited cancer in prostate needle biopsies is a better predictor of clinically insignificant cancer in smaller prostate glands.

855 Preferential Association of Lichen Sclerosus with Differentiated Penile Intraepithelial Neoplasia

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Background: Lichen sclerosus (LS) is found associated with approximately 30% of penile invasive squamous carcinomas (SCCs) and up to 69% of SCCs exclusive of the foreskin. It has been postulated that LS may represent a precancerous condition and a significant association between LS and non-HPV related subtypes of penile invasive SCCs has been shown. However, little is known about a possible relation of LS and penile intraepithelial neoplasia (PeIN). PeIN can be broadly classified in differentiated type (usually HPV unrelated and affecting older patients) and PeIN of warty/basaloid types (HPV-related variants, usually affecting younger patients).

Design: This study was designed to correlate LS with subtypes of penile intraepithelial neoplasia (PeIN). A total of 115 specimens with precancerous lesions, 105 of which associated and 10 not associated with penile invasive SCCs were selected. Nomenclature used was as follows: 1- PeIN (squamous), differentiated, of low and high grade; 2- PeIN, warty, basaloid or warty-basaloid, of low and high grade. Differentiated PeIN was predominantly composed of large keratinocytes with ample eosinophilic cytoplasm; warty PeIN showed atypical cells with koilocytic changes and basaloid PeIN was predominantly composed of small cells with high nuclear/cytoplasmic ratio and round hyperchromatic nuclei. Warty-basaloid lesions had mixed features of both. Low grade lesions showed mild dysplasia affecting the lower third of the epithelium and high grade lesions included moderate and severe dysplasia affecting more than the lower third of the epithelium. LS showed vacuolar alteration of the basal layer, at least focal epithelial atrophy and the characteristic band-like hyalinization of sub-epidermal tissue.

Results:

PeIN associated with lichen sclerosus

PeIN	#Cases	Associated lichen sclerosus	%
Differentiated	75	21	28
Warty/basaloid	40	2	5
Total	115	23	20

P value 0.0034

Conclusions: There is a preferential association of LS with differentiated subtypes of PeIN when compared with PeIN of warty and basaloid types. Low and high grade differentiated PeIN were equally associated with lichen sclerosus. This study provides further evidence of the existence of two separate pathways leading to penile carcinomas and suggests that LS may be a precancerous condition involved in the HPV- independent pathway of cancer pathogenesis.

856 p16 and p53 Expression in Penile Verruciform Tumors; Correlation with Tumor Subtypes. An Immunohistochemical Analysis of 50 Cases

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Background: Penile verruciform tumors (VTs) are a heterogeneous group of lesions each representing a distinct clinicopathological entity. Warty carcinoma (WC), mixed warty-basaloid carcinoma and giant condyloma (GC) are almost invariably associated with HPV while verrucous carcinoma (VC), carcinoma cuniculatum (CC) and papillary carcinoma (PC) tend to be HPV unrelated. p16 has been shown to be specifically overexpressed in HPV-related carcinomas of the vulva and other locations and some studies suggested that p16 and p53 expression tend to be mutually exclusive. Little is known about the expression of p16 and p53 in penile VTs.

Design: We evaluated the immunohistochemical expression of p16 and p53 in 50 penile VTs including 16 VCs, 14 WCs, 6 PCs, 3 CCs, 3 GCs and 8 mixed tumors. p16 was considered to be overexpressed when a strong nuclear and cytoplasmic staining was seen in more than 25% of the tumor cells. p53 was considered positive when at least focally expressed in more than the basal layer.

Results:

p16 expression in verruciform tumors

Tumor type	Negative	Focal (<25%)	Moderate (25-75%)	Diffuse (>75%)
VC (n=16)	13	3	0	0
CC (n=3)	2	1	0	0
GC (n=3)	1	2	0	0
WC (n=14)	2	0	0	12
PC (n=6)	4	1	1	0
Mixed tumors (n=8)	4	1	2	1
Total n=50	26	8	3	13

p53 was positive in 10/50 cases (20%) including 3 WCs, 1VC and 6 mixed tumors (5 hybrid/VC and 1 warty/basaloid carcinoma). Except for the VC, p53 expression was seen in higher grade areas of the lesions. Of the 10 cases, in addition to p53, 5 also expressed p16.

Conclusions: Diffuse p16 expression correlated with high risk HPV-related neoplasms such as WC and mixed tumors containing warty/basaloid areas. GCs, which are associated with non high risk HPV types were negative for p16. Non-HPV related subtypes such as VC, CC and PC were frequently p16 negative. These two different patterns of p16 expression support the concept of bimodal pattern of carcinogenesis in penile tumors. p53 expression appeared to be independent (not mutually exclusive) from p16 at least in this subset of VTs. When positive, p53 expression was at most focal to moderate and its expression was associated with higher grade areas (independently of the tumors expressing or not p16) suggesting that p53 may be involved in later stages of cancer progression.

857 Pathologic N-Stage, and Not Primary T-Stage Is a Significant Predictor of Relapse-Free Survival (RFS) in Bladder Cancer after Radical Cystectomy for Cancer: A Multivariate Cox Analysis in 247 Patients

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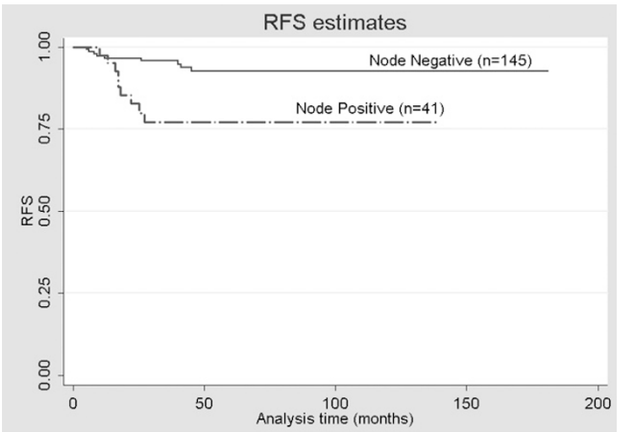
Background: Pathologic staging after cystectomy has remained a significant predictor of adverse outcome in this group of patients. The aim of this study was to establish to what extent relapse-free survival (RFS) is influenced by the tumor stage and nodal stage at cystectomy.

Design: Between 1995 and 2005, a total of 247 patients underwent radical cystectomy. Demographic and pathologic data were tabulated, including age, sex, pT-stage, margin status and pN-stage after cystectomy. Data on loco-regional and distal recurrence (RFS) was collected using radiology and/or, FNA/biopsy proven evidence of metastases. Cox proportional-hazards (PH) modeling was performed using Stata 10.

Results: The distribution of cases in this cohort was as follows: 43 <=T1; 55 T2 and 88 cases of >=T3, including a total of 41 node-positive and 145 node negative patients. There was a significant association between the T stage and Node positivity ($p<<0.001$). All patients were followed for a mean of 76.6 months (range: 5-198 months). On univariate modeling, tumor stage (Hazard ratio, [HR]: 1.54; 95%CI: 1.002-2.36), node involvement (HR: 2.16; 95%CI: 1.37-3.40) and positivity for urethral margin (HR: 5.93; 95%CI: 1.35-25.98) were significantly associated with RFS. Age, tumor grade and pouch type were not associated with RFS. In multivariate analyses, only node stage was significantly associated with RFS after adjusting for all other covariates (HR: 2.006 95%CI: 1.17-3.43).



Conclusions: In this cohort of radical cystectomy patients, only pathologic node involvement at cystectomy was independently associated with relapse-free survival in bladder cancer on multivariate analyses. This information will be useful in further sub-stratification of the node positive and node-negative group to help identify low- and high-risk subsets.

858 Topoisomerase IIa Expression in Renal Medullary Carcinoma: Potential Diagnostic and Therapeutic Implications

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Background: Medullary carcinomas (MCa) are aggressive renal neoplasms with no currently available effective treatment. Topoisomerase II alpha (TopoIIa) is a gyrase involved in cell proliferation, DNA maintenance and repair. Topo IIa is a target for agents such as Anthracyclines that stabilizes covalent enzyme/DNA complexes activating cell death pathway. Triggered by a recent MCa response to Topo IIa inhibitor in one of our pts, we evaluated Topo IIa expression in relation to ki67 in a group of MCa and potentially related collecting duct carcinoma (CDCa) tumors.

Design: Archival material from a total of 12 tumors, 6 MCa (including above pt with Rx response) and 6 CDCa were retrieved from our surgical files. Diagnoses were confirmed by 2 pathologists. Representative sections were immunostained with monoclonal antibodies for Topo IIa antibody (Zymed Laboratories) and Ki67 (DAKO). For Topo IIa, the percentage of cells with positive nuclear staining was assessed in highest area of expression. a previously suggested > 5% cut off (Bhargava et al Am J Clin Path 2005) was used to designate a tumor as Topo II positive.

Results: MCa pts were younger (age range: 11-32 y) compared to CDCa pts (42-87 y). **Topo IIa:** all 6 MCa tumors were positive for TopoIIa expression (median 55%, mean 49% range 15-80%). TopoIIa expression was significantly lower in CDCa (p 0.01) compared to MCa. Topo IIa was undetectable in 2/6 CDCa with only 2/6 tumors showing >5% expression index (median 3.5 %, mean 11% range 0-40%). **Ki67:** all 12 tumors revealed high ki67 expression rate of 30% or more. Although overall higher Ki67 expression index was seen in MCa (median 90%, mean 80% range 30-100%) compared to CDCa (median 50%, mean 56% range 30-90%), the difference was not statistically significant (p 0.11). ki67 index was higher than Topo IIa rate in all 12 cases. A statistically significant strong correlation between Topo IIa and ki67 expression (pairwise CC=0.89, p 0.01) was found in MCa tumors. The correlation between the two markers was weaker (CC= 0.48) in the CDCa group and did not reach statistical significance.

Conclusions: Positive Topo IIa immunohistochemical expression was demonstrated in all MCa tumors and was significantly higher in MCa compared to CDCa. If confirmed, the latter difference could be of added diagnostic utility. The potential use of Topo IIa inhibitors in MCa merits further investigation.

