SLN in these 13 pts. Separate SLNM away from the tumor site in 37 pts yielded 40 non-tumor related SLN; again, none had identifiable epithelial cells. Of 212 pts invasive tumor undergoing SLNM, 15 pts had 19 SLN positive for micrometastatic tumor less than 2 mm identified by H+E stain, while13 pts had 16 SLN positive for isolated tumor clusters seen only by IHC. Thus, in summary, for 50 total pts with SLNM either away from the main tumor or without invasive cancer, epithelial cells were absent in 79 SLN examined by MLS and IHC. In contrast, in 212 pts with invasive colorectal cancer who underwent SLNM at the primary tumor site, 28 pts had 35 nodes positive by IHC only or with micrometastasis (<2 mm). The difference between these groups is significant (Chi square; $p \leq 0.025$ comparing either pts or nodes).
Conclusions: SLNM alone does not induce BMT. While BMT in pts only with invasive tumor is not completely excluded, the rate of such passive transfer would be low. In summary, BMT is not a significant factor in SLNM of colorectal cancers.

572 Intraepithelial Lymphocytosis with Normal Villous Architecture in Proximal Small Bowel of Morbidly Obese Patients

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Background: Intraepithelial lymphocytosis with normal villous architecture (IELNVA) in the proximal small bowel may be seen in a variety of conditions besides gluten-sensitive enteropathy, including tropical sprue, food allergy, primary immunodeficiency, H. pylori gastritis, viral enteritis, Giardiasis, blind loop syndrome, Crohn's disease, autoimmune diseases, NSAIDs and irritable bowel syndrome. Morbid obesity is an increasingly common condition in Western cultures that is often treated surgically. One popular approach is the laparascopic roux-en-y gastric bypass, which entails removing a short segment of proximal jejunum after stapling the distal enteroenteric anastomosis. We have frequently observed IELNVA in these surgical specimens.
Design: We searched our surgical pathology database for patients who had jejunum removed as part of their gastric bypass surgery for morbid obesity (MO) in 2004-2005. Based on information from the treating surgeons, only one of these patients was taking any medications (NSAIDs) and another patient had irregular bowel movements. A single H+E slide from each specimen was reviewed and T-lymphocytes were stained for CD3. As controls, a similar number of Whipple procedures were similarly evaluated. The number of CD3-positive intraepithelial lymphocytes (IELs) per 100 epithelial cells was counted in three separate villi and the distribution was assessed for base-predominance, tip predominance or even distribution, as well as for epithelial sublocalization (subnuclear, internuclear or both).
Results: Slides from 16 patients who underwent gastric bypass surgery and 15 controls were evaluated. Eleven (69%) in the MO group had IEL counts >25. Of these, 6 (38%) had counts of 40-60 and 4 (25%) had counts >60. The IELs were evenly distributed throughout the villi. Most of the IELs were subnuclear. In the control group, 13 (87%) had counts <25 and 2 (13%) had counts of 25-40 (p <0.01).
Conclusions: IELNVA is frequently encountered in the proximal small bowel of a subset of morbidly obese patients. The etiology is uncertain but warrants further investigation. Its elucidation may contribute to our understanding of the link between inflammation and morbid obesity.

573 Barrett's/Cardiac High Grade Dysplasia Is Not a Strong Marker for Concurrent Carcinoma, Unless Architectural Changes Suspicious for Adenocarcinoma Are Also Present

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Background: Recommendations for therapy of high grade dysplasia (HGD) in Barrett's esophagus are based on the historical finding of carcinoma (CA) in 30-50% of esophagi resected for HGD, implicating HGD as a marker for concurrent CA. This implication was confirmed recently, but based on biopsies that were not reviewed. Studies of conservative management of HGD have found CA developing in about 15%—lower than expected based on resection data. This study was undertaken to ascertain the prevalence of CA in esophagi resected for HGD using current diagnostic thresholds, and to evaluate histologic features in HGD that may be predictive of concurrent CA.
Design: 127 esophagectomies performed for columnar HGD, or high grade dysplasia suspicious for carcinoma (HGD/S), were studied. The corresponding biopsies in 69 cases were reviewed and reclassified as either negative for dysplasia, low grade dysplasia, HGD, HGD/S or CA by consensus of a group of gastrointestinal pathologists using established criteria, and a set of architectural features, including complexity, necrotic debris in tubules, and irregular dilated tubules, to classify biopsies as HGD/S.
Results: Based on original diagnoses, CA was present in the resection specimen of 16.9% (15/89) patients with HGD, and 73.7% (28/38) of those with HGD/S. Based on reclassification of 69 cases, only one of 21 (4.8%) patients with HGD on biopsy was found to have carcinoma in the subsequent esophagectomy specimen, and this carcinoma was only intramucosal. Of the 25 patients whose biopsies were reclassified as HGD/S, 18 (72%) had carcinoma in the resection specimen, as did 73.9% (17/23) of patients whose biopsies were reclassified as adenocarcinoma.
Conclusions: Based on current criteria, fewer than 10% of esophagectomies for Barrett's HGD harbor CA. In contrast, when HGD/S is diagnosed based on architectural features, CA is found in nearly 75%, comparable to the rate in patients with diagnoses of CA. Management guidelines for patients with Barrett's HGD should be based on up to date resection and follow up data. The lower rate of concurrent CA in patients with Barrett's HGD, and our reclassification of biopsies from HGD to HGD/S or CA highlights the evolution of diagnostic criteria for Barrett's dysplasia that has resulted from experience with these cases and their outcomes.

Genitourinary

574 Plasmacytoid Urothelial Carcinoma of the Urinary Bladder

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Background: Plasmacytoid urothelial carcinoma (PUC) is a rare tumor of the urinary bladder. Its clinical and histopathological features have not yet been characterized. In this study, we report six cases of PUC at one instituition.
Design: Cases of invasive urothelial carcinomas (UC) at the Ottawa Hospital over a period of 7 years were reviewed. Those cases with plasmacytoid features were selected for review of the slides, along with the clinical history. Representaive sections from each case were submitted for immunohistochemical studies.
Results: There were total of seven cases of PUC among 260 cases of invasive UC. In five cases, common UC was either absent, or present in focal areas. In the remaining two cases, common UC formed the predominant component. Cases with extensive PUC showed coarse mucosal foldings and indurations of the thickened bladder walls, with no grossly identifiable tumor. Catheterized urine showed scanty number of atypical single cells, frequently without tumor diathesis, leading to a shortfall in the positive cytological diagnosis. Histologically, PUC appeared as dyshesive, plasmacytoid cells with eccentric nuclei, extending widely into the bladder walls and extensively into adjacent pelvic organs. The diagnosis of the entity in biopsy was based on the presence of urothelial carcinoma in situ, and by immunocytochemistry typical of UC. The typical clinical presentation for PUC included advanced disease at presentation with the absence of hematuria, late onset of lower urinary tract symptoms, indurated mucosal surface on endoscopy, and radiographic findings of bladder wall thickening and hydronephrosis. The disease followed an ominous course with recurrence in all the patients, and death in 6 patients.
Conclusions: PUC is a distinct clinical and pathological entity of urothelial carcinoma, unique by its frequently late clinical presentation, plamacytoid appearance and poor clinical outcome.

575 Separation of Chromophobe Renal Cell Carcinoma from Oncocytoma and Clear Cell Carcinoma: An Optimal Immunohistochemical Panel for Differential Diagnosis

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Background: The separation of chromophobe renal cell carcinoma (chromophobe RCC), oncocytoma, and conventional (clear cell) renal cell carcinoma (clear cell RCC) using light microscopy remains problematic. The aim of this study was to determine a useful immunohistochemical panel for the differential diagnosis of chromophobe RCC.
Design: After reviewing the literature, we chose a panel of markers for the differential diagnosis of chromophobe RCC, including vimentin, GST-alpha, CD10, CD117, CK7, and EpCam. We employed the tissue microarray technique to study the immunohistochemical profile for these markers in 20 chromophobe RCCs, 11 oncocytomas and 36 clear cell RCCs
Results: We found that vimentin and GST-alpha expression were exclusively observed in clear cell RCC. CD10 staining was more frequently detected in clear cell RCC (92%, 33/36) than in chromophobe RCC (45%, 9/20) and oncocytoma (27%, 3/11). CD117 was preferentially expressed in chromophobe RCC (80% of cases, 16/20) and oncocytoma (100 %, 11/11), whereas none of the cases of clear cell RCCs was immunoreactive. Diffuse CK7 staining was observed in 17 of 20 chromophobe RCCs (85%) while focal staining was noted in the remaining 3 cases (15%). All CK7-positive oncocytomas (64%, 7/11) showed only single scattered cell positivity. EpCam protein was diffusely expressed in all 20 chromophobe RCC (100%) whereas all EpCam-positive oncocytomas (82%, 9/11) displayed only scattered and focal positivity in single cells or small cell clusters. EpCam showed diffuse staining in 6 of 36 clear cell RCCs (17%) and focal staining in 8 of 36 clear cell RCCs (22%). Using the combination of three markers (vimentin, GST, and EpCam), we achieved 100% sensitivity and 100% specificity for the differential diagnosis of chromophobe RCC, oncocytoma, and clear cell RCC. The pattern of "Vimentin-/GST-" effectively excludes clear cell RCC, and diffuse EpCam expression confirms the diagnosis of chromophobe RCC rather than oncocytoma. CD117 and CK7 were also useful markers and could be used as second line markers for the differential diagnosis, with high specificity (100%) and high sensitivity (87%, 85%).
Conclusions: We identified three markers (vimentin, GST, and EpCam) for the differential diagnosis of chromophobe RCC, oncocytoma, and clear cell RCC. These markers appear to provide complete accuracy in separation of these renal neoplasms.

576 Clusterin Expression Differentiates Wild Type-*VHL* from *VHL*-Defective Sporadic Clear Cell Renal Cell Carcinomas

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Background: Approximately 70% of sporadic clear cell renal cell carcinomas (CCRCC) show somatic inactivation of both Von Hippel Lindau (*VHL*) gene alleles. The most well characterized function of *VHL* is its ability to regulate HIF-2α (hypoxia inducible factor). Clusterin is a ubiquitously expressed glycoprotein known to regulate apoptosis. Recently, our group showed that clusterin expression is decreased in CCRCC cell lines that lacked wild type *VHL,* including a line transfected to express Type 2C pVHL which is associated with normal HIF function and no increased risk for CCRCC. The results suggest that clusterin may serve as a marker for HIF-independent *VHL* activity.

We correlated the pattern of clusterin protein expression in sporadic *VHL*-mutant and wild-type *VHL* CCRCC with the findings obtained *in vitro*. We also characterized the *VHL* alterations observed in sporadic CCRCC.

Design: The specimens were obtained from the archives of Brigham and Women's Hospital and Kyoto University Hospital. *VHL* genotyping of 63 CCRCC was performed on freshly frozen tumors by PCR and *VHL* hypermethylation assays. Immunohistochemical analysis with anti-clusterin antibody (clone 41D) was performed on formalin-fixed and paraffin-embedded tissues. The cytoplasmic immunoreactivity in ≤ or >10% of cells was assessed. Statistical significance was determined by the Fisher's exact test.

Results: Thirty-four out of 63 tumors had *VHL* alterations (33 mutations and 1 hypermethylation). Frameshift mutations accounted for 66.6 % (22) and mutations leading to amino acid (AA) substitution for 27.2 % (9) of the cases. Of the latter, four each occurred in exons 1 and 2, and one in exon 3. Splice site errors were seen in three cases. Histologically, *VHL* mutant tumors displayed a profound decrease in clusterin expression relative to wild-type *VHL* ($p<0.0001$): 91.1% (31 out of 34) of *VHL* mutants showed staining in ≤ 10% of cells; 79.3% (23 out of 29) of WT-*VHLs* were positive in >10% of tumor cells (37.8% positivity >50% cells).

Conclusions: The results obstained *in vivo* mirrored those noted in cell culture with respect to clusterin expression. We show data supporting clusterin as a potential biomarker for HIF-independent *VHL* function. Importantly, clusterin expression may have prognostic implications since *VHL* alterations have been described as an independent prognostic factor in sporadic CCRCC.

577 Alpha-Methylacyl Coenzyme A Racemase Immunoreactivity in Partial Atrophy of the Prostate

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Background: Partial atrophy (PA) is a variant of benign prostatic glandular atrophy with features mimicking prostate cancer. In addition, partial atrophy may show variable numbers of basal cells, adding to the diagnostic dilemma. Recently, Herawi et al reported 15 of 19 cases of partial atrophy seen in consultation were positive for alpha-methylacyl coenzyme A racemase (AMACR) (*Am J Surg Pathol.* 2005;29:874). However, the cases were highly selected consultation cases with variable AMACR staining methods from different institutions. The purpose of the current study was to investigate AMACR immunostaining in a large series of 120 foci of partial atrophy with standard AMACR staining from a single institution.

Design: One hundred-twenty foci of partial atrophy on needle core biopsy specimens from 61 patients were retrieved from the archived files at one institution. Immunohistochemistry for p63, high molecular weight keratin (34betaE12), and AMACR was performed with the PIN-4 antibody cocktail (Biocare). AMACR staining intensity was scored from 0 to 3+ and the percentage of positive glands was estimated in partial atrophy (n=120), adjacent benign glands (n=120), and prostatic carcinomas (n=28). In addition, the percentage of basal cells in partial atrophy stained with p63/34betaE12 was evaluated.

Results: Fifty-eight of 120 foci of partial atrophy showed AMACR immunoreactivity. Typically the AMACR staining in these cases was weak or moderate with only 6 of 120 foci showing strong diffuse expression. Overall, the mean AMACR immunostaining intensity of partial atrophy was similar to that of adjacent benign glands but less than that of adenocarcinoma. Furthermore, 80 of 120 foci of PA showed patchy distribution of basal cells, with 34 of 120 foci having scattered clusters of completely negative glands.

Conclusions: Our study demonstrates partial atrophy may show mild to moderate AMACR immunoreactivity using the PIN-4 staining method. Compounding this problem, focal lack of basal cells may also be seen. However, the AMACR staining pattern of partial atrophy is usually focal, weak and comparable in intensity to adjacent benign glands. Our study indicates caution when interpreting AMACR and basal cell stains in partial atrophy on prostate needle biopsy. It is essential to compare AMACR immunostaining in partial atrophy with that of adjacent benign glands in conjunction with thorough histologic evaluation.

	AMACR Expression		
	PA	Benign glands	CA
PA w/ CA, n=28	0.90+/-1.18	0.79+/-1.12	2.65+/-0.61
PA w/o CA, n=92	0.91+/-1.05	0.80+/-1.06	

578 Anterior Prostatic Tumors: A Morphologic Analysis

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Background: Aggressive screening and prostate needle biopsy protocols have been successful in early detection of low-volume posterior tumors. Consequently, we have observed an increased incidence of anterior-dominant prostate cancers (AT). Zone of origin, pattern of spread, and pattern of extraprostatic extension of this important group of tumors have not been well studied.

Design: We identified 52 patients with dominant (largest) tumors anterior to the urethra in whole-mounted radical prostatectomy specimens and studied their histopathologic features.

Results: Of the 52 AT, 28 (53.9%) were located in the peripheral zone (PZ), 23 (44.2%) in the transition zone (TZ), and 1 (1.9%) was of undetermined zone. **PZ tumors:** 15/28 (53.6%) were Gleason score (GS) 6 and 13/28 (46.4%) were GS 7; 24/28 (85.7%) were located predominantly in the apical portion of the gland; 15/28 (53.6%) involved anterior fibromuscular stroma (AFMS); 21/28 (75%) were organ confined (OC) and 3/28 (10.7%) had extraprostatic extension (EPE), with invasion of anterior adipose tissue; one of the EPE cases and one additional case had a positive anterior surgical margin (M+); 18/28 (64.3%) invaded the apex with 3 cases showing apical M+; 25/28 (89.3%) showed additional PZ tumors, 1 of which had EPE. **TZ tumors:** 11/23 (47.8%) were GS 6 and 12/23 (52.2%) were GS 7; 22/23 (95.7%) were located predominantly in the apical portion of the gland; 18/23 (78.3%) involved AFMS; 19/23 (82.6%) were OC and 2/23 (8.7%) showed extraprostatic extension (EPE), with invasion of anterior adipose tissue (2/23) or bladder neck (1/23); 18/23 (78.3%) invaded the apex, with one tumor showing apical M+; 19/23 (82.6%) showed additional PZ tumors, 1 of which was present at a posterior inked margin, without EPE. The 1 tumor of undetermined zone was located apically, situated in the AFMS without a clear relationship to either TZ or PZ, and OC; it was associated with an additional PZ tumor which was GS 6.

Conclusions: PZ tumors, conventionally considered posterior-predominant, are more prevalent than TZ tumors in the anterior prostate. Unlike previous studies comparing TZ tumors with posterior PZ tumors, AT of both zones show similar apical location and GS. Anterior PZ tumors tend to show EPE/M+, while TZ tumors more commonly invade AFMS/prostatic apex. Tumor extension into anterior adipose tissue may represent definitive EPE in the anterior prostate, where no clear 'capsule' is present. Prostates with predominant AT frequently contain additional PZ tumors which may occasionally impact on stage.

579 Hypoxia Response Pathway Markers in Clear Cell (Conventional) Renal Cell Carcinoma: A Tissue Microarray Based Immunohistochemical Study

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Background: The majority of clear cell (conventional) renal carcinomas (CRCC) display genetic abnormality in *VHL* gene pathway. A mutated, non-functional VHL complex results in accumulation and over-expression of hypoxia inducible factor HIF1-α. This leads to over-expression of a number of downstream factors including vascular endothelial growth factor (VEGF), glucose transporter1 (GLUT1), and carbonic anhydrase IX (CA9), some of which may have diagnostic, therapeutic and prognostic applications in CRCC. These hypoxia response pathway markers have not been adequately studied together in CRCC.

Design: Ninety eight cases of CRCC with a spectrum of nuclear grades (NG) were assembled in a tissue microarray block. Immunohistochemistry was performed using antibodies against HIF1-α, CA9, VEGF, and GLUT1. Semiquantitative analyses were performed and the staining was graded from 0 to 3+ based on the percentage of immunoreactive cells (0, no staining; 1+, 1-25% positive cells; 2+; 26-50%; and 3+, >50%). Only cytoplasmic expression of CA9, GLUT1 and VEGF and nuclear expression of HIF1-α were considered positive.

Results: Detailed results are shown in the tables below. All 4 markers were expressed together in only 38 (40%) of the tumors, with CA9 being the most commonly expressed marker (93% of tumors). Of 98 tumors, 54 (55%) showed similar (± 1) staining grades for HIF1-α, CA9 and GLUT1, with VEGF being the outlier in most cases. The other 46% of cases showed variable staining (2 or more grade difference) for these 3 markers.

Staining Grades for the Immunohistochemical Markers

	Staining Grade (%)			
Antibody	0	1+	2+	3+
HIF1-α (n=98)	23 (23)	15 (15)	11 (11)	49 (50)
CA9 (n=96)	2 (2)	1 (1)	4 (4)	89 (93)
GLUT1 (n=98)	17 (17)	23 (23)	14 (14)	44 (45)
VEGF (n=92)	45 (50)	6 (7)	16 (17)	24 (26)

Staining for CA9 and VEGF, but not for HIF1-α and GLUT1, showed significant association with tumor NG ($p=0.04$ and 0.005, respectively).

Conclusions: Overall, the majority of CRCC express HIF1-α, CA9 and GLUT-1. These markers are more likely to be co-expressed than to be differentially expressed. The marked difference in expression of these hypoxia-response pathway markers in a significant proportion of cases indicates that, although upregulation of HIF1-α may be a central factor, it is neither necessarily required, nor does it always result in over-expression of downstream hypoxia-related markers. Among these markers, CA9 is the most consistently expressed in CRCC and its expression shows significant correlation with NG.

580 Prostatic Involvement by Urothelial and Prostatic Carcinoma: A Clinicopathological Analysis of 120 Consecutive Cystoprostatectomies

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Background: The prostate is not infrequently involved by urothelial carcinoma (UC) in patients with bladder cancer, which influences pathologic stage as well as treatment options. Prostatic apical sparing is desirable at time of continent diversion in order to safeguard urinary continence. Prostatic carcinoma (PC) discovered at the time of cystoprostatectomy for UC is common with a substantial number being incidental. The presence of prostatic involvement by UC, its location and its association with PC, has not been well studied. We studied a large series of cystoprostatectomies to investigate these findings.

Design: One hundred twenty consecutive cystoprostatectomy specimens were studied in which the whole mount prostate sections were available. Slides from the bladder and the prostate were examined. Cases were assessed for the presence of UC and PC, their location, grade, margin and lymph node status. Clinical follow-up was obtained for all patients.

Results: Of 120 cases, 38 (32%) had prostatic involvement by UC, all of which were high grade. Of the 38 cases, 28 (74%) had invasion into prostatic stroma, including 4 as direct extension through perivesical soft tissue, and 25 (66%) were associated with at least muscle-invasive UC. Ten of 38 cases (26%) had UC involvement of the prostatic urethra and/or periurethral ducts without invasion into prostatic stroma. Of the 38 cases, 27 (71%) had bladder neck (BN) or trigone (TG) involvement by UC, 30 (79%) were associated with flat UC in-situ in the bladder and 25 (66%) with multifocal invasive bladder cancer, compared to 25 (30%), 30 (37%) and 25 (30%) of the remaining 82 cases, respectively ($p=0.001$). There is significant difference in the rate of distant metastasis

between cases with and without invasion of prostatic stroma (13/28 vs. 13/92, p=0.001). PC was identified in 56 cases (47%), 17 of which were present in the apex (30%).
Conclusions: Prostatic involvement by UC is relatively common in patients with bladder UC and seems to be a manifestation of advanced and multifocal disease and high-grade tumor. The presence of UC at BN/TG increases the chance of having prostatic involvement by UC. Invasion of the prostatic stroma by UC is associated with a higher rate of metastasis from UC. PC is a common incidental finding in prostates removed at the time of cystoprostatectomy for bladder UC and the presence of PC in the apex (30%) may have profound clinical implications in selecting the type of diversion to perform.

581 Basaloid Carcinoma (BC) of the Prostate: A Clinicopathologic Study of 27 Cases

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Background: BC of the prostate are rare tumors.
Design: We studied 27 cases of BC (collected over 20 yrs).
Results: Mean age 69 yrs. (42-89). Total specimens examined: TURP (n=22), radical prostatectomy (RP) (n=6), enucleation (n=1), and needle bx (n=6). Histology: 26/27 cases' slides were re-reviewed with 22 having >1 pattern: 1) adenoid cystic-like (AC) n=16 (61.5%); 2) small solid nests with peripheral palisading n=16 (61.5%); 3) resembling basal cell hyperplasia n=8 (31%); 4) small tubules +/- cords of cells occasionally lined by a hyaline rim n=7 (27%); 5) large solid nests often with central necrosis n=6 (23%). 12 cases of small nests/tubules were centrally lined by eosinophilic cells. Desmoplasia was noted in 19 (73%) cases. Infiltration around benign glands was seen in 10 (38%) cases, predominantly small nests and AC. Perineural invasion was noted in 3 cases with AC. Perineural and vascular invasion was seen in a BC with large solid nests. Mitoses ranged from 0-60/10hpf (mean=4). Collagenous globules and micro-calcifications were noted in 3 (11.5%) and 2 (8%) cases, respectively. 1 case also had stromal sarcoma. 1 case also had small cell and sarcomatoid carcinoma. 4 (15%) cases also had acinar carcinoma, 1 GS=4, 2 GS=6, and 1 GS=8. Immunohistochemistry: BCl2 was diffusely positive in 20/22 (91%). Ki67 ranged from 2-80% (mean=22%). Basal cell markers (HMWCK, p63) highlighted multiple cell layers in 15/24 (68%) cases, sparing the inner most luminal layer. In 6/24 (27%) cases, just the outermost layers were positive. In 3/24 (14%) cases, scattered cells stained (2 with large solid nests with central necrosis, 1 with tubules and cords). 6/22 (27%) cases showed AMACR staining and only 1/22 case was focally labeled with p53. Findings at RP and TURP: 6 patients had RP with 4/6 showing extraprostatic extension (EPE) with 1/4 showing seminal vesicle invasion and 1/4 a positive margin; 1/6 was organ confined; and 1/6 had no residual disease. An additional 10 cases showed EPE with bladder neck invasion on TURP. Follow-up (FU): 19/27 cases had FU> 1 year with a mean of 4.3 years (1-19 years). 14/19 (77%) had no evidence of disease after 1-19 (mean 5.8) years. 2/19 had local recurrence following TURP. Distant metastasis developed in 3/19 men.
Conclusions: BC have a broad morphologic spectrum. Although most are indolent, some behave aggressively with local recurrences and distant metastases. The most common morphology with aggressive behavior was large solid nests often with central necrosis, high Ki67 %, and less staining with basal cell markers.

582 NKX3.1 as a New Tissue Marker of Prostatic Adenocarcinoma

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Background: NKX3.1 is a prostatic tumor suppressor gene. While PSA and PSAP are markers of prostate cancer, at times they may be only focally or weakly expressed in high grade prostate tumors. Thus, the diagnosis of prostate cancer in TUR specimens, biopsies and in metastatic sites can be difficult at times even with these markers. The purpose of this study was to determine the performance of NKX3.1 immuostaining (IHC) as a marker of prostate cancer using a novel antibody.
Design: NKX3.1, PSA, and PSAP IHC was performed on 19 poorly differentiated adenocarcinomas of the prostate (PDPCa), Gleason score 5+5=10 (18 TUR and 1 needle biopsy) and a TMA of metastatic prostate cancer. Staining was scored as the percentage of tumor cells with any positivity and the percentage of tumor staining strongly. For specificity we used TMAs from various tumor sites: bladder, breast, colon, salivary gland, stomach and thyroid and standard paraffin sections of carcinomas: adrenal cortical (n=10), renal cell (n=13: 6 papillary, 5 clear cell and 2 chromophobe), hepatocellular (n=14), lung (n=13: 7 squamous cell, 4 adeno, 1 brochioloalveolar and 1 adenosquamous), and carcinoids (n=15: 8 GI, 3 lung and 4 metastatic to liver). 6 testicular germ cell tumors (1 teratoma, 1 pure yolk sac, 1 embryonal + yolk sac, 2 seminomas, 1 embryonal+ yolk sac+teratoma) were also stained.
Results: For PSA: Only 8 of 19 (42%) PDPCa cases stained (% positive [Pos] ranged from 1-80 and % Strong ranged from 0-70). For PSAP: 17 of 19 (89%) stained (% Pos ranged from 10-90 and % Strong ranged from 5-60). For NKX3.1: 18 of 19 (95%) stained (% Pos ranged from 3 cells to 100 and % Strong ranged from 1-90), with most cases showing strong diffuse nuclear staining. Metastatic prostate cancer was positive in 52 of 61 cases (85%). The non-prostate tumors did not stain with NKX3.1, or with PSA. PSAP showed patchy staining of two rectal carcinoids. NKX3.1 showed patchy strong staining of benign salivary glands and bronchial submucosa. Fetal seminiferous tubules showed moderate nuclear staining with NKX3.1 as did some sertoli cells in 2 adult testes.
Conclusions: Most PDPCa stained diffusely and strongly with NKX3.1. This is surprising since the pathogenesis of prostate cancer is related to the loss of NKX3.1. If validated in additional studies, IHC with NKX3.1 may prove valuable to definitively identify PDPCa.

583 The Incidence and Significance of the Invaginated Extraprostatic Space (IES) Involvement by Prostatic Carcinoma (PCA) in a Series of 310 Radical Prostatectomies

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Background: The awareness and clinical significance of the invaginated extraprostatic space (IES) in the prostate is largely unknown to most Pathologists. IES is a loose vascularized connective space that enters the prostate gland along with the seminal vesicles and extends up to the verumontanum, where it fuses with another extraprostatic space that completely encircles the prostatic urethra. In our previous study, we have shown that a high proportion of pT3b prostatic carcinomas involve the IES or extend very close to it. In this study we attempt to assess the true incidence of IES involvement by PCa, in a series of 310 consecutive radical prostatectomies.
Design: 310 consecutive cases of radical retropubic prostatectomy performed in a single institution, over a two year period (1991-1992) were included in this study. Wholemount sections of all cases of prostatectomy were reviewed, and PCa involvement of IES was recorded with respect to involvement of verumontanum/periurethral space (veru) and ejaculatory ducts (ED). PCa that extended close to the IES (< 1 mm) was also recorded. Seminal vesicle involvement was recorded separately and correlated at a later stage.
Results: Pca involved IES in 44 cases (14%), and of these 30 cases (68%) showed SVI by Pca. Pca involved only the veru in 14% cases, only ED in 57% cases, and involved both veru-ED in 29% cases. Pca was close to the IES in 23 cases (7%), and of these, 9 cases (39%) showed SVI by Pca. SVI by Pca was noted in 49 cases out of 310 (16%), and of these IES positivity was noted in 30 cases (61%), with Pca being close to IES in another 8 cases (16%).
Conclusions: The incidence of IES positivity by Pca in our series is 14%, and two-thirds of these show SVI by Pca. IES invovlvement by PCA, is a definite route of direct tumor spread to SV. IES invovlement may be a surrogate marker for PCA invovlement of SV, and may indicate a need to comprehensively evaluate SV for accurate pathology staging.

584 Detection of Chromosomal Abnormalities in Voided Urine and Bladder Washes by FISH: A Close Look at 3784 Cases

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Background: The UroVysion Bladder Cancer Kit is designed as an aid for initial diagnosis and monitoring recurrence of bladder carcinoma. The kit is intended to be used on voided urine specimens fixed on microscope slides and employs four fluorescence *in situ* hybridization (FISH) enumeration probes specific for chromosomes 3, 7, 17, and the 9p21 region.
Design: Voided urine or bladder washing was submitted from 3784 patients for testing with the UroVysion kit. The specimens were processed and prepared as outlined in the UroVysion Kit. Scoring was performed by microscopic inspection of at least 25 morphologically abnormal cells. Specimens were categorized as abnormal if more than 3 of the 25 abnormal cells showed gains for 2 or more chromosomes in the same cell, or if more than 11 of the 25 cells showed complete loss of the 9p21 signal. Likewise, specimens were categorized normal if less than 4 cells displayed a supernumerary chromosome count, or if less than 12 cells displayed a complete loss of the 9p21 signal. If less than 25 abnormal cells could be identified in a single specimen, the cell yield was deemed as insufficient for testing. The collected data were then separated based on specimen type and results.
Results: A total of 3412 urine specimens and 372 bladder washes were reviewed. Normal cases numbered 2810 for urine specimens and 192 for washes. Abnormal cases numbered 565 for urine specimens and 174 for washes. The cell yield in 43 cases (37 from urine specimens and 6 from bladder washes) was insufficient for testing.

Distribution of FISH results

Specimen Type	#Cases	Normal	Abnormal	No Result
Urine	3412	2810	565	37
Washes	372	192	174	6
All	3784	3002	739	43

Conclusions: The distribution of abnormal cases differs almost 3-fold between voided urine and bladder washes (17% compared to 47%). The cause of these discordant results could be attributed to selection bias. The procedure for collecting voided urine is non-invasive by nature. Bladder washes, however, are usually reserved for individuals presenting with relatively more advanced symptoms, thus positively selecting for those with bladder cancer.

585 Benign Mimickers of Renal Malignancy: Pathologic and Radiologic Appraisal with Clinical Correlation

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Background: Radiology plays increasing role in detection of renal tumors. Cystic lesions are typically defined on CT according to Bosniak classification which is widely used to predict malignancy. For solid lesions, hyperdensity and enhancement are predictors of malignancy on CT. We sought to evaluate how specific are these predictors.
Design: We searched for lesions which were radiologically classified as suspicious for renal cell carcinoma (RCC) but pathology showed benign lesions. There were 18 patients: 4 patients with 4 cystic lesions (Bosniak category 3 and 4) and 14 patients with 17 solid lesions which were isodense or hyperdense. We reviewed pathologiy and correlated with radiology and the clinical picture.
Results: There were 21 lesions in 18 patients; 15 females & 3 males; female : male ratio 5:1. All 4 cystic lesions were diagnosed as mixed epithelial and stromal tumor of the kidney (MEST) ranging in size from 1.7-10 cm. The stroma was strongly positive for smooth muscle actin (SMA), estrogen (ER) & progesteron receptor (PR). All MEST were found in females; age range 36-43 & all patients had a history of hormonal treatment.

Among the solid lesions there was one capsuloma (2.3 cm, in 35 year old female) & 16 angiomyolipomas (AML) which measured 1.5-6 cm. There were 10 females & 3 males (age range for females: 28-83, for males 44-50). All AMLs showed predominantly smooth muscle proliferation & contained <25% of fat [designated as fat-poor (FP)AML]. All AMLs were strongly positive for SMA & HMB-45 and in 9 lesions were positive for ER of which in 7 were also positive for PR. Four patients had uterine leiomyomata, several were obese. All except one lesions were sporadic. One patient with tuberous sclerosis and lymphangioleiomyomatosis had 1 typical and 1 FP-AML. All lesions were benign.

Conclusions: MEST, FP-AML and capsuloma are benign mimickers of malignancy on imaging studies. They are predominantly seen in women, frequently at the perimenopausal age and are frequently associated with hormonal therapy or hyperestrogenic state (i.e. obesity). It remains to be seen whether the incidence of these lesions will increase in patients on various hormonal treatments and with increased intensity of radiologic examinations. These smooth muscle proliferations must be differentiated from other spindle cell lesions on pathology which may be particularly challenging in small biopsies.

586 Angiomyolipoma with Epithelial Cysts (AMLEC): A Distinct Cystic Variant of Angiomyolipoma

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Background: Renal angiomyolipoma (AML) is typically a solid lesion, composed of adipose, vascular, and muscular tissue, lacking an epithelial component. While entrapped renal tubular elements may be observed in AML, presentation as a cystic mass has not been previously reported.

Design: We identified four AML with epithelial cysts (AMLEC) and studied their clinicopathologic features.

Results: Lesions were from 2 male and 2 female patients, ranging in age from 37 to 76 years, without history of hormonal therapy. One patient had known tuberous sclerosis and two presented with bilateral cystic lesions. Two lesions each were located in the right and left kidneys and measured from 1.3 to 4.5 cm. All lesions had three components: a) epithelial cysts lined by cuboidal to hobnail cells; b) a compact, subepithelial "cambium-like" cellular stroma resembling endometrial stroma; and c) muscle-predominant AML with associated dysmorphic blood vessels external to the cellular stroma. All lesions labeled with HMB-45, Melan-A, ER, PR, and CD10, with greatest intensity in cellular subepithelial stroma. In contrast, smooth muscle actin and desmin labeled with greatest intensity in the peripheral muscle-predominant AML component. Cyst lining cells were positive for pancytokeratin and soy bean agglutinin, the latter characteristic of distal nephron epithelium, but did not label for any of the other markers listed above.

Conclusions: AMLEC should be considered in the differential diagnosis of cystic renal lesions, particularly mixed epithelial-stromal tumor (MEST). The cyst lining most likely represents entrapped, dilated, native renal tubules. The condensed, subepithelial HMB45-positive AML cells show mullerian differentiation, as evidenced by their morphologic similarity to endometrial stroma and corresponding CD10 immunoreactivity, and their enhanced ER/PR immunoreactivity. While mullerian differentiation has not previously been described in AML, it has in other cystic renal lesions such as MEST. The capacity for renal lesions to show mullerian differentiation may reflect the common embryological origin of the kidney and genital systems from the urogenital ridge.

587 Urothelial Carcinoma in Patients ≤ 30 Years of Age: A Clinicopathologic Analysis of 8 Cases

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Background: Urothelial carcinoma is uncommon in the first three decades of life with previous studies suggesting an indolent disease course. Young adults with neurogenic bladder due to CNS malformations represent a small subset of these patients. We reviewed the clinicopathologic findings in 8 cases of urothelial carcinoma diagnosed in patients ≤30 years of age.

Design: The archives of the University of Iowa Department of Pathology were searched to find cases of urothelial carcinoma in patients ≤ 30 years of age. Eight cases were identified through medical records, although only 6 had glass slides available for review.

Results: The age of the 8 patients at diagnosis ranged from 20-30 years (mean = 25) and 6 were male. None of the 8 patients had a family history of genitourinary cancer, personal history of cancer, chemoradiation, or known exposure to arylamines. Two of 8 patients smoked cigarettes, smoked marijuana, and used methamphetamines. Meningomyelocele with neurogenic bladder was present in 3 patients diagnosed at 20, 22, and 23 years of age. All three presented with high-grade muscularis propria invasive tumors. Two of these three patients were treated by cystectomy with one death due to metastatic disease. The third patient with neurogenic bladder was diagnosed at autopsy as an unexpected finding. The patients with neurogenic bladder had frequent infections, intravesical antibiotics, did not smoke, and performed self catheterization. One had recurrent vesical calculi. The remaining 5 patients presented with gross hematuria. Pathologic diagnoses included two high-grade noninvasive papillary urothelial carcinomas, one high-grade papillary urothelial cancer with muscularis propria invasion, and one high-grade urothelial carcinoma with lamina propria invasion and multifocal carcinoma in situ. One patient had low-grade papillary carcinoma in the bladder and renal pelvis along with multifocal carcinoma in situ of the ureter. These five patients are alive with one that has biopsies and bladder washings showing high-grade urothelial carcinoma.

Conclusions: Chronic inflammation within a neurogenic bladder may be a risk factor for the development of urothelial carcinoma. Bladder tumors in these patients may be high-grade and high stage at presentation contrary to most published reports. Young adults with normally functioning bladders in our cohort appear to have risk factors and clinical outcomes similar to older patients.

588 Non-Specific and Paradoxical Effects of Neo-Adjuvant PS341 after Radical Prostatectomy in High-Risk Patients

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Background: New therapies for prostate cancer are necessary as currently no effective therapy is available for patients at high risk of recurrence. The advent of new targeted therapy drugs opens the door to new intervention options. PS341 (Bortezomib, Velcade) is a first generation, reversible, potent inhibitor of the 26s proteasome, that binds to and inhibits the 20S catalytic core.

Design: Twenty one patients with locally advanced/high grade prostate cancer received neo-adjuvantly PS-341 by IV push 1.6 mg / m^2 once weekly for four weeks followed by radical prostatectomy within 72 hours of the last dose. Specimens were fully embedded, whole mounted and digitized. Areas of tumor with therapy related effect were mapped and quantified using image analysis. Tissue microarrays were created with non-neoplastic prostates, BPH and prostate cancer. Gleason matched controls were obtained from a large outcomes array database. Preoperative biopsies from the same patients were also obtained. Controls and PS341 arrays were immunostained with antibodies against P-Akt, GSK, Cyclin E, NFkB, Ki67 and Tunnel.

Results: Toxicity was moderate. Tumoral effect defined as pyknosis/necrosis was identified, but was modest and located mostly in the periphery of the tumors. As expected, nuclear NFkB decreased in patients treated with PS341 while cytoplasmic NFkB increased (p=0.000). Consequently, the apoptotic ratio was increased in patients treated with PS341 than controls (p=0.000). To our surprise, the proliferation ratio was also increased (p=0.000), as well as levels of P-Akt (p=0.000). These results were true when compared both to the master database and the preoperative biopsies.

Conclusions: Cytoplasmic entrapment of NFkB and subsequent increase in apoptosis demonstrates proteasome inhibition by PS341. However the paradoxical increase in proliferation and levels of Akt in the PS341 treated patients are paradoxical and might correspond to activation of alternate pathways. These are novel preliminary findings that warrant further exploration.

589 Podoplanin: A Histological Marker for Seminoma

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Background: Podoplanin is a newly identified 44 kDa sialoglycoprotein overexpressed on the surface of tumor cells. Its physiological role has yet to be determined and its function in tumors is not well characterized. However, it was recently proposed that its mouse homologue, Aggrus, functions as an adhesion molecule that promotes platelet aggregation in pathological conditions. Furthermore, Aggrus expression has recently been reported in testicular seminomas but not in embryonal carcinomas, suggesting that it may be a specific marker for seminomas.

Design: 50 cases of testicular germ cell tumors (GCTs) were selected from our surgical pathology archives; these cases had been previously analyzed for the expression of OCT-3/4. The expression of podoplanin was evaluated by immunohistochemical staining of 4m sections using a polyclonal antibody (Angiobio, Del Mar, CA, dilution 1:100). Stained sections were graded in a semi-quantitative/qualitative fashion as follows: negative (<10% of tumor cells staining), 1+ (mild intensity), 2+ (moderate intensity), 3+ (intense staining).

Results: Diffuse cytoplasmic expression with membrane accentuation of the seminoma cells was seen in all cases. Lymphocytes and interstitial cells were negative. In mixed germ cell tumors, expression was seen in small clusters of ambiguous tumor cells adjacent to foci of embryonal carcinoma and in rare, admixed trophoblastic cells. There was no significant staining of any non-trophoblastic, non-seminomatous elements.

Conclusions: Podoplanin is a specific marker for recognition of seminomas. In contrast to OCT-3/4 it does not stain embryonal carcinoma cells. The availability of a specific marker for this tumor will enable better recognition of these tumors, particularly in non-gonadal sites. The focal expression of this marker in non-descript tumor cells adjacent to foci of embryonal carcinoma gives credence to the hypothesis that the latter arise from seminomas as a part of "differentiation".

590 Immunohistochemical Profile of Renal Epithelial Neoplasms: An Institutional Experience with 92 Consecutive Cases

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Background: Accurately sub-classified, renal epithelial neoplasms (REN) have different prognostic implications. Historically, immunohistochemical stains (IHC) have an important role in differentiating between the various subtypes of REN. We offer our institutional experience with 92 consecutive cases evaluated with immunohistochemistry.

Design: Of 154 cases of partial, total, and radical nephrectomies, 92 renal epithelial neoplasms, [62 clear cell renal cell carcinomas (CRCC), 12 chromophobe renal cell carcinomas (CHRCC), 7 papillary renal cell carcinomas (PRCC), 6 oncocytomas (OC), 3 renal epithelial oncocytic neoplasms (REON), and 2 renal cell carcinomas, not otherwise specified (RCC, NOS)] were evaluated by a panel of IHC including Vimentin (Vim), Renal Cell Carcinoma Antigen (RCC), CD10, Cytokeratin 7 (CK7), E-cadherin (E-cad), Ber-EP4, C-kit, Inhibin, Ki-67, and histochemical Hale's colloidal iron (HCI).

Results:

CRCC	CHRCC	PRCC	OC	REON	RCC, NOS		NP
Vim	61(98)	7(58)	7(100)	2(33)	0	2(100)	
RCC	47(77)	2(18)	7(100)	0	0	1(50)	2
CD10	61(100)	9(75)	7(100)	6(100)	3(100)	1(50)	
CK7	25(41)	9(81)	6(100)	3(50)	1(33)	1(50)	3
Inhibin	28(49)	8(73)	5(83)	6(100)	3(100)	2(100)	7
Ber-EP4	7(14)	8(80)	3(43)	1(17)	2(67)	2(100)	14
E-cad	35(73)	11(100)	4(67)	6(100)	3(100)	0	16
C-kit	5(25)	2(100)	3(75)	1(17)	0	NP	65
Ki-67	0-75	0-5	1-20	<1-<5	1-3	NP	
HCI	1(2)	9(90)	0	1(17)	0	0	32

NP- not performed. (_) indicate percentages

1. CRCC: Vim+/CD10+/RCC(maj+)/E-cad(maj+)/CK 7 (41% +)/Ber-EP4(maj-) 2. PRCC: Vim+/RCC+/CD10+/CK7+/E-cad(maj+) 3. CHRCC: E-cad(maj+)/Ber-EP4(maj+)/CK7 (50% f+)/CD10 (50% w.cyto+)/ RCC and Vim(f/ w.cyto gr+) 4. OC: E-cad+/CD10 w.cyto gr +/CK7 (50%+)/RCC-/Vim- 5. Inhibin and C-kit are not differentially expressed in the subtypes of RCC. 6. HCI is generally weakly positive in all CHRCC. Key: maj-majority; f-focal;w.-weak;cyto-cytoplasmic;gr-granular

Conclusions: 1. IHC profile shows cross-over in patterns of expression; however staining patterns (membrane/ cytoplasmic/ extent of expression) are more important than positive/ negative results and add significant value. 2. Immunohistochemical stains did not help in the classification of cases diagnosed as REON and RCC NOS, since no specific pattern was seen.

591 Fluorescence In-Situ Hybridization (FISH) Profile of Renal Epithelial Neoplasms (REN): An Institutional Experience with 92 Consecutive Cases

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Background: Chromosomal (chr) alterations in clear cell renal cell carcinomas (CRCC), chromophobe renal cell carcinomas (CHRCC), papillary renal cell carcinomas (PRCC), and oncocytomas (OC) have been reported. Cytogenetic abnormalities supplement histology and immuno-histochemistry (IHC) information, and help subtyping these neoplasms. We offer our institutional experience with 92 consecutive REN evaluated by FISH.

Design: FISH with centromere probes for chr 1, 2, 7, and 17 was performed on 92 REN [62 CRCC, 12 CHRCC, 7 PRCC, 6 OC, 3 renal epithelial oncocytic neoplasms (REON), and 2 renal cell carcinomas, not otherwise specified (RCC NOS)]. An average of 60 cells were analyzed. Chr losses were considered significant if present in greater than 30% of cells, indeterminate/ borderline (bor) if present in 20-30% of cells, and insignificant if seen in less than 20% of cells. Chr gains were considered significant if present in greater than 20% of cells, bor if present in 10-20%, and insignificant if seen in less than 10% of cells.

Results: 16 cases (16%) had inconclusive results by current knowledge of the various REN subtypes. Their subclassification here is reported according to histologic impression.

REN	All	CRCC	CHRCC	PRCC	OC	REON	RCC, NOS
+1	1	0	0	1	0	0	0
-1	30	16	7	1	3	2	1
+2	0	0	0	0	0	0	0
-2	29	13	9	2	2	1	1
+7	8	3	1	1	1	2	0
-7	20	14	5	1	1	0	1
+17	2	0	0	0	0	2	0
-17	45	33	8	1	1	0	1

No significant chr abnormality (NSCA): 6 CRCC & 1RCC, NOS

Half the cases of CRCC were monosomic for chr 17, about 25% of cases had monosomies for chr 1, 2, and 7 each; 10% of CRCC had NSCA. Monosomy 2 was the most prevalent (75% of the cases) chr alteration in the CHRCC; monosomies of 1, 7, and 17 were observed in 42 to 67% of the cases. Monosomy of chr 1 was present in about half the cases of OC. No consistent chr alterations were present in the PRCC. FISH was useful as it helped classify 2 REON as solid variants of PRCC (+7 and +17).

Conclusions: 1. Majority of CRCC show significant chromosomal losses, especially of chr 17. 2. FISH has diagnostic value in evaluating REON as it helps discriminate OC. 3. Some of the tumors reported as REON and RCC, NOS may represent variants of known subtypes of REN or cases with divergent histologic and IHC patterns; alternately these may represent yet uncharacterized subclasses of REN.

592 UroVysion Fluorescence In-Situ Hybridization (FISH) on Upper Urinary Tract Samples

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Background: UroVysion FISH is an accepted modality for surveillance of urothelial carcinoma (UC) of the bladder. The utility of UroVysion FISH for upper urinary tract (UUT)has not been explored.We present our experience with ten patients (pts) and twenty-three samples.

Design: FISH testing was performed, using commercial UroVysion kit. The probes in the kit are CEP3 (3p11.1q11.1), CEP7 (7p11.1q11.1), and CEP17 (17p11.1q11.1) and LSI 9p21. Four cells with gains (minimum of two out of the three chromosomal gains for 3, 7, and 17 required) and twelve cells with homozygous 9p21 del are required for a positive diagnosis. Upto twenty-five cells were evaluated.

Results: 10 pts (6 males and 4 females) underwent UUT (renal pelvis/ ureteral) washing with cytology and concurrent FISH. 3 pts had history of previous UC of the urinary bladder (surveillance); 1 pt with hematuria had a remote history of contralateral nephrectomy for UC; 6 pts were being evaluated to rule out UC. A total of 23 procedures were performed. 1 sample was acellular; 8 samples were positive; 13 were negative; 1 was inconclusive (3 trisomic cells of a total of 40 evaluated). 4 positive FISH results had concurrent positive cytology/ bx; 4 positive FISH results had concurrent atypical/ suspicious cytology and no biopsy (bx); 4 negative FISH results had concurrent positive cytology/ bx; 9 negative FISH results had concurrent atypical/suspicious cytology/ bx; 1 negative FISH result had concurrent negative cytology and bx; 1 inconclusive FISH result had concurrent atypical cytology. Of note, 1 patient with positive FISH and negative cytology had subsequent positive bx; 1 pt with subthreshold trisomy had a recent positive bladder wash with trisomy on FISH. The positive predictive value of the FISH on this limited set of samples is 62.5%. The false negative rate is 28.6%.

Conclusions: 1. FISH aimed at defined chromosomal abnormalities might help in detecting UC of the upper urinary tract. 2. There is a significant false negative rate. FISH should therefore always be combined with current standard biopsy/ cytology evaluations. 3. FISH false positive results may subsequently convert to true positives and therefore require close follow-up. Additional longer-term studies are needed to address this issue. 4. The set of false negative cases might have chromosomal abnormalities other than those detected by UroVysion FISH.

593 Pitfalls in the Diagnosis of Prostate Cancer. A Review of 1861 Cases

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Background: A large number of pathological mimics of prostate cancer have been described. However there have been very few studies examining the possible reasons for the misdiagnosis of prostate cancer with follow-up data available. The Trans Atlantic Prostate Group was set up to examine the natural history of clinically localised prostatic carcinoma diagnosed in the UK between 1990 and 1996 which had been treated conservatively. This entailed pathological review, allowing an analysis of the false positive error rate in this period.

Design: We examined 1,861 cases of prostate cancer diagnosed in the UK between 1991 and 1996. All cases were clinically localised at presentation, treated by non-curative methods and detailed follow-up including PSA levels were available. The pathology on all the cases where the diagnosis was doubtful was reviewed by at least 2 genito-urinary pathologists. Where possible, reasons for the reassignment were given.

Results: 137 cases (7.4%) were reassigned to a non-malignant diagnosis. 34% (47) of these cases, the largest group, showed no obvious reason for the malignant diagnosis. 17% (23) showed sclerosing adenosis, 11% (15) showed inflammatory induced atypia, 11% (15) showed atyical adenomatous hyperplasia. 10% (14) showed florid basal cell hyperplasia. The remainder showed a variety of changes including atrophy (5), clear cell hyperplasia (4), granulomatous inflammation (2), and one example each of granulation tissue, seminal vesicle epithelium, squamous metaplasia, prostatic intraepithelial neoplasia, high-grade urothelial carcinoma, a ganglion, atypical basal cell hyperplasia and a metaplastic urethral polyp. Follow-up of 9.73 years (range 7.26 to 14.95 years) revealed that death from prostate cancer occured in 8 of these cases (6%), compared with 8% of those with Gleason score 5 or less and 40% of those with Gleason 9 or more in the series.

Conclusions: Many morphological entities may mimic prostate cancer and may be responsible for misdiagnosis in TURPs and in biopsy specimens. Continuing education in morphological mimics of cancer and immunohistochemistry including basal cell markers and racemase may help to reduce this error rate, and the potential for overtreatment.

594 Gleason Score and Survival in Conservatively Treated Localised Prostate Cancer

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Background: The treatment of clinically localized prostate cancer is controversial. The Trans-Atlantic Prostate Group investigated the natural history of prostate cancer in the era of PSA measurement and modern concepts of Gleason grading.

Design: Men under 76 years old and diagnosed with prostate cancer in the UK between 1990 and 1996 were identified through 6 cancer registries. The notes were abstracted and those with clinically localised cancers, and treated by watchful waiting or by hormonal therapy alone were identified. Serum PSA values were recorded. The pathology was centrally reviewed by a genito-urinary pathologist and a Gleason score assigned. The diagnostic Gleason score, if given in the records, was recorded.

Results: 2,419 men were eligible for evaluation. Of these, 1,680 were managed by watchful waiting and 739 by hormone therapy. With a median potential follow-up of 9.73 years (range 7.26 to 14.95), 539 (22%) had died from prostate cancer**,** 675 (28%) had died from other causes and 574 (24%) were alive, but with evidence of disease progression. The slides of 1724 cases were available for review. An initial diagnostic Gleason score had been provided on 1148 cases. 464 cases initially diagnosed as Gleason score 4 or less were reassigned to Gleason score 6 or more. The revised Gleason score was the most important prognostic factor (X^2 trend of 189 for cancer death and 116 for progression) followed by PSA level at diagnosis. Cox regression analysis showed these were largely independent and use of both of them gave substantially more predictive power than either one alone. The initial Gleason score was much weaker predictor of progression.

Conclusions: The revised Gleason score had strong independent predictive effect on prostate cancer mortality in a unique series of conservatively treated prostate cancers. It also revealed a substantial shift in the grade from the original to the revised grade. Accurate Gleason grading provides valuable prognostic information which is essential for informed decision making at diagnosis. Predictive models of untreated cancers can now be constructed to give a more reliable indication of the natural history of prostate cancer.

595 Inflammatory Atrophy in Prostate Needle Biopsies: Relationship to Cancer?

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Background: Chronic inflammation of longstanding duration has been linked to the development of carcinoma in several organ systems. It is controversial whether there is any relationship of inflammatory atrophy to prostate cancer. In a recent study, atrophy in an asymptomatic population undergoing screening was not associated with greater prostate cancer or high-grade PIN incidence during subsequent screening rounds. The aim of this study is to compare in needle biopsies showing cancer the frequency and extent of atrophy with and without inflammation as well as clinicopathologic findings in the two groups of patients subsequently submitted to radical prostatectomy.

Design: The frequency and extent of atrophy with and without inflammation was studied in 172 needle biopsies showing cancer. The data were analyzed using the Mann-Whitney test for comparison of independent samples and either the chi-square or Fisher's exact test for evaluating differences between proportions.

Results: From a total of 172 needle biopsies, 56/172 (32.55%) biopsies showed no atrophy; and, 116/172 (67.44%) showed atrophy. From the total of 116 biopsies with atrophy, 46/116 (39.65%) showed atrophy with inflammation; and, 70/116 (60.34%) atrophy without inflammation. Atrophy without inflammation was significantly more extensive (seen in a higher number of cores) (p=0.0314). There was no statistically significant difference between biopsies showing atrophy with inflammation and biopsies showing atrophy without inflammation in patients submitted to subsequent radical prostatectomy for: preoperative PSA (p=0.9067), Gleason score (p=0.7194), positive surgical margins (p=0.1697), tumor extent (p=0.9981), extraprostatic extension (p=0.5480), and seminal vesicle invasion (p>0.9999).

Conclusions: The result of our study seems not to favor the hypothesis that inflammatory atrophy predisposes to prostate cancer. Atrophy is a frequent finding in biopsies showing cancer (67.44%). Furthermore, atrophy without inflammation is more fequently found and more extensive than atrophy with inflammation; and, no difference in clinicopathologic findings was seen between patients subsequently submitted to radical prostatectomy.

596 Seminal Vesicle Invasion in Radical Prostatectomies: Which Is the Most Common Route of Invasion?

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Background: There are conflicting studies as to whether (i) extraprostatic extension (EPE) into soft tissue adjacent to the seminal vesicle; or, (ii) invasion via the sheath of the ejaculatory duct, is the most common route of seminal vesicle invasion (SVI). The aim of this study was to find which is the most frequent of 3 possible routes of SVI: 1) EPE into soft tissue adjacent to the seminal vesicle and then into the wall of the seminal vesicle; 2) invasion via the sheath of the ejaculatory duct and then extending up the ejaculatory duct into the seminal vesicle muscle wall; and, 3) discontinuous metastases (neither via the sheath of the ejaculatory duct nor EPE).

Design: The surgical specimens of 230 consecutive patients submitted to radical prostatectomy were histologically evaluated by complete embedding and whole mount processing . SVI was defined as tumor infiltrating the muscular coat of the seminal vesicle. EPE was diagnosed whenever cancer was seen in adipose tissue or, in case of desmoplasia, whenever an irregular bulge was seen in the surface contour of the prostate. Involvement of the ejaculatory duct was diagnosed whenever the neoplastic tissue involved the sheath of the duct.The Mann-Whitney test was used for comparison of means.

Results: From a total of 230 patients, 28/230 (12.17%) surgical specimens had either unilateral or bilateral SVI. The frequency of the possible routes was 0/28 (0%), 3/28 (11%), 6/28 (21%), and 19/28 (68%), respectively, for: 1) only via the sheath of the ejaculatory duct; 2) discontinuous metastases; 3) both EPE and via the sheath of the ejaculatory duct; and, 4) only EPE. From the total of 28 seminal vesicles involved, half (14/28, 50%) had unilateral invasion. In most of these cases (42.85%), EPE was unilateral and ipsilateral. The mean number of sections showing EPE was 4.95 and 2.16, respectively, for specimens with SVI and seminal vesicle without invasion (p=0.0008).

Conclusions: In our study, the most fequent route of seminal vesicle invasion was extraprostatic extension. No case showed only involvement of the ejaculatory duct sheath. The findings in unilateral seminal vesicle invasion and the evaluation of the number of sections showing extraprostatic extension, strongly favor that the most probable sequence of events is extraprostatic extension, involvement of the soft tissue adjacent to the ipsilateral seminal vesicle, and then into the wall of the seminal vesicle.

597 Extent of Prostatic Atrophy in Needle Biopsies and Serum PSA Levels (Free, Total and Free/Total Ratio): Is There Correlation?

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Background: There is evidence that age associated prostatic atrophy may be a manifestation of chronic ischemia due to local arteriosclerosis. In autopsies, there was a positive and statistically significant correlation between intense local arteriosclerosis and presence and extent of atrophy. The aim of this study was to find any correlation between extent of atrophy in biopsies and serum PSA levels.

Design: The study was based on 136 needle prostatic biopsies corresponding to 123 patients. The only diagnosis in all biopsies was focal prostatic atrophy without presence of cancer, high-grade PIN, suspicious for cancer, or prostatitis. The data were analyzed subdividing the patients into lesser and higher probability of harboring cancer not shown in the biopsy: with free/total serum PSA ≥0.15 (Group 1, 61 biopsies), and free/total PSA<0.15 (Group 2, 75 biopsies). The extent of atrophy was evaluated considering either the absolute number or the percentage of cores showing the lesion. Regression or simple correlation were applied using in each analysis the most suitable function (linear, parabolic, sinusoidal, logistic or fourth degree polynomial) that best fitted to the distribution of the data.

Results: The mean absolute number of cores showing prostatic atrophy was 4.8 (range:1-15); and, the mean percentage 59.8% (range:12.5%-100%). The mean free, total, and free/total serum PSA level was 1.44, 6.83, and 0.22, respectively. Group 1: there was a positive and statistically significant correlation between extent of atrophy and either free (p=0.0076 and p=0.0210 for parabolic and linear functions, respectively) or free/total PSA (p=0.0068 and p=0.0085 for fourth degree and parabolic functions, respectively); no correlation was found for total PSA. Group 2: no significant correlation was found between extent of atrophy and free, total or free/total PSA. Prostatic nodular hyperplasia is a cause of free PSA serum elevation; in our series, no significant correlation was found between extent of atrophy and the volume of the prostate.

Conclusions: Considering that age associated prostatic atrophy may be a manifestation of chronic ischemia due to local arteriosclerosis, the results suggest that chronic ischemia may be involved in serum free PSA elevation in patients with several needle biopsies showing only prostatic atrophy and free/total PSA ≥0.15.

598 NANOG, a Sensitive and Specific Marker for Seminoma and Embryonal Carcinoma in Retroperitoneal Lymph Node Dissection Specimens

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Background: Testicular germ cell tumors are the most common malignancies in men in the second and fourth decades of life. These tumors are highly treatable and therefore a correct diagnosis is critical for patient management. Diagnosis of metastatic germ cell tumor in the retroperitoneal lymph nodes could be challenging, therefore a sensitive and specific germ cell marker will be of help in such situation. NANOG, a homeobox transcription factor, plays a crucial role in the second embryonic cell fate specification following formation of the blastocyst. Recently, NANOG has been shown to be expressed in the testicular germ cell tumors. In this study, we examined the diagnostic value in the differential diagnosis of retroperitoneal neoplasm.

Design: Retroperitoneal lymph node dissection specimens are stained for NANOG expression. The specimens included 21 cases of testicular germ cell tumors; 10 cases of metastatic melanoma, 14 cases of lymphoma, 12 cases of metastatic poorly differentiated carcinoma.

Results: All of the seminoma (N=10) and embryonal carcinoma (N= 11) cases show strong nuclear staining. No nuclear staining was observed in yolk sack tumor (n=3). Pathological lesions that may present differential diagnostic possibilities were also examined. Metastatic poorly differentiated carcinomas and melanomas are negative for NANOG expression. No positive staining was seen in malignant lymphoma.

Conclusions: NANOG is a sensitive and specific marker for metastatic seminoma and embryonal carcinoma, which may facilitate in the differential diagnosis of retroperitoneal lymph node tumor.

599 Apoptosis in Spermatocytic and Usual Seminomas: A Light Microscopic and Immunohistochemical Study

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Background: Despite its alarming appearance, spermatocytic seminoma (SS) virtually never metastasizes. We hypothesized that this paradox may be related to increased apoptosis compared to metastasizing germ cell tumors. We therefore compared apoptosis and its regulators in spermatocytic and usual seminomas (US).

Design: 17 SSs from our consultation files and 18 USs had either some unstained sections or blocks available for study. 2 SSs also had a sarcoma component. Apoptotic cells were quantified by light microscopy by averaging the number of cells with homogeneously dense chromatin or karyorrhectic nuclear fragments in photographs of 5 randomly selected high power fields (hpf) fields. Immunostains for caspase-3, p53, bcl-2, bcl-xl, FADD, FAS and survivin were performed using standard methods and appropriate controls. Either counts of positively staining cells (for caspase-3), averaged over 5 hpfs, or estimates of the percent of positively staining tumor cells were performed.

Results: The average number of apoptotic cells per hpf and immunostaining results are shown in the table. There were significantly greater numbers of apoptotic cells and caspase-3 positive cells in SS compared to US (p=0.0003 & p=0.024, respectively). There was over a 10-fold range in apoptotic cells in US but only a 4-fold variation in SS. SS had decreased p53 expression compared to US, with marked variation in bcl-2 expression and increased FADD. The two sarcomas in SS, however, showed decreased apoptosis and caspase-3 reactivity, with upregulation of p53 and bcl-2 and decreased FADD expression.

Conclusions: Apoptosis, caspase-3 and FADD expression are increased in SS compared to US. Apoptotic parameters are decreased in sarcomatous transformation of SS. The increased apoptosis of SS, possibly mediated by FAS independent activation of the death receptor pathway, may have a role in its excellent prognosis. The variation in apoptosis of USs merits investigation as a prognostic parameter.

Apoptosis in SS and US (average with range and # studied)

	SS	Sarcoma in SS	US
avg. # apototic cells	101 (51-183) (n=13)	30 (42 & 17)	32 (7-78) (n=13)
caspase-3 (+cells/hpf)	18 (3-54) (n=17)	3 (4 & 2)	9 (3-24) (n=18)
p53 (%)	<1 (0-5) (n=16)	60 & 90	7 (1-25) (n=18)
bcl-2 (%)	12 (0-70) (n=14)	95 & 80	0 (n=18)
bcl-xl (%)	0 (n=14)	0 (n=2)	0 (n=18)
FADD (%)	71 (40-95) (n=12)	20 & 15	46 (30-60) (n=18)
FAS (%)	1 (0-10) (n=13)	0 & 10	<1 (0-1) (n=17)
survivin (%)	0 (n=9)	0 (n=2)	0 (n=9)

600 c-kit Protein Immunoreactivity in Renal Cell Carcinoma with Sarcomatoid Dediffrentiation

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Background: The c-kit expression in human neoplasms has generated tremendous interest because of the possible response of these neoplasms to tyrosine kinase inhibitors such as Gleevec. A recent European report of strong c-kit expression in limited cases of sarcomatoid renal cell carcinoma has suggested the possibility of using Gleevec to treat this highly aggressive tumor (*J Urol. 2004 171:2176*). However, the following study of these KIT positive tumors failed to show c-kit mutation suggesting that Gleevec therapy might not work. We previously have reported the KIT immunoreactivity in a large series of renal cell neoplasms. Using a standard immunohistochemical method, we analyzed KIT immunoreactivity in sarcomatoid components of renal cell carcinoma to evaluate this controversy.

Design: We examined nephrectomy specimens from 25 patients diagnosed with renal cell carcinoma with sarcomatoid dedifferentiation at two teaching hospitals between 1996 and 2005. Sixteen of 25 cases were associated with clear cell components. Formalin-fixed paraffin embedded sections were evaluated for c-kit expression using routine immunohistochemical analysis with a polyclonal rabbit antibody specific for KIT (A4502, DakoCytomation, California). The KIT immunoreactivity staining was graded for intensity (negative, weak and strong) and focality (negative, focal and diffuse) in sarcomatoid tumor components.

Results: Only 1 (4%) case showed strong but focal KIT cytoplasmic staining, 2 (8%) demonstrated diffuse but weak KIT positivity and 4 (16%) showed weak focal staining. The remaining 18 (72%) cases lacked KIT immunoreactivity.

Conclusions: In contrast to what was reported by the European study, we found the majority of sarcomatoid components of renal cell carcinoma lack significant KIT immunoreactivity using the standard immunohistochemistry protocol. Our findings challenge the notion that tyrosine kinase inhibitors such as Gleevec can be effective in treating patients with renal cell carcinoma with sarcomatoid dedifferentiation.

601 Number of Positive Cores but Not Gleason Score Is Predictive of High Stage Disease in Radical Prostatectomy of Patients with Gleason Score ≥ 8

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Background: The presence of Gleason score (GS) ≥ 8 is considered a contraindication for radical prostatectomy (RP) by some urologists. We sought to investigate if GS or other needle biopsy parameters are related to extraprostatic disease (stage pT3) in a group of patients with GS ≥ 8 in RP.

Design: 83 RP from two institutions with GS ≥ 8 were available for analysis. Data from corresponding biopsies were available for 74 RP. Captured needle biopsy parameters included patients' age, GS, number of cores, number of positive cores, tumor length, serum PSA, PSA density, and digital rectal examination. RP parameters included GS, pathological stage, surgical margin status, and the presence of lymphovascular invasion.

Results: 42%, 30% and 24% of cases had pT2, pT3a and pT3b stage of disease, respectively. Lymph node positive disease was present in only 8.4% of cases. Only 40.5% (30/74) of patients had GS ≥ 8 in needle biopsy, while remaining (44/74) or 59% of patients showed GS < 8 on biopsy. Logistic regression analysis demonstrated that the number of positive cores, but not GS, was the only needle biopsy parameter significantly associated with extra-prostatic disease in RP (p=0.002).

Conclusions: The number of positive cores but not the presence of GS ≥ 8 was associated with high stage disease in RP in our patients. The mere presence of GS ≥ 8 in needle biopsy should not be used as a sole predictor of advanced stage disease at RP, as significant number of patients still show organ-confined disease.

602 Is Higher Gleason Pattern More Likely To Be Detected in the Same Number of Needle Biopsies (NB) Sampling Smaller Compared to Large Prostate Glands? A Study of 331 NB and Corresponding Radical Prostatectomy Specimens (RPS)

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Background: The accuracy of the NB in reflecting the Gleason Score (GS) of the RPS is dependent on variables such as the number and anatomic distribution of the cores obtained and likely, the volume of the gland sampled. The objective of this study was to investigate if the number of biopsy cores is controlled for, the correlation between the GS of the NB and RPS would be more accurate in smaller versus larger glands and if a higher GS pattern is more likely to be detected in smaller glands.

Design: All patients treated by RP on whom NB information was available in our institution between 2000 and 2004 were considered for this study. Only patients who had 6 or more NB were included. The RPS weight as documented by gross exam was divided into intervals of 10 grams. The age, race, pre-op PSA, GS of the NB and RPS were recorded for all patients.The correlation between gland volume and the NB ability to detect higher grad tumor in different age, race and PSA strata was analyzed.

Results: In 208 of 331 cases (63%) the GS of the NB and the RPS was identical. Using a logistic regression approach, the probability of the biopsy GS to predict that of the RPS did not correlate with gland weight OR = 0.9, p=0.9). When RPS were divided into two weight groups, same GS was found in the NB and RPS in 35/51 (68.6%) of glands weighing < 30g rams compared to173/280 (61.8%) of RPS >30 grams, p=0.3. Considering GS of >=7 only, there seem to be a better correlation with gland weight. In a multivariate analysis controlling for number of cores, the probability for correctly predicting Gl score vatied inverse with gland weight OR = 0.9, p=0.2. No significant differences in PSA between the cases with correct and incorrect prediction 6.83 and 6.96 p=0.8. The mean age for cases with same GS for NB and RPS was slightly lower than for the other group 58 vs 60 years, p=0.02.There are were no significant differences in race distribution between the group with correct and incorrect grade prediction.

Conclusions: Gland weight had no apparent impact on the correlation of GS of the NB and RPS when the number of biopsies is controlled for. While there was a better tendency for NB sampling smaller glands to reflect a more accurate GS and to detect higher grade Pca, the trends wwre not statistically significant in our study.

603 Optimized Prostate Biopsy Sampling Model

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Background: The number of prostate biopsy cores obtained in routine practice has risen from 4 to 10 or more in the past decade in order to increase cancer detection; however, a significant number of cancers remain undetected. The optimal number of biopsies has not been defined, resulting in wide variation in clinical practice. Further, the probability of cancer yield according to core number has not been previously determined. We sought to create an optimized model for prostate biopsy sampling based on volumetric measures.

Design: We developed a practical easy-to-use web-based empirical mathematical sampling computer model to determine the minimum number of needle biopsies for optimal detection of prostate cancer. The user specifies the volume of the prostate, the cancer volume to be detected, and the desired level of probability for cancer detection. The optimization model assumes that biopsies are discrete, non-overlapping, and randomly distributed in the peripheral zone; biopsy cores are uniform and optimal in volume; cores are exclusively from the peripheral zone; and the peripheral zone accounts for 40% of total prostate volume.

Results: The probability of cancer detection increased exponentially with an increase in prostatic peripheral zone volume, number of needle cores, and cancer volume. This increase in chance of cancer detection was greatest in small prostates. For example, in a 10 cc prostate, there was 90% probability of detection of 1.5 cc cancer with 14 cores. By comparison, in a 35 cc prostate, there is only a 46% probability of detecting the same 1.5 cc cancer with 14 cores.The probability curves for the number of cores needed for cancer detection as a function of prostate peripheral zone volume and cancer volume were exponential, with convergence with the largest cancer volumes. For example, in a large (35 cc) prostate, the number of cores needed to predict 90% or 95% chance of identifying a 1.0 cc cancer was well over 50 cores.

Conclusions: Sampling by biopsy can be optimized by considering prostate volume, cancer volume, and desired level of probability for cancer detection. Current sampling methods with 10-12 cores have disappointingly low detection accuracy for prostate cancer of any size. To our knowledge, this is the first biopsy sampling model of an organ such as the prostate based on volumetric measures. The computer program is freely available at www.cancerhome.com.

604 Atypical Small Acinar Proliferation and Serum PSA Provide Additive Information about Presence of Prostatic Cancer in Follow-Up Biopsies

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Background: A previous diagnosis of high-grade prostatic intraepithelial neoplasia (HGPIN) or atypical small acinar proliferation (ASAP) is considered a sufficient reason for repeat prostate biopsy. We sought to investigate which diagnosis is significantly associated with prostate cancer (PCa) diagnosis in subsequent biopsies independent of serum PSA.

Design: Between 2000 and mid 2005, 190 patients' biopsies with an initial diagnosis of HGPIN, ASAP, or both, followed by at least another biopsy, were reviewed. Captured parameters included serum PSA, presence or absence of HGPIN or ASAP, number of foci involved, total number of cores and number of cores in the final biopsy. A logistic regression analysis - after controlling for serum PSA - was performed.We also compared different tumor parameters in cancer patients with and without a previous diagnosis of HGPIN or ASAP.

Results: A total of 442 biopsies were evaluated. Mean patients' age and serum PSA was 67 years (38-82) and 7.8 ng /ml (0.3-58) respectively. 79%, 53% and 32% had an initial diagnosis of HGPIN, ASAP or both. 35% had a diagnosis of PCa in follow-up biopsies. Mean number of cores in the previous and final biopsies with PCa was 11 and 9.4 respectively. Incidence of PCa following a diagnosis of HGPIN, ASAP, or both was 34%, 47%, and 54% respectively. Logistic regression analysis showed that, whereas serum PSA and prior ASAP were strongly associated with a final result of cancer (p<0.0001), prior HGPIN, number of foci with ASAP or HGPIN, and total number of cores or biopsies were not. There was also no statistical difference in tumor parameters between cases with and without a previous diagnosis of HPIN or ASAP.

Conclusions: In patients with HGPIN, ASAP, or both in initial biopsy – and after controlling for effect of serum PSA – presence of ASAP, but not HGPIN, is strongly associated with PCa diagnosis in final biopsy. Moreover, total number of affected areas does not signify higher cancer rate in subsequent biopsy.

605 The Frequency and Clinicopathological Significance of Tertiary Gleason Pattern (TGP) in Radical Prostatectomy Specimens (RPS): Comparative Analysis of 78 Patients with and 1140 without TPG

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Background: The presence TGP in a small but not rare percentage of RPS is becoming more recognized in recent years. The clinico/pathological settings in which this phenomenon occur and its prognostic significance are not well defined. The objective of this study was to investigate the potential correlation between the presence and the amount of TGP with morphological parameters and patients outcome.

Design: All RPS performed at our institution between January 1997 and July, 2005, were considered for this study. We used the 2005 CAP definition to determine the presence TGP. Two cohorts of RPS that did not or did have TPG (based on review of pathology reports) were established. For the latter, all H&E slides were reviewed to determine the percentage of each Gleason pattern. The patients age, race, pre op PSA, the RPS pathologic stage and tumor volume and the biochemical recurrence data were extracted from our RP database. we correlated these parameters between the two cohorts.
Results: Of the 1218 patients considered for this study, 78 (6.8%) had RPS with three different Gleason patterns including 2 whose RPS had 4 patterns. Clinically, patients with TPG presented with higher pre op PSA values (12.9 vs 6.8 p=0.008), and older age at diagnosis (63 vs 59, p=0.001). There were no significant differences between the African American (AA) and Caucasian© patients with and without a TPG AA : C = 47.8% : 41.3% and AA: C = 48.7% : 41% respectively (p=0.9). RPS with TPG had lower proportion of pT2 tumors 19.2% vs 60.3% (p=0.001), more frequent pT3a 32.1% vs 10.8% and pT3b 29.5% vs 5.1% and a larger tumor volume: 6.6 vs 2.45 p=0.001. While this was true when TGP cases were compared to patients without TGP of Gleason score <= 7, there were no significant differences between non TGP with Gleason score >= and TGP group in any of the parametrs analyzed.
Conclusions: Patients with TPG seem to have more clinically and pathologically advanced disease when compared with the rest of the RP cohort at large but is similar to the high-grade Gleason prostate subgroups.