859 Benign Nerve Sheath Tumors Involving the Urinary Bladder

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Background: Benign neural tumors (schwannoma and neurofibroma) involving the urinary bladders are rare with only case reports and limited studies.

Design: We identified 6 neurofibromas and 2 schwannomas involving the urinary bladder. Of the 8 cases, 7 were sent to one of the authors in consultation and the remaining came for treatment to The Johns Hopkins Hospital.

Results: Patients (3 male, 5 female) ranged in age from 2.5 to 69 years old (average 36 yrs. old). Presenting symptoms included recurrent infection in 6 patients, hematuria in 3 patients, and irritative symptoms in 3 patients (some patients with 2 symptoms). Three patients had a pelvic mass on physical examination. Four patients (4/8) had a solitary lesion in the bladder, 3 patients (3/8) had multifocal lesions, and 1 patient had no information available. Five patients underwent local resection. One patient, who was 3 years old, had multiple plexiform neurofibromas within the bladder and did not have surgical treatment and another patient is also undergoing surveillance. One patient had no treatment information available. Seven patients had clinical follow-up information available. The length of follow-up varied from 2 to 124 months (average 47 months). Three out of 7 neurofibromas with follow-up information had neurofibromas in other sites, including the skin, uterus, mesentery, and ureter. Both cases with schwannoma had only bladder involvement. A clinical family history of neurofibromatosis was not noted and genetic studies were not performed on any of the patients. Four neurofibromas were of the diffuse type with the remaining 2 cases plexiform. None of these cases on follow-up demonstrated malignant transformation.

Conclusions: The importance of accurately diagnosing plexiform neurofibromas of the bladder is that about one-half of these patients will have neurofibromatosis. If the lesion is focal, local conservative excision is the choice of treatment with a low risk of recurrence. Diffuse neurofibromas can be difficult to diagnose leading to delay of treatment and potentially the need for a more extensive excision. Once recognized as a neurofibroma, it is important to recognize diffuse neurofibroma, given its different relationship to neurofibromatosis.

860 Partial Atrophy on Prostate Needle Biposy Cores: A Morphological and Immunohistochemical Study

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Background: Partial atrophy is the most common benign mimicker of prostate cancer on needle biopsy.

Design: Of 3,916 prostate needle core biopsy cases received in our consultation service from 3/1/07 to 5/31/07, 170 cases (4.3%) with partial atrophy were diagnosed as atypical glands by outside pathologists and prospectively identified. We supplemented our material with 108 cases of partial atrophy sent to our consultation service in 2006 from a single institution which frequently uses a triple cocktail stain [p63, high molecular weight cytokeratin (HMWCK), alpha methyl acyl-Coa racemase (AMACR)]. The morphological features of the 278 cases and immunohistochemistry of 236 cases (198 with prostate cocktail and 38 with only basal cell makers) were analyzed.

Results: 48/278 (17.3%) of partial atrophy cases were mixed with post atrophic hyperplasia. Enlarged nuclei were visible in 43/278 (15.5%) cases, with prominent nucleoli seen in 58/278 (20.9%) cases (30 cases associated with nuclear enlargement). Of 198 cases with a prostatic cocktail stain, 48 (24.2%) had a cancer pattern for both basal cells and AMACR (p63-, HMWCK-, AMACR+), 14 (7.1%) had a cancer pattern for basal cells (p63-, HMWCK-, AMACR-), 89 (44.9%) had a cancer pattern for AMACR (p63+, HMWCK+, AMACR+), and 47 (23.7%) had a totally benign pattern (p63+, HMWCK+, AMACR-). Of the 198 cases using the cocktail stain, 136 (68.7%) had positive basal cell staining. The percentage of basal cells labeled with the combination of p63/HMWCK was: <5% in 42 (21.2%) cases; 5%-75% in 58 (29.3%) cases; and >75% in 36 (18.2%) cases. An additional 38 cases immunostained only for p63 and/or HMWCK was negative in 2 (5.2%) cases, <5% in 5 cases (13.1%), 5-75% in 19 (50%) cases, and >75% in 12 (31.6%) cases.

Conclusions: Partial atrophy mimicks adenocarcinoma on H&E and on IHC stains. IHC can help confirm the diagnosis if basal cells are demonstrated, yet basal cells may be very focal, labeling only a couple of cells in a few of the glands. Negative basal cell staining is still consistent with the diagnosis. AMACR stains are also potentially misleading as it is often positive in partial atrophy. Recognizing the H&E features of partial atrophy is still the most critical aspect in prevent a misdiagnosis of carcinoma. Partial atrophy remains a particularly difficult lesion for pathologists on prostate needle biopsy and additional reports on this topic will hopefully increase the awareness of this lesion's morphological and immunohistochemical profile.

861 Testicular Carcinoid Tumor: A Study on 13 Cases

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Background: Testicular carcinoid tumors are rare with only limited studies.

Design: We identified 13 testicular carcinoid cases from 5 academic institutions.

Results: Patients ranged from 16-65 yrs. old (mean 38). Of 13 cases, 12 were testicular primaries and 1 was a metastasis from an ileal primary. Presenting symptoms included testicular mass (n=10), incidental positive PET scan (n=1), incidental finding during vasectomy (n=1), and scrotal pain in the patient with a history of ileal carcinoid (n=1). 2 patients had carcinoid syndrome. Treatments include focal excision in 3 patients (1 with metastatic carcinoid tumor) and radical orchiectomy in 10 patients. Of the 12 primaries, 8 were pure carcinoid tumors, 2 were associated with cystic teratoma, 1 was associated with an epidermoid cyst which showed a lack of isochromosome 12p on FISH studies done on paraffin, and 1 had extensive nodular calcification suggestive of a calcified epidermoid cyst. Mitotic figures were rare in the primaries with only 2 cases showing 1/50 HPF; necrosis was found in only 1/13 cases. Occasional nuclear pleomorphism was seen in 4/13 cases. The metastatic carcinoid tumor was similar to primaries with the exception of a slightly increased mitotic rate (2/50 HPF). All patients had follow-up information ranging from 1-114 mos. (mean 31 mos.). Of the 12 primary tumors, 11 (including the 2 cases treated with focal excision) have had no recurrence with 1 patient experiencing a local recurrence. None developed nodal or distant metastases. The patient with secondary testicular carcinoid tumor also had metastatic tumor in liver and bone and died from carcinoid tumor 9 years after his primary and 2 years after his testicular metastasis.

Conclusions: Within the literature, pure testicular carcinoid tumors and those with teratoma have metastasized. However, our study shows that testicular carcinoid tumors have a very low risk of recurrence after orchiectomy, even in the presence of necrosis, epidermoid cysts, or atypia. None of our cases were atypical carcinoids, such that our findings only apply to usual carcinoid tumors. Rarely, carcinoid tumor in the testis reflects metastatic disease where the prognosis is poor reflecting advanced disease.

862 PSMA Expression in Schwannoma: Potential Diagnostic Pitfall for Metastatic Prostate Carcinoma by Radioimmunoscintigraphy Imaging Studies

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Background: Radioimmunoscintigraphy using a radiolabeled prostate-specific membrane antigen (PSMA) antibody is frequently used to detect prostate carcinoma

(PCa) recurrence and metastasis to lymph nodes, soft tissues and bone. PSMA expression has been shown in occasional non-prostatic neoplasm (eg. urothelial adenocarcinoma) and neovasculature of other malignancies. PSMA expression has not been described in benign neoplasms. Recently, during evaluation of a PCa patient, we encountered a"false positive" PSMA radioimmunoscintigraphy scan in a pathologically confirmed Schwannoma (SCH) lesion. The current study further evaluates PSMA expression in Schwannoma.

Design: 11 soft tissue SCH were retrieved from our surgical pathology archives. Representative sections were immunostained with monoclonal antibody for PSMA (Clone 3E6, DAKO, CA). PSMA expression was evaluated in tumor cells and lesional vessels. Extent of staining was calculated as percent of positive cells in highest areas of expression. Positive staining was considered focal, multifocal or diffuse based on percent of positive cells: <5%, 5-75% and >75% respectively.

Results: all 11 SCH showed tumoral and or vascular staining. 7 displayed both vascular and tumoral cell staining. Remaining four had only vascular staining (2) or tumor cell staining(2). The extent of tumoral cell and vascular staining varied widely among lesions (tumor cells: focal in 8 and diffuse in 1; Vascular: focal in 7, multifocal in 1 and diffuse in 1 lesion).

	Number of Cases (%)			
PSMA Expression	Negative	Pos Focal	Pos Multifocal	Pos Diffuse
Tumor Cells	2/11(18%)	8/11(72%)	0	1/11(9%)
Tumor Vessels	2/11(18%)	7/11(63%0	1/11(9%)	1/11(9%)

Conclusions: This the first report of PSMA expression in a benign neoplasm. Given our finding of frequent expression of PSMA in Schwannomas, they should be considered in the differential diagnosis of a lesion that is positive on PSMA radioimmunoscintigraphy studies during a metastatic work up of PCa patient.

863 Sarcomatoid Carcinoma of the Upper Urinary Tract

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Background: Sarcomatoid carcinoma (carcinosarcoma) of the upper urinary collecting system is a rare aggressive malignancy composed of malignant epithelial and stromal components. Due to the paucity of reported cases, the clinical behavior, molecular alterations and potential therapies for this malignancy are not well understood.

Design: Four cases of sarcomatoid carcinoma involving the renal pelvis, calyces and/or upper ureter were studied. Immunohistochemical expression of EGFR, HER2/neu, cytokeratins, vimentin, actin, desmin, CD31, CD34, S-100, HMB-45, c-kit and p53 was analyzed in each case. Evaluation for amplification of EGFR and HER2/neu genes was performed by FISH. Each tumor was also examined for gains of chromosomes 3, 7 and 17 and for loss of chromosome 9p21 by UroVysion FISH.

Results: All four patients were females with an age range of 60 to 78 years and mean age of 70 years. Presenting symptoms included gross hematuria, fever, sepsis and flank mass. Radical nephroureterectomy was performed on all patients. Lymph node metastases were present in 2 cases at the time of surgery. All patients died within 1.5 years after surgery (mean survival, 5.6 months). EGFR immunostaining was moderately to strongly positive in all 4 cases. Her2/neu immunostaining was weakly positive in 1 case. c-kit was negative in all cases. p53 was positive in 2 of 4 cases. EGFR polysomy was demonstrated in 3 of 4 cases; disomy was present in the remaining case. No amplification of HER2/neu was present in any case. UroVysion FISH was positive in all 4 cases.

Conclusions: Our study indicates that gains of chromosome 3, 7 and 17 and loss of chromosome 9p21 are the common alterations in sarcomatoid carcinoma of the upper urinary tract, consistent with urothelial origin. Moderate to strong EGFR expression was demonstrable in all 4 examples of this neoplasm that we studied, and was associated with EGFR polysomy at the molecular level in the majority of cases. The expression of EGFR raises the possibility that anti-EGFR therapy may be a rational therapeutic option for this highly aggressive cancer.

864 EGFR Protein Expression and Gene Amplification in the Chemo-Refractory Metastatic Embryonal Carcinoma

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Background: Testicular cancer is the most common cancer in young males. The combination of cisplatin-based chemotherapy and surgery has resulted in high cure rate. However, a small group of patients who have late relapses are often refractory to chemotherapy and have poor prognoses. The treatment options are very limited. Further studies are needed to explore the molecular pathogenesis and the potential therapies for this group of highly aggressive tumors.

Design: We analyzed 21 patients who presented with metastatic nonseminomatous germ cell tumor and were treated with chemotherapy and subsequently underwent retroperitoneal lymph node dissection. Histologically, all the cases had metastatic embryonal carcinoma or mixed germ cell tumor with embryonal components at retroperitoneal lymph node dissection. Immunostaining for epidermal growth factor receptor (EGFR) was performed on the paraffin-embedded tissue sections using the avidin-biotin-peroxidase method. Result was recorded as positive expression when >=10% of tumor cells demonstrated membranous staining. EGFR gene amplification was performed by interphase fluorescence in situ hybridization (FISH). Correlation between EGFR protein expression and gene amplification was evaluated statistically using chi-square test.

Results: Immunohistochemically, 9 of 21 (43%) cases demonstrated positive EGFR staining; 12 of 21 (57%) cases had absent or very limited EGFR expression. Polysomy/amplification was revealed in 16 of 21 (76%) cases (15 cases: polysomy; 1 case: amplification); normal disomy FISH pattern was present in 5 of 21 cases (24%). We found significant correlation between EGFR protein expression and gene polysomy/amplification (p=0.02).

Conclusions: Our study indicates that EGFR protein is frequently expressed in a subset of patients with chemo-refractory metastatic embryonal carcinoma. EGFR gene polysomy/amplification may be one of the mechanisms that cause increased EGFR protein expression. The anti-EGFR therapy might be useful in the management of this subset of patients.

865 Renal Oncocytoma: A Clinicopathological Analysis of Thirty-Six Cases

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Background: Renal oncocytoma is the most common benign neoplasm of the kidney with excellent prognosis. However, the diagnosis of oncocytoma is not always clear-cut due to overlapping morphological characteristics with other renal oncocytic tumors such as chromophobe renal cell carcinoma (RCC) and oncocytic type of conventional RCC. Some oncocytomas show certain degree of atypia, such as invasion into peri-nephric adipose tissue and nuclear atypia or mitosis, which still cause concerns among pathologists and urologists, even though these features are considered compatible to the diagnosis of oncocytoma.

Design: In this retrospective study, we evaluated the clinical and pathological features of renal oncocytomas with a focus on the "atypical" types, from a collection of 50 benign renal neoplasms with primary resections in our hospital from 1995 to 2007.

Results: Of the 50 benign renal neoplasms, 36 were oncocytomas treated either with partial or total nephrectomy (40% and 60%, respectively). There were 17 female and 19 male patients with an age range of 20-87 years (mean age 60). Overall, 27% were multifocal tumors. Size of the tumors ranged from 1.1-15.0 cm (mean: 4.5cm). Eighteen cases were classic oncocytomas, the rest of the cases had one or more "atypical" features included: 8 with features resemble but not diagnostic of chromophobe RCC; 5 with nuclear atypia and/or mitosis; two with focal papillary change (one was confirmed by immunohistochemistry to be a small papillary RCC arising within a oncocytoma); 6 with peri-nephric fat extension. Seven cases had lymph nodes dissection with no metastasis identified (none had tissue diagnosis prior to surgery). With clinical follow-up from 0.5-8 years among 20 cases, recurrence of oncocytoma was identified in one patient twice after 4 and 8 years of the first resection, whose original tumor was multifocal and involving peri-nephric fat. No other recurrence or metastasis was identified. No tumor related death occurred.

Conclusions: In our series, presence of the "atypical" features is rather common (50%). There is no significant difference in sex, age, and size of the tumor between classic and "atypical" oncocytomas. The clinical outcome, classic or "atypical", appears excellent. Tumor multifocality with peri-nephric fat extension may be associated with future tumor recurrence (metachronous tumor), but not malignant transformation. Our data provides further evidence that oncocytomas, even with "atypical" features, are benign and should be treated as such.

866 Assessment of Pathologic Risk Factors for Understaging in Patients with Clinical T1 Bladder Cancer

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Background: For the lamina propria invasive (T1) bladder cancers, recurrence and progression rate is highly variable. Which patients in this group should be offered an aggressive early cystectomy remains an important and difficult management decision. The objective of this study was to assess pathologic risk factors which would predict understanding at the time of cystectomy in patients diagnosed with T1 bladder cancer at transurethral resection of bladder tumor (TURBT).

Design: Ninety five patients with T1 disease at TURBT who underwent cystectomy within 3 months of the diagnosis were identified. Adequacy of the clinical staging was confirmed by presence of muscle or repeat TURBT. Several clinicopathological parameters including grade, tumor multifocality, necrosis, mixed histological differentiation, depth of invasion above or below the muscularis mucosae, associated CIS, growth pattern (papillary versus inverted), urethral involvement and lymphovascular invasion (LVI) seen at TURBTs were compared using univariate and multivariable statistical models between patients who were upstaged (≥ pT2 or pT any N+ or pTN any with M+) versus those staged ≤ pT1 at cystectomy.

Results: Of 95 patients, 26 (27%) were upstaged at cystectomy while remainder were ≤pT1. All tumors were high grade. Statistically significant difference was observed for muscularis mucosae involvement (p=0.01), urethral involvement (p=0.02), presence of mixed histology (p=0.01) and gender (p-0.01) for patients under staged versus not under staged at cystectomy. In multivariate analysis urethral involvement had odds 4.7 (95% confidence interval (CI) 1.2, 18.3) of under staging at cystectomy.

Conclusions: Our results suggest that clinical under staging remains an important problem. Several pathologic features when present at TURBT, including mixed histologic differentiation, invasion of the muscularis mucosae, and involvement of urethra are associated with significant risk of under staging at cystectomy. Patients with these pathological features at TURBT may benefit from an aggressive early cystectomy.

867 Expression of PAX8 in Nephrogenic Adenoma and Clear Cell Adenocarcinoma of the Lower Urinary Tract: Evidence of Related Histogenesis?

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Background: Recent evidence has shown that nephrogenic adenoma (NA) is a true "nephrogenic" lesion derived from exfoliated renal tubular cells that have subsequently implanted in the lower urinary tract. This new concept has led to the identification of PAX2 as a reliable marker for NA. PAX8 is a protein structurally and functionally

related to PAX2, and they are both expressed in renal tubular cells. In this study, we used immunohistochemistry (IHC) to investigate the expression of PAX 8 in NA, clear cell adenocarcinoma (CCA) of the lower urinary tract, and other mimics of NA.
Design: The study group included 35 NA, 7 CCAs of the lower urinary tract, 100 prostate adenocarcinomas constructed into 3 tissue microarrays (TMA), and 62 radical cystectomy specimens (TMA or tissue sections) that provided samples of 30 urothelial carcinoma (UC) NOS, 10 micropapillary variants of UC, 3 clear cell variants of UC, 2 sarcomatoid variants of UC, 2 neuroendocrine variants of UC, 1 nested variant of UC, 5 squamous cell carcinomas (SCC) and 9 adenocarcinomas of the urinary bladder. The uninvolved urothelium, prostate glands and stroma served as normal tissue controls. IHC of PAX8 (Proteintech Group), PAX2 (Zymed), ER and PR were performed with the avidin-biotin peroxidase method after antigen retrieval.
Results: Distinct nuclear staining of PAX 8 was detected in all NAs and in all CCAs of the lower urinary tract. The staining was diffusely positive in six of the CCAs and focally positive in one. PAX8 was not detected in the normal urothelium, prostate glands, and stroma, nor was it detected in the samples of prostate adenocarcinoma, UC of the bladder and its variants, SCC, or adenocarcinoma of the urinary bladder. PAX 2 nuclear staining was detected in 2 of the 7 CCAs of the lower urinary tract. ER and PR staining was performed in 5 of the CCAs of the lower urinary tract and was negative.
Conclusions: PAX8 may be an additional diagnostic marker for differentiating NA and CCA from other common tumors in the lower urinary tract. In addition, since PAX8 and PAX2 are cell lineage restricted transcription factors usually expressed in normal and neoplastic tissues of nephric origin, detection of these markers in both NA and CCA may indicate that these two entities have a related histogenesis.