606 Expression of Ezrin-Radixin-Moesin Proteins, a Membrane Cytoskeletal Crosslinker Complex, in Prostate Adenocarcinoma

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Background: Ezrin–Radixin–Moesin (ERM) complex links the actin-containing cytoskeleton to the plasma membrane. ERM is a target of signaling molecules involved in the regulation of cell survival, proliferation and migration. In a comprehensive gene expression analysis of 80 cases of prostate adenocarcinoma (PCa), including 20 foci of metastases, using the Affymetrix U95a, U95b, and U95c chip sets we showed a strong decrease of radixin expression in PCa.
Design: We evaluated the expression of ezrin, moesin and radixin in 268 cases of PCa, prostatic intraepithelial neoplasia (PIN), normal prostate tissue adjacent to tumor (NAT), true benign prostate tissue from organ donors, and benign prostatic hyperplasia (BPH). Paraffin Tissue Microarray (TMA) slides were immunostained with antibodies to ezrin, radixin and moesin and scored using a 0-4 semi-quantitative system for both intensity and intracellular distribution of these proteins.
Results: ERM complex is localized in the cytoplasm of secretory cells of normal prostate, BPH, PIN and PCa with varying intensity and in distinct cytoplasmic compartments. Ezrin, Radixin and Moesin expression is decreased in PCa. The most dramatic changes are seen in ezrin expression. Ezrin expression is significantly lower in PCa and PIN when compared to normal tissues (median 0.66 vs.2 vs. 3.3, respectively; $p < 0.001$). Also, ezrin expression in normal prostate tissue adjacent to PCa is lower than in normal donor prostate tissue (median 2.75 vs. 3.3; $p < 0.001$). Ezrin expression correlates with Gleason score (GS) ($p<0.035$) and presence of metastases ($p < 0.021$).
Conclusions: Decrease in ERM expression parallels the progression of PCa. Loss of ezrin expression indicates an aggressive PCa.

607 B-RAF and K-RAS Mutations in Prostatic Adenocarcinoma

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Background: Constitutive activation of the kinase cascade involving RAS, RAF, MEK, ERK, and MAPK is common to human cancers. Mutations of RAS and BRAF are mutually exclusive and serve as alternatives to activate RAS/RAF/ERK signaling pathway. RAS mutations have been known to occur in prostate adenocarcinomas but little is known about BRAF mutations in prostate adenocarcinomas.
Design: In the present study, an extensive characterization of BRAF and KRAS mutations has been performed in 206 prostate adenocarcinomas using enhanced PCR-RFLP and direct sequencing. The results of KRAS or BRAF mutations were analyzed in relation to preoperative serum PAS level and Gleason score, tumor stage.
Results: Mutations in codon 600 of BRAF were identified in 21 (10%) of 206 prostate adenocarcinomas. The most frequently found mutation in codon 600 of BRAF gene was a GTG->GCG muatation, resulting in a valine to alanine amino acid shift, which was evident in 12 (57.1%) of 21 mutations. The second most frequent mutation was a GTG->ATG (valine to methionine) mutation, found in 8 (38.1%) of 21 mutations. The rest one mutation was a GTG->GAG mutation (valine to glutamic acid). 20 tumors carried transition mutations and one tumor showed a transversion mutation. KRAS mutations in codons 12 or13 were found in 15 (7.3%) / 206 prostate adenocarcinomas. Six tumors carried a mutation of codon 12 and eight tumors had a mutation of codon 13. One tumor showed mutations at both codon12 and codon13. All of the nine mutations in codon13 were transition mutation and 5 of 7 mutations in codon12 were transition. BRAF and KRAS mutations were not observed in the same specimens. Prostate adenocarcinomas with BRAF mutation tended to show higher preoperative serum PSA, Gleason score, and tumor stage than prostate adenocarcinomas with KRAS mutation.
Conclusions: The results showed that BRAF mutations were as uncommon as KRAS mutations in prostate adenocarcinoma. Although BRAF and KRAS are members of the same RAS/ERK signaling pathway, prostate adenocarcinomas with BRAF mutations showed different clinicopathologic features from those of prostate adenocarcinoma with KRAS mutation.

608 Dicarbonyl/L-Xylulose Reductase (DCXR): A New Potential Marker for Prostate Adenocarcinoma Identified by LCM-microSAGE

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Background: Serial analysis of gene expression (SAGE) assesses the expression of thousands of genes in a single experiment and also provides an unbiased view of expression. Laser-capture microdissection (LCM) allows one to reliably procure specific cells from a histologic slide. We combined these techniques to construct cell-specific, large-scale gene expression profiles of prostate cancer (PCA). Our goal was to identify novel diagnostic and prognostic markers and therapeutic targets.
Design: The use of the LCM-microSAGE technique has been reported (Mod Pathol 2005,18:577-84). Two LCM-microSAGE libraries from paired cancer and normal cells from a patient with PCA (Gleason score 3+4=7, $T_2N_0M_0$) were sequenced for a total 1253 concatemer clones. Sequence data processing and tag extraction strategy were determined using SAGE 2000 and Monte Carlo simulation. For the validation of candidate genes, we used quantitative real time RT-PCR (QRT-PCR) and virtual Northern blot (VNB) of 189 SAGE libraries. For confirmation of protein expression, we performed immunohistochemical staining using a tissue microarray composed of 46 organ-confined, early stage PCA and 29 chemotherapy/hormonally-treated PCA.
Results: After computational comparison of two libraries, a total 6462 unique tags were procured. Fifty-six transcripts revealed significant differential expression ($p<0.05$): 31 were upregulated and 25 were downregulated in cancer cells. In the upregulated group, we identified dicarbonyl/^{L}-xylulose reductase (DCXR), an enzyme catalyzing α-dicarbonyl and L-xylulose, as a novel marker. Specificity of DCXR upregulation was confirmed by QRT-PCR and VNB. Immunohistochemistry using a polyclonal anti-DCXR antibody (Taisho Pharmaceutical Co., Japan) showed that DCXR is expressed at low levels in most normal epithelial cells and is mostly confined to the cytoplasmic membrane. By contrast, DCXR is strikingly overexpressed in all grades of early stage PCA as well as in virtually all chemotherapy/hormonally-treated PCA. In cancer cells, DCXR shows altered subcellular localization with mostly cytosolic and nuclear expression. Stromal and inflammatory cells are negative for DCXR.
Conclusions: These findings suggest that DCXR is a potentially useful diagnostic marker for PCA and may be involved in carcinogenesis.

609 Vesicular Connexin 26 Staining Correlates with Aggressive Grade and Stage Prostate Cancer

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Background: Modulation of gap junction proteins, in particular connexins, has been seen in various cancers. We have previously shown that upregulation of connexin 26 occurs in both prostate cancer cell lines and primary prostate tissues. This upregulation correlates with aggressive features in the prostate cancer cell lines, and is associated with changes in cell adhesion. We have sought to correlate connexin 26 staining and cellular localization in prostate specimens with clinical, pathologic, and outcomes features.
Design: The 299 patient NCI CPCTR prostate cancer tissue miocroarray set was analyzed for staining of connexin 26 with attention to staining pattern and intensity. Pattern of staining was evaluated as cytoplasmic, nuclear, membranous, or vesicular. The vesicular staining is intracytoplasmic and perinuclear, reminiscent of the ER/golgi apparatus. Staining intensity and background staining were measured on a 0 (no staining) to 3 (strong brown staining) scale. This data was then correlated with the associated patient clinical, pathologic, and outcomes data. Statistical analysis was performed using Fisher's exact or Chi squared tests.
Results: Of the 299 patients on the tissue microarray set, 267 (89%) yielded evaluable data. Of these 267 cases, 265 (99%) demonstrated positive staining. Two patterns of staining were seen, diffuse cytoplasmic staining (179 cases) and intracytoplasmic perinuclear dot-like staining corresponding to ER/golgi type vesicles (189 cases). In 103 cases both patterns were present. The presence of vesicular connexin 26 staining was significantly correlated with high Gleason grade (7-9, p=0.0040), and advanced tumor stage (pT3 or pT4, p=0.0041). In addition, vesicular connexin staining was present in 8 of 9 cases with lymph node metastasis. No significant correlation was seen with PSA recurrence or patient vital status.
Conclusions: Recent studies have suggested that abnormal gap junction proteins are present in cancers. We have previously shown that connexin 26 is upregulated in prostate cancers, and now demonstrate a correlation between vesicular pattern connexin 26 staining and aggressive prostate cancer. This data supports the dysreulated expression of gap junction proteins in advanced cancers.

610 Distinguishing Poorly Differentiated Prostatic Adenocarcinoma from Invasive Urothelial Carcinoma in Transurethral Biopsy/Resection Specimens: Utility of p63

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Background: Poorly differentiated prostatic adenocarcinoma (PDPC) may have significant morphologic overlap with invasive high-grade urothelial carcinoma (UC), particularly in transurethral biopsy or resection (TUR) specimens where thermal artifact and small tumor volume may limit the evaluation. Although PSA and PSAP are well-established immunohistochemical markers of PDPC, there is no consensus regarding the optimal use of urothelial markers. Immunohistochemistry for p63 has recently been suggested as a potential urothelial marker in this setting.
Design: TUR specimens from 17 PDPC and 18 UC with sheet-like, solid growth or marked nuclear pleomorphism were identified. Representative formalin fixed, paraffin embedded tumor sections were stained with p63 (1:50; DAKO), Uroplakin III (1:5; Research Diagnostics), Thrombomodulin (1:50; DAKO), high molecular weight cytokeratin (1:50; DAKO), PSA (1:1400; DAKO), and PSAP (1:100; Cell Marque). Antigen retrieval was used for all antibodies except PSA and Thrombomodulin. The

percentage of neoplastic cells demonstrating immunoreactivity was scored for each antibody.
Results: All 18 UC showed nuclear immunoreactivity for p63 with a specificity of 76% (4/17 PDPC were focally positive). The percentage of positive tumor cells in UC ranged from 5-100% (mean:70%, median:85%), while the reactivity in PDPC ranged from 5-15% (mean:9%, median:8%). Other urothelial markers compared as follows: Uroplakin III (sensitivity:39%; specificity:100%; percent positive cells:10-60%, mean:18%, median:10%), Thrombomodulin (sensitivity: 83%; specificity:100%; percent positive cells: 5-60%, mean:29%, median:25%), and high molecular weight cytokeratin (sensitivity:100%; specificity:94%; percent positive cells:10-100%, mean:72%, median:80%). For the diagnosis of PDPC, PSA had a sensitivity and specificity of 88% and 100%, while PSAP was 100% and 94%, respectively.
Conclusions: Although p63 had lower specificity than Thrombomodulin and Uroplakin III (76% vs. 100% and 100%, respectively), it had higher sensitivity (100% vs. 83% and 39%) and a more diffuse pattern of immunoreactivity (mean % of positive cells 70% vs. 29% and 18%). Cytokeratin 34BE12 had the most optimal sensitivity, specificity, and extent of tumor reactivity. In the differential diagnostic setting of distinguishing PDPC from UC in TUR specimens, p63 is a useful adjunct to other prostatic and urothelial markers given its high sensitivity and typically robust immunoreactivity.

611 Histologic Grade Is More Important Than Tumor Thickness as Predictor of Nodal Metastasis in Squamous Cell Carcinoma (SCC) of the Penis Invading 5-10 mm

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Background: Low risk for regional metastasis has been demonstrated in tumors invading lamina propria/ corpus spongiosum to a depth inferior to 5 mm whereas SCCs involving corpora cavernosa have high metastatic potential. A frequent clinical problem is to perform or not inguinal dissection in tumors invading 5-10 mm. This study was designed to compare incidence of metastasis according to histologic grade and thickness in 5-10 mm thick tumors.
Design: 48 partial penectomies with 5-10 mm thick SCCs were evaluated. Inguinal dissection materials were also studied. Histologic grades were classified as low and high. The tumor was considered to be high grade if any proportion of anaplastic cells was present. Measurement was performed on HE stained slides from the non-necrotic non-keratinized tumor surface to deepest point of invasion.
Results:

Histologic grade and nodal metastasis (48 cases)

Histologic grade	Negative nodes	Positive nodes # (%)	p value
Low grade	11	4 (27)	0.03
High grade	13	20 (61)	

Tumor thickness and nodal metastasis (48 cases)

Histologic grade	Negative nodes Av thickness mm	Positive nodes Av thickness mm	p value
Low grade	7.4	7.8	0.8
High grade	8	7.5	0.4
All grades	7.7	7.5	0.7

Av thickness: average thickness
Conclusions: 42% of tumors invading 5-10 mm were not associated with metastasis and may be spared from a lymph node dissection. Thickness alone appears to be not sufficient for the decision since we found no differences in thickness in cases with negative and positive nodes. In this subset of patients, histologic grade is more useful than thickness to predict metastasis.

612 Prognostic Index in 110 Invasive Squamous Cell Carcinoma of the Penis. A Useful Guide for the Management of Inguinal Lymph Node Dissections

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Background: Because histological grade and depth of tumor invasion are among the most important prognostic parameters in penile squamous cell carcinoma (SCC), an index combining the 2 factors have been proposed. The aim of this study was to evaluate nodal metastasis according to penile cancer Prognostic Index (PI) in a new larger series.
Design: Pathologic materials from 110 patients with primary surgical and inguinal node dissections for penile SCC were evaluated. The Prognostic Index, from 1 to 6, consisted in the addition of numerical values given to histological grade (1, 2 and 3) and the anatomical level involved by cancer. In the glans: lamina propria (LP), 1; corpus spongiosum (CS), 2 and corpus cavernosum (CC), 3. In the foreskin, LP, 1; dartos, 2 and skin, 3. Grade 1 SCCs showed minimal to mild atypia, grade 2 showed moderate atypia and tumors showing any proportion of anaplastic cells were classified as grade 3.
Results:

Prognostic index	Negative nodes	Positive nodes	% of positive nodes	Total
1-3	12	0	0	12
4	8	2	20	10
5	31	23	43	54
6	10	24	71	34

Conclusions: Low-grade tumors invading LP or CS (PI: 1-3) were not associated with metastasis in this study. Indexes 5 and 6, which usually correspond to high-grade deeply invasive carcinomas, are frequently associated with lymph node metastasis, especially PI 6. Index 4, usually low-grade tumors involving CS or Dartos, are associated with a low risk of metastasis. The prognostic index is a useful guide to the clinicians in the often difficult decision to perform or not an inguinal dissection.

613 SEMA4F Overexpression in Perineural Invasion In Vitro Model of Prostate Cancer

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Background: SEMA4F belongs to a family of proteins expressed in the embryo that regulates the development of the nervous system. But its role in perineural invasion (PNI) remains unknown. In this study, we detected SEMA4F gene expression level in PNI in vitro model of prostate cancer (PCa), and then verified the effect of SEMA4F gene knockdown by RT-quantitative PCR and neurite outgrowth in SEMA4F-knockdown PNI in vitro model.
Design: The PNI in vitro model (DU-145/DRG coculture) was created using PCa DU-145 cells and mouse dorsal root ganglion (DRG) embedded in EHS matrigel for 14 days, DU-145 alone as control. Total RNA was isolated from PNI in vitro model. Gene profiling was performed by cDNA microarray analysis. Quantitative RT-PCR analysis was used to measure expression of target genes identified by cDNA microarray. To create a SEMA4F knockdown PNI of PCa, siRNA to SEMA4F, Cyclophilin B (positive control) and Non-Targeting Duplex siRNA (Negative control) for eliciting RNAi were transfected separately into DU-145 cells. The PNI in vitro model was set using transfected DU-145 cells. Total RNA was extracted and purified from SEMA4F knockdown-PNI in vitro model 48 hours later. The extent of SEMA4F knockdown level was evaluated by measuring mRNA copies using quantitative RT-PCR. Neurite outgrowth, cell colony growth were photographed and quantitated with Optimas imaging system.
Results: SEMA4F gene expression level increased more than 2-folds in PNI in vitro model comparing to the control. The results of its validation demonstrated the very similar expression pattern of the microarray analyses. 88% of SEMA4F gene has been knockdown from DU-145/DRG in vitro model 48 hours after transfection comparing to negative control samples. Neurite outgrowth in SEMA4F knockdown PNI in vitro model was 2-fold less than the control.
Conclusions: We identified SEMA4F gene over-expression in PCa PNI in vitro model through cDNA microarray. SEMA4F gene might be an important gene associated with the development of PNI.

614 Pathology of 68 Renal Epithelial-Stromal Tumors

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Background: The renal tumors now classified as "epithelial-stromal tumors" incorporates those which we had previously coded as renal pelvic hamartomas or renal cortical hamartomas depending upon their dominant location. Upon reviewing both groups it became apparent that they were not separable.
Design: A total of 68 cases were reviewed using H & E slides and immunohistochemistry.
Results: 26 cases were initially classified as renal "pelvic hamartomas" with ages ranging from 23-73 years, a female dominance of 8:1 and a median and mean age of 47 and 48. The "cortical hamartomas" numbered 42 with ages ranging from 11-79 years, a 4:1 female dominance and median and mean ages of 44. Of the entire group tumor size varied from microscopic up to 30 cm, mean 5.0cm. Pelvic cases typically bulged into or formed a polypoid mass in the pelvic cavity. The cortical lesions sometimes extruded into perinephric soft tissue as a polypoid lesion. The pelvic cases were more often grossly cystic but they were identical in the cortex. Some of the tumors involved the full thickness of cortex and medulla leaving no doubt that they are the same type tumor. Microscopically, some of the cortical lesions especially are largely or entirely smooth muscle, although there are usually peripheral tubular and tubulopapillary formations which likely are neoplastic. Commonly, smooth muscle consists of scattered fascicles admixed with a stroma which is acellular or cellular collagen. Rarely aggregates of fat cells and usually small primitive appearing cells are seen. The epithelial element consists of bland cuboidal cells occurring as isolated and scattered aggregates of tubules with an adenoma-like appearance. Many of the cysts are lined by eosinophilic hobnail cells identical to the cystic nephromas. It should be noted that these tumors are not encapsulated. Peripherally they usually admix with renal parenchyma-making it sometimes difficult to distinguish tumor and entrapped elements. The differential diagnosis includes angiomyolipoma. Unlike the latter, aggregates of prominent blood vessels lack the advential features of the AML and the muscle cells lack the vacuolated or clear cell features of AML. Both, of course, take the smooth muscle markers but these are negative for the melanoma markers. Intrarenal leiomyomas are rare but they, too, do not react with the latter. In the few cases tested estrogen and progesterone markers have been positive in the epithelial-stromal tumors.
Conclusions: The renal epithelial-stromal tumors are benign tumors (we have seen no recurrences), seen usually in adult females with features distinctive from cystic nephromas, angiomyolipoma and leiomyomas.

615 Expression of Caveolin Proteins in Prostatic Adenocarcinoma

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Background: Caveolin (cav) proteins 1-3 are the principal components of caveolae membrane invaginations and have been implicated in cell signaling, proliferation, differentiation, and apoptosis. Recent studies have suggested increased caveolin-1 protein as a serum marker of prostate cancer (PCa), yet only a small number of studies have addressed mRNA and cav protein expression in benign and malignant prostatic tissues.
Design: RNA from fresh frozen tissue was isolated after laser capture microdissection of epithelial cells from tumor (Gleason score 6-9, n = 10) or matched normal tissues (n = 9). mRNA was amplified by in vitro transcription, labeled with Cy3-dUTP during reverse transcription, mixed with similarly amplified Cy5-dUTP-labeled common reference BPH RNA, and hybridized to cDNA microarrays on glass slides. Sections of tissue microarrays containing Gleason pattern (GP) 3, 4, and 5 PCa or metastatic PCa were stained by immunohistochemistry against cav-1, -2, and -3 using monoclonal antibodies (Transduction Labs). Staining intensity was graded 0-3+ for both epithelium and stroma.

Results: **RNA studies:** There was marked down-regulation of both cav-1 and cav-2 mRNA in both normal prostatic epithelial cells as well as tumor cells when compared to mRNA from reference BPH. **Cav-1 Protein:** 110 cores (53 cases) of GP3, 36 cores (16 cases) of GP4, 10 cores (5 cases) of GP5, and 107 cores (48 cases) of normal prostatic tissue demonstrated no epithelial staining and 2-3+ stromal staining. Metastatic PCa, including pelvic lymph nodes (136 cores; 55 cases), soft tissue (24 cores; 9 cases), and bone (10 cores; 4 cases) also showed no epithelial staining. **Cav-2/Cav-3 Proteins:** 60 cores (31 cases) of GP3, 37 cores (16 cases) of GP4, 10 cores (5 cases) of GP5, and 57 cores (26 cases) of normal prostatic tissue showed no staining of tumor or secretory luminal cells. 2-3+ cav-2 staining was seen in basal cells and prostatic stroma. 3+ staining for cav-3 was seen in skeletal muscle only.

Conclusions: Cav-1 and -2 are immunohistochemically undetectable in normal and cancerous (primary and metastatic) prostatic epithelia and strongly expressed in prostatic stroma. Down-regulated expression of cav-1 and -2 mRNA in microdissected benign and malignant prostatic epithelial cells when compared with BPH is consistent with expression of both mRNAs predominantly/completely in prostatic stroma, supporting the immunohistochemical findings. Detection of increased serum caveolin in PCa relative to BPH may be related to invasion of and release of caveolin from prostatic stroma.

616 Expression of Topoisomerase II Alpha in Testicular Germ Cell Tumors

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Background: Topoisomerase II alpha (Topo IIa) is an enzyme which plays a crucial role in DNA synthesis and repair. Etoposide, a potent inhibitor of Topo IIa, is a component of the chemotherapy regimen for non-seminomatous testicular germ cell tumors. Despite the high success rate of current chemotherapy, a significant number of patients experience late relapse. The molecular events related to chemosensitivity of testicular germ cell tumors are not well understood. We analyzed Topo IIa expression in a large number of testicular germ cell tumors in order to ascertain which histological components may be chemoresponsive.

Design: Specimens from 99 patients with testicular germ cell tumors were subjected to immunohistochemical study using monoclonal antibody against Topo IIa (Vector, 1:40). Each histological component was analyzed independently (73 seminomas, 40 embryonal carcinomas, 24 yolk sac tumors, 17 immature teratomas, 15 mature teratomas, and 7 choriocarcinomas). Nuclear immunoreactivity was seminquantitatively evaluated as negative (< 5% of cells stained), focally positive (5-10% of cells stained), or positive (>10% of cells stained).

Results: The majority of embryonal, seminoma and yolk sac components were Topo IIa immunoreactive (positive), while all mature and most immature components were either negative or focally positive. (See table)

Conclusions: Topo IIa expression was demonstrated in the majority of embryonal, seminoma, and yolk sac histological components of testicular germ cell tumors, suggesting their sensitivity to chemotherapy composed of Topoisomerase II inhibitors, such as etoposide. Mature teratomas and immature teratomas, the most common components of late relapsing germ cell tumors, were found to express low levels of Topo IIa. Our findings imply a molecular basis to the variable chemoresponsiveness of testicular germ cell tumor components.

Topo IIa	Seminoma	Embryonal	Yolk Sac	Immature Teratoma	Mature Teratoma	Choriocarcinoma
Positive	56 (77%)	39 (98%)	19 (79%)	1 (6%)	0	4 (57%)
Focally positive	8 (11%)	1 (2%)	4 (17%)	9 (53%)	0	0
Negative	9 (12%)	0	1 (4%)	7 (41%)	15 (100%)	3 (43%)
Total	73	40	24	17	15	7

617 Immunohistochemical Antibody Cocktail Staining (p63/HMWCK/AMACR) of Ductal Adenocarcinoma and Gleason Pattern 4 Cribriform and Noncribriform Acinar Adenocarcinomas of the Prostate

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Background: Overexpression of AMACR in combination with absence of basal cell markers, i.e p63 and HMWCK, is typical of classic acinar prostatic adenocarcinoma (PCa). We studied the expression and diagnostic utility of p63/ HMWCK/AMACR immunohistochemical cocktail staining in ductal adenocarcinoma and cribriform Gleason pattern 4 acinar PCa and compared it to noncribriform Gleason pattern 4 acinar PCa.

Design: One to four representative formalin fixed paraffin embedded archival tissue blocks from 62 radical prostatectomy specimens harboring PCa of ductal (n: 51), cribriform Gleason pattern 4 acinar (n: 27), and noncribriform Gleason pattern 4 acinar adenocarcinoma (n: 48) were included in this study. Immunohistochemistry was performed using a triple stain of AMACR, p63 and HMWCK. Only staining that was moderate or strong was considered positive. The percentage of staining intensity as well as the presence of occasional basal cells positive with p63/HMWCK were recorded in each histological type of prostatic carcinoma.

Results: 77% of ductal PCa, 67% of cribriform acinar PCa and 81% of noncribriform acinar PCa showed positive staining for AMACR. There was no statistically significant difference between AMACR staining amongst the 3 histological types, although there was a trend for noncribriform acinar PCa to have greater expression of AMACR than cribriform acinar PCa (p=0.07). Staining was often heterogeneous varying in staining intensities within the same histological type of carcinoma. Basal cells were detectable by p63 and HMWCK in a patchy fashion in 31.4% (16/51) of ductal and 29.6% (8/27) of cribriform acinar carcinomas compared to 2.1% (1/48) of noncribriform acinar carcinomas.

Conclusions: 1) The majority of prostatic ductal and cribriform acinar carcinomas expressed AMACR strongly, however, subpopulations of these PCa were either completely negative or only weakly positive. 2) Staining was often heterogenous in intensity in the same histological type of tumor, even within the same case. 3) As expected, we demonstrated that patchy basal cell staining in noncribriform acinar PCa is rare. In contrast, remnants of basal cells identified by p63/HMWCK were seen in a patchy fashion in a significant minority of both ductal and cribriform acinar PCa, which most likely represents intraductal spread of tumor.

618 Inverted Urothelial Papillomas of the Prostatic Urethra

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Background: Inverted papillomas (IP) are benign urothelial neoplasms most commonly seen in the bladder and upper urinary tract, where their association with urothelial carcinoma (UC) has been controversial. IP originating in the prostatic urethra have not been well characterized.

Design: We identified 21 IPs of the prostatic urethra and studied their clinical and histopathologic features.

Results: Patients had a mean age of 65.1 years (range: 30 to 89 years), with 10/21 (47.6%) presenting with gross hematuria (n=8) or irritative symptoms (n=2) related to IP and 11/21 (52.4%) detected incidentally during work-up/treatment of prostate cancer (n=6) or BPH (n=5). 14 cystoscopically evaluated lesions measured 0.1 to 2.0 cm, and were polypoid (n=9), papillary (n=4), or an enlarged median lobe (n=1). Lesions were diagnosed on TUR (n=8), biopsy/polypectomy (n=6), radical prostatectomy (n=4), or biopsy unrelated to the lesion (n=3). Histologically, 14/21 cases (67%) displayed classic IP architecture. The remaining cases showed foci of squamous metaplasia with moderate atypia (n=4), rare true papillary fronds in a classic IP background (n=2), or both (n=1). 11 cases with associated prostate tissue in addition revealed: adenocarcinoma of the prostate Gleason score 6 (n=3) and 7 (n=3); prostatic intraepithelial neoplasia (n=1); BPH (n=3); or adenosis (n=1). No patients had prior history of IP or UC, while two patients were diagnosed with high grade UC of bladder synchronous with their IP diagnosis. None of the 18 patients with available follow-up have had IP recurrence in the prostatic urethra or other locations in the urinary tract (mean follow-up 39.9 months; range: 3 to 120).

Conclusions: IP of the prostatic urethra are benign lesions that may be detected incidentally and are not associated with a history of urothelial malignancy. Although UC elsewhere in the genitourinary tract may occur simultaneously, malignant transformation or recurrence as a malignant lesion has not been identified in IP of the prostatic urethra.

619 Microscopic Seminal Vesicle Invasion in Radical Prostatectomies

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Background: Seminal vesicle invasion (SVI) is an ominous finding in prostate cancer. This discovery in radical prostatectomies (RP) is accidental in most cases because patients with clinical or histological evidence of extraprostatic disease are not surgically treated in most protocols. Nevertheless, microscopic SVI escapes the clinical and radiologic evaluations and appears with some frequency in the routine studies of RPs. This study aims to quantify this histological finding and to evaluate its significance in a large and homogeneous series.

Design: Over a 7-year period (1998-2004), a total of 290 patients with prostate cancer underwent RP. The combination of clinical staging, serum PSA levels and core biopsy (CB) data selected the candidates for surgery. The histological study showed SVI (pT3b stage) in 29 cases (10%). The presence of SVI in RPs has been correlated (Spearman's correlation) with several histological parameters both in CBs and in RPs.

Results: Microscopic SVI correlated with total millimeters of cancer (average of 8,4 mm vs. 17,1 mm, ρ=0.297), number of tumor foci (average of 2,1 vs. 3,3, ρ=0.283), bilateral invasion (ρ=0.256), Gleason Index (GI) >7 (ρ=0.306), perineural invasion (ρ=0.318), and HGPIN (ρ=0.142) in CBs, and with GI >7 (ρ=0.357), HGPIN (ρ=0.211), and margin (ρ=0.287), perineural (ρ=0.447), and apex (ρ=0.307) invasions in RPs.

Conclusions: Microscopic SVI is a frequent finding in RPs, even after a correct selection of patients for surgery. SVI correlates with tumor volume parameters, bilateral invasion, and other morphologic parameters of bad prognosis both in CBs and in RPs.

620 Minute Cancer in Core Biopsies. Histological Spectrum and Correlation with Findings in Radical Prostatectomies

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Background: Minute carcinoma (MC) is a common finding in prostate core biopsies (CB), but its exact significance may be unreliable because a diagnosis of MC does not necessarily imply low Gleason index (GI) or organ confined disease in all patients. The present study aims to review the histological findings that allow the diagnosis of MC and to correlate these histological features in CBs with those parameters of prognostic significance that appear in their subsequent radical prostatectomies (RP).

Design: Over a 7-year period (1998-2004), a total of 290 consecutive RPs were performed in our Institution and 30 (10,3%) of them had MC on their previous CBs. MC was defined as "one focus of cancer <0.5 mm in length" in the whole biopsy material (6 to 8 tissue cores). MC was morphologically characterized and its presence in CBs correlated with pT category, GI, status of the surgical margins and apex, HGPIN, perineurial invasion, and extraprostatic extension on RPs.

Results: Nuclear enlargement (28/30), infiltrative pattern of growth (27/30), nucleoli (25/30), associated HGPIN (25/30), GI≤7 (25/30), intraglandular secretions (16/30), and crystalloids (16/30), were the most consistent findings in CBs. The correlations of MC with organ confined disease (pT2a=9, pT2b=19, pT3a=1, pT3b=0, pT4=1) and with GI≤7 (<7=12, 3+4=10, 4+3=3, >7=0) were evident. Positive margins (8/30), and apex (5/30) and perineurial (14/30) invasions were variably found.

Conclusions: MC is not an uncommon finding in CBs. MC displays a wide range of histological findings and may be associated to some parameters of bad prognosis in RPs.

621 Predictors of Adverse Outcomes on Radical Prostatectomy (RP) Following Positive Ten-Core Biopsy

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Background: Biopsy predictors of adverse RP outcomes have been studied previously, largely based on studies using six-core biopsies. Biopsy predictors have not been explored in a contemporary larger study based on ten-core biopsies.

Design: Sample size included 1150 ten-core biopsies matched with RP. All biopsies and RP were performed in our institution between 07/2000 and 04/2005. The following biopsy variables were studied: total percent cancer (TPC), cancer length in mm, number of positive cores, Gleason Score (GS), neural invasion, PSA and PSA density. We studied the following adverse RP outcomes: stage pT3, positive margins, positive seminal vesicles (SV) or lymph nodes (LN), and tumor volume >10% gland involvement (by semi-quantitative assessment). Univariate analysis was used to determine cut-off values for individual predictors, based on odds ratio, p value, and C-statistic. Multivariate logistic regression models were developed using predictors with the strongest correlation for each outcome.

Results: On univariate analysis, multiple predictors of cancer extent on biopsy, GS 7 or >7, PSA >10 ng/ml and PSA density >0.3 strongly correlated with the adverse RP outcomes. Multivariate model for stage pT3 included: GS≥8 (odds ratio 10.5; p< 0.0001), TPC >30 % (odds ratio 4.35; p=0.0013), and PSA density (odds ratio 2.37; p=0.0013). C-statistic for the pT3 model was 0.79. Positive margins model included following predictors: positive cores >3 (odds ratio 2.6; p<0.0001), GS 7 (odds ratio 1.68; p=0.0008) and neural invasion (odds ratio 1.4; p=0.0485). C-statistic for the positive margins model was 0.65. Best predictors for SV or LN invasion were: GS≥8 (odds ratio 15.01; p< 0.0001), TPC >30% (odds ratio 5.05; p=0.0048, and PSA density (odds ratio 2.95; p=0.0029). C-statistic for SV or LN model was 0.81. Tumor volume >10% gland involvement was associated with these top predictors: TPC >30% (odds ratio 6.33; p=0.0248, positive cores >3 (odds ratio 3.27; p<0.0001), and PSA density >0.3 (odds ratio 4.33; p<0.0001). C-statistic for the tumor volume model was 0.78.

Conclusions: 1.) Adverse RP outcomes correlated with cancer extent and GS on ten-core biopsy and preoperative PSA density. 2.) Best predictors for stage pT3 and SV or LN invasion included TPC >30%, GS≥8, and PSA density >0.3. 3.) Positive margins were best predicted by a model that included GS 7, >3 positive cores, and neural invasion. 4.) Best predictors in a multivariate model for tumor volume >10% were TPC >30%, positive cores >3, and PSA density >0.3.

622 With Two or More Needle Biopsies (NB) Containing Prostate Cancer (Pca) of Different Gleason Score, What Predicts Better for the Radical Prostatectomy Specimen (RPS) Gleason Score (GS)?

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Background: The GS discrepancy between the NB and RPS is well recognized. The more complex encounter of having two or more NB positive for Pca but with a different GS between/among the positive cores poses further uncertainty with respect to predating the GS of the prostate gland. The objective of this study was to analyze the pathological findings of the NB and RPS of this subset of prostate cancer patients.

Design: All patients diagnosed with Pca in whom at least two different cores harbored two different GS and subsequently underwent radical prostatectomy in our institution between 1999 and 2005 were included in this study. The number of NB with Pca and the percentage of NB involvement for the different GS components were documented. The findings were correlated with the GS of the RPS.

Results: One hundred and sixteen patients qualified for analysis. When the highest GS in the NB was used as a predictor, in 59 of 116 cases (50.8%) the NB score was the same as that of the RPS p<0.0001. Alternatively, when the GS representing the highest percentage of core involvement in the NB(s) was used as a predictor, the same GS was present in the NB and the RPS in 58 (50%). Finally, if the GS encountered on more cores was used as a predictor, the number RPS that had the same GS decreased 37 (31.8%), p=0.16. Furthermore, the highest Gleason score tends to correctly predict high grade cancers in RPS (53%) whereas the percentage of core involvement or accounting for the most common GS on the cores collectively appear to predict with more accuracy low-grade cancers (46%) Even when highest GS represents a smaller proportion of the tumor (30/116), 27% of the cases, its capacity to predict the grade in RPS decreases only slightly (correct prediction in 40% versus 43.3% for percentage estimates). A multivariate analysis using multinomial logistic regression shows that highest GS predicts the GS of the RPS independent of the percentage of core involvement or accounting for the most common GS on the cores collectively. (p=0.007).

Conclusions: Our data suggest that the best prediction for GS in the RPS is provided by using the highest needle biopsy GS. This appears to be true even in the presence of a more extensive lower-grade Pca reflected either by a higher number of positive cores or by a higher percentage of core involvement.

623 Clinical Significance of Minute Foci of Gleason Score 8-10 Prostate Cancer on Needle Biopsy

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Background: Prostate needle biopsies (NB) with high grade cancer usually contain abundant tumor. The clinical significance of minute high grade cancer on NB is unknown.

Design: We identified 108 patients with a minute (≤1 mm) focus of high grade prostate cancer, defined as Gleason score (GS) 8 (4+4 only), 9, or 10, from the consultative files of one of the authors. Progression free survival (PFS) was calculated actuarially using Kaplan-Meier curves.

Results: Of the 108 patients, 37 had GS 8, 41 had GS 9, and 31 had GS 10 on NB. 44 patients each underwent RP and radiation therapy (RT; either external beam or brachytherapy), 15 underwent hormonal therapy (HT), and 5 opted for surveillance. 5 patients who underwent RP showed evidence of anti-androgen therapy on RP and were not graded, but were staged when feasible. 17/39 (43.6%) of the remaining RP patients had GS ≤ 7 on RP. Of 42 patients that could be pathologically staged, 28 (66.7%) had organ-confined (OC) disease, 7 (16.7%) had extraprostatic extension with negative margins (EPE/M-), 5 (11.9%) had EPE with a positive surgical margin (EPE/M+) and 2 (4.8%) had either seminal vesicle or lymph node positivity (SV/LN+). 40/44 patients with RP had available follow-up (mean: 38.7 months; range 3-113 months). Overall, RP patients had a 90.2% 2-yr PFS and 78% 5- and 10-yr PFS. Patients with GS ≤7 on RP had 100% PFS, while those with GS >8 on RP had 87.5% 2-yr PFS & 65.6% 5-yr PFS. Patients with OC disease or EPE/M- tumor had 100% 2-yr PFS & 86% 5-yr PFS, while those with EPE/M+ or SV/LN+ had 40% 2-yr PFS. Of the 44 RT patients, 32 had at least 1 yr of follow-up. Overall, RT patients had 70.8% 2-yr PFS & 63% 3-yr PFS. 10/15 HT patients had available follow-up with 60% still hormone-sensitive at a mean of 48.5 months (range: 26-92 months).