868 Fluorescence *In Situ* Hybridization (FISH) of Chromosome 12p in Germ Cell Tumors: Clinical Validation of a Bacterial Artificial Chromosome Clone 12p Probe on Paraffin-Embedded Tissue

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Background: 80-90% of germ cell tumors (GCT) have isochromosome 12p i(12p) by metaphase cytogenetics with FISH detection of 12p overrepresentation being even more sensitive. Non-germ cell malignancies arising in GCT, epsecially in metastatic sites, can be difficult to diagnose based only on morphology.
Design: We developed for clinical use an interphase FISH assay for detection of overrepresentation of 12p in GCT. The designed probe covers almost 400 kbp and consists of 3 sequenced, contiguous, bacterial artificial chromosome (BAC) clones on chromosome 12p12.1. Paraffin-embedded specimens (1984-2006) included 1 ovarian dysgerminoma and 1 testicular mixed GCT which had an i(12p) by metaphase cytogenetic studies. In total, 23 blocks from 14 tumors and 18 controls (lymph nodes, lung, mediastinal tissue) were studied by FISH. Tumors included 7 testicular malignant mixed GCT and 2 ovarian dysgerminomas. In addition, we analyzed 4 non-GCT (undifferentiated sarcoma, Wilms tumor, PNET, and a case with both rhabdomyosarcoma and carcinoma) arising in GCT. A separate mediastinal adenocarcinoma in a patient with a history of a testicular GCT was also studied. For each tumor and control the number of red (12p) and green (centromere) signals were counted and averaged. The ratio of the average red to green signals was calculated, and a ratio of 1.3 was considered positive.
Results: All GCT and non-germ cell malignancies arising in GCT were positive for 12p overrepresentation. The 12p to 12 centromere ratios ranged from 1.26-2.70 for the tumors. The controls had ratios ranging from 0.96 to 1.16 with a mean of 1.04 +/- 0.064. Based on a Gaussian distribution of the data, >99.7% of normal controls would have a ratio of less than 1.3. The mediastinal adenocarcinoma arising in a patient with a GCT was negative for 12p overrepresentation and may represent a second primary tumor. In only one case did testing of multiple blocks result in a difference in 12p positivity with 2/3blocks for 1 dysgerminoma positive while a 3rd negative, suggesting that an initial negative result may warrant testing of additional blocks.
Conclusions: Since GCT may metastasize with non-GCT morphology, the use of interphase FISH is critical in distinguishing de-novo malignancy from GCT recurrence. The use of BAC cloned probes which hybridize to large 12p regions yielded positive results in all tumor cases studied including all cases where non-GCT arose in a GCT.

869 Correlation of Biopsy and Radical Prostatectomy Gleason Score in Contemporary Extended ≥12 Core Biopsies Practice: Improved Correlation with Biopsy Worst Gleason Score

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Background: With sextant prostate biopsies, there is up to a 1-in-3 chance that the underlying pathologic Gleason score (GS) is higher. Knowledge of underlying GS has important prognostic and therapeutic relevance. To improve the cancer detection, an extended biopsy sampling methods are increasingly being utilized. Objective of the current study was to analyze correlation of biopsy and radical prostatectomy (RP) GS in pure extended (≥12) core biopsies practice by stratifying them using various biopsy and RP reporting methods utilized in practice.
Design: Two hundred six consecutive patients who underwent an extended biopsies and subsequent RP at our institution were included for this study. Extended biopsy Global (gestalt or overall), Worst, and Largest volume GS was compared with final RP index (largest size nodule in multifocal cancers) and Global (gestalt or overall) GS. Weighted kappa statistic was used to determine agreement between biopsy and RP GS with 1, 0 and value below 0 indicating 100%, no agreement, and worse than chance agreement respectively.
Results: Biopsy Global GS had weighted kappa agreement of 0.61(range 0.51, 0.71) for Global RP GS, and 0.59 (0.48, 0.70) for index RP GS; biopsy Worst GS had weighted kappa agreement of 0.64(0.54, 0.73) for Global RP GS, and 0.61(0.51, 0.71) for Index RP GS; and biopsy Largest volume GS had weighted kappa agreement of 0.54(0.44, 0.65) for Global RP GS, and 0.52(0.41, 0.63) for Index RP GS. Correlation between biopsy parameters (number of positive cores, volume etc) and RP GS nor the biopsy GS and RP GS were predicted by multifocality or index tumor size.
Conclusions: Our results suggest that even in extended biopsy practice, overall correlation with RP GS is at the best in the range of 64%. Best correlation is observed when Worst biopsy GS is utilized. There is very good agreement between Index and Global GS at RP. Additional correlation analyses focusing specifically on clinically significant upgrading or under grading between biopsy and RP GS are currently being investigated.

870 Gene Expression Signature Predicting Prostate Cancer Progression

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Background: In prostate cancer management, differentiating relatively indolent from more aggressive disease is a key clinical issue. The Gleason grading system, based on microscopic features, is one of the strongest predictors of many clinical endpoints, including PSA recurrence following radical prostatectomy or radiotherapy. However, the Gleason grading system is subject to inter-observer variation. Among Gleason scores 6 or 7 cancers, the ability of Gleason score to predict the clinical course is limited. New prognostic factors will be helpful to assist clinical decision making.
Design: We studied 191 patients with localized prostate cancer treated with radical prostatectomy in our hospital between 1993 and 1995. The clinical endpoint of progression was PSA recurrence and the median follow-up was 7.2 years. The samples were divided randomly into a training set of 124 tumor samples (53 recurrences) and a validation set of the other 67 samples (24 recurrences). Formalin-fixed paraffin-embedded tumor sections were analyzed for the expression of 1536 specifically selected prostate cancer related genes using the DASL gene microarray platform.
Results: A 62-gene predictor was developed and strongly prognostic for PSA recurrence in both the training set (hazard ratio=8.3, 95% CI 3.9-17.7, $p < 0.0001$) and the validation set (hazard ratio=6.3, 95% CI 2.45-16.0, p=0.00013). After adjusting for Gleason score, the 62-gene predictor remained a powerful predictor with hazard ratios of 5.2 (95% CI 2.1-12.9, $p < 0.0001$) and 4.6 (95% CI 1.1-18.4, p=0.03) in the training set and validation set, respectively. Importantly, the 62-gene predictor separated patients with Gleason scores 6 and 7 cancers into good and poor prognosis groups in the training set ($p < 0.0001$) and the validation set (p=0.0015).
Conclusions: A set of 62 predictive genes has been identified that strongly separates men with Gleason score 6 and 7 prostate cancers into good and poor prognosis groups for PSA recurrence following prostatectomy. If further validated, this gene expression-based signature may provide strong prognostic information beyond the Gleason grading system.

871 Metabolomic Imaging of Prostate Cancer

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Background: Diagnosis and management of prostate cancer is limited by lack of sensitive and specific imaging technique to detect prostate cancer in vivo. In order to provide biochemical basis for improved in vivo MR spectroscopic (MRS) imaging of prostate cancer, we hypothesized that the metabolite processes (pathways), or metabolomics, are specifically altered in prostate cancer. We studied 200 tissue samples from radical prostatectomy specimens. By analyzing the chemical shifts via MR spectroscopy, we were able to identify a specific metabolomic profile of prostate cancer (*Cancer Research, 2005; 65: 3030-4*). In the current study, we attempted to apply the metabolomic profile of prostate cancer in ex in vivo MRS imaging of prostate cancer.
Design: Five radical prostatectomy specimens from prostate cancer patients were analyzed for this study. Two- and three-dimensional MRS chemical shift imaging (CSI) analyses were conducted at room temperature, on a 7.0 T scanner. The specimens were subsequently submitted for whole mount histological evaluation and mapping of the prostate cancer. Metabolite intensities (n=36) from CSI data for each voxel were processed individually, and used to construct prostate cancer specific metabolomic profiles based on the published prostate cancer results (*Cancer Research, 2005; 65: 3030-4*). The values of the calculated profiles for each voxel were used as indices in the color-map to determine the color and displayed transparently on an overlay of the anatomic MRI image. The final metabolomic image is the anatomic map of the prostate with the voxel grid overlaid - each voxel's color indicates that voxel's status on the metabolomic cancer-index. The Metabolomic images were then compared to the current conventional MRS images based on phospho choline and the histological mapping of the prostate cancer.
Results: Metabolomic images demonstrated high intensities of cancer specific profiles in or around the regions where tumors are identified by histopathological mapping of the prostate cancer. Using the level of the tested metabolomic profiles, we were able to detect prostate cancer that extended into the voxel rim and with an overall size of more than twice the MRS voxel sizes (18 mm^2), with an overall accuracy of 93%.
Conclusions: Our results demonstrate the potential of detecting cancer in human prostate by the novel concept of metabolomic imaging. With additional systemic developments, we consider the application of the concept in in vivo clinical diagnosis to be imminent.

872 Tubulocystic Carcinoma of the Kidney – Clinicopathological, Immunohistochemical and Molecular Characterization

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Background: Tubulocystic carcinoma, a rare form of renal neoplasm, is characterized by the presence of tubules and cystic structures lined by atypical epithelial cells. The nature of this tumor is unclear, although it has been suggested that it may represent a low grade collecting duct carcinoma.

Design: We examined the clinicopathological and immunohistochemical features of thirteen cases of tubulocystic carcinoma of the kidney. Furthermore, using gene expression microarray analysis, we analyzed the molecular signature of this tumor by comparing it with other renal tumors in our established molecular profile database.

Results: Pathologically, all 13 tumors were composed of closely packed tubules and cysts of varying sizes lined by epithelial cells with abundant eosinophilic cytoplasm and prominent nucleoli. Clinically, one of the 13 cases developed pelvic lymph node metastasis. Five of the 13 cases coexisted with papillary renal cell carcinoma (n=3) or papillary adenoma (n=2). The immunoreactivity of tubulocystic carcinomas included AMACR (100%), CK7 (88%), and CD10 (100%). The Ki67 proliferative index was low (<0.5%) in all the cases studied. In addition, the molecular profile of tubulocystic carcinoma was similar but not identical to those of papillary renal cell carcinoma by clustering analysis. Comparative genomic microarray analysis showed gains of chromosome 17, but not chromosome 7.

Conclusions: Based on its unique clinicopathological features and molecular signature as well as its biological behavior to develop metastasis either by itself or by associated papillary renal cell carcinoma, tubulocystic carcinoma of the kidney should be recognized as a distinct subtype of renal cell carcinoma and be distinguished from other benign and malignant cystic lesions of the kidney.

873 Tissue Factor and VEGF Expression in the Benign and Malignant Prostate: A Tissue Microarray Study

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Background: Tissue factor (TF) is a transmembrane protein that is the principal physiologic initiator of coagulation. It also plays an important role in tumor growth and metastasis possibly by contributing to angiogenesis. Our study aims to document the differential expression of TF in benign and malignant prostate tissue and correlate it with the expression of the pro-angiogenic protein, vascular endothelial growth factor (VEGF).