Conclusions: For minute foci of GS 8-10, there is poor correlation between GS on NB and either GS or stage at radical prostatectomy. RP is not often considered for patients with more abundant GS 8-10 on NB. However, given that 83.4% of patients with minute, high grade disease on NB had at RP either OC or EPE/M- disease which was associated with an excellent prognosis, surgery may well be an option in a subset of these patients. The 43.6% of patients with GS 8-10 on NB yet GS ≤7 on RP underscores the potential for sampling error that is incurred by prostate needle biopsy, where pattern 3 is missed on the initial biopsy.

624 Correlation of Prostate Needle Biopsy and Radical Prostatectomy Gleason Scores: A Contemporary Update

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Background: We previously reported on 499 radical prostatectomies (RP) performed at our institution in 1994 and correlated needle biopsy (NB) and RP Gleason score (GS) depending on whether the NB grade was assigned at our or another institution.

Design: To determine if contemporary Gleason grading patterns have changed, we repeated the study on 1004 men who underwent RP at our institution from 2002-2003.

Results: In the current series, other institutions made a NB diagnosis of GS 2-4 in 1.9% of cases as compared to 22.3% in the previous series; of the 19 GS 2-4 cases, 31.6% revealed GS 7-10 on subsequent RP. No GS 2-4 was diagnosed at our institution. We diagnosed GS 6 on NB with 80% accuracy to the RP GS, as compared with 63% in the older data, likely due to increased RPs with GS 6 (66.3% current v. 50% in past). Overall, outside NB diagnoses showed 69% agreement (kappa-statistic: 0.32) with RP GS vs. 78% agreement for our NB diagnoses (kappa-statistic: 0.48). Comparing kappa statistics from the older and newer studies, showed a larger proportionate increase in outside NB-RP GS agreement (from 0.1 to 0.32) than for our agreement (from 0.32 to 0.48). In case of GS discrepancy between institutions, our GS correlated better with RP GS (e.g. outside NB GS 6 and ours >6, 83% (39/47) of RP had GS >6). Comparison of ours and outside NB GS revealed 88% agreement for GS 6, 73.2% agreement for GS 7, and 44.4% agreement for GS 8-10. When outside NB GS = 8-10, our diagnosis was ≤ 7 in 50%. Conversely, if our NB GS = 8-10, outside diagnosis was ≤ 7 in 55.5%. Furthermore, for our NB GS 6, 7, and 8-10, incidence of non organ-confined disease at RP was 18.3%, 48.3%, and 53.8%, whereas for the same NB GS at outside institutions the incidence was 18.4%, 45.7%, and 34.8%.

Conclusions: In the last decade, undergrading of NB, once prevalent in the pathology community, has significantly diminished. Still, nearly 1/3 of outside cases assigned GS 2-4 revealed GS 7-10 at RP and all were GS ≥ 5, reaffirming that GS 2-4 is a NB diagnosis that rarely if ever should be made. With aggressive screening and biopsy protocols detecting earlier, lower grade cancer, the percentage of RP with GS 6 has increased significantly. Finally, the over- and undergrading of GS 8-10 and concurrent poor correlation with stage is now a significant issue. The significant improvement in grading in more recent years, especially less undergrading of GS 6, underscores the need for further education and refinement of Gleason grading for GS 8-10.

625 Expanding the Morphologic Spectrum of Mucinous, Tubular, and Spindle Cell Carcinomas: Emphasis on "Mucin-Poor" Variants

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Background: Mucinous, tubular, and spindle cell carcinomas (MTSC) are polymorphic neoplasms characterized by small, elongated tubules lined by cuboidal cells and/or cords of spindled cells separated by pale mucinous stroma. Non-classic morphologic variants and features of MTSC have not been well studied.

Design: We studied the morphological and immunohistochemical (IHC) features of 17 MTSCs from Surgical Pathology and consultative files and compared their IHC to that of MTSC mimickers, including 5 sarcomatoid carcinomas (SARCCA) and 4 papillary renal cell carcinoma, solid variant (SVPRCC).

Results: 8/17 MTSCs were "mucin-poor", with 5 cases showing equal tubular and spindled morphology, 2 cases with spindle cell predominance (70%; 95%), and 1 case of tubular predominance (90%). In 6/8 mucin-poor cases, alcian blue staining showed scant (<5%) mucin in cellular areas, not appreciable by H&E. 8/17 cases were "classic", with 6/8 showing foci of tubular predominance. Alcian blue staining revealed abundant (>50%) mucin in all classic cases. 1/17 cases showed mixed spindled and tubular areas with extracellular hyaline matrix and blue-tinged mucin within tubules. This case also contained solid areas with epithelioid cells, heterotopic bone and calcification, and focal moderate atypia (enlarged nuclei/prominent nucleoli). Unusual histologic features identified in the 17 cases were: necrosis [n=3], oncocytic cells [n=1], multinodular growth with lymphocytic cuffing [n=1], and numerous small vacuoles [n=1]. An exceptional case contained well-circumscribed HMB45-positive AML within the MTSC. Claudin 7, reportedly a marker of distal nephron tumors, labeled 11/15 MTSCs, 3/5 SARCCA and 2/4 SVPRCC. CK7, expressed in normal distal nephron and in papillary RCC stained 3/4 SVPRCC, 3/5 SARCCA, and 14/15 MTSC, while AMACR, a purported marker of papillary RCC, labeled 4/4 SVPRCC, 2/5 SARCCA, and 13/15 MTSC.

Conclusions: MTSC may show a predominance of any one of its principal components, potentially mimicking SARCCA (spindle-cell predominant) or SVPRCC (tubular-predominant). Furthermore, although MTSCs are thought to be low grade tumors with only rare reported metastases, worrisome features such as atypia and necrosis may occasionally be observed. Immunohistochemical staining for claudin 7, CK7, and AMACR are not helpful in this differential diagnosis. Pathologists must be aware of the spectrum of histologies within MTSC to ensure their accurate diagnosis.

626 Evaluation of Serum SELDI-TOF Proteomic Information and Tumor Volume in Prostate Cancer Patients

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Background: As the diagnostic utility of serum surface enhanced laser desorption/ionization time-of-flight (SELDI-TOF) proteomics for early detection of prostate cancer (CaP) is supported by recent studies, the underlying nature of peaks on the proteomic spectral signatures remains a mystery. It is being proposed that these are either low abundant proteins that are leaked from the malignant prostate or products of host response to the tumor. Serum markers originating from the prostate that may predict a low tumor burden may aid in selection of CaP patients wherein conservative management is appropriate. We investigate whether protein markers defined by peaks on the SELDI-TOF spectra in a large cohort of CaP patients correlate with the size of the tumor in their corresponding diseased prostates.

Design: Serum samples from 281 CaP patients who underwent radical prostatectomy were processed in duplicate using a Biomek 2000 robotic workstation. Serum SELDI-TOF profiles were generated using IMAC3-Cu and WCX2 arrays by a Ciphergen Protein Biological System II ProteinChip Reader. Significant peaks from the 2 - 50 kD range were clustered using the Biomarker Wizard of Ciphergen's ProteinChip Software on both array types. Tumor volume was measured using paraffin-embedded prostate whole mounts. Statistical analysis of serum SELDI-TOF information and tumor volume was carried out using Pearson correlation analysis and t-test.

Results: A total of 115 significant SELDI-TOF peaks were identified on IMAC3-Cu and WCX2 array spectral data. Median tumor volume was 4.0 cc ranging from 0.013 to 38.63 cc. Tumor volume and SELDI-TOF peak data did not exhibit normal distribution and had to be log transformed prior to statistical analysis. Pearson correlation analysis did not demonstrate a relationship between tumor volume and SELDI-TOF peaks. No significant correlation was identified by t-test even when tumor volume was stratified ($TV < 0.5$cc vs. $TV \geq 0.5$cc) and treated as a categorical variable.

Conclusions: The intensities of serum protein peaks as elucidated by SELDI-TOF mass spectrometry on the WCX2 and IMAC3-Cu arrays did not correlate with CaP tumor volume in this study. Further investigations, which may yield better markers of CaP tumor volume utilizing different array surface chemistries, are warranted.

627 Gene Expression / Biochemical Pathway Signatures of Benign Prostatic Glands of Patients with Well and Poorly Differentiated Carcinomas

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Background: The focus of the current study is to identify prostate cancer (CaP) associated gene expression signatures in specific cell types of the human prostate gland with a goal to carefully define the pathobiology of epithelial cell components in prostate tumorigenesis and cancer progression. Do benign prostate epithelial cell-derived gene expression signatures have the potential to distinguish patients with poorly differentiated (PD) and well differentiated (WD) carcinomas?

Design: Laser capture micro-dissected (LCM) epithelial cells from prostate glands of carefully selected CaP patients were analyzed by Affymetrix U133a GeneChips. The benign epithelial cells are from two groups: one group with PD carcinoma (9 patients) and PSA recurrence and one with WD carcinoma (11 patients) without PSA recurrence. The bioinformatics analysis was enhanced by using a pathway analysis software (Ingenuity), which applies knowledge-based network relation between the differentially expressed genes.

Results: Multidimensional scaling (MDS) plots using supervised analysis of nine normal samples from the PD group and eleven from the WD group were generated. A subset of 200 genes were obtained, which can serve as a classifier for the samples. Among the top ten genes were CXCL2, EPOR, PPFIBP1, RUNX1, BTN3A3 (p-Value range from 0.000988 to 0.024952) that differed between the PD and WD groups. Utilizing the pathway analysis, genes from several different pathways were identified that differentiated the PD and WD groups. Genes like chemokine, erythropoietin, transcription factor and those with the function of glucose homeostasis (CXCL2, EPOR and RUNX1) were significantly up regulated in the PD group and down regulated in the WD group.

Conclusions: Gene expression signatures in benign prostate tissues separated patients with WD form those with PD. Significant changes in the expression level of these subset of genes might provide early warning of tumorigenesis. It is not clear if these changes are inherent or due to a paracrine effect from the adjacent tumor.

628 Atypical Small Acinar Proliferations and High-Grade Prostatic Intraepithelial Neoplasia in Incidental Prostatectomies

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Background: Atypical small acinar proliferation (ASAP) has been utilized in needle core biopsies (NCB) as a diagnosis of uncertainty; however, its predictive value for prostatic adenocarcinoma (PCa) exceeds that of high-grade prostatic intraepithelial neoplasia (HGPIN). Application of immunohistochemical stains for basal cells (high-molecular weight cytokeratin (CK 903) and/or p63) and for α-methylacyl coenzyme A racemase (AMACR) may resolve lesions that are large enough to survive subsequent levels. Those that are too scant to exclude non-neoplastic conditions remain ambiguous. Nevertheless, given the association with a subsequent diagnosis of PCa, ASAP is considered by some to represent marginally sampled PCa, leading to aggressive rebiopsy and even radical prostatectomy. We reviewed asymptomatic prostates for the presence of ASAP and evaluated the foci in a manner analogous to a diagnostic NCB.

Design: Reports from cystoprostatectomies for bladder carcinoma (60), colorectal carcinoma (3), prostatic sarcoma (1), and a benign urological condition (1) were reviewed. Those prostates with incidental PCa were documented and excluded from further review. Archived slides from the remaining prostates were examined for the presence of HGPIN and ASAP. Foci of ASAP were further evaluated with immunohistochemical stains (CK903, AMACR), and three additional H&E levels.

Results: Twenty four of 65 prostates (37%) contained PCa. Of the 41 benign prostates, 3 (7.3%) contained 4 foci of ASAP, 6 (14.6%) contained both ASAP and HGPIN, and 14 (34%) contained only HGPIN. Of the 10 foci of ASAP, two were not present on any additional level. All of the remaining eight lesions had absent peripheral CK903 staining. Of these, three were not present on the AMACR stained slides. All five remaining lesions demonstrated granular cytoplasmic staining. Two of these five foci developed into larger lesions on subsequent H&E levels, which were considered histologically diagnosable PCa.

Conclusions: Almost a fourth of the otherwise benign prostates contained foci of ASAP. All of those that persisted had a staining pattern which, on NCB, would support a diagnosis of PCa; however, on deeper levels, only 2 of 10 lesions were quantitatively sufficient for PCa. A similar percentage likely represents PCa where levels moved out of the lesion, while the remainder are atypical glands that are quantitatively insufficient for a diagnosis of invasive PCa.

629 COX-2 Expression in Prostate Needle Core Biopsies – Comparison of Prostatis and Prostate Cancer

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Background: Chronic prostatitis is a world-wide problem affecting 15% of men in a lifetime. Basic, clinical and epidemiologic evidence suggests a link between inflammatory mediators such as COX-2 in prostatitis and in the development of prostate cancer.

Design: IHC for COX-2 (Clone M-19 Santa Cruz Biotechnology) was performed on 53 core needle biopsies from 32 patients. Cytoplasmic staining was scored as 0-4+ intensity. The data was analyzed using a proportion odds model of statistical analysis to compare normal, prostatitis and cancer

Results: 44 samples had "normal" areas, 27 prostatitis and 17 carcinoma. Inflamed tissue expressed a highly significant level of COX-2 staining compared to both normal and cancer ($p<0.001$). Expression in cancer was less than prostatitis but greater than normal ($p<0.001$). Grade 3 tumors were higher than Grade 4 ($p<0.025$) and there were too few grade 5 tumors for meaningful comparison.

Conclusions: Chronic prostatitis is commonly seen in prostate core biopsies performed for increased PSA. COX-2 expression as demonstrated in diagnostic specimens is significantly increased in prostatitis compared to both normal and cancer. COX-2 may be an early marker of prostatic cell injury. Investigation of both upstream and downstream pathways for COX-2 expression may lead to better understanding the mechanisms of early carcinogenesis and to the identification of chemopreventive and therapeutic targets

630 Do Patients with Small Tumor Volume Have Prostate-Specific Antigen Expressing Epithelial Cells in Blood?

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Background: Detection of circulating prostate-specific antigen (PSA)-expressing cells (CPECs) in blood of patients with prostate cancer has been demonstrated by a number of investigators. The purpose of this study is to determine if CPECs can be detected in tumors less than 0.5 cc.

Design: Between 1993 and 2001, 717 patients had radical prostatectomy at the Urology Service of WRAMC. Patients with prior hormonal therapy were excluded. All specimens were totally embedded as whole-mounts. 140 patients had information about CPECs in preoperative blood samples. Twenty-one had tumor volumes less than 0.5 cc. CPECs from these patients were compared to 119 patients with tumor volumes greater than 0.5 cc.Epithelial cells from peripheral blood of all patients were isolated using antiepithelial cell antibody, Ber-EP4-coated magnetic beads, and total RNA specimens from these cells were analyzed for PSA expression by RT-PCR.

Results: In 21 of the patients with less than 0.5 cc tumors, the mean age was 60 years (range 41-73 years). The mean preoperative PSA was 5.3 ng/ml (range 0.9-22 ng/ml). The mean index tumor volume was 0.19 cc (range 0.01-0.44 cc). Most of the tumors were multifocal. The mean number of tumors was 5 (range 1-13). All cases were pT2. The distribution of Gleason scores was 4 (1 case), 5 (3 cases), 6 (14 cases), 7 (3 cases). None of the case had a primary Gleason pattern 4 or 5. In 119 patients with greater than 0.5 cc tumors, the mean age was 60 years (range 39-76 years). The mean preoperative PSA was 6.5 ng/ml (range 0.6-43 ng/ml). The mean index tumor volume was 5.37 cc (range 0.5-32.6 cc). Most of the tumors were multifocal. The mean number of tumors was 5 (range 1-14). The distribution of Gleason scores was 5 (1 cases), 6 (57 cases), 3+4 (36 cases), 4+3 (8 cases), 8 (9 cases), 9 (7 cases), 10 (1 case). Seventy cases were pT2 and 49 cases pT3. Twenty-six cases had positive and 93 had negative surgical margins. Peripheral blood specimens of 17 (81.0%) of 21 were positive and 4 (19.0 %) of 21 were negative in small tumor volume CaP patients by ERT-PCR/PSA assay. In 119 men from

the comparison group, 97 (81.5 %) of 119 were positive and 22 (18.5 %) of 119 were negative in ERT-PCR/PSA assay.
Conclusions: Patients with CPECs do not differ with respect to pathological parameters including tumor volume. The quantitative evaluation of CPECs is in progress.

631 Renal Lymph Nodes: Appraisal of 221 Nephrectomies with Microscopic Examination of Hilar Fat

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Background: The role of lymphadenectomy in the management of renal cell carcinoma (RCC) is emerging. While sentinel lymph node(LN) detection is the standard of practice in several cancers, mapping of sentinel LNs has not been utilized in the management of RCC. Studies in porcine models have shown that lymphatic drainage is not uniform. Thus, sampling of LNs during nephrectomy for malignancy is an open issue. Although gross examination of hilar tissue to assess the nodal status is performed routinely, it is not known whether this approach is adequate. Our aim was to evaluate the LN status in nephrectomy specimens.
Design: All radical nephrectomies performed over a 5 year period were reviewed, with particular attention paid to the identification and number of hilar and other LN and their tumor involvement. As per protocol, the entire hilar adipose tissue was submitted for histology. If hilar LNs were grossly visible, they were submitted separately by the surgeon or by the pathologist. We also reviewed cases with regional lymphadenectomy. The metastases were correlated with the grade and stage of the tumor; staging was adjusted to the current classification.
Results: There were 221 nephrectomies performed for renal mass, including several cytoreductive procedures. 1-15 hilar LNs were identified in 41 cases (18.5%); of which LNs were grossly seen in 9 cases. Metastases were detected in all 9 cases with enlarged LN on H&E and all of these cases had a higher tumor stage [pT2 x1; pT3 x7; pT4 x1]. The tumor grade was 2(x3),3(x2), 4(x4). In 32 nephrectomies (15%) microscopic hilar LNs were found. All these LNs were negative for metastases. 39 (18%) patients had regional lymphadenectomy: periaortic, paracaval and aorto-caval. The number of identified LNs ranged from 1-17. In 7/39 cases (18%) there were metastases. All 7 cases with positive LNs were pT3; nuclear grade 2(x3),3-4(x4). Cases with negative LN were pT1a(x4), pT1b(x14), pT2(x5), pT3(x9).
Conclusions: Renal hilar LNs are relatively uncommon. Microscopic hilar LNs were seen in 15% of nephrectomies and all were benign. In contrast, grossly visible hilar LN were invariably positive (100%, 9/9). LN positivity correlated with tumor stage but not grade. Thus, searching for occult LNs is not practical. In patients who are not at high risk for LN metastases and do not have grossly visible LNs at the time of surgery, regional lymphadenectomy and its associated morbidity may be avoided.

632 Adenomatoid Tumors of the Female and Male Genital Tract Express D2-40

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Background: Adenomatoid tumors are benign neoplasms of the female and male genital tract. A number of studies have demonstrated strong support of mesothelial derivation of these lesions. Calretinin and WT1 are expressed in mesothelial cells and have been identified in adenomatoid tumors. Recently D2-40 has been demonstrated in mesothelial cells. The aim of this study is to assess the expression of D2-40 in adenomatoid tumors and compare it with the expression of calretinin and WT1 in these tumors.
Design: Twenty cases of adenomatoid tumor of the female (15) and male (5) genital tract were obtained. These included 12 from myometrium, 3 from fallopian tube, 4 from epididymis and 1 from paratesticular region. Immunohistochemical staining using antibodies D2-40, calretinin and WT1 was performed on formalin fixed, paraffin embedded tissue sections.
Results: D2-40, calretinin, and WT1 were positive in all 20 adenomatoid tumors. D2-40 was expressed on the luminal surface of the tubules whereas calretinin showed diffuse positive cytoplasmic and nuclear staining of the tumor cells. WT1 was positive in the tumor cell nuclei.
Conclusions: These results provide further support for the mesothelial origin of adenomatoid tumors and suggest that D2-40 expression may be used in identifying these neoplasms.

633 Papillary Renal Cell Carcinoma: Assessment of Clear Cell Change and Clinicopathologic Correlation

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Background: Papillary renal cell carcinoma with clear cell change and chromosome 3p21 aberration has been described. The significance of this finding, however, remains unclear. We perform the first study to investigate the significance of clear cell change and its clinicopathologic correlation.
Design: Nineteen cases of papillary renal cell carcinoma between 1992 and 2005 were retrieved from the slide archives in the Department of Pathology, Westchester Medical Center. Cytogenetic findings were obtained in 2 cases. All tumors were subclassified as type 1 or 2 and were evaluated for clear cell change and Fuhrman nuclear grade. American Joint Committee on Cancer TNM Staging of Renal Cell Carcinoma (2002) was used and clinical charts were reviewed retrospectively to obtain clinical stage.
Results: The patient age ranged from 11 to 77 years (mean 56). Sixteen patients were males and 3 were females. Tumor size ranged from 1.8 to 10 cm (mean 4.6 cm). All tumors contained clear cells ranged from 0 to 85%. Of the 12 tumors with 0 to 25% clear cells, 9 cases presented with stage I, 2 with stage II, and 1 with stage III disease. Seven tumors possessed clear cell change ranged from 30 to 85%. Of these 7 patients, 2 cases presented with stage I, 1 with stage II, 3 with stage III, and 1 with stage IV. Cytogenetics findings in a tumor with 30% clear cells revealed 49-50X,-X, der(3)add(3)(p21),+7,+17,-19,+21 and the case with 5% clear cells showed 57,XXY,+2,+3,+4,+7,+8,+12,+16,+17,+20. Nine cases (47%) were classified as type 1 and 10 cases (53%) type 2. Of the 9 type 1 tumors, 2 cases had grade 1 nuclei, 6 grade 2, and 1 grade 1. Six of these patients presented with stage I, 2 with stage 2, and 1 with stage IV. In comparision to type 1, 5 cases of type 2 lesions had a nuclear grade of 2 and 5 had grade 3 nuclei. Five patients presented with stage I, 1 with stage II, and 4 with stage III disease.
Conclusions: Type 2 papillary renal cell carcinomas have higher nuclear grade and stage than that of type 1 lesions. Type 2 lesions have poorer prognosis than type 1. Patients bearing tumors with greater than 30% clear cells present with higher stage of disease. Therefore, clear cell change may be a useful pathologic prognosticator in evaluating clinical behavior of these tumors.

634 Defective DNA Repair Gene, hOGG, in Clear Cell Renal Cell Carcinoma

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Background: One of the mechanisms involved in carcinogenesis is somatic mutation of tumor suppressor genes. A possible mechanism for the production of mutations is through DNA damages by free radicals. The kidney is subjected to DNA oxidative damage from reactive oxygen species generated by free radicals and toxic metabolites, leading to formation of DNA base lesions. One such DNA lesion is 8-oxoquanine, which, if not sufficiently removed, is potentially mutagenic because it can cause G:C to T:A transversion in subsequent DNA replication. The human 8-oxoquanine DNA glycosylase 1 (hOGG1) gene encodes an enzyme that specifically repair 8-oxoguanine in DNA. This gene is located at chromosomal region, 3p25-26, which showed frequent loss of heterozygosity in clear cell renal cell carcinoma (CC-RCC). To further explore the role of hOGG1 gene in the development of CC-RCC, we characterize hOGG1 mRNA expression levels in 10 CC-RCC and adjacent benign renal parenchyma, using real-time RT-PCR technology.
Design: Total RNA was extracted from 15 fresh CC-RCC tissue samples and their matched benign renal parenchyma. hOGG1 gene expression was analyzed by real-time RT-PCR Quantitation with ABI Prism 7000, using hOGG1 primers and fluorescent dye-labeled Taqman hOGG1 probe (ABI Applied System) and Taqman GAPDH as an internal controls for normalization. The wilcoxon signed ranks test was used for statistical analysis.
Results: Four of these 15 cases did not yield satisfactory PCR amplification for either the hOGG1 or the GAPDH gene. Thus only 11 cases were included in the final analysis. In 8 of 11 cases (72%), hOGG1 mRNA expression levels were significantly lower in CC-RCC than those seen in adjacent normal parenchyma, ranging from 20% to 51% of the normal tissues. In 3 cases, however, the hOGG1 levels were higher in CC-RCC than those in normal tissues, varying from 135% to 190%. Overall, the hOGG1 mRNA expression in CC-RCC is about 61% of those seen in normal renal tissues. A mean relative hOGG mRNA level for all 11 cases was 0.47 as compared to a mean of 0.76 for normal renal parenchyma. The difference in hOGG1 mRNA expression between CC-RCC and adjacent normal tissues is statistically significant ($p = 0.041$).
Conclusions: Majority of CC-RCC showed significantly lower hOGG1 mRNA expression as compared to normal kidney. This result, in conjunction with the loss of hOGG1 protein that we previously showed, strongly supports the role of hOGG1, as a tumor suppressor, in the development of CC-RCC.

635 Loss of Heterozygosity of DNA Repair Gene, hOGG1, in Clear Cell Renal Cell Carcinoma but Not in Renal Cortical Adenoma

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Background: The kidney is constantly exposed to free radicals (oxidative stress) due to its active metabolism and process of toxic metabolites. Among 20 or so free radical-induced DNA lesions, 8-oxoquanine is the most abundant and is potentially mutagenic if not sufficiently removed. The human 8-oxoquanine DNA glycosylase 1 (hOGG1) gene specifically repairs 8-oxoguanine. This gene resides at chromosomal region 3p25-26, which shows frequent loss of heterozygosity (LOH) in clear cell renal cell carcinoma (CC-RCC). At present, the diagnostic criteria for renal cortical adenoma without clear cell component are arbitrarily defined, depending on the size (less than o.5 cm in diameter) of the lesion. To explore the relationship between CC-RCC and renal cortical adenoma, we perform LOH analysis of hOGG1 gene in both CC-RCC and renal cortical adenomas.
Design: 11 cases of CC-RCC and 5 cortical adenomas were included in this study. DNA samples from CC-RCC and cortical adenomas were obtained from tissue sections collected using laser-capture microdissection (LCM). All DNA samples were subjected to PCR amplification using 4 fluorescent-labeled microsatellite makers adjacent to the hOGG1 locus (3S1297, 3S1289, 3S1300, 3S1274), followed by fragment analysis using ABI PRISM 3100 Genetic Analyzer.
Results: Nine of 11 (81.8%) cases of CC-RCC are informative for at least one of the 4 markers used and among these 9 cases, 8 (88.8%) showed evidence of hOGG1 LOH. Four of 5 cases of cortical adenomas are informative for at least one of the 4 markers used and in these 4 cases, none (0%) showed evidence of hOGG1 LOH.
Conclusions: Loss of heterozygosity (gene loss) of the hOGG1 gene frequently occurs in CC-RCC (88.8%), indicating that this major repair gene for oxidative DNA damage is critical in carcinogenesis of CC-RCC. Renal cortical adenomas are distinctively different from CC-RCC at the genetic level.

636 Renal Chromophobe Carcinomas with Focal Oncocytic-Like Morphology: Cytogenetic Analysis of 5 Cases

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Background: Renal chromophobe carcinomas and oncocytomas pose diagnostic difficulty to surgical pathologists. Both of these neoplasms, which each constitute 6% of renal cortical tumors, share similar morphology. Many studies have focused attention on identifying genetic aberrations in each of these neoplasms. Chromophobe tumors can have multiple chromosomal losses including Y, 1, 6, 10, 13, 17 and 21 while oncocytomas commonly have loss of chromosomes Y and 1. Because these tumors share morphologic features, it has been suggested that they may be genetically related, and some hypothesize that oncocytomas may undergo additional genetic mutations and progress to chromophobe carcinoma. In this study, we attempt to test this hypothesis by genetically analyzing renal chromophobe carcinomas which demonstrate oncocytic-like foci within the main tumor mass.

Design: A retrospective review of renal chromophobe carcinomas from January 2003 through April 2005 was undertaken. Thirteen renal chromophobe carcinomas were reviewed for focal oncocytic-like morphology, and the 5 selected cases had cytogenetic analysis via fluorescent *in situ* hybridization (FISH) using commercially available centromere probes Y, 1, 2, 6 and 10. Between 100 and 125 non-overlapping nuclei were scored in the chromophobe and oncocytic-like areas by 2 independent evaluators. The percent mono-, di- and tri-somy for each probe was calculated.

Results: Three patients were female and 2 patients were male. Only 1 of 5 tumors demonstrated monosomy of chromosome 1 within the oncocytic focus. In both the cases from men, the oncocytic-like foci within the main chromophobe mass demonstrated loss of Y. FISH analysis of the chromophobe regions demonstrated monosomy as follows: Y (2 of 2 cases), 1 (3 of 5 cases), 2 (1 of 5 cases), 6 (3 of 5 cases), and 10 (2 of 5 cases). No clear cytogenetic relationship between the oncocytic-like foci within the chromophobe carcinomas was evident in these 5 cases.

Conclusions: Cytogenetic FISH analysis of chromophobe carcinomas with focal areas of oncocytic-like morphology does not demonstrate similar genetic alterations. Multiple chromosome losses in chromophobe tumors identified in prior studies were not evident in this current study, and thus FISH analysis on paraffin embedded tissue may not represent the most accurate methodology to test this hypothesis. Nonetheless, this study does not support the hypothesis that an oncocytoma represents a precursor tumor which evolves into a renal chromophobe carcinoma.

637 Urachal Carcinoma: A Clinico-Pathologic Study of 24 Cases

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Background: Urachal carcinomas (UC) occur mostly in the bladder dome, comprise 22 to 35% of vesical adenocarcinomas and are generally treated by partial cystectomy with en bloc resection of the median umbilical ligament and umbilicus. Detailed pathologic studies with clinical outcome correlation are few.

Design: We reviewed histologic material and clinical data from 24 cases selected from a database of 56 dome-based tumors diagnosed and treated at our institution from 1984 to 2005. Follow-up information was available in 23 patients.

Results: The mean age at diagnosis was 52 years (range 26-68). 15 patients were male and 9, female. Location was the dome in 23, and dome and anterior wall (DAW) in 1. 13 cases were pure adenocarcinoma (AC), NOS, 3 were AC with focal areas of micropapillary carcinoma, lymphoepithelioma-like carcinoma and clear cell carcinoma component, and 8 were enteric type AC. Signet ring cell features were focally seen in 2 cases.Cystitis cystica (CC) was seen in 4, and cystitis glandularis (CG), in 2 cases. Urachal remnants (UR) were identified in 14 cases: the urachal epithelium was benign transitional in 8, dysplastic in 5, and in one instance, showed adenocarcinoma in situ. The invasion pattern was 'infiltrative' in 20 and 'pushing' in 2 cases; tumor was confined to the urachal remnant in 2 cases. The overlying urothelium was colonized by AC in 3 cases. In all three, urachal remnants were identified, and showed transition from benign to dysplastic epithelium. The Sheldon pathologic stage was pT1 in 2, pT2 in 2, pT3a in 6, pT3b in 13 and pT3c in 1 patient. One patient had a positive surgical margin. The mean follow-up period was 40 months (range 0.3-157.6). The clinical outcome data is as follows: Local recurrence (LR): 6/23 (26%); mean time interval: 2.4 years; Alive with no evidence of disease (NED): 7/23 (30%); Alive with disease (AWD): 10/23 (44%); Dead of disease (DOD): 5/23 (22%). LR occured both in the bladder and urethra. 1 patient, who died of unrelated causes, had pT1 disease followed by 5 episodes of 'seeding' of the urothelial tract. 1 patient (AWD-pT3b) had 'seeding'of the prostatic urethra and seminal vesicles two years after diagnosis. All patients who were DOD were Stage pT3: pT3a (1) and pT3b(4).

Conclusions: Pathologic stage is an important prognostic factor in UC. Surface urothelial involvement by carcinoma and presence of CC and CG do not necessarily exclude the diagnosis of UC. LR can occur, even in non-invasive tumors, by 'seeding' within the urothelial tract.

638 Testicular Mixed Germ Cell Tumors: A Morphological and Immunohistochemical Study using OCT3/4, SOX2 and GDF3, with Emphasis on Morphologically Difficult-to-Classify Areas

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Background: OCT3/4 -a stem cell marker- is widely used in the differential diagnosis of testicular germ cell tumors (GCT). SOX2 and GDF3 are two other stem cell markers that we observed were highly expressed in some GCTs in a cDNA microarray study.We studied the immunohistochemical (IHC) expression profile of different GCT components for these markers, and investigated their utility in the differential diagnosis of otherwise morphologically difficult-to-classify (DTC) components of GCTs.

Design: Fifty mixed GCT, most also containing DTC areas, were investigated. In these areas morphologic details (nuclear features, nuclear overlap, distinctness of cell borders, apoptosis, and architecture) were noted. Nuclear details similar to those in embryonal carcinoma (EC) in the same section were considered "high-grade". We performed IHC staining for OCT3/4, c-kit, CD30, SOX2, and GDF3 on sections from one representative paraffin block from each tumor, and graded it in each component of GCT from 0 to 3+ (0-negative, 1+- 1-25% positive staining cells, 2+- 26-50%, 3+- >50%).

Results: The tumors contained the following components: seminoma (8), EC (50), yolk sac tumor (YST) (40), mature teratoma (MT) (26), immature teratoma (IT) (14), choriocarcinoma (CC) (3), syncytiotrophoblasts (22), and intratubular germ cell neoplasia (IGCNU) (35).The positive staining patterns were as follows: c-kit–all seminoma and IGCNU (3+), 14/40 YST (1+), 3/50 EC (1+); OCT3/4–all seminoma, ICGNU and EC (3+); CD30– 49/50 EC (mostly 3+, but weak); SOX2 – all EC (3+), 11/14 IT (1+, in primitive neuroectoderm); GDF3 – All IGCNU, seminoma (3+), all YST (2 or 3+), 40/50 EC (1 or 2+), all MT (3+, in all components), all CC (3+ in cytotrophoblast). 43 of the tumors contained DTC areas, and 34/ 43 of these were 3+ positive for OCT3/4, CD30 and Sox2, supporting the diagnosis of EC. "High grade" nuclear features were the most important criterion for the diagnosis of EC.

Conclusions: SOX2 is expressed in EC and primitive neuroectodermal component of IT, and unlike OCT3/4, not in ICGNU and seminoma.Therefore, it may be useful in the diagnosis of early carcinomatous differentiation in seminoma. GDF3 positivity combined with morphologic features, in the absence of OCT3/4 positivity, are helpful in the diagnosis of YST. High grade nuclear features in DTC are the most important morphologic criterion for the diagnosis of EC.

639 The Impact of the 2005 International Society of Urological Pathology (ISUP) Consensus Conference on Standard Gleason Grading of Prostatic Carcinoma

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Background: The application of the standard Gleason grading varies considerably in contemporary surgical pathology practice due to several reasons. The goal of the ISUP consensus conference was to achieve consensus amongst leading urological pathologists in specific areas of Gleason grading, including areas where there is currently either a lack of data or scant information as to the optimal method of grading. The aim of this study was to describe the impact of the consensus recommendations on a series of needle biopsies previously graded by an experienced uropathologist according to the standard Gleason system.

Design: The study was based on 172 needle prostatic biopsies. The new grading of adenocarcinoma, not previously considered by the same reviewer, included: 1) cribriform pattern 3 was only diagnosed for well circumscribed glands of the same size of normal glands; 2) ill-defined glands with poorly formed glandular lumina also warrant the diagnosis of Gleason pattern 4; 3) in high-grade cancer lower grade patterns were ignored if they occupied less than 5% of the area of the tumor; 4) high-grade tumor of any quantity was included within the Gleason score; and, 5) for tertiary Gleason patterns, both the primary pattern and the highest grade were recorded. Four prognostic Gleason grading groups were considered: Gleason scores 2-4, 5-6, 7, and 8-10.

Results: For Gleason primary pattern, secondary pattern and score there was an exact concordance between the standard Gleason reading and the reading according to the consensus recommendations, respectively, in 83.14%, 63.37%, and 68.02% of the cases; -1 unit in 0%, 14.53%, and 4.07%, respectively; +1 unit in 16.28%, 19.77%, and 24.41%, respectively; and, +2 units in 0.58%, 2.33%, and 3.48%, respectively. In 4/172 (2.33%) of the cases there was a change toward a lower Gleason grading group, and in 46/172 (26.74%) of the cases a change toward a higher group: 27/172 (15.7%) of the cases changed from the group 5-6 to the group 7; 17/172 (9.88%) from 7 to 8-10; 1/172 (0.58%) from 2-4 to 5-6; and, 1/172 (0.58%) from 5-6 to 8-10.

Conclusions: The highest impact of the ISUP consensus recommendations for Gleason grading was seen on the secondary pattern which had the lowest percentage of concordance. It reflected in a change toward a higher Gleason grading group in 46/172 (26.74%) of the cases.

640 Intraductal Carcinoma of the Prostate: Histologic Features and Clinical Significance

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Background: We use "intraductal carcinoma of the prostate" (IDC-P) on prostate biopsy for rare intraductal glandular lesions characterized by marked pleomorphism or frequent central comedonecrosis, beyond that typically seen with HGPIN.

Design: 40 cases of IDC-P on biopsy were identified from our consult files with 29 cases of pure IDC-P and 11 cases of IDC-P with <10% infiltrating prostatic carcinoma (mean 5.8%).

Results: Morphology: Numbers of cores with IDC-P ranged from 1-12, with >1 core in 27 cases. Summed length of IDC-P averaged 13.8 mm (2-102 mm.). Predominant patterns of IDC-P were cribriform (38/40), solid (14/40), and micropapillary (6/40). Usual HGPIN also observed in 12 cases. In 12 cases, glands of IDC-P were crowded, closely mimicking infiltrating carcinoma. Marked pleomorphism was present in 18/40 cases, central comedonecrosis in 24/40 cases, and mitoses in 27/40 cases. Basal cells were observed on the regular stained slides in 26/40 cases; basal cells were confirmed by IHC for high molecular weight cytokeratin (n=33) and/or p63 (n=5). Treatment and Prognosis: Radical Prostatectomy (RP) (n=10): All 10 cases had infiltrating cancer at RP with 7 also revealing prominent IDC-P. Of 8 cases with only IDC-P on biopsy, RP showed Gleason score 8-9 in 7/8 cases and Gleason score 7 in 1/8 cases. Extraprostatic extension (EPE) of carcinoma was observed in 5/8 cases and vascular invasion in 1/8 cases. 1 man with IDC-P and 1% infiltrating carcinoma on biopsy had Gleason score 8 organ confined tumor at RP; he subsequently developed bone and lymph node metastases. Another man with IDC-P and 5% infiltrating cancer on biopsy had Gleason

score 7 cancer at RP with EPE. Radiation Therapy (n=12): 1 man developed metastases to bone. Hormonal Therapy (n=5): 3 men developed metastases to bone. Combined Radiation and Hormonal Therapy (n=5): 1 man had a PSA recurrence. Watchful Waiting (n=8): 1 man developed metastases to a lymph node and 1 man had a bone scan suspicious for metastases. Mean follow-up times for men free of recurrence without RP was relatively short, typically 2-3 years.

Conclusions: IDC-P has distinctive morphological features compared to HGPIN. IDC-P is frequently associated with high-grade cancer and poor prognostic parameters at RP and potentially advanced disease following other therapies. These findings support prior studies that IDC-P is an advanced stage of tumor progression with intraductal spread of tumor. We feel that it is justified to treat patients with intraductal carcinoma on biopsy aggressively even in the absence of documented infiltrating cancer.

641 Prognostic Significance of Non-Invasive Squamous Lesions in the Urinary Bladder

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Background: With the exception of invasive squamous cell carcinoma and nonkeratinizing squamous metaplasia, squamous lesions are uncommon in the urinary bladder.

Design: 29 cases of transurethral biopsies or resections of the bladder were studied from the consult files of one of the authors. Cases included extensive keratinizing squamous metaplasia (5), nonkeratinizing hyperplastic squamous epithelium (5), squamous papilloma (5), squamous cell carcinoma in situ without invasive carcinoma (8), and condyloma acuminatum (3).

Results: Extensive Keratinizing Squamous Metaplasia: Invasive urothelial carcinoma with squamous features developed in 2 cases, keratinizing squamous metaplasia remained in 1 case, and no follow-up information was available in 2 cases. Nonkeratinizing Hyperplastic Squamous Mucosa: 1 patient developed invasive squamous cell carcinoma, 1 developed squamous cell carcinoma in situ, 1 had persistent hyperplasia on rebiopsy, and 2 had no further follow-up information. Squamous Papilloma: 1 patient developed low-grade urothelial carcinoma, 2 were free of lesions, and 2 were lost on follow-up. Squamous Cell Carcinoma In-Situ: 3 patients developed invasive squamous cell carcinoma, 1 developed invasive urothelial carcinoma with squamous features, 1 developed high-grade urothelial carcinoma (not otherwise specified), and 3 were lost on follow-up. Condyloma Acuminatum: 1 patient developed squamous carcinoma in situ, 1 had persistent condyloma, and 1 was lost on follow-up. Immunohistochemistry: In the 23 cases that were immunostained, strong signals for EGF-R were observed in 20 cases, absent in 2 cases, and not evaluable in 1 case. HPV signal was positive in 1 case of condyloma acuminatum and 1 case of squamous cell carcinoma in situ.

Conclusions: With the exception of squamous papilloma, the above studied squamous lesions of the urinary bladder are associated with either invasive squamous carcinoma, invasive urothelial carcinoma with squamous features, or the development of squamous cell carcinoma in situ. Patients with extensive keratinizing squamous metaplasia, nonkeratinizing hyperplastic squamous epithelium, squamous cell carcinoma in situ, and condyloma acuminatum of the urinary bladder should be closely followed for development of in-situ or invasive cancer. As seen in head and neck squamous lesions, the enhanced expression of EGF-R in squamous lesions of the bladder may provide a treatment strategy for bladder lesions that are difficult to manage clinically.

642 Expression of Kidney-Specific Cadherin in Chromophobe Renal Cell Carcinoma and Renal Oncocytoma

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Background: Differentiating benign renal oncocytoma from chromophobe renal cell carcinoma (RCC) can be a diagnostic challenge. Mazal PR et al (Hum Pathol, 2005) recently proposed the use of kidney-specific cadherin (Ksp-cad) immunostaining to differentiate these two tumors, reporting 97% expression in chromophobe RCC but only 3% expression in oncocytoma. However, Shen et al (Mod Pathol, 2005) showed uniform Ksp-cad expression in both tumors. We attempted to evaluate the Ksp-cad expression in these two renal tumors using cDNA microarrays and immunohistochemistry.

Design: Ksp-cad mRNA levels from cDNA microarrays containing 50,000 genes were examined in 15 chromophobe RCCs and 15 oncocytomas and compared to those of normal kidney tissues (n=12). Tissue microarray blocks containing 25 chromophobe RCC and 28 oncocytomas and were constructed with 1.5 mm cores of formalin-fixed, paraffin-embedded archival tissue. Immunohistochemistry was performed using a monoclonal antibody for Ksp-cadherin. Staining intensity was scored from 0 to 3+ and the percentage of positive cells was estimated.

Results: Based on cDNA microarrays, the average Ksp-cad mRNA levels in chromophobe RCCs was 90% of normal kidney expression compared to 70% in oncocytomas. By immunostaining 21of 25 (84%) chromophobe RCCs showed moderate to strong expression of Ksp-cad compared to 26 of 28 (93%) oncocytomas. The mean intensity of Ksp-cad immunostaining was 2.24 for chromophobe RCC and 2.21 for oncocytoma without obvious differences.

Conclusions: Both mRNA and protein expression levels of Ksp-cadherin were similar in chromophobe RCCs and oncocytomas. Our study provides evidence that Ksp-cadherin immunohistochemistry is not particularly useful in differentiating chromophobe RCC from oncocytoma

643 Stratified Epithelium in Glands of Prostatic Carcinoma. A Potential Mimic of High-Grade Prostatic Intraepithelial Neoplasia

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Background: Several architectural and cytological variants of prostatic adenocarcinoma (PC) have been described in recent years, including foamy gland, pseudohyperplastic, and atrophic variants. Typically glands of PC have a single cell lining, although stratification can be seen in invasive carcinomas with a cribriform architecture, including ductal carcinoma. The presence of stratified cells within non-cribriform carcinomatous prostatic glands has not been well addressed.

Design: The morphological features and immunohistochemical profile of cases of PC showing stratified epithelium within carcinomatous glands were analyzed. These cases were selected from needle biopsy cases from the consultation files of one of the authors, are well as from a review of 150 consecutive in-house needle biopsy cases of PC (diagnosed between 1996 and 1998), identified via computer database file search. Any degree of stratification was sufficient for inclusion in the study, while cases displaying a cribriform architecture were excluded. Immunohistochemistry was performed utilizing antibodies against 34βE12, p63 and α-methylacyl-coenzyme-A racemase (AMACR).

Results: A total of 6 cases were identified, including 2 from consecutive in-house cases (1.3%). In two cases, the focus with glands having stratified epithelium was the sole carcinomatous component in the biopsy, while such a component represented 5-30% of the invasive PC seen elsewhere in the remaining cases. The main attribute in all these foci was the presence of glandular profiles lined by several layers of epithelial cells with cytological and architectural features resembling flat or tufted high-grade prostatic intraepithelial neoplasia (HG-PIN), but lacking basal cells as confirmed by immunohistochemical negativity for 34βE12 and/or p63 in all cases. The AMACR staining profile of these foci was variable, with 3 foci showing positivity, a profile similar to the adjacent PC without stratification, and the remaining foci being negative, including two cases with AMACR positivity in the adjacent PC.

Conclusions: Stratified epithelium in non-cribriform glands of PC can be focally identified in prostate needle biopsy specimens with PC and can resemble HG-PIN glands. Rarely, these PIN-like carcinomas can present in pure form. Recognition of this fact, as well as occasional immunohistochemical evaluation of stratified glands, may be indicated to correctly diagnose these glands as PC.

644 Sarcomatoid Carcinoma of the Prostate: A Study of 42 Cases

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Background: Sarcomatoid carcinomas of the prostate are rare.

Design: We examined TURP, needle bx, and radical prostatectomy specimens from 42 men with sarcomatoid carcinoma of the prostate, all received in consultation. Clinical information was obtainable on 32 men. 5 were lost to follow-up and information could not be obtained on the 5 remaining men.

Results: Prior Prostatic Adenoca.: 65% of men (n = 21) had a prior history of acinar adenoca. of the prostate with reported Gleason scores of 6 (n = 7), 8 (n = 4) and 10 (n = 3). 11 men presented with de novo sarcomatoid carcinoma. The time between the original diagnosis of acinar adenoca. and diagnosis of sarcomatoid carcinoma ranged from 6 mos. to 16 yrs. (mean 6.8 yrs). Concurrent Adenoca: Most men had concurrent high grade adenoca. with Gleason scores 7 (n = 3), 8 (n = 9), 9 (n = 10), 10 (n = 10). A subset of men had admixed ductal adenoca. (n = 4), small cell carcinoma (n = 3), squamous cell carcinoma (n = 3) or other unusual patterns of prostate cancer (n = 3). In 1 case, the diagnosis was based on IHC evidence of epithelial differentiation along with the history of prior adenoca. Morphology Sarcomatoid Component: Sarcomatoid growth ranged from 5% to 99% (mean 65%). Bizarre atypia with giant cells was present in 55% of cases. Heterologous elements were seen in 10 cases (29%), including osteosarcoma (n = 7), chondrosarcoma (n = 5), and rhabdomyosarcoma (n = 2). In 8/12 cases received with cytokeratin IHC, the sarcomatoid component was at least focally positive. Prognosis: Approximately 50% of men developed metastases either at presentation or subsequently. Of men with longer follow-up, 6/7 died within 1 yr. of the diagnosis of sarcomatoid carcinoma; another 20 were alive yet with short follow-up (median 1 yr.; mean 2.3 yrs). Kaplan-Meier analysis revealed an actuarial risk of death at 1 yr. following diagnosis of sarcomatoid carcinoma of 20%. No correlation was identified between patient survival and morphologic features, prior radiation or hormone therapy, or concurrent high grade prostate cancer.

Conclusions: Sarcomatoid carcinoma demonstrates diverse spindle and epithelial cell morphologies. The sarcomatoid component often has heterologous elements and in one case no epithelial component was seen on routine stained sections. The epithelial component is typically high grade acinar adenoca., yet other aggressive tumor subtypes such as ductal adenoca. and small cell carcinoma may also be seen. Sarcomatoid carcinoma is an aggressive form of prostate cancer, the prognosis of which is dismal regardless of other histological or clinical findings.

645 Neoplasia in Bladder Diverticula: A Clinicopathologic Study of 88 Cases

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Background: Bladder diverticula are defined as outpouching of the urinary bladder either with or without a muscular wall.They could be congenital or acquired.The reported incidence of neoplasia arising in bladder diverticula ranges from 0.8-10.8%.Staging invasive neoplasms in bladder diverticula is complicated by the altered anatomy, such as absence of muscularis propria.Our intent was to study a large cohort of patients who presented with urothelial neoplasms arising in bladder diverticula.

Design: One hundred consecutive cases with resection for bladder diverticulum were reviewed.88 cases with carcinoma arising in the diverticulum were identified from 67 patients.Histologic slides and medical records were reviewed for morphologic features,demographic and clinical data.

Results: The mean age was 68 years (41-90y). 63 patients were men and 4 were women. Multiple diverticuli were present in 21 (31%) of 67 patients. The diverticula were present in all anatomical locations,but the most common sites were the right lateral wall (29 cases) and left lateral wall (25 cases). Muscularis mucosae (MM) was identified in 39 cases. In 25 of these cases MM was hypertrophic. Muscularis propria was present in 16 cases. 40 cases were non-invasive and 48 cases had invasive carcinoma.The non-invasive neoplasms included high grade (23) and low grade (9) papillary carcinomas,CIS (7), and papillary urothelial neoplasm of low malignant potential (1).The invasive urothelial carcinomas (UC) included 34 invasive UC, NOS (16 cases had associated HG papillary urothelial carcinoma),8 UC with squamous differentiation, 3 cases of sarcomatoid carcinoma, 2 pure squamous cell carcinoma and 1 small cell carcinoma. The depth of invasion was classified as invasion into lamina propria (13), into MM (9), through the MM (8), into peridiverticular adipose tissue (9), and into muscularis propria (3). 6 cases were at least invasive into the lamina propria. CIS was present in 43 patients. Extradiverticular bladder tumors were noted in 46% of the patients. Follow-up was available for 31 patients and ranged from 1-168 months (mean: 39m). At last follow-up 8 (26%) of the patients died of disease, 7 (22%) were alive with no evidence of disease and 5 (16%) were alive with disease.

Conclusions: Bladder diverticula with neoplasia are most commonly seen in males. Urothelial carcinomas of all grades can be seen in diverticula. Contrary to the general belief muscularis propria is present in a subset of bladder diverticula. Almost 50% of patients have tumors involving extradiverticular urothelium.

646 Laparoscopic Versus Open Radical Prostatectomy: Analysis of 1000 Cases at Massachusetts General Hospital

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Background: Traditionally, patients with prostate cancer are treated surgically with open retropubic radical prostatectomy (RP). Recently, laparoscopic radical prostatectomy has become a popular trend because it is less invasive. However, there has been no large-scaled study directly comparing these two types of surgeries. Here, we compared the clinical and pathologic data of 286 laparoscopic and 714 open radical prostatectomies (total 1000 cases) at the Massachusetts General Hospital (MGH) from 2001 to 2005.

Design: We collected general, clinical and pathological information from 1000 consecutive patients who underwent radical prostatectomy and enrolled in the prostate cancer tissue bank at the MGH from 2001 to 2005. The information includes clinical stages, pre-operative PSA levels, Gleason scores on biopsy, pathological stages, Gleason scores on RP as well as margin status.

Results: 89% of patients had clinical stage T1c caner. Over 90% of patients had a pre-operative PSA level of less than 10ng/ml and a Gleason score on biopsy of 4-7/10. Similar distributions of clinical stages, pre-operative PSA levels and Gleason scores on biopsy were seen between the two groups. After RP, Over 80% of the patients showed pathological stage pT2 cancer and over 90% of the patients had Gleason scores of 5-7/10. Again, similar distributions were seen between the two groups in pathological stage and Gleason scores ($p>0.05$). Most importantly, there was no statistical difference in the margin status between the two groups. The rates for positive margins were 15.0% and 17.4% for laparoscopic and open surgery groups, respectively. Different **clinical** stages did not influence the margin status. However, patients with **pathological** stage pT3 or Gleason score of 8-9 cancers had an increased chance of having a positive margin (over 30%) and it is similar in both groups. The positive margins were at peripheral and distal aspects of the prostate in both groups.

Conclusions: There was no significant difference between laparoscopic and open surgery in selecting patients based on clinical stage, pre-operative PSA level and Gleason score on biopsy. After surgery, there was no significant difference in final pathological findings including Gleason score, pathological stage, margin status as well as the location of positive margin. In conclusion, we provide here the direct evidence that with an experienced laparoscopic urologist, laparoscopic radical prostatectomy is able to achieve similar surgical margin status as traditional open radical prostatectomy.

647 Expression of CDX2 in Adenocarcinoma of the Prostate (PCa)

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Background: CDX-2 is a transcription factor involved in proliferation and differentiation of intestinal epithelium. Numerous studies have claimed its relatively high specificity and sensitivity in establishing a gastrointestinal origin in metastatic tumors of unknown origin. We have recently seen 2 cases of adenocarcinoma of the prostate on needle biopsies with diffuse strong nuclear staining for CDX-2 sent for consultation. One case was a prostatic duct adenocarcinoma with positive immunostains for PSA in a man who presented with a PSA value of 320 ng/ml. The second positive case for CDX-2 was PCa Gleason score 4+4=8 in a man with a PSA value of 15 ng/ml. A Gleason score 3+3=6 adenocarcinoma from the contralateral side did not express CDX-2. As documented examples of this phenomenon are exceptionally rare, we investigated the immunoexpression of CDX2 utilizing tissue microarrays (TMAs).

Design: Three slides of TMAs were used to stain 708 tissue samples (0.6-mm-diameter) containing either benign or malignant prostate tissue, as well as control tissues from various anatomic sites including colon. Tissues were from 70 radical prostatectomies (RP) and 68 metastatic PCa to lymph nodes, bone and soft tissues. In total, 195 samples of primary PCa, 195 samples of benign prostate tissue, and 185 samples of metastatic PCa were studied. Of the primary prostate cancers, Gleason scores were: 6 (n=41); 7 (n=21), and 8 (n=8).

Results: Of 70 RPs examined for carcinoma in TMAs, 4 (5.7%) were positive for CDX-2. Three of the 4 positive cases were Gleason score 6, with the other positive case being Gleason score 4+3=7. Additional focal moderate positive staining was seen in benign prostate in 7 of 60 RPs (11.7%). None of the metastatic prostatic PCa expressed CDX-2.

Conclusions: CDX-2 may uncommonly be focally expressed in benign prostatic glands. Staining in PCa is less common and appears independent of Gleason grade. The staining is usually patchy and focal and of lesser intensity than in colonic tissue, however, rarely strong and diffuse staining may be seen. A positive CDX-2 staining in high grade PCa (ductal, cribriform and solid) may be confused with secondary carcinoma of colonic origin. Routine histopathology, positive PSA immunostaining, and clinical findings can confirm the correct diagnosis.

648 Clear Cell Carcinoma of the Bladder and Urethra Closely Mimicking Nephrogenic Adenoma (NA)

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Background: Classic clear cell carcinoma (CCA) consists of solid and tubopapillary patterns. Clear cells, often with hobnailing, are common although cells with eosinophilic cytoplasm may predominate. Although CCA may focally mimic NA, we studied cancers where almost the entire lesion closely resembled NA.

Design: 12 CCA, 7 NAC (adenocarcinomas closely resembling NA), and 10 NA were studied.

Results: Clinical: Female:male ratio was: 10:2 in CCA; 5:2 in NAC; 2:8 in NA. Mean age of all patients was 62 yrs. (36-91), with no differences between groups. 5/12 (42%) CCA, 3/7 NAC (43%), and 1/10 (10%) NA were in the urethra with remaining cases in the bladder. Histology: Although in both CCA and NAC, tubulopapillary patterns predominated, a solid pattern was seen in 7/12 CCA and none of NAC. 7/12 CCA had aeras of marked pleomorphism versus 1/7 NAC. All but one CCA had areas of clear cytoplasm ($\geq$50% clear cells in 7 cases); 4/7 NAC had no clear cells and 3 cases $\leq$10% clear cells. Necrosis was in 8/12 (67%) CCA and 3/7 (43%) NAC. Mitoses per 10 HPF averaged 16 (5-36) for CCA and 7 (2-11) for NAC. Necrosis and mitotic figures were absent in NA. In 5 CCA and 4 NAC, some specimens had extensive muscle invasion. Vascular invasion was noted in 3/12 CCA and 1/7 NAC. CCA was associated with urothelial carcinoma (n= 2) and endometriosis (n= 1) versus none of NAC. Hobnail morphology, stromal fibrosis, inflammation was similar in both CCA and NAC. Immunohistochemistry: ki67 proliferation rate: CCA mean 46%; median 45%; range 20%-90%. NAC mean 33%, median 20%, range 10-80%. NA mean 1.6%, median 1, range 0%-5%. p53: CCA mean 20%, median 5%, range 2-90%. NAC mean 4.4%, median 3%, range 0-15%. NA mean 0.2%, median 0%, range 0-1%. The following antibodies were not helpful in distinguishing CCA, NAC and NA: CD10, ER, p63, HMWCK and AMACR.

Conclusions: There is a subset of clear cell carcinomas with infrequent clear cells, no solid growth, less nuclear pleomorphism, and infrequent mitotic figures, closely resembling the tubulopapillary pattern of NA. Feature favoring NAC include occasional clear cells, greater pleomorphism, occasional necrosis, extensive muscle invasion, mitotic figures, and elevated ki67 and p53 rates. Recognition of NAC and its features can help distinguish it from NA. In cases where the distinction can not be made with certainty, close follow-up and repeat biopsy with more extensive sampling can help to resolve the dilemma.

649 Solitary Fibrous Tumor (SFT) on Prostate Needle Biopsy or TURP

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Background: One of the least commonly encountered spindle cell tumors seen on biopsy or TUR of the prostate is SFT.

Design: We studied 12 cases of SFTs identified on either prostate needle biopsy (n=7) or TURP (n=5).

Results: Mean patient age at diagnosis was 62 years (range 46 to 75 years). 11 tumors presented with urinary tract symptoms and 1 patient was biopsied during work-up of bone metastases. 9 cases were SFTs involving the prostate, 2 cases arose between prostate and rectum extending to prostate (n=2), and 1 case was a pelvic mass without involvement of the prostate. In 7 cases the tumor was resected by cystoprostatectomy (n=2), radical prostatectomy (n=3), pelvic exenteration (n=1), and pelvic tumor resection (n=1) with tumor sizes ranging from 8.5 to 12 cm. Mitotic rates were 3 per 10 HPF in 3 cases with the remaining cases having either rare or no mitoses identified. 8 cases demonstrated areas of necrosis. Based on a combination of cellularity, necrosis, pleomorphism, and infiltrativeness, 4 prostatic SFTs were malignant, 4 benign, and 1 borderline. Of three non-prostatic SFTs, 1 was malignant and 2 were borderline. All tumors but one were immunoreactive for CD34 (n=11). Material for additional immunohistochemistry was available in 10 cases with positive stains for Bcl-2 (10/10), c-kit (0/10), CD99 (7/10), and beta-Catenin (5/10). 3 SFTs demonstrated >5% p53 immunoreactivity including one tumor with 50% positivity, and 3 cases had Ki-67 rates of $\geq$20%.

Conclusions: Although all SFTs were initially clinically considered to be of prostatic origin, some of the cases arose in the pelvis with secondary involvement of the prostate. Approximately 50% of prostatic SFTs were malignant. Even in the prostatic and nonprostatic SFTs with no overt malignant features, sometimes it was necessary to remove the prostate and in some instances the adjacent organs due to tumors' large size. SFTs must be differentiated from other spindle cell neoplasms of the prostate especially from GISTs that may arise from the rectal wall with invasion of the prostate or from the region between the rectum and the prostate.

650 S100p: A Marker for Transitional Epithelium and Urothelial Carcinoma

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Background: The morphologic distinction between prostate and urothelial carcinoma can be difficult. Difficulties also arise in distinguishing urothelial carcinoma arising in the renal pelvis from some renal cell tumors. The number of useful markers that aid in these distinctions is limited. Markers that positively stain urothelial carcinoma are particularly limited.]S100P is a member of the S100 family of proteins. It is distinct in its expression pattern from the proteins recognized by the polyclonal antiserum against "S100" in clinical use as a marker for melanoma and other neoplasms.

Design: We analyzed expression patterns in 5 kidney and 5 bladder cancer tissues using complementary DNA microarrays and compared these to 5 prostate carcinoma samples that have previously been publised by our group. S100P and GATA3 were highly expressed in the urothelial tumors. Expression of these proteins was analyzed by immunohistochemistry using tissue microarrays with 337 urothelial carcinomas. Additional arrays containing 267 prostate carcinomas, 148 renal tumors, and 459 other tissues were also studied. Immunohistochemistry was also performed on these arrays for p63 and CK5/6 in order to permit a comparison with urothelial markers currently in clinical use.

Results: Together with our prior studies on renal neoplasms and normal kidney, the cDNA array studies suggested that the gene S100P is specifically expressed in benign and malignant urothelial cells. A polyclonal antiserum against S100P protein stained 85% of urothelial carcinomas while only 3% prostatic adenocarcinomas and 1% of renal cell carcinomas stained. Similar to S100P, p63 stained 86% of urothelial carcinomas while CK5/6 stained 54% and GATA3 stained 59%. Importantly, when S100P and p63 were combined 93% of urothelial carcinomas were labeled by one or both markers.

Conclusions: We conclude that detection of S100P protein expression may help distinguish urothelial carcinomas from other genitourinary neoplasms that enter into the differential diagnosis. GATA3 showed less promise than S100P, both in terms of the number of urothelial carcinomas stained, but also in terms of the robustness of its staining.

651 Functional Characterization of the Metastasis-Associated Protein 1 (MTA1) in Prostate Cancer Cell Lines

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Background: The metastasis-associated gene 1 (MTA1) is overexpressed in several human cancers. Recent reports suggest that MTA1 may play a role in cancer progression through transcription repression. We previously reported an association of MTA1 expression and prostate cancer progression. Aim of this study was to identify a functional correlate for this observation.

Design: We cloned the human MTA1 gene into the plasmid pcDNA3.1 and generated stable MTA1 expressing LNCaP prostate cancer cell lines. Two MTA1-expressing clones and one vector control clone were isolated by selection with Geneticin. Migration and invasion of MTA1-overexpressing LNCaP cells were assessed using modified Boyden chamber assays. Proliferation was determined by cell number increase over 8 time points every 24h and calculation of the doubling time. To characterize the putative function of MTA1 as transcription repressor, we used Affymetrix U133APlus2.0 expression arrays. Arrays were performed in duplicate for each cell clone. The raw data was normalized, filtered, and clustered with dChip software and significantly dysregulated genes determined with SAM analysis.

Results: MTA1-transfected cell clones showed an approximately 8-fold expression of MTA1 compared to vector-transfected cells as determined with quantitative real-time PCR and Western blots at the mRNA and protein level, respectively. Transwell assays showed an up to 100-fold increase in migration and invasion ability in MTA1 over-expressing cells. The doubling time of MTA1 over-expressing cells decreased to 30h compared to 46h in the mock-transfected control cells. Genes upregulated in MTA1 overexpressing LNCaP cells included neurotensin (52-fold overexpression), matrix metalloproteinase 7 (21-fold), and alpha-methylacyl-CoA-racemase (2.6-fold). Downregulated genes included inhibitor-of-DNA-binding 1 and 3 (0.2-fold and 0.3-fold, respectively) and quiescin Q6 (0.38-fold).

Conclusions: Our data suggests that overexpression of MTA1 is associated with an increase in migration and invasion as well as in proliferation of LNCaP cell lines. Although MTA1 has been previously suggested to be involved in transcription dysregulation we have now identified potential downstream target genes of MTA1. Many of these genes have been found to be involved in cancer cell migration, invasion, and proliferation. This study may provide a mechanistic explanation of how the increased MTA1 expression levels found in metastatic prostate cancer initiate and/or propagate metastasis.

652 Neo-Adjuvant Hormone Ablation Therapy Confounds Post-Prostatectomy Risk Assessment

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Background: Neo-adjuvant (pre-operative) hormone ablation therapy is used to reduce tumor size prior to surgery or in cases where the prostatectomy is delayed. Previous studies have reported decreased stage and surgical margin status in neo-adjuvantly treated patients as well as an increase in Gleason grade. This study examines the effect of neo-adjuvant hormone ablation therapy on pathology parameters and evaluates their ability to predict PSA recurrence.

Design: Between 1984-2002, 1118 patients underwent radical prostatectomy at our instituion. 167/1118 (15%) of patients received pre-operative hormone ablation therapy based on suspected tumor volume or advanced stage as part of a routine practice.

Results: Neo-adjuvant hormone ablation therapy was associated with a higher incidence of extra-prostatic extension (EPE, 61% vs. 54% in untreated patients, p=0.04) and seminal vesicle invasion (SVI, 61% vs. 25%, p=0.01). The surgical margin status (SM) was similar to untreated patients (53% vs. 54% positive). Pre-operative PSA levels was lower in treated patients (25% with PSA 4-10 ng/ml and 35% with >10 ng/ml vs. 38% and 48% in untreated patients, respectively; p=0.01). Pathology parameters such as pT-Stage, pN-Stage, number and density of positive lymph nodes, Gleason Scores (GS), nuclear grade(G), as well as PSA recurrence rates showed no significant difference between the treated and untreated groups. In untreated patients, 212/951 (22%) experienced PSA recurrence and EPE, SVI, SM, GS, G, T- and N-stage and preoperative PSA were significantly associated with time to PSA recurrence (p<0.05). In contrast, none of these parameters predicted PSA recurrence in the 44/167 (26%) of patients treated with neo-adjuvant hormone ablation therapy.

Conclusions: Understaging is of concern in patients with neo-adjuvant hormone ablation therapy as tumor cells are often not identifiable because of treatment effects. In our cohort, patients receiving neo-adjuvant hormone ablation therapy have a higher rate of EPE and SVI which may be due to more advanced tumors at time of diagnosis. We did not observe a significant decrease in SM or GS. However, it remains important for pathologists to be notified of neo-adjuvant hormonal therapy when a specimen is evaluated. In our cohort, parameters predicting PSA recurrence in untreated patients seemed to be of little value for patients having received pre-operative hormonal therapy.

653 Temporal Trends and Cancer Prediction Rates for Atypical Small Acinar Proliferation (ASAP) with or without Associated High Grade Prostatic Intraepithelial Neoplasia (PIN)

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Background: Historically ASAP with or without PIN predicts for subsequent detection of prostatic adenocarcinoma (PAC) in 34-60% of cases. The purpose of this study was to assess the frequency of ASAP, ASAP + PIN and PIN-ASAP (PIN-ATYPIA) and their subsequent cancer detection rates in a large needle biopsy database .

Design: From May1999 to December 2004, 9013 sextant and extended sextant prostate needle core biopsy specimens were reported by three urologic pathologists. 123 isolated ASAP cases, 119 ASAP + PIN (topographically separated) and 31 PIN-ASAP were identified. The diagnosis of ASAP included categories of ASAP suspicious for malignancy and ASAP of uncertain significance. The mean follow up was 18.4 months (mo) for ASAP, 18 mo for ASAP + PIN and 12.9 mo for PIN-ASAP (range 1-48 mo).

Results: Follow up biopsy data was available in 79 cases of isolated ASAP (64%), 74 ASAP + PIN (63%) and 17 PIN-ASAP(55%) and the cancer detection rates on follow up biopsies were 49%, 59% and 41% respectively with most PAC cases identified within 12 mo. Of initial ASAP diagnosis. The PAC rates for ASAP, suspicious and ASAP, of uncertain significance were 60% and 36% respectively. The rates of ASAP and ASAP + PIN declined from 1999 to 2004. The overall frequency of PAC cases in the database remained relatively constant averaging 43%, however the rate of minimal PAC (1 core, < 5%) rose from 3% in 1999 to 7.2% in 2004.

Table 1

Dx/ YEAR	1999	2000	2001	2002	2003	2004
Isolated ASAP	2.6%	1.9%	1.8%	1.2%	0.8%	0.9%
ASAP + PIN	4.1%	2.2%	1.1%	1.0%	0.7%	0.8%
PIN- ASAP		0.2%	0.2%	0.4%	0.6%	0.4%
Minimal PAC	3%	5.2%	5.0%	5.0%	6.1%	7.2%

Conclusions: ASAP with or without PIN is highly predictive of PAC on follow up biopsy. The declining rates of ASAP +/- PIN are accompanied by increasing rates of minimal PAC likely related to increased core sampling and the use of racemase which was introduced into our laboratory in mid- 2003.

654 Immunohistochemical Expression of P63, GLUT1 and XIAP in Micropapillary Urothelial Carcinoma

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Background: Micropapillary urothelial carcinoma (MPC) has been shown to have aggressive behavior with early lymphovascular infiltration, metastasis and poor prognosis. Molecular pathways involved in its pathogenesis are not well studied. We studied immunohistochemical expression of P63, GLUT, a Glucose transporter , induced by hypoxia driven pathways and XIAP, an X linked inhibitor of apoptosis in MPC and compared these findings in normal urothelium (NU), carcinoma insitu (CIS), low grade (LG) and high grade urothelial carcinoma (HG).

Design: Sections were deparaffinized, subjected to citrate based antigen retrieval (P63&XIAP) and immunostained with anti XIAP (BD bioscience) anti P63 (Santa Cruz) and anti GLUT1 (Chemicon) and developed using streptavidin-biotin kit (Biogenex, San Ramon, CA or Envision plus, DAKO).

Results: p63: 9/10 MPC were p63 negative. Diffuse p63 expression was seen in all CIS, LG and HG including 5/8 HG regions associated with MPC . Staining was basally localized in all NU. GLUT1: Rare staining was observed in 1/10 cases of MPC, however MPC associated HG component was positive in 5 cases. Staining in CIS, LG and HG increased with grade (table1). XIAP: Highest level of expression was seen in MPC (60%) which ranged from focal to diffuse. HG regions associated with MCP stained in 5/6 cases in which MPC was positive. Staining in CIS, LG and HG increased with increasing grade (table1).

	Immunostaining pattern		
Lesion	p63	GLUT1	XIAP
CIS	10/10 (100%)	2/4 (50%)	1/9 (11%)
LG	12/12 (100%)	3/5 (60%)	1/12 (8%)
HG	12/12 (100%)	9/12 (75%)	6/12 (50%)
MPC	01/10 (10%)	1/10 (10%)	6/10 (60%)

CIS; urothelial carcinoma insitu, LG; urothelial carcinoma insitu, HG; high grade urothelial carcinoma, MPC; micropapillary urothelial carcinoma.

Conclusions: p63 expression has been shown in normal urothelium as well as urothelial carcinoma and reportedly decreases with higher grade. Loss of expression in MPC is a novel finding emphasizing its aggressive nature. In our study, increase in GLUT1 expression was shown with increasing grade of urothelial carcinoma. However almost no expression of GLUT1 in MPC suggests different pathogenesis for MPC. XIAP expression was highest in MPC possibly relating to its role in tumor progression and suggesting potentially new therapeutic approaches. Supported by Estate of Hilda Leveen.(D.E.B)

655 Immunohistochemical Analysis of the mTOR Pathway in Prostatic Cancer. p4EBP1 as a Potential Marker of High Risk HGPIN

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Background: Activation of the PI3K/Akt/mTOR signal transduction pathway contributes to the development of tumors by prevention of apoptosis and deregulation of cell cycle. mTOR controls the translation machinery via activation of the p70S6K and via inhibition of 4EBP1, and constitutes a main controller in cell growth. 4EBP1 plays a crucial role in regulating translation and progression in cell cycle, by control of cyclin D1 and c-myc. The aim of this study was to analyze the mTOR pathway in prostatic cancer (PC) and High Grade PIN (HGPIN), using a Tissue Microarray design.

Design: We have analyzed the immunohistochemical expression of phosphorilated (p) proteins pAkt, p4EBP1, pp70S6K and pS6 in 55 radical prostatectomies with PC and 8 cistoprostatectomies with vesical cancer and concomitant HGPIN but without PC. The levels of expression were evaluated as percentage and intensity of stained tumor cells (Hscore). The clinicopathological data included pTNM, Gleason, PSA and follow-up (mean 22 months)

Results: Activation of Akt/mTOR cascade was detected both in PC and HGPIN areas around it, with similar pAkt (63%) and pP70S6K (PC 15%; HGPIN 21%) expression. p4EBP1 trends to be high in HGPIN (96% vs. 42%, pNS), with HScores high in HGPIN (85 vs. 50, p=0.006). The pS6 expression was focal and scarce. We found a significant correlation between pP70S6K and p4EBP1 in PC (p=0.047), and between pAkt and pP70S6K (p=0.026) and p4EBP1 (p=0.033) in HGPIN. Interestingly, 4EBP1 expression in HGPIN with PC was higher than in HGPIN without it (p<0.001), with an inverse pattern for pP70S6K (p=0.044). Expression was no related to any clinicopathological data

Conclusions: Immunohistochemical detection of p molecules of the Akt/mTOR pathway in PC and preneoplastic lesions is feasible. The higher p4EBP1 expression in HGPIN around cancer than in the tumor suggests a predominant role of this molecule in malignant transformation. Importantly, p4EBP1 expression was much higher in HGPIN associated with carcinoma than in isolated HGPIN. This finding may reflect its more aggressive behavior. Further studies have to be done to confirm whether p4EBP1 expression can be used as a potential marker of progression risk in HGPIN

656 Papillary Renal Cell Carcinoma

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Background: Renal cell carcinoma (RCC) consists of several histologic subtypes with distinct morphologic and genetic characteristics. Pathologic features and patient outcome differ significantly by subtype. Papillary RCC, the second most common subtype, accounts for 10-15% of RCCs in surgical series. Others have reported that papillary RCC may be further divided into type I (basophilic) and type II (eosinophilic). We describe our experience with these tumors in the largest review to date of papillary RCC.

Design: A retrospective review of 339 patients with sporadic, unilateral papillary RCC who underwent a nephrectomy at our institution between 1970 and 2002. The medical records, pathology reports and pathology slides were reviewed by two urologic pathologists. Associations of pathologic features with death from RCC were evaluated using Cox proportional hazards regression.