Design: Tissue microarray (TMA) was constructed from archival prostatectomy specimens. Core samples were collected from benign (BP), high-grade prostatic intraepithelial neoplasia (PIN) and carcinoma (PCa) areas. TMA sections were stained with an immunopurified polyclonal TF antibody (anti-sTF; 0.5mg/ml) and a rabbit polyclonal VEGF antibody (Santa Cruz, sc-507) with placental tissue as positive control. Two pathologists manually scored staining in epithelial cells in TMA sections using the German Immunoreactive Score (% staining cells X intensity). Cores containing less than 50% of the sampled area after processing were disregarded. Positive cores were defined as score 2 or higher and negative as 0-1. Fisher Exact test was used to test for significance.

Results: Positive staining for TF was seen predominantly in PCa (43/92, 47%) with rare positive glands in BP (7/73, 9%) and PIN (2/25, 8%). Positive staining for VEGF was seen in PCa (63/93, 42%), BP (35/77, 45%) and PIN (13/26, 50%). Rates of TF expression were significantly lower in BP vs PCa specimens ($p<0.001$) and in PIN vs PCa specimens ($p<0.001$). Rates of VEGF expression were also significantly lower in BP vs PCa specimens ($p=0.003$) but not in PCa vs PIN ($p=0.430$). Majority of PCa samples positive for TF were also positive for VEGF (34, 79%).

Conclusions: TF expression is markedly increased and correlates with VEGF expression in PCa compared to BP and PIN. VEGF expression appears to occur earlier in malignant transformation. Currently clinical research in anti-VEGF therapy (bevacizumab) is being investigated in prostate cancer. Studies are needed to determine whether the association and co-localization of TF and VEGF in malignant prostate epithelium may have prognostic or predictive significance in addition to being therapeutic targets.

874 Differential Expression of Angiopoietin Receptor Tie-2 in the Benign and Malignant Prostate: A Tissue Microarray Study

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Background: Angiopoietins are important factors in angiogenesis and Tie-2 receptor (Tie-2) is a member of the endothelial cell-specific receptor tyrosine kinase family through which angiopoeitin 1 & 2 regulate angiogenesis. Overexpression of Tie-2 has been implicated with increased microvessel density in tumors. Targeted tyrosine kinase inhibitors for Tie-2 are being studied for possible clinical therapeutic use. Here we describe a study that documents the differential expression of Tie-2 in benign and malignant prostate tissue.

Design: The prostate tissue microarray (TMA) was constructed from archival prostatectomy specimens. Multiple core samples were collected from benign (BP), high-grade prostatic intraepithelial neoplasia (PIN) and carcinoma (PCa) areas. TMA sections were stained with Tie-2 abntibody (Santa Cruz, SC324) and manually scored using the German Immunoreactive Score (% staining cells X intensity). Cores containing less than 50% of the sampled area after processing were disregarded. Positive cores were defined as score 2 or higher and negative as 0-1. Fisher Exact test was used to test for significance.

Results: Cytoplasmic staining for Tie-2 was seen in prostatic glandular epithelium. There was positive staining in 19 of 96 (20%) BP samples, 15 of 24 (62%) PIN samples and 87 of 92 (94%) PCa samples. There was a statistically significant difference between the percentage of positive staining in BP and PCa ($p<0.001$); between BP and PIN ($p<0.001$); and between PIN and PCa ($p<0.001$).

Conclusions: Our study demonstrates that Tie-2 is differentially expressed in the epithelial cells of BP, PIN and PCa, with higher expression in PCa. Further studies of Tie-2 are warranted to determine its role in prostate carcinogenesis and metastasis; its prognostic/predictive importance in PCa; and its possible use as a target for directed antibody therapy in PCa.

875 Intraoperative Frozen Section Analysis of Apical Margin Biopsies during Radical Prostatectomy: Ten Year Experience from a Single Institution

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Background: The ability to accurately diagnose adenocarcinoma of prostate by frozen section during radical prostatectomy has not been widely accepted. Additionally the clinical value of intraoperative biopsies of the apical margin remains controversial. Since 1997, apical margin biopsies during radical prostatectomy have been routinely submitted for frozen section diagnosis at our institution.

Design: We reviewed a consecutive series of radical prostatectomy cases (n = 1669) performed between 1997 and 2007, in which apical soft tissue margins were routinely evaluated by frozen section. Sensitivity and specificity of frozen section evaluation was assessed specifically addressing issues contributing to false negative or positive diagnoses. Additionally, isolated positive apical margins were evaluated for risk of PSA recurrence.

Results: Among 1669 patients who underwent frozen section examination of apical margins, 56 cases (3.4%) of a positive apical margin were diagnosed at frozen section. The frozen section diagnosis of a positive apical margin had accuracy rate of 97.5%, sensitivity of 59.6%, specificity of 99.8%, and positive and negative predictive values of 94.9% and 97.6%, respectively. The low sensitivity was due to a substantial false negative rate (n =38). The false negative cases were evaluated individually and explained as follows: limited focus of carcinoma located deeper in the block (29 cases, 76%) or misinterpretation as benign (9 cases; 24%). The deferment rate of frozen diagnosis was 2.4% (40 cases). Only in a minority of patients with positive apical margin on frozen section (20 of 56; 36%) was additional apical tissue resected allowing conversion to a negative margin. Five patients who were converted to negative apical margins had positive surgical margins elsewhere in the specimen. The association of positive apical margins with PSA recurrence currently is not statistically significant however awaits greater follow-up interval (mean = 30 months) for conclusive determination.

Conclusions: Although the yield of intraoperative biopsy of apical margin is low, the high positive predictive value and accuracy of frozen diagnoses suggest that frozen section can be routinely performed with an accurate yield. The clinical impact of the intraoperative strategy awaits longer follow-up of the subset with positive apical margins.

876 Rete Testis Involvement in Testicular Germ Cell Tumors (TGCT): Pattern of Involvement Is Associated with Tumor Extension into Paratesticular Tissues

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Background: Rete testis consists of network of channels at the hilum of the testis. Hilum has previously been described as the most likely site for extratesticular extension in testicular germ cell tumors (TGCT). The relationship between rete testis involvement (RTI) and extratesticular extension into paratesticular tissues is not well established in (TGCT). The objective of the study is to examine the frequency and morphological patterns of RTI in TGCT and to investigate their association to paratesticular tissue extension (PTE).

Design: We studied a total of 225 consecutive orchiectomy specimens for malignant TGCT performed in our center between 2000-2005. Pathology slides and reports were reviewed in all cases. Status and pattern of RTI were examined and categorized into two major groups as pagetoid vs. direct. Pagetoid RTI was defined as pure pagetoid involvement of rete epithelium. Extension beyond pagetoid spread with invasion into the hilar stroma was defined as direct RTI. Extent of direct RTI was further subdivided into focal (minimal invasion of hilar stroma with only a few entrapped ducts) or established (more than focal). PTE was defined as tumor extension beyond the rete into the paratesticular tissues including connective tissues, epididymis and spermatic cord.

Results: Of 225 cases of TGCT histological status of rete testis could be assessed in 206 (91%) cases. Of those 127 (62%) showed RTI. Table 1 summarizes the patterns and frequency of RTI. PTE was identified in 45/206 (23%) cases and of those 91% (41/45) also showed RTI. Significant statistical correlation was found between RTI and PTE ($p<0.0001$). When data was further analyzed according to the patterns of RTI, only direct established RTI revealed significant statistical association with PTE ($p<0.0001$).

Table 1: Patterns and Frequency of RTI in TGCT:

	RTI Present	Direct	Pagetoid
All TGCT (n=206)	62% (127/206)	85% (108/127)	15% (19/127)
Seminoma (n=122)	61% (74/122)	93% (69/74)	7% (5/74)
Non-seminomatous (n=84)	63% (53/84)	74% (39/53)	26% (14/53)

Conclusions: This study demonstrates the significance of the pattern of RTI with respect to PTE. PTE strongly correlates with direct established RTI; no association is present

with pagetoid or direct focal pattern. Frequency of RTI is similar in all GCT and in its histological subtypes. Direct RTI is the most common pattern both seminomatous and non-seminomatous GCT.

877 Testicular Seminoma: Clinicopathological Study of 132 Cases

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Background: The objective of the study is to present single-center experience on testicular seminomas over a five-year period. We investigated the incidence of clinical and histopathological parameters, and correlated them to clinical stage at presentation.

Design: We studied a total of 235 consecutive orchiectomies for TGCT performed at our center between 2000 and 2005. Pure seminoma was identified in 132 (56%) cases. Pathology slides and reports were reviewed in all cases. Clinical and morphological features examined included; age, tumor size, intratubular germ cell neoplasia (ITGNU), presence of coagulative tumor necrosis, vascular invasion, rete testis invasion (RTI) and pattern (pagetoid vs. direct). Extratesticular tumor extension beyond tunica albuginea; into tunica vaginalis (TV), paratesticular tissues (hilar soft tissues or epididymis) and spermatic cord was recorded. Histological parameters were correlated with stage at presentation.

Results: Mean patient age was 39 years (20-67). Mean tumor size was 4.1cm (0.5-18). ITGNU identified in 82% (108/132). Vascular invasion was detected in 14% (18/132). RTI was seen in 61% of cases, (93% direct, and 7% pure pagetoid). Coagulative necrosis was present in 29% (38/132). Extratesticular extension was identified in 22% (29/132); of those, 96% (28/29) represented paratesticular soft tissue or epididymis extension (PTE). Spermatic cord was involved in 4% (5/132). TV invasion was seen in only one case. Clinical stage was obtained in majority of the patients 93% (123/132). In our cohort 91% of patients presented as stage I and 9% presented with stage II and III. 61% of stage I patients was initially managed by surveillance. Follow-up was obtained in 88% (116/132) of our cases with a mean follow-up of 39 months (1-87). Relapse rate in stage I patients was 10% and all were initially managed by surveillance. Tumor size and necrosis showed statistically significant association with stage at presentation (Fisher's Exact Test, p= 0.012, Student T test, p= 0.007, respectively). Overall and disease-free survival was 100% during the follow-up period.