Results: The average age at diagnosis was 64 years (84% male). Thirty-one patients died of RCC at an average of 4.3 years following nephrectomy. The average follow-up for the patients who were still alive was 9.3 years. The majority of tumors were pT1a (49%) or pT1b (27%). Patients with high-stage tumors were more likely to die of RCC compared with patients with low-stage tumors (risk ratio [RR] 8.1; p<0.001). Positive regional lymph nodes and distant metastases were present in 1.5% and 2.4% of patients, resulting in RRs of 24.7 (p<0.001) and 24.1 (p<0.001). The majority of tumors were grade 2 (64%) and grade 3 (33%). Patients with high-grade (3 and 4) tumors were more likely to die from RCC compared with patients with low-grade tumors (RR 4.1; p<0.001). Cases with foam cells (73%) were less likely to die from RCC (RR 0.22; p<0.001). Coagulative tumor necrosis was present in 45% of cases, but was not significantly associated with death of RCC (RR 1.0; p=0.994). Type I or II differentiation was not significantly associated with outcome unless 100% type II differentiation was present (RR 3.0; p=0.004).

Conclusions: We confirm that the pathologic features of stage and grade are important prognostic indicators for patients with papillary RCC. In addition, the presence of foam cells or macrophage infiltration was associated with an improved outcome. The subclassification of papillary RCC into type I and type II contained no prognostic information unless 100% type II differentiation was noted.

657 Clonal Origin of Metastatic Mature Teratomas

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Background: Testicular teratomas in adult patients are histologically diverse tumors that frequently coexist with other germ cell tumor components. These mixed germ cell tumors often metastasize to retroperitoneal lymph nodes where multiple tumor components are frequently found in metastatic lesions. The genetic relationships of the different components in metastatic lesions have not been elucidated.

Design: We examined 25 patients who underwent retroperitoneal lymph node dissection for a metastatic testicular germ cell tumor. All patients had metastatic mature teratoma with one or more different germ cell tumor components present. Genomic DNA samples from each coexisting component were prepared from formalin-fixed, paraffin-embedded tissue sections using laser-assisted microdissection. Loss of heterozygosity (LOH) assays for 7 microsatellite polymorphic markers on chromosome 1p36 (D1S1646), 9p21 (D9S171, IFNA), 9q21 (D9S303), 13q22-q31 (D13S317), 18q22 (D18S543), and 18q21 (D18S60) were performed to assess clonality. A total of 25 mature teratomas, 10 embryonal carcinomas, 7 seminomas, 7 yolk sac tumors, and 7 choriocarcinomas were included in this study.

Results: Twenty-three of 25 (92%) cases showed allelic loss in one or more components of the metastatic germ cell tumors. Twenty-three of 25 mature teratomas showed allelic loss in at least one of seven microsatellite polymorphic markers analyzed. The frequency of allelic loss in informative cases of metastatic mature teratoma was 25% (6 of 24) with D1S1646, 35% (8 of 23) with D9S171, 36% (8 of 22) with IFNA, 25% (6 of 24) with D9S303, 48% (11 of 23) with D13S317, 27% (6 of 22) with D18S543, and 36% (8 of 22) with D18S60. Completely concordant allelic loss patterns between mature teratoma and all of the other metastatic germ cell tumor components were seen in 20 of 23 cases in which the mature teratoma component showed loss of heterozygosity. Nearly identical allelic loss patterns were seen in the 3 remaining cases.

Conclusions: Our data support the common clonal origin of metastatic mature teratomas with other components of metastatic germ cell tumors.

658 MUC-4 Expression in Prostate Cancer

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Background: The role of MUC-4, a ligand for Her2, in prostate cancer is not yet understood. Recently, MUC-4 upregulation has been found in prostate cancer cell lines.The aim of this study is to determine the protein expression of this gene in untreated prostate cancer.

Design: One hundred and thirty radical prostatectomy specimens performed for primary untreated prostate cancer were selected for this study. The immunohistochemical staining was carried out using MUC-4 antibody (Invitrogen, Carlsbad, CA) at a 1:200 dilution using the L-SAB detection system (DakoCytomation,Carpinteria, CA). Same slides were also immunostained for the detection of Her2 using HercepTest Kit (DAKO).

Results: Five (4%) tumors were positive for MUC-4. Three of them were high-grade and two low-grade tumors. Strong and focal cytoplasmic membrane positivity for this antibody was seen in all but one positive case which showed diffuse staining. Benign prostatic tissue was uniformly positive for this antibody. Ten (8%) cases were Her2 positive, and most of them were high-grade tumors. They all show focal and moderate intensity of staining. Co-expression of MUC-4 and Her2 was not seen.

Conclusions: 1- MUC-4 is expressed in a minority of untreated primary prostatic carcinomas. 2- When present, MUC-4 shows a strong cytoplasmic membrane positivity. 3- There is no co-expression of MUC-4 and Her2 protein in untreated primary prostatic carcinomas.

659 Microvessel Density in Prostatic Adenocarcinoma: How Relevant Are "Hot Spots"?

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Background: Microvessel density (MVD)- a measure of tumor angiogenesis, has emerged as a strong prognostic marker in various tumors including prostate carcinoma (PrCa) and remains a potentially important therapeutic target. However, MVD in tumors may vary and areas with increased vascularity due to neoangiogenesis ('hot spots') are recognized, that may predict tumor behavior more accurately. Variability of MVD in different areas of a PrCa may limit its clinical application in prostate needle biopsies. In this study we aim to establish extent of MVD variability in transuretheal resection specimens (TURP) by analyzing multiple randomly chosen areas of PrCa in a given case.

Design: 42 TURP specimens from an ongoing PrCa study were included in this analysis. Hematoxylin and eosin stained slides were reviewed in each case to identify and grade (Gleason's grade) the tumors. Slides obtained from formalin fixed, paraffin embedded tissues were stained immunohistochemically with antibody against factor VIII related antigen. MVD was counted in 5 arbitrarily chosen high power fields (40X magnification) using an Olympus BH-2 microscope in a representative slide in each case. The mean, standard deviation and range of MVD was calculated in each case.

Results: The Gleason's score (total of 2 predominant Gleason's grades) and corresponding MVD are shown in the table. In general, poorly differentiated tumors with a higher Gleason score had greater MVD. However, within each group there was considerable variability. Tumor volume did not have a direct relationship with MVD and within each tumor the variability was mild. (Mean standard deviation<5)

Conclusions: Earlier it has been shown that MVD is an independent and strong prognostic factor in PrCa. Our study shows that MVD correlates with tumor differentiation in general. More importantly, there is only mild variation in MVD in a given case despite different histologic patterns in different areas. Hence, MVD counting can be used as a valuable prognostic indicator in cases of diagnostic needle biopsies in PrCa.

Gleason score	MVD Range	No. of cases	Mean+S.D
3	15-27	1	19.2±4.21
4	7-26	11	13.16±4.26
5	4-24	9	13.16±4.56
6	8-28	5	17.52±4.91
7	6-48	14	19.54±7.94
8	14-32	1	21.8±6.08
9	34-40	1	35.8±2.23

660 Immunohistochemical Detection of Aquaporin 1 as an Aid in Identifying Renal Cell Carcinoma in the Setting of Metastatic Carcinomas of Unknown Primary

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Background: Aquaporin 1 is a water channel protein identified in different segments of the renal tubulary system. Recently it has been detected by immunohistochemistry (IHC) in conventional and papillary renal cell carcinomas. We assessed the potential utility of aquaporin 1 in identifying renal cell carcinomas in the setting of metastatic carcinoma of unknown primary by assessing its expression in a series of known carcinomas.

Design: A series of 18 conventional renal cell carcinomas, 8 resection specimens and 10 tissue microarrays (TMA), and non-renal carcinomas were tested for aquaporin 1 expression by IHC using a rabbit polyclonal antibody (Chemicon International). The scoring was based on the percentage of positive tumor cells exhibiting cytoplasmic membrane staining: negative (0%), rare cell (<1%), focal (1-25%), variable (25-75%), and uniform (>75%).

Results: Fifteen out of 18 (83%) conventional renal cell carcinomas tested were positive for aquaporin 1. No expression of aquaporin 1 was identified in the TMA sections of non-renal carcinomas from colon (0/10), breast (0/8), ovary (0/9), and urothelium (0/8). A small number of cases of TMA sections of carcinomas of the pancreas, 1/10 (10%); liver, 1/10 (10%); and stomach, 2/9 (22%) were positive for aquaporin 1.

Conclusions: Immunohistochemical detection of aquaporin 1 in conventional renal cell carcinomas exhibited a sensitivity of 83% and a specificity of 94%. Use of aquaporin 1 in the appropriate IHC panel may aid in the diagnosis of renal cell carcinoma in the context of metastatic carcinoma of unknown primary.

661 Inflammatory Myofibroblastic Tumors of Ureter: A Clinicopathologic and Immunohistochemical Study of 3 Cases

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Background: Inflammatory myofibroblastic tumors (IMT) have been described in a variety of genitourinary sites including urinary bladder, prostate, kidney and paratestis. Only rare isolated examples of IMT involving ureter have been reported.

Design: Three cases of ureteric IMT were identified in the consultation files of the authors. Clinicopathologic features of these 3 cases were documented along with immunohistochemistry for cytokeratins (CAM 5.2, AE1/ AE3), vimentin, desmin, HHF35, smooth muscle actin, CD34, S100 protein and ALK-1.

Results: The 2 males and 1 female had a mean age of 38.6 years (range 30- 53). Clinical presentation included hydronephrosis (3 patients), complete ureteric obstruction (2), flank pain (1), a mass in the region of uretropelvic junction (UPJ) (1) and gross hematuria (1). In each case imaging studies showed a mass lesion obstructing the ureter. The main differential diagnoses included urothelial malignancy and idiopathic retroperitoneal fibrosis. One patient underwent nephrectomy with partial ureteric resection and two patients nephroureterectomy. Gross features included a fibrotic mass involving the UPJ and proximal ureter (1 case) and symmetrical or asymmetrical thickening of ureter producing a mass-like lesion (2 cases). The histologic patterns included a fibro-inflammatory lesion composed of lymphocytes and plasma cells admixed with hypocellular dense keloid- like fibrosis. One case showed a predominantly cellular spindle cell pattern. Poorly formed granulomas were evident in one case. The spindle cell component showed the following immunophenotype: cytokeratins (CAM 5.2, AE1/ AE3) -, vimentin+, HHF35- focal +, smooth muscle actin- focal +, desmin -, CD34 -, S100 - and ALK-1-. No recurrences have been noted on short term follow up.

Conclusions: IMT rarely affects the ureter where it clinically and grossly simulates a malignant neoplasm. The process has diverse morphologic patterns similar to those seen in other genitourinary sites and displays an imunophenotype suggesting derivation, at least in part, from myofibroblasts.

662 Fat Tissue Can Be Seen within Prostatic Parenchyma: A Study 139 Whole Mount Radical Prostatectomy Specimens

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Background: In the literature, fat tissue is reported to be absent in prostatic parenchyma. Therefore, when one sees a cancer within the fat tissue, it indicates extraprostatic extension. During our routine histologic evaluation of prostatic needle biopsies, we encountered a case showing fat tissue in the prostatic parenchyma. This case prompted us to review the whole mount sections of the prostate to systematically evaluate the presence or absence of fat tissue in the prostatic parenchyma as well as the location and amount of fat if it is present.

Design: To investigate the presence of fat tissue within the prostatic parenchyma, we reviewed 139 cases of whole mount sections from radical prostatectomy specimens treated for prostatic cancer.

Results: Of these, 5 cases (3.5%) showed fat tissue within the prostatic parenchyma. Patient's age ranged from 39 to 79 years old (mean, 61.9 years). Fat tissues were present in the peripheral zone of right lateral wall just beneath the prostatic fibromuscular tissue (capsule) in 3 cases. Fat tissue was also identified in one case each from the peripheral zone of left lateral wall just beneath the capsule and in the central zone near the anterior wall of prostatic verumontanum. The fat tissue in all cases was present 1~3 mm within the prostatic capsule and between the prostatic glands. The fat tissues were ranged in size from 0.1 mm to 3 mm with a mean of 1 mm. Fat tissue was not observed in the deep prostatic parenchyma.

Conclusions: Fat tissue can occur rarely within the prostatic parenchyma and always is seen near the prostatic capsule. Because of its small amount and peripheral location, its presence does not create problems of interpretation in the evaluation of radical prostatectomy specimens. However, when fat tissue is present within the prostate glands and if tumor is present within the fat in a core biopsy specimen, a special caution is warranted before the interpretation of extraprostatic extension of prostate carcinoma is given.

663 Predictive Value of High-Grade Prostatic Intraepithelial Neoplasia (HGPIN) for Prostatic Adenocarcinoma (CaP) on 12-Core Prostate Needle Biopsy (Bx)

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Background: Isolated HGPIN on a sextant prostate bx historically confers a 20-30% risk of synchronous CaP on immediate repeat bx. We postulated that the predictive value of isolated HGPIN for an unsampled CaP on a 12-core bx would be lower, due to more thorough prostate sampling and a lower risk of missing small CaPs on initial bx. The aim of our study was to 1) determine the incidence of CaP on repeat bx in pts with isolated HGPIN on initial 12-core bx and to 2) correlate with CaP size.

Design: We searched the medical record for 12-core prostate bxs performed by one surgeon (1/99 and 7/05). Inclusion criteria were: diagnosis (dx) of isolated HGPIN and absence of any prior bxs. We reviewed all bxs to confirm dxs, determine the number (%) of cores with HGPIN, and correlate clinical features and pathologic outcomes on follow-up bxs and radical prostatectomies (RPs). Pt groups with follow-up benign / HGPIN and CaP were statistically compared.

Results: Of 577 men undergoing 12-core bx, 48 (8.3%) had isolated HGPIN, including 5 (0.9%) with concurrent atypical small glands. All had one (n=37; 77.1%) or multiple (n=11; 22.9%) repeat bxs. On initial repeat bx, 27 (56.3%), 11 (22.9%) and 10 (20.8%) had benign, HGPIN and CaP, respectively, at a median (range) of 31 (14-459) days. At last follow-up, CaP was detected in 15 (31.3%) pts, including 10 with bx Gleason score $\leq$6; 33 (68.8%) had persistent HGPIN or benign dxs. The initial PSA levels for these groups were 8.8 and 5.4 ng/ml (p=0.03), and the % cores with HGPIN were 28.5% and 15% (p=0.003), respectively. Mean (range) CaP size for 11 pts undergoing RP was 0.7 cm (0.2-1.8).

Conclusions: Isolated HGPIN detected on a 12-core bx confers a 31.3% risk of an unsampled synchronous CaP. This is similar to the predictive value for synchronous CaP on repeat bx using a standard 6-core bx. However, most CaPs in pts having RPs were $\leq$ 0.7 cm, suggesting that initially unsampled CaPs in these pts are smaller than CaPs in pts with isolated HGPIN in a 6-core bx. It is also likely that 12-core bxs detect more pts with small volume HGPIN and smaller CaPs on the initial bx. Age, race, and DRE did not correlate with CaP on repeat bx. However, PSA and % cores with HGPIN were significantly greater for pts with eventual CaP. Isolated HGPIN on initial bx requires close follow-up for unsampled synchronous and metachronous CaP.

664 Ductal Adenocarcinoma of the Prostate. A Clinicopathological Study of 50 Cases

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Background: Ductal adenocarcinoma of the prostate, composed of large glands with tall columnar cells, is an uncommon tumor accounting for 0.2-0.8% of prostate cancer. There have been only a few reports with limited cases studied and controversies exist regarding its pathogenesis and prognosis. Most of the studies used transurethral resection (TUR) or biopsy specimens, further complicating the issue with sampling errors. A study from a larger cohort with radical prostatectomies may provide better understanding of this entity.

Design: From the Surgical Pathology archives of two teaching hospitals we retrieved 50 cases of ductal adenocarcinoma mostly from radical prostatectomies (biopsies, n=5; TUR, n=3; RP, n=42). Fifteen cases of ductal adenocarcinoma were further analyzed with immunohistochemistry using a rabbit monoclonal antibody specific for alpha-methylacyl coenzyme A racemase (AMACR).

Results: The age of the patients ranged from 51 to 94 (mean:65). The most common presentation was elevated PSA levels detected in an asymptomatic man. The PSA value ranged from 2.78 to 28.9 in the cases with PSA levels documented. Concomitant acinar adenocarcinoma was found in 94% of cases with only 3 cases defined as "pure" ductal adenocarcinoma, 7 as dominant, 32 as mixed and 8 as minor component. The Gleason score of the associated acinar adenocarcinomas was 7 in 37 cases (78.8%), 8 in 5 cases (10.6%), and 9 in 5 cases (10.6%). Twenty six of 42 RP cases (61.9) were of advanced stage including 23 cases in stage III, 3 cases in stage IV and only 16 cases in stage II. By immunostaining, 11 of 15 cases (73.3%) of ductal adenocarcinoma showed decreased AMACR immunoreactivity compared to the acinar adenocarcinoma.

Conclusions: The strong association between ductal adenocarcinoma and acinar adenocarcinoma in the majority (94%) of cases in this study suggests their common etiology and pathogenesis. The coexistence of ductal adenocarcinoma with high grade adenocarcinoma with high volume and high stage disease indicates a poor prognosis. Furthermore, the frequent decrease in the AMACR immunoreactivity in ductal adenocarcinoma signifies caution in interpretation of the immunostaining in diagnosis of prostate cancer. Based on these findings the identification of ductal adenocarcinoma in biopsy specimens should be considered as poor prognostic factor.

665 Hybrid Renal Cell Carcinoma with Clear Cell and Papillary Cytomorphological Features

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Background: Conventional or clear cell renal cell carcinoma (CRCC) and papillary or chromophil renal cell carcinoma (PRCC) are the two most frequent subtypes of renal cell carcinoma (RCC). In the present study, cytohistopathological features of RCC, containing admixture of papillae and clear cells, are described.

Design: Over a period of 12 years, 532 consecutive cases of RCC were collected and reviewed to identify RCC with admixture of papillae and clear cells. The neoplasms were grouped based on common morphological features. Biological behavior of tumors in each group was also noted.

Results: Of the 532 RCC cases reviewed, 75 cases with admixture of papillae and clear cells were grouped into: Group 1 (18 cases), CRCC with solid architecture and focal to extensive areas of papillary architecture; Group 2 (28 cases), papillary architecture without solid areas of CRCC, divided into 2 subgroups; Group 2a (9 cases), papillae without chromophil cells but with extensive clear cell change with areas of foamy epithelial cells and/or foamy macrophages; Group 2b (19 cases), papillae with chromophil cells (typical PRCC) and with clear cells; Group 3 (29 cases), papillary architecture, with features of groups 2a and/or 2b, and areas of CRCC with solid architecture. There was a high rate, 12 of 75 cases (16%), of sarcomatous transformation. In comparison to pure PRCC, all groups had a tendency of low rate of lymph node metastasis, high rate of vascular invasion, high rate of distant metastasis, and worse prognosis. This tendency was stronger in Group 1 compared to Group 2 and 3.

Conclusions: Group 1 of this study does not represent a hybrid RCC but rather papillary differentiation in the progression of CRCC. Group 2 and 3 represent a hybrid RCC between CRCC and PRCC with characteristic cytohistopathological features and with distinct biological behavior. Recognition of this hybrid tumor may facilitate the classification of RCC with ambiguous morphology.

666 Expression of Kidney-Specific Cadherin (Ksp-cad) in Rare and Unusual Renal Epithelial Neoplasms (REN). Diagnostic and Histogenetic Implications

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Background: Ksp-cad is a recently characterized calcium-dependent cell]adhesion molecule that appears to be kidney-specific in its distribution with expression localized primarily in the distal nephron. Two studies in the literature have analyzed its immunohistochemical (IHC) expression in the more common REN. IHC expression of this marker in rare and recently described subtypes of REN is unknown.

Design: Standard sections and tissue microarray (TMA) specimens including a wide spectrum of REN, but specifically weighted to include recently described tumors, were analyzed for Ksp-cad (Zymed Laboratories,dilution 1:75). IHC expression was analyzed in normal and tumor areas; staining was quantitated on a scale of 0-3+.

Results: Immunoexpression was confirmed in tumors of distal nephron derivation - chromophobe renal cell carcinoma (RCC) and renal oncocytoma; with predominately negative immunoreactivity in tumors histogenetically related to the proximal convoluted tubules - clear cell and papillary RCC. There was no immunoreactivity in 23 mucinous, tubular and spindle cell carcinomas (MTS RCC), 3 high grade collecting duct carcinomas and 2 renal medullary carcinomas. One of four (25%) t(x,1) translocation associated RCC (3 proven cytogenetically and all 4 by IHC for TFE3) showed 3+ positivity. Ten of fourteen tubulocystic carcinomas showed 1 to 2+ staining of weak to moderate intensity. Staining in the normal kidney was 3+ and restricted to the distal convoluted tubule (DCT) and ascending thick limbs.

Conclusions: 1) This study documents immunoreactivity for Ksp-cad in the range of contemporarily classified REN. 2) Immunohistochemical panels to subtype REN and constructed (from among CK7, RCC antigen, CD10, parvalbumin, alpha-methylacyl-CoA-racemase) based on the specific differential diagnostic situation should include Ksp-cad for tumors related to distal nephron differentiation (chromophobe RCC and oncocytoma). 3) Negative Ksp-cad immunoreactivity in MCT argues against distal portion of nephron origin for the tumor.

667 The Role of Frozen Section in the Management of Intrascrotal Mass Lesions

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Background: With increasing use of imaging, there has been a rise in the number of small intrascrotal lesions identified that require frozen section examination to elucidate their nature.

Design: The total number of intra-operative frozen sections requested on intrascrotal lesions in 2001-5 was ascertained and information noted regarding clinical presentation, reason for FS request, result of FS and paraffin section examination and subsequent operative management.

Results: In the four-year period from 2001-5, a total of 12 frozen sections were requested on the basis of either solitary testis, clinical uncertainty regarding site or small size of lesion. Patients ranged in age from 22-62 years (mean 35 years) and the lesions varied from 3mm to 40mm in size. Nine of the 12 cases resulted in a benign frozen section diagnosis, resulting in testis-sparing surgery. A comparison of frozen and paraffin sections upheld the accuracy of this technique in all cases.

Conclusions: Frozen section is a useful tool in the intra-operative diagnosis of intrascrotal masses where there is clinical and radiological uncertainty with regards to the nature of the lesion. The accuracy of the modality relies on good communication between the urologist and the pathologist, and ideally a pathologist with experience or a special interest in urological pathology. In nine of 12 cases in our series, a benign frozen section diagnosis allowed testis-conserving surgery to be performed.

Case	Age	Presentation	Size	FS diagnosis	Management
1	29	mass	5mm	Leydig	Local excision
2	37	mass	15mm	Cyst	Local excision
3	37	mass	5mm	Leydig	Local excision*
4	42	mass	15mm	Scar	Local Excision
5	22	solitary testis	40mm	Lymphoma	Orchidectomy and chemotherapy
6	32	incidental finding	3mm	Leydig	Local excision
7	39	mass	15mm	Scar	Local excision
8	62	mass	8mm	Adenomatoid	Local excision
9	43	mass	N/A	Cyst	Local excision
10	38	mass	40mm	Seminoma	Orchidectomy
11	30	mass	10mm	Leydig	Local excision
12	34	mass	12mm	Teratoma	Orchidectomy

N/A = not available, * = postop complication resulted in orchidectomy

668 Size, Location and Number of Nerves with Perineural Invasion (PNI) for Prostate Cancer on Biopsy – Do They Matter?

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Background: The significance of PNI on needle biopsy has been studied as presence or absence in most of the previous studies. However, when PNI is identified on biopsy, the significance of the nerve size, location and number has not been studied previously.

Design: We investigated size, location and number of nerves with PNI in a cohort of 101 consecutive biopsies, reported initially as positive for PNI. Nerve diameters were measured using computer image analysis. The findings were correlated with the prostatectomy (RP) stage: pT2 (78) vs pT3 (23) and the biopsy Gleason score and cancer extent. We also studied a separate group of 23 biopsies that had an advanced pT3 stage with periprostatic fat invasion (pT3 PF) on biopsy, in addition to PNI .

Results: Mean and median nerve sizes did not differ by stage. Greatest nerve diameter (Max D) measured 413, 517, and 737 µm for stages pT2, pT3 and pT3 PF, respectively (all medians, $p<0.001$; Kruskal-Wallis). The number of nerves with PNI was also associated with the stage, with medians of 2, 5, and 11 for stages pT2, pT3 and pT3 PF, respectively ($p<0.001$; Kruskal-Wallis). The number of nerves with PNI showed stronger correlation for biopsy Gleason Score (0.36) and cancer extent (0.57) (both $p<0.0001$, Spearman), than the Max D (Gleason Score 0.18, $p=0.05$; cancer extent 0.26, $p=0.004$). A Max D > 500 µm was associated only with positive margins on RP ($p=0.002$; Pearson), but not with other RP specific outcomes. Patients with > 3 nerves with PNI were more likely to have seminal vesicle invasion ($p=0.027$; Pearson), but no association was found with other RP outcomes. On multivariate analysis, the number of nerves with PNI was associated with stage pT3 after controlling for biopsy Gleason score and cancer extent, but Max D was not an independent stage predictor. Nerves with PNI were most often identified in the cores from the base (69.1%), followed by the mid-zone (23.6%) and the apex (7.2%). The distribution of PNI did not vary by stage or Max D. In pT3 PF group the distribution of PNI was more uniform (base 45.8%, mid-zone 33.2%, apex 21%), due to larger cancer extent and higher Gleason scores on biopsy.

Conclusions: 1.) Max D and number of nerves with PNI on biopsy correlated with biopsy Gleason score, cancer extent and the stage on RP 2.) The number of nerves with PNI, but not Max D, was an independent predictor for stage. 3.) PNI was most frequently identified in the cores from the base, except in cases with advanced stage and higher Gleason score.

669 Clinical, Radiographic, and Pathologic Characteristics of Cystic Nephroma (CN) and Mixed Epithelial Stromal Tumor of the Kidney (MEST)

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Background: CN and MEST are grouped together as "mixed mesenchymal and epithelial tumors" in 2004 WHO classification; however, it is uncertain whether they are two distinct, or related entities. In this study, we compared the clinical, radiographic, and pathologic features of 31 such cases.

Design: Cases diagnosed as CN or MEST were retrieved from a single center nephrectomy database. Clinical and radiological information were retrieved pathology was re-reviewed.

Results: 31 tumors from 29 patients were identified from 1982 to June 2005. CN and MEST had similar age and gender distribution with a remarkable female predilection (Table). Radiologically, CN was more commonly Bosniak 3, often included a renal pelvic component, and the diagnosis was suspected by on imaging in 42%. Pathologically, CN and MEST both were composed of epithelial and stromal components, although MEST had more exuberant epithelial elements of many types and expansile stromal elements that often included smooth muscle. Ovarian type stroma was present in both CN and MEST, but absent in male patients. Bilateral tumors were present in 1/21 patients with CN (5%) and 1/5 patients with MEST (20%). 2 patients with CN had concomitant RCC, including 1 with multifocal ipsilateral clear cell RCC and 1 with bilateral papillary RCC. At median follow-up of 2.4 years (0.1 – 17 years), no patients with CN or MEST developed novel or recurrent tumors. Three patients had sarcomas arising in CN or MEST, including 2 in CN and 1 in MEST. Two of those patients are dead of disease and 1 is NED.

Conclusions: With similar clinical and pathological features, CN and MEST are probably related benign tumors. They are benign tumor per se; however, malignant transformation occurs in small number of patients.

Clinical and radiological characteristics of CN and MEST

	CN (21 pts)	MEST (5 pts)	Malignant MEST (3 pts)
Clinical			
Median Age(range)	55 years(39-79)	56 years(39-61)	36 years(14-36)
Female:Male	19:2	5:0	2:1
Hormonal Use or Disorder	4 / 21 (21%)	3 / 5 (60%)	0
Median Follow-up (Range)	2.5 years(.1-17)	2.5 years(1.7-3.8)	2.1 years(.7-6.8)
No evidence of disease	21 (100%)	5 (100%)	1 / 3 (33%)
Radiographic	(22 tumors)	(6 tumors)	(3 tumors)
Bosniak 3/4/solid	12/2/3	1/2/3	0/2/1
Median Size(range)	6.7 cm(2.3-18)	3.7 cm(1.9-14)	9.0 cm(2.9-10)
Extend to renal sinus (central)	15 / 18 (83%)	100%	100%
Extend into pelvis	5 / 18	0%	0%
Dx suspected by radiologist	8 / 19 (42%)	0 / 6 (0%)	0/3(0%)

670 Is Microalbuminuria Associated with Prostate and Bladder Cancer?

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Background: Proteinuria is associated with solid tumors. We attempted to associate proteinuria with prostate and bladder cancer by testing the urine for microalbuminuria.

Design: Urine samples from 1235 patients were tested for the presence of microalbuminuria using the Dade-Behring analyzer. These urine samples were then tested with a standard dipstick chemistry strip to rule out interference in microalbumin concentrations from glucosuria, acute inflammation, and hematuria. The results of microalbuminuria testing were compared with the findings in prostate needle biopsies and the results of urine cytology and fluorescence in situ hybridization (FISH) testing.

Results: Of the 1235 patients, 85 underwent prostate needle biopsies. The sensitivity and specificity of microalbuminuria for prostate cancer detection was 18.2% and 77.0%, respectively. Twenty-five of 1235 urine samples were evaluated by urine cytology and FISH for bladder cancer. Of these 25 urine samples, microalbuminuria had a sensitivity of 80% and a specificity of 40% for bladder cancer detection.

Conclusions: This preliminary study suggests that microalbuminuria is a sensitive marker for bladder cancer detection and relatively specific marker for prostate cancer detection.

671 Primary Renal Sarcomas: A Clinicopathologic Study

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Background: Primary renal sarcomas are extremely rare. Only very limited clinical and pathological information is available in the literature. This study reports the clinical and pathological features of this group of rare disease that we encountered during a 13-year period.

Design: Primary renal sarcomas were defined as sarcomas arising primarily in the kidney parenchyma, intrarenal vessels, renal pelvis and renal capsule, excluding those arising in the perinephric regions and involving the kidney secondarily. Pediatric sarcomas as well as angiomyolipomas, renal cell carcinomas with sarcomatoid differentiation were excluded in this study. Cases that satisfied the above criteria were retrieved and reviewed. Clinical information was obtained by IRB-approved patient contact and chart review. Additional immunohistochemical or fluorescent in situ hybridization chromosomal studies were performed when deemed appropriate to confirm the original diagnosis.

Results: Between 1992 and 2005, 21 out of 2342 (0.90%) radical or partial nephrectomies were confirmed to have primary renal sarcomas. The mean age at diagnosis was 53 years (range 14 to 82), with a male to female ratio of 1:1.2. Seven patients (32%) presented with metastasis. The mean tumor size was 10.7cm (range 2.5 to 17 cm). Renal vein invasion was present in 7/21 (33.3%). Adrenal involvement was present in 2 (9%) patients. Histological types included 8 (38.1%) leiomyosarcoma, 5 (23.8%) primitive neurectodermal tumor, 3 (14.3%) malignant fibrous histiocytoma, 3 (14.3%) sarcomas arising in cystic nephroma or mixed epithelial and stromal tumor, and 1 (5%) poorly differentiated sarcoma. All tumors were histologically high grade. No lymph node metastasis was identified. Follow-up was available in 81% (17/21) of patients with a mean follow-up of 2.7 years (range 0.2- 9.6 years). 8 (47.1%) patients were never rendered disease free with surgery, 3 (17.6%) have no evidence of disease at 3.9 years (mean) after surgery, and 6 (35.3%) had systemic recurrence with a mean time to recurrence of 5.9 years. Sites of metastasis included lung (33.3%), bone (21.4%), liver (20%), subcutis (14.3%) and brain (7.1%). 10/17 patients succumbed to cancer-specific death, with a mean of 2.24 years in survival (range 0.3 to 7.1 years). The 5-year survival is 45.7%. The prognosis did not correlate with the histologic types.

Conclusions: Primary renal sarcomas are a rare malignancy and account for < 1% of all renal neoplasms. The most common type is leiomyosarcoma. These tumors often have a poor prognosis.

672 Xanthogranulomatous Epididymitis: Identification of Bacterial Agents Using a PCR Method

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Background: Xanthogranulomatous inflammation is an uncommon benign process, characterized by tissue destruction and accumulation of abundant foamy macrophages mixed with lymphoplasma cells. Of those cases in genitourinary tract, especially xanthogranulomatous epididymitis has been seldom reported. We identified the pathogen in three cases of xanthogranulomatous epididymitis through a PCR method using 16s rRNA primers.

Design: We made 3 different primers with which we could amplify 16s rRNA of bacterial agents. DNA was extracted from formalin-fixed and paraffin-embedded tissue and amplified by PCR using 16s rRNA primers. Then the PCR products were sequenced and compared with the public database of NCBI using BLAST algorithm. After selecting common final candidates from those sequences, we performed PCR using another pairs of pathogen-specific primers.

Results: E. coli and Shigella were the common candidates from the 16s rRNA sequences. We confirmed that all those cases contained E. coli gene after further PCR amplification using E. coli- and Shigella-specific primers.

Conclusions: These results indicate that E. coli was the pathogenic agent in newly reported three cases of xanthogranulomatous epididymitis. PCR method using 16s rRNA primers can be useful in identifying pathogens of microbial culture-negative cases.

673 Proteomic Analysis of Protein Expression in Prostate Cancer

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Background: Few studies have investigated protein expression in prostatic cancer by proteomics using 2-dimensional gel electrophoresis (2-DE) and mass spectrometry. Conventional harvesting of tissue blocks for proteomic analysis has been used in previous studies and has the disadvantage that it is then difficult to distinguish true overexpression from overrepresentation of a tissue compartment such as fibromuscular stroma. Here, we have applied a novel technique for cytological harvesting of epithelial cells for proteomics.

Design: Cells were collected from 29 peripheral zone tumors and from benign tissue of the same zone by scraping cut surfaces of radical prostatectomy specimens. Samples were suspended in a medium with protease inhibitors and prepared for 2-DE. Gels were analyzed and protein spots that differed quantitatively between tumor and benign tissue were identified via mass spectrometric fingerprinting of tryptic fragments and tandem mass spectrometry sequence analysis.

Results: In total, 63 spots differed between cancer and benign samples ($p<0.01$), 56 were overexpressed (more than 1.5 fold) in cancer and 7 underexpressed (less than 0.6 fold). Among overexpressed proteins were transcription factors (nucleoside diphosphate kinase 1) and enzymes involved in gene silencing (chromobox protein), protein synthesis (39S ribosomal protein L12, BiP protein, protein disulfide isomerase), degradation (cytosol aminopeptidase, endopeptidase Clp, inorganic pyrophosphatase) and energy metabolism (acyl-CoA dehydrogenase, isocitrate dehydrogenase, NADH-ubiquinone oxidoreductase, pyruvate dehydrogenase), heat-shock proteins (60 and 70 kDa), structural proteins (cytokeratins) and membrane proteins (stomatin-like protein 2).

Conclusions: The protein profile of prostate cancer differs from that of benign tissue. Several potential target proteins for detection or for evaluation of prognosis in prostate cancer were identified.

674 Expression of Multiple Survival-Associated Markers in Prostate Cancer with Perineural Invasion

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Background: We previously suggested that a survival strategy might be critical to the development of perineural invasion (PNI) in prostate cancer (PCa). However, the regulatory pathway for an enhanced survival in PNI remains a mystery. In this regard, a panel of survival-associated markers was examined for their clinicopathologic significance in PCa with PNI.

Design: 1210 patients who underwent radical prostratectomy at Baylor College of Medicine affiliated hospitals were enrolled for this study. All specimens were fixed in formalin and then cut into wholemount serial tissue sections, which were subsequently embedded in paraffin to produce wholemount blocks. Wholemount H&E slides were examined for PNI with 226 cases selected for constructing 2mm PNI tissue microarray. The PNI array slides were immunostained with a large panel of antibodies (NFκB, cyclin-D1, cyclin-E, GSK-3, pim-2, FKHR, P-FKHR, Akt, C-MYC, P27, β-catenin, androgen receptor, and Ki-67).

Results: C-MYC was correlated with clinical stages (r^2=-0.223, p=0.024), lymph node metastasis (r^2=-0.248, p=0.014), and extracapsular extension (ECE) (r^2=-0.217, p=0.029) while androgen receptor correlated with Gleason score (GS) (r^2=-0.273, p=0.0022) and seminal vesicle invasion (SVI) (r^2=-0.212, p=0.0184). β-catenin correlated with ECE (r^2=-0.251, p=0.0105); NFκB, P-FKHR correlated with GS (r^2=0.166, p=0.0468; r^2=-0.257, p=0.010, respectively); and Pim-2, P27 and GSK-3 correlated with SVI (r^2=0.194, p=0.035; r^2=-0.214, p=0.022; r^2=-0.199, p=0.0314, respectively). Akt, cyclin-D1, cyclin-E, FKHR, Ki-67 did not correlate with any clinicopathological variables. NFκB, Ki-67, cyclin-D1, cyclin-E, GSK-3 were all associated with biochemical recurrence by univariate analysis. C-MYC and cyclin-E were the independent predictors of biochemical recurrence.

Conclusions: Our data demonstrated that many biomarkers were correlated with prognostic factors and/or associated with disease outcome in PCa with PNI while some were not at all. The up/down-regulated expression of these factors might reflect a complicated regulatory system that uniquely facilitates PNI.