Conclusions: Our study confirms the excellent prognosis in testicular seminomas. Presence of coagulative tumor necrosis and tumor size was the only histological parameters associated with clinical stage at presentation. PTE through the hilum is the most common site of extratesticular tumor extension in seminomas. TV invasion is a rare finding.

878 Residual Tumor Potentially Left Behind after Local Ablation Therapy in Prostate Adenocarcinoma

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Background: Unilateral cryosurgery has recently been applied as a new treatment of predicted clinically insignificant prostate cancer (PCA) on biopsy that is confined to one prostate lobe. However, PCA is recognized as a multifocal and bilateral disease with older studies showing on average 6 cancers per prostate. Whether in the modern screening era this data is still valid and to what extent multifocal contralateral tumors left behind by focal therapy may be clinically insignificant has not been addressed.

Design: We investigated 100 consecutive serially sectioned and completely embedded radical prostatectomies (RP) where the needle biopsy predicted limited disease in the resection specimen (<3 positive cores; ≤ 50% involvement of any positive core; Gleason score ≤ 6) and where all the positive needle cores were restricted to one side of the gland.

Results: The mean age was 57.6 and the mean preoperative PSA level was 5.5 ng/mL. The number of positive cores was 1 in 66 cases and 2 in 34. The mean percentage of tumor in each positive core was 13.9%. The mean number of separate tumor nodules per RP was 2.9. In 63 cases, there were tumor nodules in the RP on the opposite side to the biopsy (1 in 26 cases, 2 in 25, 3 in 10, and 4 in 2). The total tumor volume in the RP on the opposite side of the biopsy averaged 0.2 cm^3 (largest 1.3 cm^3). In 23 cases, the total tumor volume on the opposite side to the biopsy was larger than that on biopsy side. All together, 13 cases had clinically significant prostate cancer (>0.5cm^3) on the opposite side. Volume on the opposite side from the biopsy could not be predicted by the number of positive cores (1 vs. 2) or maximum percent of the core involved.

Conclusions: In a highly selected population of men with potentially insignificant PCA localized to one side of the gland on biopsy, there were still on average about 3 multifocal tumors per prostate. In the majority of cases, contralateral tumor was small where unilateral focal therapy could have still been warranted. However, in 13% of cases more significant cancer would have been left untreated in the contralateral lobe, despite negative biopsies from that side of the prostate. Urologists and patients must be aware of these risks prior to undergoing this experimental therapy.

879 Claudin-7 and Claudin-8: Immunohistochemical Markers for the Differential Diagnosis of Chromophobe Renal Cell Carcinoma and Oncocytoma

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Background: Claudin-7 (CLDN7) and claudin-8 (CLDN8) code for tight junction proteins expressed in distal nephron epithelium. In a recent oligonucleotide microarray study, we identified CLDN7 and CLDN8 as candidate markers to distinguish chromophobe renal cell carcinoma (RCC) from other renal tumors, including oncocytoma. Distinction of these lesions can be difficult by light microscopy, but is clinically important because chromophobe RCC has malignant biological potential, while renal oncocytoma is benign.

Design: CLDN7 and CLDN8 expression was studied by immunohistochemistry (IHC) in 11 chromophobe RCC and 17 oncocytoma, using formalin-fixed paraffin-embedded tissue sections of tumor with adjacent non-neoplastic kidney. Steam antigen retrieval was performed prior to IHC. Specificity was verified by negative control reactions without primary antibody, and appropriate membranous staining patterns in positive control tissues (colon carcinoma and adjacent non-neoplastic kidney).

Results: CLDN7 protein was expressed in a membranous pattern in 10/11 chromophobe RCC and 4/17 oncocytomas ($p < 0.01$). CLDN 8 was expressed in multiple patterns: In oncocytoma, 11/17 cases showed cytoplasmic, 4/17 membranous, and 2/17 negative reactions. In chromophobe RCC, 0/11 cases showed cytoplasmic, 3/11 membranous, and 8/11 negative reactions ($p < 0.01$). The IHC pattern of membranous CLDN7 and negative CLDN8 was seen in 7/11 chromophobe RCC and 1/17 oncocytoma (63% sensitivity, 84% specificity, 88% positive predictive value for chromophobe RCC). Negative CLDN7 and cytoplasmic CLDN8 were observed in 10/17 oncocytomas and 0/11 chromophobe RCC (59% sensitivity, 100% specificity and positive predictive value for oncocytoma).

Conclusions: The distal nephron proteins CLDN7 and CLDN8 have potential utility as IHC biomarkers in the differential diagnosis of chromophobe RCC and oncocytoma. Expression of CLDN7 and CLDN8 may reflect the relationship of chromophobe RCC and oncocytoma to intercalated cells of the cortical collecting duct. To improve diagnostic sensitivity and specificity, it may be necessary to identify additional biomarkers to include with CLDN7 and CLDN8 in a larger IHC panel.

880 PAX8: An Additional Marker for Kidney Tumors

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Background: The cell lineage determining transcription factors (TF) have specific (or limited) expression patterns in normal and neoplastic tissues, and have emerged as a group of valuable diagnostic markers for human tumors. Both PAX2 and PAX8 are two important TFs for the development of nephric lineage cells during embryonic stages. PAX2 has been shown to be a useful marker for kidney tumors and renal related lesions. However, little is known about the expression of PAX8 in kidney tumors. In this study, we used immunohistochemistry (IHC) to investigate the expression of PAX8 in various types of kidney tumors.

Design: Regular tissue sections or tissue microarray sections from 144 specimens of radical or partial nephrectomies were used, including 59 clear cell renal cell carcinomas (cRCC), 21 papillary RCC (pRCC), 11 chromophobe RCC (chRCC), 7 sarcomatous RCC (sarRCC), 23 oncocytomas, 21 urothelial carcinomas (UC) of the renal pelvis, and 2 renal medullary carcinomas (RMC). The uninvolved renal parenchyma of 15 nephrectomies, the renal pelves of 5 cases, 5 ureters, 5 urinary bladders, and 40 UC of the urinary bladder were used as control tissues. IHC of PAX8 (Proteintech group, Chicago) was performed using the avidin-biotin-peroxidase method with antigen retrieval. The nuclear staining of PAX8 was evaluated.

Results: PAX8 was detected in the epithelial cells of the proximal tubules, distal tubules, collecting ducts, renal papillae, and the parietal cells of the Bowman's capsule. PAX8 was also detected in scattered cells in mid or basal layers of the urothelium of the renal pelvis and the ureter, but not in the urothelium of the urinary bladder. PAX8 was detected in 98% (58/59) of cRCC, 71% (15/21) of pRCC, 82% (9/11) of chRCC, 71% (5/7) of sarRCC, 95% (21/22) of oncocytomas, and 100% (2/2) of RMC. Interestingly, PAX8 was detected in 19% (4/21) of UC of the renal pelvis, whereas none of the 40 UC of the urinary bladder was positive PAX8.

Conclusions: PAX8 is widely expressed in various types of renal tumors and may be used as an additional diagnostic marker. Expression of PAX8 in sarRCC and RMC supports their renal epithelial cell origin. In addition, detection of PAX8 in the normal urothelium and some UC of the renal pelvis reflects the ureteric bud origin of the epithelial cells in the renal pelvis and ureters, similar to renal tubular cells.

881 Utility of a Fluorescence In Situ Hybridization (FISH) Assay for *ERG* Gene Disruption in Prostate Needle Biopsies (PNBxs) with Prostatic Adenocarcinoma (CaP), Prostatic Intraepithelial Neoplasia (PIN), and Partial Glandular Atrophy (PGA)

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Background: Lower serum PSA thresholds for PNBx have led to more PNBxs with small foci of CaP, which can be difficult to distinguish from PGA and high-grade (HG) PIN. Fusion of *TMPRSS2* gene with transcription factor gene *ERG* due to intrachromosomal deletion and resulting in *ERG* disruption was recently identified as a common genetic event in CaP. The aims of our study are: 1) to determine the sensitivity and specificity of *ERG* disruption in CaP in PNBxs, 2) to determine the frequency and significance of *ERG* disruption in PIN and PGA, and 3) to identify variant *ERG* disruption patterns.

Design: Formalin-fixed, paraffin-embedded sections of 35 PNBxs from 27 pts (4 pts had multiple PNBxs) containing small foci of CaP (N=10), PGA (N=10), HG PIN (N=8), and LG PIN (N=7) were obtained from the University of Pittsburgh's case files. Dual-color FISH using an *ERG* break-apart probe was performed on targets identified on H&E sections. Cases were considered positive if >20% of targeted cells showed either translocation or unbalanced disruption of *ERG*. Immunostains for α-methylacyl CoA racemase (P504S) were performed on corresponding sections.

Results: *ERG* disruption was present in: CaP 9/10 (90%), HG PIN 3/8 (37%), LG PIN 2/7 (29%), and PGA 0/10 (0%). Of the pts with CaP and *ERG* disruption, 7 had Gleason score (GS) 3+3=6 and 2 had GS 3+4=7. All CaPs showed strong P504S reactivity. Of the 15 pts with LG or HG PIN, 4/8 with concurrent CaP had *ERG* disruption, compared to

1/7 without concurrent CaP. One pt with 3 LG PIN foci and 1 HG PIN focus had *ERG* disruption in single foci of LG PIN and HG PIN; the LG PIN focus had unbalanced *ERG* disruption in 59% of cells. Another pt with 2 HG PIN foci had 1 focus of *ERG* disruption and nondisrupted *ERG* hyperploidy.
Conclusions: *ERG* disruption is common in CaP and was not related to GS in our pts. *ERG* disruption distinguishes CaP from PGA, but not from PIN (although less common in PIN). PIN foci in pts with concurrent CaP have more *ERG* disruption. LG PIN with *ERG* disruption may indicate a higher risk of CaP in some pts. Classic translocation and unbalanced *ERG* disruption patterns were noted but the significance of these are unclear. We are in the process of assessing morphologically normal glands in PNBxs with CaP to determine if *ERG* disruption occurs in normal-appearing cells.