675 High-Level Cyclin D1 Is Associated with High Gleason Grade and Predicts Worse Biochemical-Free Survival in Prostate Cancer

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Background: Cyclin D1 is an important regulator of the G1 restriction point of the cell cycle and has been implicated in disease progression and prognosis of human malignancies. However, its prognostic significance has not been well established in human prostate cancer (PCa).

Design: We used 640 PCa cases with radical prostatectomy to build tissue microarrays. Normal prostate tissue and index tumor were cored in triplicate (0.6 mm). Slides were

immunostained with an antibody to cyclin D1 and then digitized using a slide scanner. Spearman test was used for the correlations between cyclin D1 expression and clinicopathological variables and biological markers (Ki-67, p-Akt, pim-2, p-FKHR, Skp-2, C-MYC, and apoptotic index) that were available in the same tissue microarray database. Kaplan-Meier analysis and Cox proportional hazard regression were used to test the prognostic value of cyclin D1.

Results: Cyclin D1 was predominately expressed in nuclei of benign and cancerous epithelia of the prostate. Cyclin D1 expression index was higher in PCa compared to normal prostate (2.05±2.09 *vs.* 0.51± 1.14). Increased nuclear cyclin D1 in PCa was associated with high preoperative PSA (rho=0.097, p=0.0415), and high Gleason scores (rho=0.149, p=0.0016). High level expression of nuclear cyclin D1 was correlated with increased expression of Ki-67(rho=0.149, p=0.0016), p-Akt (rho=0.158, p=0.0011), pim-2(rho=0.323, p=0.0000), p-FKHR (rho=0.212, p=0.0001), Skp-2 (rho=0.128, p=0.0218), C-MYC(rho=0.251, p=0.0000) and inversely correlated with apoptotic index (rho=-0.127, p=0.0123). High level of nuclear cyclin D1 was associated with early biochemical recurrence in PCa. Significantly, cyclin D1 was an independent predictor of biochemical recurrence in PCa (HR 5.979 (1.937-18.542)), P=0.0019).

Conclusions: Our data suggested that expression of cyclin D1 is associated with preoperative PSA and Gleason scores as well as many biological markers that involve proliferation and survival in PCa. These findings provide evidence that increased cyclin D1 promotes proliferation and disease progression. Cyclin D1 might become a potential biomarker for the prediction of biochemical recurrence in PCa.

676 Expression of Proteasome Activator 28γ in Human Prostate Cancer

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Background: Proteasome activator (PA) 28γ is a proteasome activator that stimulates the hydrolysis of peptides. Increasing evidence has shown that PA28γ functions as an anti-apoptotic factor. Its expression and significance in human prostate cancer (PCa) has not been documented.

Design: 640 PCa cases with radical prostatectomy were collected to build tissue microarrays. Normal prostate tissue and index tumor were cored in triplicate (0.6 mm). Slides were immunostained with an antibody to PA28γ and then digitized. Spearman test was performed for the correlations between PA28γ expression and clinicopathological variables (preoperative PSA, clinical stages, lymph node metastasis, extracapsular extension, seminal vesicle invasion, surgical margin, and Gleason score) and several biological markers (P27, FKHR, p-FKHR, pim-2, C-MYC, Ki-67, apoptotic rate) that were available in the same tissue microarray master database. Kaplan-Meier analysis and Cox proportional hazard regression were used to test the prognostic value of PA28γ.

Results: Nuclear PA28γ was decreased while cytoplamic PA28γ was increased in PCa compared to normal prostate (6.54±1.73 *vs.* 8.8±0.75; 2.16±1.72 *vs.* 5.28±1.99). High level expression of PA28γ was correlated with increased expression of pim-2 (rho=0.218, p=0.0000), FKHR (rho=0.221, p=0.0001) p-FKHR (rho=0.154, p=0.0035), C-MYC (rho=0.203, p=0.0001), P27 (rho=0.132, p=0.0063) and p-Akt (rho=0.229, p=0.0000). but not with Ki-67 and apoptotic index. Expression of PA28γ was not associated with clinicopathological factors or biochemical recurrence in PCa.

Conclusions: Our data suggest that increased cytoplasmic expression of PA28γ might involve the activation of prosurvival mechanism since PA28γ in cytosol was upregulated in PCa and was also correlated with overexpression of survival-associated markers as shown in our data.

677 Expression of Human Kidney Injury Molecule-1 (hKIM-1) in Renal Cell Neoplasms

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Background: Human Kidney Injury Molecule-1 (hKIM-1) is a type I cell membrane glycoprotein, which has been recently shown to be expressed in injured renal proximal tubules, in a high percentage of renal cell carcinoma (RCC), and absent in nearly all 485 non-renal tumors (Han WK et al. J Am Soc Nephrol 2005;16:1126-1134). The current study attempts to confirm and extend this finding in a large series of defined subtypes of renal cell tumors and metastatic renal cell carcinoma.

Design: Tissue microarray (TMA) sections containing 184 renal cell tumors (73 clear cell RCC, 30 papillary RCC, 16 chromophobe RCC, 15 oncocytoma, and 50 metastatic RCC) were included in this study. One 1.5mm tissue core or two 1.0mm punch tissue cores were obtained from each case. TMA sections were incubated with AKG7 anti-hKIM-1 monoclonal antibody using an EnVision-HRP kit (Dako). The scoring system was classified into negative - no staining; 1+ - < any tumor cells with positive staining -10% of tumor staining; 2+ - 11-49% of tumor staining; and 3+ - >50% of tumor staining.

Results: The results demonstrated that granular cytoplasmic and membranous staining pattern for hKIM-1 was observed in 54 of 73 (74%) conventional (clear cell) RCCs, 28 of 30 (93%) papillary RCCs, 1 of 16 (6%) chromophobe RCCs, and 2 of 15 (13%) oncocytomas. Among the 73 cases of clear cell RCC, positivity for hKIM-1 protein was seen in 36 of 49 (73%) and 18 of 24 (75%) cases with Furhman nuclear grade I/II and III, respectively. Similar staining pattern was observed in 39 of 50 (78%) metastatic RCCs. Strong positivity (3+) was seen in 37% of clear cell RCC, 50% papillary RCC, and 38% metastatic RCC, respectively.

Conclusions: These data indicate that hKIM-1 is a highly sensitive and specific marker for papillary RCC and has a high specificity and relatively good sensitivity for metastatic RCC, although it has little value in differentiating chromophobe RCC from oncocytoma.

678 Expression of S-100 Protein in Renal Cell Neoplasms

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Background: Polyclonal antibody to S-100 protein has been routinely applied for initial screening of various types of tumors, including melanocytic tumors and neurogenic tumors. S-100 protein has been shown to have a broad distribution in human tissues, including renal tubules. The potential utility of antibody to S-100 protein in renal cell neoplasms has not been extensively investigated.

Design: Using an EnVision-HRP kit (Dako), we evaluated the diagnostic value of S-100 protein on tissue microarray sections from 175 cases of renal epithelial neoplasm (145 primary renal neoplasms and 30 metastatic renal cell carcinomas) and 24 non-neoplastic renal tissues. Immunohistochemical stains for pancytokeratin, HMB-45 and Mart-1 were also performed. The staining intensity was graded into weak and strong. The distribution was recorded into negative (no staining), 1+(<25%), 2+(26-50%), and 3+ (>50%). Western blot using the same antibody (anti-S-100 protein) was performed on 4 cases of renal cell neoplasm.

Results: The results demonstrated that nuclear and cytoplasmic staining pattern for S-100 protein was observed in 56 of 81 (69%) conventional (clear cell) RCCs, 10 of 33 (30%) papillary RCCs, 1 of 16 (6%) chromophobe RCCs, and 13 of 15 (87%) oncocytomas. Among the 81 cases of clear cell RCC, positivity for S-100 protein was seen in 41 of 58 (71%) and 15 of 23 (65%) cases with Furhman nuclear grade I/II and III, respectively. Focal immunostaining was present in 22 of 24 (92%) normal renal tubules. Similar staining pattern was observed in 21 of 30 (70%) metastatic RCCs. Western blotting demonstrated the S-100 protein expression in both renal cell neoplasm and normal renal tissue. Importantly, 20% of each set of clear cell RCC (16/81) and metastatic RCC (6/30) revealed an immunostaining profile of pancytokeratin (-)/S-100 protein (+).

Conclusions: These data indicate that caution should be taken in interpreting an unknown primary with S-100 positivity and cytokeratin negativity. In addition, it suggests that S-100 has a diagnostic value in differentiating oncocytoma from chromophobe RCC.

679 Expression of S-100A4 Protein in Renal Cell Carcinoma Associated with High Tumor Grade and Metastasis

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Background: S-100A4 is a calcium-binding protein and has been implicated in tumor metastasis and carcinogenesis of the pancreas, breast and thyroid. Renal cell carcinoma (RCC) is notorious for distant metastasis years after the initial diagnosis; and there are not reliable molecular markers for prediction of a metastasis. Expression of S-100A4 in conventional (clear cell type) RCC (CRCC) which accounts for 70% of total cases, has not been explored. In this study, we attempt to evaluate the correlation of expression of S-100A4 protein in CRCC with tumor differentiation and metastasis.

Design: Using an EnVision-HRP kit (Dako), we evaluated expression of S-100A4 (1:100 dilution; LabVision) on tissue microarray sections from 94 cases of CRCC (58 primary CRCCs and 36 metastatic CRCCs) and 20 non-neoplastic renal tissues. The study cases were divided into four groups, including Group one (G1): 36 metastatic CRCCs, Group two (G2): 27 CRCCs with history of metastasis, Group three (G3): 31 CRCCs without history of metastasis, and Group four (G4): 20 cases of non-neoplastic renal tissue. The staining intensity was graded into weak and strong. The distribution was recorded into negative (no staining), 1+ (less than 25%), 2+ (26-50%), and 3+ (>50%). Western blot using the same antibody (anti-S-100A4) was performed on 2 cases of CRCC.

Results: The results demonstrated that cytoplasmic and /or nuclear staining pattern for S-100A4 was observed in 31 of 36 (86%), 25 of 27 (92%), and 7 of 31 (23%) of G1, G2, and G3, respectively. Among the 58 cases of primary CRCC, positivity for S-100A4 was seen in 13 of 36 (36%) and 19 of 22 (86%) cases with Furhman nuclear grade I/II and III, respectively. In Group four, focal immunostaining (1+) in renal distal tubules was present in 6 of 20 (30%) cases of G4. Statistical significance (p=0.001) was observed in 1) G1 versus G3; 2) G2 versus G3; and 3) Furhman nuclear grade I/II versus grade III. In contrast, no statistical significance (p=0.69) was noted in between G1 versus G2. Western blotting demonstrated the overexpression of S-100A4 in both cases of CRCC, with an average of 12 fold increase over the corresponding normal kidney.

Conclusions: These data suggest that the expression of S-100A4 in clear cell RCC correlates with tumor differentiation and may serve as a biomarker in predicting aggressiveness of RCC and clinical outcome.

680 Loss Expression of VHL Protein in Metastatic Renal Cell Carcinoma Associated with Overexpression of p53

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Background: Inactivation of von Hippel-Lindau (VHL) tumor suppressor gene by mutation or hypermethylation has been implicated in initiation and progression of clear cell renal cell carcinoma (CRCC). p53 has also been shown to be a poor prognostic indicator for CRCC. Data on the regulatory role of VHL gene product (pVHL) on p53 expression are discordant and findings on subcellular localization of pVHL in CRCC are inconsistent. Immunodetection of both pVHL and p53 in CRCC has not been well documented. In this study, we use a well-characterized anti pVHL antibody to evaluate the expression of pVHL in primary and metastatic CRCCs and to assess the association with p53 expression.

Design: Using an EnVision-HRP kit (Dako), we evaluated the expression of pVHL [1:50 dilution; VHL (FL-181); Santa Cruz Biotechnology] and p53 on tissue microarray sections from 83 cases of RCC (47 primary CRCCs and 36 metastatic CRCCs) and 20 non-neoplastic renal tissues. The staining intensity was graded into weak and strong. The distribution was recorded into negative (no staining), 1+(less than 25%), 2+(26-

50%), and 3+ (>50%). Fisher's Exact text was performed to evaluate the statistical significance of the immunostaining profile between primary and metastatic RCCs.

Results: The results demonstrated that cytoplasmic and membranous staining pattern for pVHL was observed in 47 of 47 (100%) primary RCCs and 28 of 36 (78%) metastatic RCCs with a strong staining (3+) on the majority of cases. Strong p53 staining was observed in 10 of 36 (28%) metastatic RCCs, but not in primary RCCs and normal kidney. Seven of 36 (19%) metastatic RCCs showed an immunostaining profile of pVHL-/strong p53 +; and an inversed correlation (r) between two markers was 0.707 (p=0.0001, n=36). Strong and diffuse (3+) cytoplasmic/membranous staining for pVHL was present in 20/20 (100%) normal renal proximal and distal tubules. Statistical significance was observed between primary and metastatic RCCs as shown in Table 1.

MAKERS	PRIMARY RCC	METASTATIC RCC	FISHER'S EXACT TEXT
VHL +	47/47 (100%)	28/36 (78%)	P=0.001
VHL -	0/47 (0)	8/36 (22%)	P=0.001
STRONG p53 +	0/47 (0)	10/36 (28%)	P=0.001
VHL-/STRONG p53+	0/47 (0)	7/36 (19%)	P=0.002

Conclusions: Our preliminary data suggest that concurrent loss of pVHL expression and overexpression of p53 due to the compromised p53 pathway (if it is not mutation) may contribute to the promotion of a metastatic process in RCC.

681 Molecular and Immunohistochemical Analysis of the Prognostic Value of Cell-Cycle Regulators in Urothelial Neoplasms of the Bladder

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Background: Molecular alterations of tumor suppressor genes implicated in the G1 to S-phase cell cycle transition are very important molecular events in tumor pathology. Our main objective is to evaluate the prognostic and predictive value of molecular and immunohistochemical markers related to cell-cycle control, in terms of recurrence, progression and survival in urothelial neoplasms of the bladder (UNB).

Design: Clinical and pathological findings of 84 patients with UNB were assessed. Stage and grade distribution were as follow: 8pTa, 55pT1 and 21pT2-4; 15 G1, 43 G2 and 26 G3. Homozygous deletion (HD) and promoter methylation of p14ARF, p15INK4B, p16INK4A; LOH of the locus 9p21 (D9S157, D9S162, D9S942); p53 mutations; and immunohistochemical expression of p53, p16INK4A, p14ARF, p21WAF1, p27KIP1, pRb, Ki67, MDM2, cyclin D1 proteins were evaluated in relation to overall survival (OS), recurrence-free survival (RFS) and progression-free survival (PFS).

Results: In the univariate analysis, RFS was shorter in those cases with p14ARF (p=0.006), p15INK4B (p=0.003), p16INK4A (p=0.03) HD and Ki67 immunoreactivity index (IRI) (p=0.04); HD of the 9p21 locus genes remained as an independent prognostic factor for early UNB recurrence (p=0.006) whereas tumour stage (p=0.00001) and cyclin D1 IRI (p=0.049) were related to worse PFS in the multivariate analysis. Furthermore, IRI for Ki67 (p=0.002), cyclin D1 (p=0.06), p53 (p=0.00008), p16 (p=0.02), p27 (p=0.0005) MDM2 (p=0.01) and p53 mutations (p=0.03) were related to poor OS, and only the Ki67 and cyclin D1 IRI kept their independent value in the multivariate analysis.

Conclusions: 9p21 HD and cyclin D1 IRI constitute independent predictive factors for UNB recurrence and progression respectively. In addition, Ki67 and cyclin D1 IRI are independent prognostic factors for overall survival in UNB. These findings should be taken into considertaion in the pathological staging and clinical management of the UNB of the bladder. Supported with a grant of the European Association of Urologists and with a grant from the Fundacion Instituto Valenciano de Oncologia.

682 In Situ Adenocarcinoma of the Urinary Bladder (ISAB). Clinicopathological Features and Immunophenotype

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Background: In situ adenocarcinoma of the urinary bladder (ISAB) is a rare variant of carcinoma in situ (CIS) first described in 1980's. The clinical significance and histogenesis of ISAB remains uncertain since most reported cases are associated to urothelial carcinoma (UC) or small cell carcinoma (SCC) and its immunophenotype remains unsettled. Our aim is to illustrate clinicopathologic features and the immunophenotype of ISAB.

Design: Eleven patients with ISAB were collected. Papillary, cribriform, and flat were the architectural patterns of ISAB (AJSP 2001, 25:892-899). To study the histogenesis of ISAB and to determine its association with conventional CIS we have performed immunohistochemistry using 16 monoclonal antibodies including CDX-2, PTEN, p53, ki67-MIB1, p16, CKs 20, 7, 34ßE12, and AE1/AE3, cerb-B2 (HER2-neu), CD44v6, MUC6, MUC5A, MUC2, MUC1-core, and MUC1.

Results: ISAB had cribriform (n=1), flat (n=4), cribriform+papillary (n=1), flat+cribriform (n=2) or flat+cribiform+papillary (n=3) architecture. Four cases had conventional CIS; eight had UC stage Ta (n=1), T1 (n=1), T2 (n=4) or T3 (n=2) showing glandular differentiation (n=2), discohesive morphology (n=1) or micropapillary carcinoma (n=1). All were high grade UC (ISUP/WHO). None of ISAB was associated to adenocarcinoma, villous adenoma, or SCC. At immunohistochemistry, ISAB showed high ki-67 labeling index and p53 accumulation, high nuclear and cytoplasmic p16 expression and diffuse PTEN expression; a phenotype that also characterized conventional CIS. CDX-2 was present in 9 cases of ISAB ranging 5%-to-60% of cells. In addition, CK20 diffusely stained all ISAB cases; CK7 and 34ßE12 were focally present in 9 and 8 cases, respectively. All cases had c-erbB2 (range 1+ to 3+) and CKAE1/AE3 immunorreactivity. MUC5A (range 50%-75%) and MUC2 (20%-40%) were positive in all ISAB cases; MUC1 was focally present in 9 cases. CD44v6, MUC6 and MUC1- core were negative.

Conclusions: 1) ISAB is a distinctive rare variant of CIS showing a level of proliferation and p53, PTEN and p16 alterations similar to the observed in conventional CIS, 2) a phenotype of CK20+/CDX-2+/MUC5A+/MUC2+/cerbB2+/MUC-core - might be used to differentiate ISAB from conventional CIS, and 3) the ISAB immunorreactivity to CK 7 and 34ßE12 might suggest an urothelial origin for ISAB hence supporting a histogenetic link.

683 Tumor Suppressor Gene PTEN in Prostate Cancer: A Potential Therapeutic Target

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Background: Despite the initial response to androgen deprivation, most advanced prostate cancer (PCa) becomes hormone-refractory. Novel pharmacological and chemotherapy regimens are under investigation to improve survival and response. Taxotere is a microtubule depolymerization inhibitor impairing mitosis and cell proliferation. Granulocyte-macrophage colony stimulating factor (GM-CSF) can influence the recruitment and activation of dendritic cells and tumor-specific cytotoxic T lymphocytes. Thalidomide is an anti-inflammatory agent with antiangiogenic activity. The tumor suppressor gene PTEN negatively regulates cell growth and/or proliferation. Loss of PTEN protein occurs frequently in PCa and may be a useful independent prognostic marker.

Design: Using high-throughput tissue microarrays, we examined the expression of PTEN by immunohistochemistry in 157 Gleason score 7 (GS7) PCa, 49 concomitantly metastatic PCa (Met), and 44 clinically advanced PCa treated with neoadjuvant therapy: 17 patients had received GM-CSF and thalidomide (GM/thal), and 27 received taxotere (Tax) prior to tissue harvest. P-values were obtained using Chi-squared and Fisher's Exact tests, as appropriate.

Results: PCa were initially divided into positive, mixed and negative. PTEN protein expression was lost in 47.8% of GS7 and 44.9% of Met, and reduced in 42.7% of GS7 and 51.0% of Met (Tables 1). PTEN expression was significantly different between GS7 and Tax (p=0.0043) and Met and GM/thal (p=0.0504). In contrast to GS7 and Met, most GM/thal and Tax PCa had positive and mixed PTEN expression. The cases were subsequently divided into those showing positive staining (positive + mixed) and negative, and the results were compared. PTEN expression was significantly higher after taxotere (GS7 vs Tax, p=0.0040; Met vs Tax, p= 0.0213) and higher but not statistically significant after GM/thal treatment (GS7 vs GM/thal, p=0.06; Met vs GM/thal, p=0.13).

Conclusions: PTEN expression was increased by neoadjuvant therapy suggesting that treatment may either preferentially be effective on and kill PTEN negative cells, or may restore its expression. PTEN functional status may have important therapeutic and prognostic implications.

Expression of PTEN in different PCa groups

PTEN	GS7	Met	GM-CSF/thal	Tax
Positive	15 (9.5%)	2 (4.1%)	4 (23.5%)	1 (3.7%)
Mixed	67 (42.7%)	25 (51%)	9 (52.9%)	21 (77.8%)
Negative	75 (47.8%)	22 (44.9%)	4 (23.5%)	5 18.5%)

684 Pathologic Features of Single-Nodule Prostatic Carcinoma

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Background: Prostate cancer (PCa) is considered to be a multifocal and diffuse process, and no data is available in the literature regarding the incidence and characteristic features of single-nodule PCa.

Design: For 130 consecutive prostate glands obtained at radical prostatectomy (RP) for PCa from August 2004 to July 2005 and reviewed by a single pathologist, we mapped the tumor outline, determined the number (#) of separate foci/nodules of cancer and the extent of high grade prostatic intraepithelial neoplasia (HGPIN). When a single nodule of PCa was identified, the tumor volume (TV), zone of origin, Gleason score (GS), histologic features, extraprostatic extension (EPE), and seminal vesicles (SV) invasion were also recorded.

Results: The age and preoperative PSA (iPSA) of the general patients population ranged from 38 to 74 years (mean 58.7 years) and 1.5 to 29.0 mg/ml (mean 6.1 mg/ml), respectively. A single-nodule PCa was identified in the peripheral zone (PZ) of 17 (13.1%) RP. Two and 3 distinct PCa foci/nodules were found in 35 (26.9%) and 33 (25.4%) cases, respectively. The # of PCa foci ranged from 4 to >10 in the remaining RP. The extent of HGPIN (focal, multifocal, absent) in the general population correlated with the # of PCa foci (Person correlation 0.646; p<0.0001). The age and iPSA of the single-nodule PCa patients ranged from 43 to 67 years (mean 57.9 years) and 0.9 to 12.0 mg/ml (mean 5.85 mg/ml), respectively and was not statistically different from patients with ≥2 PCa foci. In 4 (23.5%) cases the single-nodule PCa involved both lobes of the gland, in 6 (35.3%) the left and in 7 (41.2%) the right lobe. The GS was 6 in 7 (41.2%), 7 in 8 (47.0%), and 8 in 2 (11.8%) cases. The TV ranged from 2 to 400 mm^2 (mean 104.2 mm^2), with low (<0.5 cc), medium (0.5-2.0cc) and extensive (>2.0cc) TV in 8, 8 and 1 case, respectively. The GS of the single-nodule PCa correlated with the TV (Person correlation 0.813; p<0.0001). EPE was focal in 2 (11.8%) and established in 1 (5.9%) case. SV were negative in all cases. One single-nodule was a colloid (mucinous) PCa, 2 had focal mucinous features, and 1 was atrophic cancer. HGPIN was focal in 12 (70.6%), multifocal in 2 (11.8%) and absent in 3 (17.6%) cases.

Conclusions: Single-nodule PCa represents 13% of cases, predominantly involves one lobe of the gland and is associated with focal rather than multifocal HGPIN. These tumors may evolve from a single clone and be biologically different from multifocal PCa.

685 Alternative Target Genes Contiguous to RB1 and p53 Involved in Clonal Expansion of In Situ Urothelial Neoplasia

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Background: Identification of chromosomal regions containing genes involved in the development of occult in situ neoplastic lesions may provide valuable clues to the incipient events of carcinogenesis.

Design: For High-resolution mapping with SNPs, the sequence-based maps spanning approximately 5 to 27 Mb corresponding to microsatellite -defined deleted regions in 13q14 and 17p13 respectively were assembled. The integrated gene and SNP maps of 13q14 and 17p13 were used to select 661 SNPs within the 27-Mb segment around the chromosome 13q14 region and 960 SNPs within the 5-Mb segment around the chromosome 17p13 region. SNPs were genotyped by pyrosequencing. Mapping was performed on whole-organ histologic maps of eight cystectomy specimens with high-grade invasive urothelial carcinoma.

Results: We identified clusters of discontinuous loss mapping to 13q14 and 17p13 containing model suppressor genes RB1 and p53. The high density of SNP markers disclosed that loses represented clusters of discontinuous segments ranging in size from 0.1 to 4.6 Mb separated by non-deleted regions. By comparing the patterns of allelic losses from eight cystectomy specimens, we identified minimal deleted regions associated with clonal expansion of in situ neoplasia. The minimal deleted regions near RB1 included its flanking segments and contained several positional candidate genes such as ITM2B and CHC1L. Since the loss of just one RB1 allele was also an early event associated with clonal expansion, the P2RY5 gene located within intron 17 of RB1 was considered a third alternative candidate gene. The minimal deleted region near p53 was located approximately 0.5 Mb telomerically for p53 and contained DLG4 as a positional candidate gene involved in clonal expansion of in situ neoplasia.

Conclusions: The combination of whole-organ histologic maps showing the distribution of neoplastic in situ lesion with high-resolution genotyping using SNPs identified chromosomal segments associated with clonal expansion of in situ neoplasia around RB1 and p53. Mapping data provided evidence for the involvement of alternative target genes near RB1 and p53 whose inactivation precedes the loss of tumor suppressors during tumor development and may drive the initial clonal expansion of in situ neoplasia.

686 Loss of Heterozygosity (LOH) Analysis of Muscle Invasive and Metastatic Urothelial Carcinoma (UC); Can We Predict Progression/Metastasis?

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Background: In this study, we examine the genotypic profile of metastatic urothelial carcinoma (UC), its relationship to the corresponding primary muscle-invasive tumor and to morphologically normal urothelium (NU) and carcinoma in situ (CIS) in the individual patient. Our aim is to determine if allelic LOH analysis, using a UC-selective panel of primers, can be used to predict progression/metastasis.

Design: 9 cases of metastatic UC were examined. Tissue samples from primary tumor (PT), metastatic tumor (MT), NU, CIS (if present) and normal non-urothelial tissue were microdissected from formalin-fixed, paraffin-embedded tissue. The control group consisted of 5 cases of muscle-invasive, non-metastatic UC. All UCs were ISUP high-grade. DNA was extracted, PCR amplified, and genotyped with a panel of 20 primers for 1q, 3p, 10q, 11p, 7p, 9p21, 13q, 17p 17q, which target p21, FHIT, pTEN, Kai1, EGFR, p16, RB, P53 and NM23, respectively. Fractional allelic loss (FAL) was calculated for each tissue site. Likelihood ratios (LR) were calculated to examine the relatedness of the genotypic changes at the various sites. The FALs for PTs of the study and control groups were compared.

Results: A high rate of genetic instability (FAL) was present in NU in both study and control groups. The mean FALs for NU, CIS, PT and MT were 0.82, 0.75, 0.55, and 0.88 respectively, in the study group. Mean FALs in NU and PT in the control group were 0.84 and 0.71, respectively. The PT FALs were significantly higher in the control group vs. the study group ($p<0.05$). Likelihood ratios showed a significant likelihood of a clonal origin for PT and MT in 4 of 9 cases, for PT and NU in 3 of 6 cases and for PT and CIS in only 1 of 6 cases ($p<0.05\%$). Only 1 of 9 cases showed a significant genotypic association between NU, PT, CIS and MT.

Conclusions: FAL is high in NU (in both groups), CIS and MT, but is paradoxically lower in PT in patients with lymph node metastases vs. tumors confined to the bladder. This suggests a high level of genetic instability in pre-invasive lesions. Increasing LOH in high grade invasive UC may not be predictive of disease progression. No significant difference in LOH at any of the specific genetic loci examined was seen between the two groups. Mutational dissimilarity amongst NU, PT, CIS and MT in the majority of cases suggests either separate tumor clones or clonality with early divergence at various tumor sites in individual patients.

687 A Comparison of Papillary and Flat Urothelial Carcinoma Using LOH and MSI Analysis

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Background: Clinically, urothelial carcinomas (UC) occur as either superficial, papillary, recurrent tumors that progress slowly or as flat invasive lesions that behave aggressively. It is proposed that two distinct genetic pathways underlie this clinicopathologic phenomenon. In this study we examine differences in allelic LOH between papillary (P) and non-papillary (NP) UC using a panel of markers targeting genes involved in urothelial carcinogenesis and compare rates of microsatellite instability (MSI), which has been suggested to have prognostic significance, between the 2 groups.

Design: Neoplastic and normal urothelium were microdissected from formalin-fixed, paraffin-embedded tissue. DNA was extracted, PCR amplified, and genotyped with a panel of 20 primers for chromosome regions 1q, 3p, 10q, 11p, 7p, 9p21, 13q, 17p and 17q targeting p21, FHIT, pTEN, Kai1, EGFR, p16, RB, P53 and NM23, respectively. The MSI panel consisted of five loci (BAT25, BAT26, D2S123, D56346 and D17S250). Mean fractional allelic loss (FAL) was calculated for P-UC and NP-UC and for associated CIS. Likelihood ratios (LR) were calculated to examine the relatedness of UC and CIS for each group. MSI at one locus was regarded as MSI-low (MSI-L) and at ≥ 2 loci as MSI-high (MSH-H).

Results: Retrospective review identified 7 cases of P-UC (lamina propria invasion (LPI): n=4), non-invasive (n=3); 6 had concurrent carcinoma in situ (CIS). 9 cases of NP-UC were examined (LPI: n=2; muscle invasion (MI): n=7); 6 were associated with CIS. All were high grade. Mean FAL was higher in P-UC than in NP-UC (0.78 vs. 0.66) ($p>0.05$). Mean FAL was similar in CIS associated with either P-UC or NP-UC (0.85 vs. 0.87, respectively). LRs indicate a clonal origin for CIS and UC in only 2 cases of P-UC and in 1 case of NP-UC. MSI-H and MSI-L were present in 1/7 and 3/7 NP-UC and in 2/7 and 4/7 P-UC, respectively. 4 cases in each group showed MSI (H or L) in areas of CIS.

Conclusions: FAL was higher in P-UC than in NP-UC, but did not reach statistical significance. The FAL of CIS associated with either tumor type was higher than the FALs of P-UC or NP-UC . None of the individual markers evaluated showed a significant difference in fequency of LOH between the 2 groups. This panel of markers therefore fails to define distinct genotypic differences between P-UC vs. NP-UC or between CIS associated with either tumor type. MSI was more frequent in P-UC vs. NP-UC and was present in 57% of CIS foci.

688 Defining Prostate Cancer Progression by Molecular Profiling of Laser Capture Micro Dissected Prostate tissues

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Background: Expression profiling studies of prostate cancer (PCA) have traditionally used grossly dissected samples due to need of microgram quantities of RNA. However analysis of such material may yield biased results because of inherent heterogeneous cell populations. Gross dissection also precludes the profiling of entities requiring pure cell populations or small lesions like prostate intraepithelial neoplasia (PIN). We utilized laser capture microdissection (LCM) to profile a broad range of pure cell subpopulations from benign and tumor samples to gain insight into PCA progression.

Design: LCM was utilized to isolate approximately 10,000 cells from 101 samples comprising of epithelial and stromal cells from benign prostate (n=23), prostate hyperplasia (BPH) (n=11), PIN (n=13), Gleason pattern 3, 4 and 5 PCA (n=30) and metastatic PCA (n=19). Isolated RNA was subjected to Whole Transcriptome Amplification. For hybridization, 20K element cDNA microarrays were utilized and the resulting data set was subjected to unsupervised hierarchical clustering, prediction analysis for microarrays or uploaded directly into the Oncomine V3 database (www.oncomine.org) for analysis.

Results: We identified a "gene signature" whose expression correlated with progression from benign to PIN to PCA to MET. In this progression signature 851 genes were significantly over-expressed ($Q<0.05$), including SEPT11, CKS2, AMACR, TOP2A and MYC, and 1,019 genes were significantly under-expressed ($Q<0.05$). Several of highly ranked under-expressed genes, including MME and AZGP1, were masked by stromal transcripts in previous profiling studies utilizing gross samples. Genes previously identified by expression profiling as being over-expressed in BPH compared to benign prostate tissue, including NELL2, BMP5 and BMP8, were exclusively over-expressed in BPH stroma and not in BPH epithelium. PIN demonstrated similar expression pattern to PCA. Finally, we identified a "Gleason signature" of genes differentially expressed between low grade Gleason (pattern 3) PCA were differentially expressed compared to high grade Gleason (pattern 4 and 5) PCA. Gene set enrichment results demonstrated that high Gleason grade epithelium shows expression pattern consistent with androgen resistance.

Conclusions: LCM provides a unique set of pure cell populations which can be effectively utilized for molecular expression studies. Using this approach we define gene expression signatures which delineate prostate cancer progression and Gleason patterns.

689 Stathmin Is over Expressed in Metastatic Prostate Cancer: Implications in Prostate Cancer Progression

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Background: Stathmin, an intracellular phosphoprotein, plays a critical role in cell proliferation, differentiation and cell motility by promoting microtubule depolymerization and in both tumor growth and invasion. A wide variety of cancers including breast and ovarian have been shown to over express stathmin. We investigated the potential role of stathmin in prostate cancer (PCA) progression by combined cDNA expression and tissue micro array (TMA) protein analysis approach.

Design: Laser capture microscope was utilized to obtain pure population of cells of 101 samples comprising of benign, PIN, localized and metastatic PCA. Isolated RNA was subjected to Whole Transcriptome Amplification. For hybridization, 20K element cDNA micro arrays were utilized and resulting data set was subjected to unsupervised hierarchical clustering, prediction analysis for micro arrays and/or uploaded directly into the Oncomine V3 database (www.oncomine.org), a bioinformatics platform for analysis. Protein expression was correlated using immunoblot whole tissue lysates and stathmin specific mouse monoclonal antibody (BD Biosciences). Three TMAs containing 1146 cores from 80 benign, 34 PIN, 75 localized and 30 hormone refractory metatstatic PCA were evaluated for protein expression. Combined staining intensity on the scale of 1(negative), 2 (weak), 3 (moderate), 4 (strong); and the percentage cells staining on scale of 1 to 100% were analyzed for comparison.

Results: We identified a "gene signature" which correlates with PCA progression, having 851 over-expressed ($Q<0.05$) and 1019 under-expressed genes ($Q<0.05$).

Stathmin was one of the top 50 significantly over-expressed genes. Meta-analysis using Oncomine revealed stathmin to be overexpressed in localized PCA compared to benign (P=0.005, Dhanasekaran et al), and in metastastic PCA compared to localized PCA (P<0.0001, LaTulippe et al). By Western Blot, stathmin protein expression was increased in metatstatic compared to localized PCA and normal prostate tissues. Immunohistochemical staining demonstrated a significant progressive step-up in stathmin expression across benign, PIN, PCA and Metastastic PCA (Kruskal-Wallis P<0.0001).

Conclusions: Stathmin expression increases during the natural history of PCA progression, in particular with androgen-independent state and metastasis. It underscores the importance of investigating the deregulation of stathmin function and its role as a potential therapeutic target of cancer therapy.

690 Improved Filter Monolayer Method for Urine Sediment Detection of Urothelial Carcinoma by FISH

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Background: FISH of voided urine sediment is a sensitive and specific test for detecting bladder cancer; slide preparation time for the cytospin method is lengthy. We present a more cost effective and rapid alternative procedure to the cytospin method, without compromising accuracy.

Design: 451 patients with cytology and/or FISH analyses were followed with cystoscopy and/or bladder biopsies. FISH analysis was performed on all 451 cases using fluorescence-labeled probes to the centromeres of chromosomes 3, 7, and 17, and band 9p21 (p16/CDKN2A gene) (UroVysion). 11 cases had insufficient cells; cytology was performed in 440 cases. Urine was drawn up into the syringe, filtered through a Millipore filter, and the cell filtrate was subsequently transferred to a glass slide. Interlaboratory proficiency testing was performed on 15 samples. The outside laboratory used the cytospin method to prepare slides (centrifugation to concentrate the cells into a small cell suspension). A portion of this was then dropped onto the slide. This is laborious and creates difficulty in controlling the cellularity of the slide. Literature review was used to compare the cost, preparation time, specificity and sensitivity for bladder cancer detection of the cytospin method with our monolayer filter method results.

Results: A total of 75 of 451 patients (16.6%) had follow-up bladder biopsies, and 69 of these (92%) had urothelial carcinoma. The sensitivity for bladder cancer detection was significantly higher for FISH than for urine cytology (85.5% [59/69] for FISH vs. 64.6% [42/65] for urine cytology; [p<0.05]). The specificity was equivalent for FISH and urine cytology (98.9% [367/371] vs. 98.7% (370/375) for cytology). There was no difference in adequacy rate, sensitivity, and specificity between the filter method and the cytospin method. The interlaboratory concordance rate of FISH was 100% on 15 samples tested. Slide preparation time was 3 minutes per slide for our method compared to approximately 20 minutes per slide for the cytospin method. Slide preparation was $2.50 per slide for the filter method, and $5.00 per slide for the cytospin method. The filter method did not require multiple centrifugation and decantation steps or investment in new equipment; as did the cytospin method.