882 The Impact of ISUP 2005 Consensus on Gleason Grading in Contemporary Practice

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Background: International Society of Urological Pathology (ISUP) in 2005 attempted to achieve a consensus in the application of Gleason grading system in contemporary practice. We investigated how the ISUP consensus impacted the Gleason grading in a center with a large urological pathology practice.
Design: We compared the Gleason score (GS) distribution and the GS concordance on biopsy and radical prostatectomy (RP) in two patient cohorts (before and after the ISUP consensus) in our institution. Both cohorts had similar demographic, preoperative clinical, and RP characteristics. The first cohort consisted of 908 consecutive patients with matched biopsies and RP, performed from 07/2000 to 06/2004 in our institution, prior to the ISUP consensus. The second cohort consisted of 423 patients with matched biopsies and RPs, performed from 10/2005 to 06/2007, after the ISUP consensus. All biopsies and RPs were reported by one group of pathologists.
Results: GS distribution on biopsy and RP is shown in the table:

	Biopsy GS Distribution %			RP GS Distribution %		
Time Period	GS≤6	GS7	GS≥8	GS≤6	GS7	GS≥8
2000-2004	68	30	2	47	48	5
2005-2007	55	43	3	32	60	8

The ratio of GS 3+4 vs 4+3 for GS7 on biopsy and RP was similar in both cohorts. Biopsy GS 7 (3+4 vs 4+3): 24% vs 6% (2001-2004) and 35% vs 8% (2005-2007). RP GS 7 (3+4 vs 4+3): 39% vs 9% (2001-2004) and 48% vs 12% (2005-2007). Concordance data for primary Gleason, secondary Gleason, and overall GS, and concordance for GS ≤6, GS 7 and GS≥ 8 are as follows:

	Complete/ +/-1 Agreement %			Complete/+/-1 Agreement %		
Time Period	Primary Gleason	Secondary Gleason	Gleason Score	Gleason ≤6	Gleason =7	Gleason ≥8
2000-2004	90/99	62/99	63/98	59/99	76/96	48/95
2005-2007	87/99	59/98	65/96	53/98	82/93	64/82

Biopsy GS compared to RP GS were upgraded in 8% and 5% and downgraded in 29% and 30% in cohorts 2001-2004 and 2005-2007, respectively. The most common change from biopsy to RP in both patient cohorts occurred due to biopsy GS 6 upgrade to RP GS 7 (change in secondary grade from 3 to 4).
Conclusions: We document a trend for upgrading GS on both biopsy and RP in our practice after the ISUP consensus. The significance of this change for patient management and prognosis is uncertain. Although the overall GS concordance on biopsy and RP have not been significantly impacted by the ISUP consensus, the complete agreement for GS≥7 has improved after the ISUP consensus.

883 Lymphovascular Invasion in Micropapillary Urothelial Carcinoma, Diagnosis and Significance

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Background: Micropapillary carcinoma (MPC) is characterized by tight neoplastic cell clusters floating in lacunar spaces. A MPC component has been described in carcinomas of the breast, ovary, urinary bladder, lung, and colon, Its presence has been linked to a more aggressive behavior and a higher frequency of lymph node metastasis, the reasons for which, however, are not clear. This study aims to determine the nature of the lacunar spaces surrounding MPC cell nests; and to systematically evaluate lymphovascular invasion (LVI) in a large series of MPC of the urinary bladder.
Design: This study included 22 urothelial carcinoma (UC) with a MPC component accouting for at least 10% of tumor volume and 60 conventional Ucs. Consecutive tissue sections were immunostained for D2-40 (a lymphatic endothelial marker) and CD34 (a vascular endothelial marker). The findings related to the lacunae and LVI were tabulated and compared with routine sections.
Results: The lacunar spaces were almost uniformly negative for D2-40 or CD34, indicating that almost all of them are not lymphovascular channels. LVI was present in 19/22 (87%) UCs with an MPC component, but in only 17/60 (28%) conventional UCs. In UCs with MPC, LVI was noted not only in MPC areas, but also elsewhere. Within the MPC areas, LVI could only be detected by immunostain, even in retrospective examination of routinely stained consecutive sections.
Conclusions: The vast majority of the lacunae in MPC are not lymphovascular channels. Immunostain is essential for the diagnosis of LVI, especially in MPC areas, by highlighting lymphovascular channels containing tumor cells that may not be otherwise be appreciated on routine sections. LIV is much more frequent in UC with MPC component than in conventional UC, and this may in part account for its more aggressive behavior. Although LVI can be seen within or outside areas with MPC, this distinct growth pattern is a *morphologic marker* for tumor invasion into lymphatic or vasculat channels.

884 SIRT1 Expression Is Elevated in Human Prostate Cancer

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Background: Sirtuin-1 (SIRT1) is a mammalian homolog of the yeast Sir2 deacetylase among class III histone deacetylases that play a vital role in regulation of genome stability and expression. SIRT1 can directly interact with apoptotic proteins, including p53, BAX, and members of the FOXO family of transcription factors. Recent studies suggest that the antiapoptotic effects of SIRT1 may paradoxically increase the risk of cancer development and the targeted inhibition of SIRT1 can effectively limit cancer progression. In cancer cell lines and genetically modified mice, high expression of SIRT1 in human tumors (e.g. breast, colon, lung, and skin) was also reported. Our recent studies on prostate cancer cell lines showed in vitro evidence that SIRT1 may function as a transcriptional co-repressor of the prostate specific antigen promoter and promote prostate tumorigenesis during aging. The present study further investigated SIRT1 expression in human prostate cancer and its precursors.
Design: The SIRT1 immunostain was carried out in human prostate cancer tissue microarray that consisted of prostate cancer (PCa, 107 cases), high-grade prostatic intraepithelial neoplasm (HGPIN, 3 cases), and benign prostate tissue (BPT, 42 cases). PCa cases were divided into 5 groups according to Gleason scores. Groups 1-5 had Gleason scores 6 (n=28), 7 (n=12), 8 (n=42), 9 (n=9), and 10 (n=16), respectively. Fisher's exact and nonparametric correlation tests were used for statistical analysis.
Results: SIRT1 immunoreactivity significantly increased among PCa (88%, 94/107) and HGPIN (100%, 3/3) cases, compared to that in BPT (29%, 12/42). Among the immunopositive PCa cases, SIRT1 expression was observed in 68% (19/28), 100% (12/12), 93% (39/42), 89% (8/9), and 100% (16/16) of cases for Groups 1 through 5, respectively, with significant differences in immunoreactivity among the groups ($p<0.007$); in addition, SIRT1 immunoreactivity and Gleason scores were significantly correlated ($r = 0.29$; $P = 0.002$).
Conclusions: SIRT1 expression was significantly increased in human PCa and HGPIN and correlated with Gleason scores. These results suggest that SIRT1 may function as a tumor promoter in human PCa and its precursor and could be considered as a potential therapeutic target for PCa.

885 Microscopic Bladder Neck Involvement in Radical Prostatectomy Specimens: "True" or "False", and Does It Matter?

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Background: The independent prognostic significance of microscopic bladder neck involvement (BN+) by prostate cancer (PCA) in radical prostatectomy (RP) is questionable. Recent studies suggest that BN+ should not be classified as pT4 disease. Such controversy could be partially attributed to lack of clear definition of BN+. We studied a cohort of patients whom underwent RP to determine the significance of microscopic BN+.
Design: Our study group included 2,103 patients treated with RP as monotherapy between 1996 and 2005. Median age and followup was 59 years and 50.0 months, respectively. The BN has been coned from the RP and sectioned perpendicularly. BN+ was defined as PCA present within the coned BN. For this study, we further categorized the BN+ cases as "true BN+" and "false BN+". True BN+ required PCA within thick smooth muscle bundles of the coned BN without intermixed benign prostatic glands (BPT). False BN+ was characterized by PCA within smooth muscle bundles intermixed with BPT. BN+ was analyzed in relation to serum preoperative prostate-specific antigen (iPSA) level (continuous variable), and extraprostatic extension (EPE), seminal vesicle (SV) involvement, surgical margin status (SMS) and lymph node (LN) involvement (negative vs. positive).
Results: Of 2,103 patients, 90 (4.3%) were initially categorized as BN+. Those cases were further classified as true BN+ and false BN+ (63 and 27 cases, respectively). In univariate model, both BN+ (relative risk [RR] 2.34) and true BN+ (RR 2.54) was significantly associated with increased PSA-recurrence risk compared to BN negative cases ($p<0.001$), while false BN+ was not ($p=0.095$). The PSA-recurrence RR associated with EPE, SV, SMS, LN, and iPSA was 0.25, 0.15, 0.27, 0.11, and 1.07, respectively ($p<0.0001$). In multivariable model controlling for iPSA, EPE, SV, LN, and SMS, the PSA-recurrence risk associated with BN+ (true or false) was not a significant independent prognostic factor. EPE (RR 0.44, $p<0.001$), SV (RR 0.43, $p<0.001$), SMS (RR 0.36, $p<0.001$), LN (RR 0.35, $p<0.001$), and iPSA (RR 1.0, $p<0.001$) were significant independent predictors of PSA recurrence.
Conclusions: True BN+ required the presence of PCA within thick smooth muscle bundles of the coned BN without intermixed BPT. BN+ (true or false) was not an independent predictor of PSA-recurrence. In view of the data available, the staging system for bladder neck involvement should be revised.

886 Cystic Nephroma and Mixed Epitehlial and Stromal Tumor of the Kidney Are Related Lesions: Molecular and Histological Evidence

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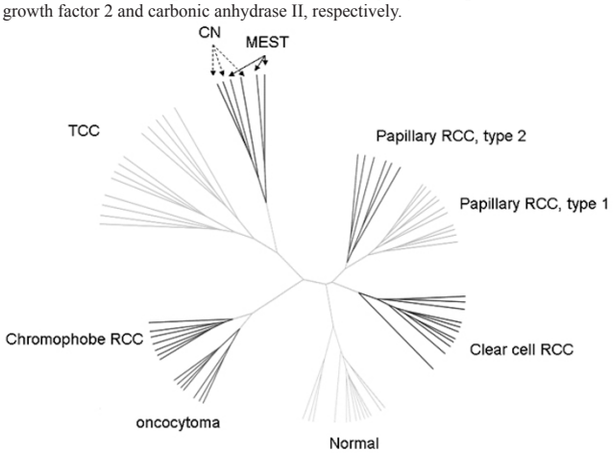
Background: Cystic nephroma (CN) and mixed epithelial and stromal tumor of the kidney (MEST) are both biphasic tumors composed of epithelial and stromal elements. They are considered as separate entities by the 2004 WHO classification of the renal neoplasms. This report studied the relationship between these two lesions using gene profiling and histological analysis.
Design: CN was defined as a multilocular cystic mass without gross solid area and cystic septa< 5 mm microscopically. MEST was cystic or partially cystic mass with solid area on gross examination and/or cystic septa >=5mm microscopically. Frozen samples of 3 CN and 3 MEST were analyzed for gene expression profile using HGU133 Plus 2.0 GeneChips (Affimatrix). The gene expression profiles of these cases were compared

to selected 48 cases of renal carcinomas of various subtypes, 7 oncocytomas and 13 normal kidney. Histological examination focused on the presence of small branching glands, stromal cellularity, ovarian-type stroma, prominent vessels and estrogen and progesterone receptors.

Results: This study included 26 CN and 13 MEST. Patient' age (53.2 vs 53.8 years) and tumor size (6.5 vs 6.8 cm) were similar between CN and MEST. CN predominantly affected women (male/female=2/24), while MEST exclusively affected female. CN and MEST had many similar histological features, including size of cysts, stromal cellularity, presence of ovarian-type stroma, calcification and hemorrhage. ER and PR were positive in 4/5 (80%) and 3/5 (60%) CN, and 5/8 (62.5%) and 5/7 (71.4%) MEST. However, prominent vessels (4/13, 30.8%) and small branching glands (53.8%) were exclusively seen in MEST (p=0.000). Clustering analysis demonstrated that CN and MEST had very similar molecular profiles (Figure 1) that were distinct from other renal tumors. Of differentially expressed genes that best distinguish CN/MEST from other subtypes of kidney tumors, the highest and lowest differentially expressed genes are insulin-like growth factor 2 and carbonic anhydrase II, respectively.



Conclusions: CN and MEST are related lesions as they share similar clinical, histological and molecular characteristics.

887 Choriocarcinoma in the Testis: A Clinicopathological Study of 65 Cases

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Background: Choriocarcinoma is a rare germ cell tumor of the testis. Previous studies have been based on small series or case reports. We aim to study pathological and clinical features in a large series of testicular choriocarcinoma.

Design: We searched our pathology files from 1986 to 2007 and identified 65 patients with choriocarcinoma involving the testis. The H and E slides and clinicopathological data including follow-up were reviewed.

Results: The average age of the patients was 29.5 years (range: 14-71). The mean tumor size was 4.5 cm (range 1.0 to 9.0 cm). Only five of these cases were pure choriocarcinoma, the other 60 cases had choriocarcinoma mixed with other germ cell tumors types. The associated germ cell tumors included embryonal carcinoma (n=48), teratoma (n=42), yolk sac tumor (n=40) and seminoma (n=16). Thirty-one tumors had lymphovascular invasion (LVI). Seventeen tumors did not exhibit any LVI. Based on the percentage of choriocarcinoma in the tumor, the tumors were divided into four arbitrary groups: group I: 0-5% (30); group II: 6-20% (13); group III: 21-50% (10); and, group IV: 51-100% (12). The mean pre-operative β-human chorionic gonadotropin (β-hCG) levels were 2919, 8679, 48366, 244394 (mIU/ml) in Groups I-IV, respectively. Clinical follow-up was available for 49 patients with an average of 39.6 months (range: 1 to 192 months). In cases where choriocarcinoma was less than 50% (Groups I to III), 20 of 40 patients developed distant metastasis. In cases where choriocarcinoma accounted for more than 50% (Group IV), 8 of 9 patients developed distant metastasis. Four patients died of disease at a mean of 18 months (range: 12 to 36); all patients had distant metastases, but the choriocarcinoma component accounted for less than 10% of the total tumor volume in these patients.

Conclusions: Choriocarcinoma is often present as a minor component (< 5%) in germ cell tumors. High volume of choriocarcinoma (>50%) in mixed germ cell tumors is associated with high β-hCG level and increased propensity for distant metastasis. However, in our series the four patients who died of disease had a low volume of choriocarcinoma.

Gynecologic

888 Overexpression of Heat Shock Protein (HSP27, HSP70 and HSP90) in Uterine Papillary Serous Carcinoma (UPSC)

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Background: Heat-shock protein (HSP) group is overexpressed in several malignancies and has been studied more extensively in uterine endometrioid adenocarcinomas (EC) than in uterine papillary serous carcinomas (UPSC). Expression of HSP70 correlates with poor prognosis while HSP90 expression is seen in well differentiated EC. This study evaluates HSP expression HSP27, HSP70 and HSP90 in UPSC.

Design: We identified cases diagnosed as UPSC or mixed EC and UPSC from 1996-2006. We included 7 EC and 6 clear cell carcinoma (CCC) as controls. 17 cases consisted predominantly of UPSC with only 1 mixed EC and UPSC. Immunohistochemical stains were performed using monoclonal antibodies to HSP27 (Abcam), HSP70 (Abcam) and HSP90 (Abcam) in all 31 cases. Staining was graded based on the intensity and percentage of tumor cells positive as negative (0), 1+, 2+ or 3+.

Results: Immunohistochemical stain results for HSP27, HSP70 and HSP90 are summarized in table 1.

TABLE1

Immunostains	Stain intensity	Uterine papillary serous carcinoma (UPSC)	Clear cell carcinoma	Endometrioid carcinoma
HSP27	0	1(5.6%)	1(16.6%)	0(0%)
HSP27	1	1(5.6%)	0(0%)	1(14.3%)
HSP27	2	6(33.3%)	0(0%)	3(42.9%)
HSP27	3	10(55.6%)	5(83.3%)	3(42.9%)
HSP70	0	0(0%)	0(0%)	0(0%)
HSP70	1	0(0%)	0(0%)	0(0%)
HSP70	2	0(0%)	0(0%)	0(0%)
HSP70	3	18(100%)	6(100%)	7(100%)
HSP90	0	2(11.1%)	0(0%)	3(42.9%)
HSP90	1	8(44.4%)	2(33.3%)	1(14.3%)
HSP90	2	8(44.4%)	4(66.7%)	3(42.9%)
HSP90	3	0(0%)	0(0%)	0(0%)

HSP27 was positive in 17 of 18 UPSC (94.4%); 5 of 6 CCC (83.3%), and 7 of 7 EC (100%). HSP70 was strongly positive in all cases of UPSC, EC and CCC. HSP90 showed mostly 1-2+ positivity in 16 of 18 UPSC (89%), 4 of 7 EC (57%) and 6 of 6 CCC (100%).

Conclusions: Our study found overexpression of HSP27 and HSP70 and comparatively weak HSP90 staining in UPSC similar to that reported in poorly differentiated endometrial carcinomas. The overexpression of HSPs in UPSC could be a potential target for therapy with anti-HSP inhibitors.

889 Serous and Endometrioid Components of Mixed Endometrial Adenocarcinoma Show Similar Immunostaining Profiles

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Background: Mixed endometrial adenocarcinoma is a tumor with two or more cell types present with each comprise at least 10% of the tumor. Mixed adenocarcinomas account for approximately 10% of cases of endometrial adenocarcinoma.

Design: To determine whether there are consistent differences in immunostaining between the endometrioid and serous components of mixed endometrial adenocarcinoma, and whether the components of mixed carcinomas are similar to pure endometrioid or serous carcinoma, ten cases of mixed endometrial adenocarcinoma with endometrioid and serous components were stained for *PTEN*, ER, p53, and ki-67. The results were compared to the staining of pure endometrioid (n=139) and serous (n=11) carcinomas of the endometrium.

Results:

Table 1- staining of endometrioid (EM) and serous(S) components of mixed endometrioid adenocarcinoma

Case	ER		P53		PTEN		Ki-67	
	EM	S	EM	S	EM	S	EM	S
1	2	2	+	+	-	-	62%	64%
2	0	0	-	-	-	-	18%	72%
3	0	0	-	-	-	-	35%	58%
4	2	0	-	+	-	-	33%	55%
5	1	0	-	+	-	-	52%	42%
6	1	1	-	-	+	+	28%	56%
7	2	0	-	-	-	-	35%	61%
8	2	0	-	-	-	-	25%	49%
9	0	0	-	+	+	+	26%	62%
10	0	0	+	+	+	+	50%	44%

PTEN immunostaining was significantly less common in the serous component of mixed endometrial adenocarcinoma (3/10) than in pure serous adenocarcinoma (10/11, p=0.007). The staining of the endometrioid and serous components of mixed adenocarcinomas were otherwise not signficantly different than pure endmetrioid and serous carcinomas, respectively.

Conclusions: The immunophenotype of endometrioid and serous components of mixed endometrial adenocarcinoma are similar in most cases, with the notable exception of high proliferative index being characteristic of the serous component. The loss of PTEN staining in the serous component is significantly different from pure serous carcinoma, and similar to that of pure endometrioid carcinoma, where loss of PTEN is a common early event during. This suggests that the serous component of mixed endometrial adenocarcinoma arises through progression from the endometrioid component.

890 Clinicopathologic Characterization of Myxoid Mesenchymal Neoplasms of the Uterus

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Background: Relatively few data exist on myxoid neoplasms of the uterus, and diagnostic criteria suggested for myxoid leiomyosarcomas are based on relatively small series. Little immunophenotypic data exist on these neoplasms.

Design: In this study, we evaluated 30 uterine myxoid neoplasms variably designated as myxoid leiomyomas, smooth muscle tumors, or leiomyosarcomas. Sixteen otherwise typical leiomyomas with minute myxoid foci and 7 inflammatory myofibroblastic