Conclusions: The improved filter monolayer method for FISH was significantly faster and less expensive than published results with the conventional cytospin method for urine cytology, with equivalent sensitivity, specificity, and adequacy.

691 The Spectrum of Morphologic Features of Renal Tumors in Hereditary Leiomyomatosis and Renal Cell Carcinoma (HLRCC) Syndrome: Our Experience with 34 Cases

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Background: Hereditary renal cell carcinoma represents about 4% of all kidney cancer. Recognition of this group of tumors is important both clinically and morphologycally because it is now known that kidney tumors may occur in familiar settings more frequently than previously contemplated. Early detection and diagnosis of these tumors are important in order to establish adequate treatment and genetic counseling. At the moment, only morphology can assist to identify and diagnose most of the newly recognized syndromes. A few cases of renal tumors in hereditary leiomyomatosis and renal cell carcinoma (HLRCC) syndrome have been morphologically described as type 2 papillary RCC. We report our experience with 34 new cases.

Design: Thirty four renal tumors from patients with known germline mutation for *FH* were morphologically assessed. Immunohistochemical staining for *Ulex europaeus* agglutinin (UEA-1), cytokeratin 7 (CK7), cytokeratin 20 (CK20), CD10, and TFE-3 was performed.

Results: The patients were 17 females and 17 males. The youngest patient was 11 and the oldest 75. There was no clear predilection for the right or left kidney. All cases presented as solitary cystic as well as solid tumor masses. Capsular and/or renal vein invasion were common. Morphologically, a variety of architectural patterns were seen; papillary, tubulopapillary, cystic and solid. All tumors showed the characteristic large orangiophilic nucleoli and prominent nuclear pleomorphism. Fuhrman nuclear grade (FNG) ranged from III to IV. Areas of focal clear cell differentiation were evident in some tumors. Metastases to adrenal, liver, and regional lymph nodes were demonstrated. Tumors were negative for mucin, UEA-1, and CK7 and 20. CD10 showed focal cytoplasmic positivity in the clear cell areas. TFE-3 was negative in all the cases.

Conclusions: Renal tumors identified as part of the HLRCC have been considered to be papillary type 2. However, a wide spectrum of morphologic patterns including tubular, tubulopapillary, oncocytic, solid and mixed patterns can also be observed. The hallmark of these tumors is the presence of the prominent nuclei and nucleoli, seen in all cases. Recognition of these tumors is of importance because of the high susceptibility to aggressive renal cancers in these families.

692 Xp11.2 Translocation Renal Cell Carcinoma with Very Aggresive Course in Five Adult Patients

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Background: Renal cell carcinomas (RCC) associated with Xp11.2 translocations (*TFE3* gene fusions) are rare tumors occurring predominantly in children and young adults. Although, thus far, only limited data is available, these tumors are believed to be rather indolent even when diagnosed at advanced stages.

Design: Five cases of *TFE3*-RCC were evaluated in patients aged 18 or older (mean age 31). Diagnosis was confirmed by IHC detection of increased TFE3 fusion protein. Morphology was examined by H&E, IHC and electron microscopy (EM) and correlated with clinical picture.

Results: H&E showed clear cells, arranged in a pseudopapillary architecture, with retention of morphology in the metastatic tumor deposits. By IHC there was strong nuclear positivity for TFE3 in all cases and focal stain for AE3 and vimentin; stains for HMB45, calretinin, pankeratin and AE1 were all negative. By EM (2/5 cases examined) there were junctional complexes and rudimentary microvilli. In one case there were abundant lipid droplets and glycogen; in a second case, rare rhomboid crystals, similar to those seen in alveolar soft part sarcoma, were present. All patients (3 Caucasian, 2 Hispanic) presented with innocuous complaints, abdominal/flank pain and hematuria, and lacked any significant prior history. All but one patient presented with distant metastases at the time of diagnosis, and all patients were diagnosed with additional metastases or tumor recurrence within 5 months of presentation. Treatments included tumor resection, interleukin-2 therapy, combination chemotherapy, and radiation therapy, all with minimal success. Patients followed a rapidly terminal course, with a mean survival of 15 months post-diagnosis (range 10-20 months). One patient is currently undergoing chemotherapy at 13 months post-diagnosis (with brain metastasis), and another patient is alive at 6 months post-diagnosis, with metastases.

Conclusions: The patients presented here were older than typically described for *TFE3*-RCC. Although tumor morphology was similar to pediatric patients, these adult patients had a very aggressive clinical course compared to pediatric *TFE3*-RCC and even to conventional, adult-type RCC. Consistent use of antibodies against TFE3 in all tumors, regardless of patient age, may expand the spectrum of Xp11.2 translocation RCC with respect to age, clinical behavior and molecular abnormalities.

693 Urovysion FISH in Monitoring for Urothelial Carcinoma Recurrence after Cystectomy with Ileal Conduit/Neobladder. Identification of an Important Technical Challenge from a Series of 3533 Total Tests

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Background: Urine cytology is used to monitor for urothelial carcinoma (UC) recurrence following local resection for superficial UC. Low sensitivity has led to molecular tests such as UroVysion (Vysis) FISH, which has significantly greater sensitivity and equally high specificity vs cytology. After cystectomy, patients are at risk for recurrence, including urethral and upper tract UC. Cytology of ileal conduit and neobladder (IC) specimens is complicated by inflammation, mucinous debris, and intestinal mucosal cells ± degeneration. Atypical diagnoses can lead to technically challenging endoscopic evaluation. Although UroVysion could be useful in these cases, we hypothesized that FISH with standard protocols may be more difficult in IC cases vs. native bladder specimens and reviewed our experience.

Design: Results for IC specimens were reviewed from a total of 3533 FISH cases from Jan 2004 to July 2005. As for native bladder specimens, IC specimens could be submitted for FISH only, cytology and FISH, or cytology/FISH if cytology non-negative.

Results: UroVysion was performed on 32 IC specimens (0.9 % total FISH cases). 14/32 (44 %) IC FISH were unsuccessful vs. only 6 % of total FISH cases. Most unsuccessful IC FISH cases were attributed to interfering substances or cell degeneration complicating hybridization. 19/32 IC FISH cases had cytology, 15 of these for non-negative cytology (9 atypical, 6 suspicious). Of these 15 IC "reflex" FISH, analysis was unsuccessful in 9 (60 %). Only 2/17 IC cases with informative FISH were positive, including 1/8 for FISH only. None of 3 informative cases with negative cytology had positive UroVysion. Only 1/6 (17 %) with non-negative cytology had positive UroVysion (vs ~ 30 % and 65 % positive FISH in native bladder specimens with atypical and suspicious cytology, respectively).

Conclusions: FISH was unsuccessful in 44 % of 32 IC cases vs. 6 % of > 3500 total UroVysion assays. UroVysion is useful to establish a positive or negative diagnosis in usual urine cases with atypical or suspicious cytology. Only 1/6 non-negative cytology IC cases had positive FISH. Although larger numbers are needed, atypical cytology diagnoses may be due in part to intestinal epithelial cells ± degeneration. However, technical modifications will likely be required for further application of FISH to augment cytology in monitoring patients post cystectomy.

694 Inflammatory Myofibroblastic Tumors of Bladder (BIMT); a Clinicopathologic Study, Including a Subset Associated with High Grade Carcinomas

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Background: BIMT, also termed postoperative spindle cell nodule, inflammatory pseudotumor, and pseudosarcomatous fibromyxoid tumor, is rare and, in the past, believed to reflect diverse entities.

Design: We reviewed a series of 39 BIMT derived from a large genitourinary consultation practice.

Results: There were 26 M and 13 F aged 3-86 years (mean, 50; median,46). Lesions were 1.2-12 cm (mean, 4.4; median 4). There was a prior instrumentation history in 6. Morphology was typical of IMT; with spindle to stellate cells and background mixed inflammation. Polypoid cystitis was present in 4 patients, all uninstrumented. Mitoses

were typically scant (0-20/10 hpf, mean, 1; median, 0) and necrosis was found in 11/39 (33%) cases. Invasion of the muscularis propria was documented in 16 (39%). By IHC, lesions at least focally expressed ALK (18/26, 69%), AE1/3 (22/26, 85%) , CAM 5.2 (9/13, 69%), CK18 (6/6, 100%), actin (22/24, 92%), desmin (13/16, 81%), calponin (6/7, 86%), caldesmon (4/7, 57%, rare cells), p53 (10/13, 77%), and most lacked S100 (0/14), CD34 (0/13), CD117 (2/13, 15%), CD21 (0/5), and CD23 (0/3). ALK gene alterations were detected by fluorescence *in situ* hybridization (FISH) in 12/15 (80%) tested cases, including 1 with prior instrumentation; 8 (66%) had concordant ALK protein by IHC. Most BIMT were managed locally, but partial cystectomy was performed in 6 and cystectomy in 1, a lesion initially misinterpreted as carcinoma. On FU in 28 cases (range, 3-120 months, mean, 37; median, 24), there were 7 patients with recurrences (one of these with 2) but no BIMT metastases. In 2 cases, biopsies showing BIMT preceded (1 and 2m, respectively) biopsies showing sarcomatoid carcinoma associated with high grade invasive urothelial carcinoma accompanied with separate fragments of BIMT; even on re-review the BIMT in these 2 cases were morphologically indistinguishable from other cases of BIMT, with FISH demonstrating ALK alterations in the BIMT areas in 1 tested case. These 2 patients both died of their carcinomas.

Conclusions: BIMT is rare and shares many features of IMT of other sites, displaying identical morphology and immunogenotypic features whether *de novo* or post instrumentation. BIMT can be locally aggressive but does not metastasize. However, BIMT may locally recur, and sometimes requires surgical resection. Uncommonly, it may be associated with sarcomatoid urothelial carcinomas. For these reasons, close follow-up is warranted.

695 Clinicopathological Significance of Multifocal Prostate Cancer

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Background: The presence of multiple foci of prostate cancer (PCa) within the same gland is a common finding. There is an index tumor (measured by largest volume), presumed to be the driver of prognosis and one or more separate accessory tumors.

Design: 947 radical prostatectomies were performed between 1983 and 1998 and processed as whole-mount specimens with all tumor foci mapped. Multifocal tumors were further analyzed in terms of number of tumor foci. Tumor volume was obtained using image analysis of the index and each of the accessory tumor foci. In 389 cases, preoperative biopsies were available, and the number and the proportion of positive cores were noted. Spearman test was used for the correlations between multifocality and tumor volumes and clinicopathological variables. Kaplan-Meier analysis and Cox proportional hazard regression were used to test the prognostic value number of foci and tumor volumes of the primary vs. accesory foci.

Results: In 73% of cases (741 cases) prostate cancer was multifocal. The mean number of foci was 2.24. The mean index tumor volume was 2.42 cc and mean accessory tumor volume was 0.61 cc. The number of tumor foci correlated with the clinical stage, extracapsular extension (ECE) and seminal vesicle invasion (SVI) ($p<0.05$). Index and accessory tumor volumes correlated with preoperative PSA, clinical stage, LN metastasis, ECE, SVI and surgical margin positivity ($p<0.05$). Upon survival multivariate analysis, number of tumor foci, index tumor or accessory tumor volume did not have any significant predictive value ($p> 0.05$). The mean of proportion of positive biopsies in unifocal tumors was 0.41, in multifocal tumors it was 0.45 and the difference was not significant ($p>0.05$).When compared with the histologic variables, the proportion of positive biopsies did correlate with the biopsy Gleason score, however. ($p=0.012$)

Conclusions: Increased number of tumor foci and larger volumes of index and accessory tumors are correlated with adverse clinicopathological parameters. However, these do not independently affect the recurrence free survival. Also the number of positive biopsies and the biopsy Gleason score did not correlate with the number of tumor foci.

696 Quantitation of Stromal Grade 0/3 in Radical Prostatectomies Is a Continuous Marker of Biochemical Recurrence Free Survival in Prostate Cancer

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Background: We previously reported that Prostatic carcinoma (PCa) with either no to little reactive stroma (RSG 0), or with abundant stroma (RSG 3) showed reduced recurrence-free survival using tissue microarrays (TMA) of radical prostatectomies and needle biopsies. Therefore they can be grouped as a prognostic unit. However the prognostic significance of quantitation of amount of RSG 0 and 3 in radical prostatectomies remains unclear.

Design: Totally embedded whole-mount radical prostatectomies specimens of 874 patients were reviewed. Reactive stroma was evaluated in the index cancer as previously described: Grade 0, area with 0 to 5% reactive stroma; Grade 1, 6 to 15%; Grade 2, 16 to 50%, and Grade 3, 51 to 100%. Furthermore, the percent of RSG 0 or 3 in the index tumors was also analyzed in percentile fashion. Correlation of RSG and the quantity of RSG 0/3 with the clinicopathological parameters and recurrence free survival was analyzed.

Results: RSG 0 was detected in 17 (1.4%) of the cases, RSG 1 or 2 in 462 (38.2%) and RSG3 in 395 (32.6%). On survival analysis, patients having RSG 1 or 2 had significantly better survival (mean 147 months) than RSG 3 (mean 108 months) and RSG 0 (mean 37 months).Higher percentages of index tumor being RSG 0/3 was associated with preoperative PSA (rho=0.115 ,p=0.0008), clinical stage (rho=0.304 ,p<0.001),LN metastasis (rho=0.195 , p<0.001), extracapsular extension (rho=0.386 ,p<0.001), seminal vesicle invasion (rho=0.302, p<0.001) positive surgical margin (rho=0.086, p= 0.011), and Gleason Score (rho=0.456, p<0.001). The higher the amount of RSG0/3, the worst the biochemical recurrence free survival became. Higher percentage of the index tumor having a RSG 0/3 was associated with early biochemical recurrence in PCa. as a continuous univariate variable (HR=1.126,p=0.000), and on multivariate analysis (HR=1.096, p<0.001).

Conclusions: Prostatic carcinoma with reactive stroma had reduced reccurence free survival. Increased quantity of RSG 0 and RSG 3 was an independent and continous marker in recurrence free survival. Quantitation of reactive stroma is becoming a very important predictor for prostate cancer of use in radical prostatectomies and biopsies.

697 Coexpression of HER2 and TOP2A in Prostate Cancer Correlates with Gleason Score and HER2 Amplification

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Background: The HER2 oncogene is amplified in many tumors, including breast cancer, where HER2 amplification is associated with poor clinical outcome. Patients with HER2 positive tumors may respond to trastuzumab, a monoclonal antibody to HER2 protein. HER2 and the adjacent TOP2A gene may be co-amplified in cancer. TOP2A encodes an enzyme (TopoIIα) involved in DNA replication which is the target of a number of chemotherapeutic agents. While TopoIIα overexpression in prostate cancer (PCa) has been reported, reports of HER2 expression are contradictory; some studies show high rates of expression in low grade PCa, and others report low rates, with expression limited to high grade tumors. In light of this conflicting data, we studied HER2 amplification and HER2 and TopoIIα expression patterns in BPH, localized and advanced PCa using tissue microarrays.

Design: Immunohistochemistry for HER2 and TopoIIα in 58 locally advanced PCa's, 41 localized PCa's and 42 cases of BPH was performed using commercially available antibodies (Dako). Staining intensity for HER2 was assessed using a 0 to 3 scoring system. Percentage of nuclei staining for TopoIIα was recorded and overexpression was defined as staining in ≥ 5% tumor cells. FISH for HER2 was performed using the PathVysion HER2 DNA Probe Kit (Vysis).

Results: Of 58 locally advanced PCa's, 18 (31%) showed TopoIIα overexpression while 9 (16%) expressed HER2 protein. 4 locally advanced tumors showed 1+ staining for HER2; 2 of these tumors co-expressed TopoIIα. 3 locally advanced tumors showed 2+ staining, and 2 showed 3+ staining for HER2; all of these tumors also showed overexpression of TopoIIα. Both tumors with 3+ staining and 2 of 3 tumors with 2+ staining showed HER2 amplification. No localized PCa showed HER2 expression or TopoIIα overexpression. Two cases of BPH (5%) showed 1+ staining for HER2; the remaining cases were HER2 negative. No BPH case showed TopoIIα overexpression. HER2 and TopoIIα correlated with tumor grade; only tumors of Gleason score ≥8 showed HER2 expression.

Conclusions: This study shows a correlation between HER2 expression, HER2 amplification, TopoIIα overexpression and Gleason score in PCa. Tumor co-expression of HER2 and TopoIIα is likely due to co-amplification of HER2 and the adjacent TOP2A gene. It is possible that combining therapies directed against HER2 and TopoIIα in patients whose tumors show such co-amplification may improve survival.

698 Intraprostatic Adipose Tissue

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Background: Prostatic adenocarcinoma (PAC) is the most frequently diagnosed cancer in American men. Tumor Gleason grade and stage provide extremely valuable prognostic information and play an important role in therapeutic decision making and patient counseling. A biopsy or radical prostatectomy specimen revealing carcinoma extending into extraprostatic tissue permits a pT3 classification. Given the lack of a true prostatic capsule, this is most easily recognized, particularly in a needle biopsy, when tumor is seen to invade the adipose tissue. The existence of intraprostatic adipose tissue is somewhat controversial and its clinical significance should be strongly emphasized.

Design: Formalin-fixed paraffin-embedded whole-mount radical prostatectomy specimens from 127 patients with PAC were evaluated for intraprostatic adipose tissue. It was defined as any collection of adipocytes amid or internal to the most peripheral glands. The amount, anatomic location and relationship to normal structures were also recorded.

Results: Intraprostatic adipose tissue was identified in 6 (4.7%) of cases. It consisted of small microscopic foci composed of 5-20 adipocytes. In two cases, the fat was intimately associated with benign glands. In another 2 cases it was associated with small nerves and in two cases was random with no specific localization. Intraprostatic adipose tissue was located in the peripheral zone along the posterolateral aspects in 4 cases and in the central zone in 2.

Conclusions: Intraprostatic adipose tissue, while uncommon, does exist. Therefore, caution must be exercised in diagnosing extraprostatic extension based only upon identification of fat invasion, especially in a needle biopsy. The small size of foci of intraprostatic adipose tissue may be a useful morphologic clue in distinguishing it from extraprostatic fat.

699 Tissue Fragments in Voided Urine are Unusual. How Concerning Is this Statement?

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Background: It is standard practice to alert the clinician that the possibility of a low-grade neoplasm cannot be excluded when tissue fragments are identified in voided urine. The cytomorphology of such a lesion cannot be reliably distinguished from a papillary neoplasm, papilloma, or papillary hyperplasia. Consequently, many patients are unnecessarily brought back for repeat cytology, cystoscopy or biopsy. The aim of this study was to determine the positive predictive value of tissue fragments in voided urine at our institution.

Design: We reviewed clinical, pathologic, and radiologic follow-up information in the electronic patient record for 174 patients who had voided urine cytology between

January 1998 and January 2000. These patients were separated into 5 groups: patients who had a previous diagnosis of urothelial neoplasia, those who had positive subsequent cytology or biopsy, those who had atypia in subsequent urines, those who had subsequent negative results, and those who had no urology follow up. The first and last categories were eliminated from analysis.

Results: Of the 183 patients originally included in the study, 24 were eliminated due to known urothelial neoplasia at the time of the case or due to lack of follow up. Of the 159 remaining cases, 24 (15%) had subsequent cytology or histology that was positive, 9 (6%) had additional atypical urine, and 126 (79%) had either subsequent negative pathology specimens or clinical follow up (sonography, intravenous pyelography, cytology, calculi, medical renal disease).

Conclusions: The results of this study indicate that the positive predictive value of tissue fragments in voided urine is lower than for non-neoplastic causes. Fifteen percent of patients with subsequent evaluation were found to have neoplasia, and only three of these (2%) were high-grade urothelial neoplasia. Rather, we found that tissue fragments most often indicate either medical renal disease or the presence of calculi, or are of no significance, having negative follow-up. One issue in the interpretation of this study is that specimens obtained with instrumentation may be mislabeled as voided, and may therefore have fragments that would have been expected had the acquisition method been known. While it is true that cytology is not an appropriate method of diagnosing low-grade urothelial neoplasia, should we even raise concern by saying that additional work-up is necessary to exclude low-grade carcinoma? The accompanying costs to patients and to the health-care system should be taken into account, and this practice revised.

700 Overexpression of Heme Oxygenase-1 in Prostate Cancer

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Background: The heme oxygenase (HO) catalyzes the degradation of heme to produce biliverdin, CO, and free iron. Three isoforms of HO have been identified. HO-1 is a 32-kDa heat shock protein found at low levels in most tissues but is highly induced by a variety of stress stimuli. HO-1 and its products possess anti-inflammatory and anti-apoptotic functions. HO-1 may also control cell proliferation. Elevated expression and activity of HO-1 have been found in various tumors. A previous study using 6 prostatectomy specimens showed increased expression of HO-1 in both benign prostatic hyperplasia (BPH) and prostate cancer (PC) (Urology 47:727-733, 1996), which has not been confirmed with more cases.

Design: Two tissue microarrays were built from prostatectomy specimens. One or more cores (average=1.5 cores/area) were taken from areas of normal prostate (N, 50 cores), BPH (82 cores), high-grade prostatic intraepithelial neoplasia (PIN, 35 cores), low-grade PC (LG, Gleason Grades 2 and 3, 104 cores) and high-grade PC (HG, Gleason Grades 4 and 5, 82 cores). They were stained immunohistochemically with a rabbit anti-HO-1 polyclonal antibody (Stressgen, Victoria, BC, Canada). Staining intensity (0 to 3+) and the percentage of positively stained cells (0 to 100) were recorded after the staining in all cores from the same case was averaged (only intact cores scored). A case was considered positive if there was 2+ or higher staining intensity in 10% or more of the cells.

Results: The results are summarized in Table 1. There was a gradual increase in the percentage of positive cases from benign (N+BPH) to PIN to PC (LG+HG). The differences between N and BPH, between benign and PIN, between PIN and PC and between LG and HG were not statistically significant. The difference between benign and PC was statistically significant ($p<0.001$).

Conclusions: 1. HO-1 is significantly overexpresed in prostatic adenocarcinoma in comparison to benign prostate tissue; 2. There is no significant difference in HO-1 expression between normal prostate and BPH as observed in the previous study using small number of cases; 3. Our data suggests that the stress-related heat shock protein HO-1 may play an important role in the carcinogenesis of PC.

Immunohistochemical study of prostate tissue microarrays for HO-1

	Pos	Neg	Total	% Pos
N	11	32	43	26
BPH	11	59	70	16
Benign (N+BPH)	22	91	113	19
PIN	12	14	26	46
LG	33	32	65	51
HG	28	14	42	67
PC (LG+HG)	61	46	107	57

701 Potential Pitfalls in Immunohistochemical Staining of Renal Oncocytoma

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Background: The differential diagnosis of renal epithelial tumors can be assisted by utilizing immunohistochemical stains. One diagnostic problem is distinguishing oncocytoma from other renal epithelial tumors with pink cytoplasm. An immunohistochemical pitfall of oncocytoma that we have observed, is due to aberrant expression of markers in tumor cells that show clear cell or atrophic changes and in entrapped normal tubules.

Design: Fifty cases of renal oncocytoma, from the Pathology Department archives were studied. The clinical data included age and gender. Microscopic examination was performed on all cases. Areas of entrapped normal tubules and tumor elements with atrophic or clear cell features were recorded and overall percent of occurrence calculated. Measurement of entrapped normal tubules from the tumor edge were also calculated for each case. Twelve cases with one or both of these features were selected for CK7 and Vimentin immunostaining. Intensity and pattern of reactivity were recorded in all cases.

Results: The mean age was 67 years (41-83 years). Twenty-one cases were from females and twenty-nine cases from males. The maximum tumor dimension ranged from 2 to 15 cm (mean, 4.2 cm). In 54% (27/50) of the cases, tumor foci with an atrophic appearance were found. These typically occurred in areas of fibrotic stroma and in association with a collapsed tubular architecture and cytoplasmic clearing. In 10/11 (91%) and 12/12 (100%) cases, the atrophic or clear tumor cells showed diffuse and intense reactivity for Vimentin and CK7 respectively. In 64% (32/50) of the cases, entrapped normal tubules were present and were located up to 10 mm from the tumor edge (mean, 3.8 mm). In all cases the entrapped normal tubules coexpressed CK7 and Vimentin.

Conclusions: Immunohistochemistry is frequently employed as an adjunct in the differential diagnosis of pink cell tumors of the kidney. Renal oncocytoma characteristically does not express Vimentin and in most cases expresses CK7 in only scattered single or small groups of cells. Aberrant expression of CK7 and Vimentin in tumor cells with an atrophic or clear cell change may result in diagnostic confusion. Positive staining of these markers in entrapped normal tubules could also be problematic if one is not aware of this common feature of oncocytoma.

702 Histologic Characteristics and Clinical Behavior of Renal Cell Carcinoma in Young Adults: A Comparison to Older Age Patients

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Background: Renal cell carcinoma (RCC) represents ~3% of adult malignancies and majority of them are sporadic cancers. The histologic features and their biologic behavior of RCCs occurring at younger age may be different from those detected at an older age group. This study describes the histologic characteristics and clinical behavior of RCC in younger adults and compares them with those of older age group.

Design: We reviewed the slides and pathology reports of 838 RCC patients treated at one institution from 1990 to 2005. The clinico-pathologic features including histologic subtype, tumor size, extension, nuclear grade, tumor stages, nodal and distant metastasis, and survival were evaluated and compared between patients who were 40 or younger and patients who were older than 40.

Results: Of the 838 RCCs, 44 (5.2%) patients were 40 years-old or younger (median 37, range 24 to 40), and 794 patients were older than 40 years (median 63, range 41 to 88). Four patients had von Hippel-Landau disease, and 1 had Birt-Hogg-Dube syndrome. The gender distribution was equal in younger patients (22 each) as opposed to the relative male predominance seen in older patients (62% males, $p=0.07$). Thirty percent of the younger patients underwent partial nephrectomy compared to 19% in older patients ($p=0.08$). Tumors in younger patients were slightly smaller (5.3 vs. 5.9 cm, $p=0.37$) and less likely to present as T3 or T4 tumors (18% vs. 31%, $p=0.09$). There was no significant difference in Furhman nuclear grade and both groups shared a similar incidence of clear cell RCC (71% vs. 79%) and papillary RCCs (14% vs. 13%). However, higher incidence of collecting duct (5% vs. 0.5%) and chromophobe RCCs (12% vs. 6%, $p=0.03$) occurred in younger patients than in older patients. Younger patients presented less frequently at higher stages (stage III and IV) than older patients (18% vs. 33%, $p<0.05$). Nodal and distant metastasis was less common in younger patients than older age group (5% vs. 8.3%, $p=0.57$). Kaplan-Meier survival analysis showed no survival difference at 5 years (77% vs. 70%), but significant difference was observed at 10 years (77% vs. 52%).

Conclusions: Younger patients have a higher incidence of collecting duct carcinoma and chromophobe RCC. Renal tumors occurring at a younger age tend to be smaller in size, lower stage, and have lower incidence of nodal or distant metastasis at the time of nephrectomy. These findings may contribute a favorable long-term survival in younger patients.

703 PSMA Expression in Different Pathologic Stages of Urothelial Carcinoma of the Bladder

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Background: PSMA (prostate specific membrane antigen) is a type II membrane glycoprotein that is expressed in benign and malignant prostate epithelium and in the neovasculature of various malignant tumors including urothelial carcinoma of the bladder. PSMA expression has not been described in vessels of normal tissues. Recently tumor-related vasculature has become a therapeutic target and several compounds that either target tumor-associated vasculature or inhibit angiogenesis are under clinical investigation. At our institution ongoing clinical trials use PSMA to target prostatic tumors and the vasculature of various non-prostatic malignancies, including urothelial carcinoma (UC). Nevertheless, the relationship between histologic grade and pathologic stage of UC and PSMA expression has not been studied.

Design: Immunohistochemical stain for anti-PSMA monoclonal antibody (13D6) was performed on 10 benign urothelium sections, 10 flat urothelial carcinoma in-situ (CIS), 10 papillary UC, low-grade (pTa,LG), 10 papillary UC, high-grade (pTa, HG), 10 UC invasive into the lamina propria (pT1), and 10 UC invasive into the muscularis propria (pT2).

Results: PSMA was not expressed in benign or malignant epithelium. Immunoreactivity was seen in the tumor associated neovasculature, but not in the vessels in relation to the benign urothelium. The results are summarized in the table.

PSMA	Benign (10)	CIS (10)	pTa, LG (10)	pTa, HG (10)	pT1 (10)	pT2 (10)
Positive	0	5 (1D)	9 (4D)	9 (6D)	9 (4D)	10 (5D)
Negative	10	5	1	1	1	0

D: Diffuse, F: Focal

Conclusions: The vessels of the benign urothelial mucosa are negative for PSMA. The neovasculature of urothelial carcinoma at all pathologic stages express PSMA, including non-invasive disease. The antibody is expressed in endothelial cells in at least 90% of the papillary urothelial carcinomas and invasive tumors while only in 50% of the CIS. PSMA is a reasonable target for monoclonal antibody based anti-neovasculature therapy in bladder cancer.

704 An Expanded Immunohistochemical Panel for Differentiating High Grade Prostatic Adenocarcinoma from Urothelial Carcinoma

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Background: Prostatic adenocarcinoma (PA) and urothelial carcinoma (UC) can usually be distinguished by morphology alone.However, at times patients present with high-grade (HG) carcinomas,especially in the bladder neck,that clinically and morphologically are not clearly UC or PA.The purpose of this study is to evaluate an immunohistochemical (IHC) panel that will be useful in this differential diagnosis.One of the IHC stains that we studied is anti-PSMA monoclonal antibody which is known to be expressed in benign and malignant prostatic epithelium but not in the associated neovasculature.

Design: Within the past year we have encountered 20 cases of HG carcinoma involving the bladder neck and trigone region.10 patients had a history of PA,5 patients had a history of both PA and UC, 4 patients had a history of UC and 1 patient presented for the first time with urinary obstruction.Morphologic features including growth pattern, were evaluated.IHC stains for PSA, PSAP,AMACR,34βE12,CK7,CK20 and anti-PSMA were performed.Also 10 benign urothelium,20 papillary UC and 10 invasive UC were stained for anti-PSMA monoclonal antibody 13D6.

Results: 14 of 20 cases were diagnosed as PA and 6 were UC.2 patients with history of UC were found to have PA and 1 with history of PA was diagnosed with UC.All tumors showed solid, cribriform and nested growth pattern while 6 of them, all of which had a final diagnosis of PA, showed focal papillary architecture.The results of IHC stains for the 20 HG carcinomas are summarized in the table.PSMA was negative in all benign and malignant urothelial epithelium and vessels underlying the benign mucosa while it was positive in over 90% of the cases in the urothelial tumor associated neovasculature.

	PSA	PSAP	Racemase	34βE12	CK7	CK20	PSMA
PA	13/14 (4F)	12/14 (9D)	12/14 (6D)	5/14 (3F)	5/14 (2F)	3/14 (F)	13 /15 *(E)(1F)
UC	0	4/6 (F)	2/6 (D)	6/6 (1F)	5/6 (5F)	1/6 (D)	5 /6 **(NV)(5D)

F: Focal; D: Diffuse; *E: Epithelium; **NV: Tumor associated neovasculature

Conclusions: Clinical history and morphologic features might not be helpful and can even be misleading in the differential diagnosis of HG PA and UC. AMACR and PSAP can be expressed in a subset of HG-UC, while 34βE12 may be positive in some PA; nevertheless they are useful components of an IHC panel.PSMA is positive in the epithelium of PA and in the tumor associated neovasculature in UC.CK20 is not a helpful marker in this setting.A panel of PSA,PSAP,AMACR,34βE12,CK7and PSMA is useful in this differential diagnosis.

705 Significance of Denuded Urothelium in Papillary Urothelial Lesions

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Background: Flat urothelial carcinoma in situ (CIS) often has prominent dyscohesion with some cases having only a few clinging CIS cells remaining on biopsy. Extensive denudation on urothelial biopsies is associated with a risk of CIS on either prior or subsequent biopsies. The significance of denudation in papillary urothelial lesions has not been formally studied.

Design: We identified from our surgical pathology files 31 specimens (from 28 patients) of papillary urothelial lesions with extensive denudation. In cases where the denudation was associated with low grade urothelial neoplasms, follow-up of subsquent cytologic and histologic specimens was obtained.

Results: Of the 28 patients, 40 to 88 years old (mean age 62), 25 (89%) were male and 3 (11%) were female. 15/31 of the biopsies were from anatomically confined areas (i.e. renal pelvis, ureter, urethra). In 22/28 (79%) patients, prominent denudation was associated with high grade papillary carcinoma, 4/28 (14%) low grade papillary carcinoma, and 2/28 (7%) papillary urothelial neoplasm of low grade malignant potential. The average extent of urothelial denudation was 82%. 61% of cases had ≥90% denudation. Associated findings in the lamina propria included: chronic inflammation in 17/31 (55%) biopsies; prominent vascularity in 30/31 (97%) cases; prominent fibrosis 5/31 (16%) biopsies; and granulomas in 1/31 (3%) biopsies. Prominent cautery artifact was present in 17/31 (55%) cases. In 13/28 patients with high grade lesions, there was a concurrent biopsy of a second urothelial lesion that was either high grade papillary urothelial carcinoma or invasive urothelial carcinoma. None of the 6 patients in which the prominent denudation was associated with a low grade papillary urothelial lesion have progressed to a high grade lesion.

Conclusions: 1) The majority of papillary urothelial lesions in our series associated with prominent urothelial denudation were high grade. 2) A significant percentage of papillary urothelial lesions with denudation occur with either prominent cautery artifact or in anatomically confined areas, suggesting both iatrogenic and mechanical contributing factors, respectively. 3) 21% of cases with prominent urothelial denudation surprisingly occurred in association with low grade papillary urothelial lesions and was not associated with progression to higher grade lesions on follow-up studies. 4) Prominent urothelial denudation in papillary lesions should prompt careful examination of these specimens for rare clinging high grade carcinoma cells, although in a minority of cases the underlying lesion will be low grade.

706 Differentiating Prostatic Adenocarcinoma (PCa) from Colorectal Adenocarcinoma (CRCa) on Biopsy Samples: The Utility of P501S (Prostein) Immunohistochemistry

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Background: Poorly differentiated adenocarcinoma identified either on prostate needle or colorectal biopsy can present a challenge in determining the exact tumor source especially in locally advanced cases where both organs are involved. Such determination will affect choice of therapy. P501S (prostein) is a newly characterized, potentially prostate tissue specific, type IIIa plasma membrane protein that was identified by high throughput cDNA microarrays analysis.

Design: A total of 27 cases of poorly differentiated carcinoma representing either PCa extending into colon or CRCa extending into prostate were identified from surgical pathology and consultations records. For current analysis, H&E sections were available for review on 13/27 cases and IHC were obtained on 8/27 pending completion of the study. Eight additional cases of poorly differentiated CRCa and PCa from routine colorectal resections and radical prostatectomy were also studied for comparison.

Results: Of the histologic parameters the presence of "dirty" necrosis, seen in 8/8 (100%) of CRCa and 1/13 (8%) of PCa, appears to be the most useful parameter. Microacinar formations, seen in 2/8 (25%) of CRCa and 11/13 (85%) of PCa, and the presence of columnar cells/ basal nuclear polarity nuclear, seen in 7/8 (88%) of CRCa and 2/13 (15%) of PCa, could be of some additional utility. The following table summarizes the rates of positive staining for all IHC markers. One PCa case was positive for P501S and negative for PSA. The single P501S negative PCa which was also negative for PSA demonstrated small cell morphology.

Primary	P501S	BCatenin	PSA	CDX2	CK7 & CK20	pCEA	P504S
PCa	8/9 (89%)	0/10 (0%)	8/10 (80%)	0/10 (0%)	4/10 (40%) & 3/10 (30%)	1/10 (10%)	8/10 (80%)
CRCa	0/6 (0%)	3/6 (50%)	0/6 (0%)	5/6 (83%)	3/6 (50%) & 5/6 (83%)	5/6 (83%)	3/6 (50%)

Conclusions: P501S is a useful marker for differentiating PCa from CRCa and should be included in IHC panels in this setting in addition to PSA, B-Catenin, CDX2. The latter has been rarely expressed in PCa. Presence of "dirty" necrosis could be useful in this differential. P501S may be more sensitive than PSA as a marker of prostatic differentiation.

707 Determination of EGFR Status in Sporadic and Hereditary Renal Tumors. Correlation between CISH and Immunohistochemistry

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Background: EGFR is a transmembranous receptor for EGF, TGF-α, and other ligands. Overexpression of EGFR is a frequent event in tumors and correlates with poor prognosis and disease progression. Different EGFR targeted therapies using agents directed toward the extracellular ligand-binding or the intracellular tyrosine kinase domains are currently in phase 3 clinical trials. High expression of EGFR has been reported as an unfavorable prognostic factor in patients with sporadic renal cell carcinoma (RCC). The EGFR status in hereditary RCCs is not known. It remains to be determined which is the best assay to evaluate EGFR status in possible candidates to undergo immunotherapy treatment. Chromogenic in situ hybridization (CISH) is a valuable tool for evaluation of EGFR gene amplification. We evaluated hereditary and sporadic renal tumors for both gene amplification and overexpression of EGFR by CISH and correlated the results with IHC to identify the technique that can assist in patient selection for molecular target based therapy.

Design: Twenty two cases of hereditary RCC, 6 BHD, 6 HLRCC, 5 papillary RCC, 5 VHL, and 13 cases of sporadic renal tumors (7 clear cell, 5 oncocytomas, and 1 chromophobe) were evaluated. CISH and IHC for EGFR were performed in paraffin-embedded tissue. EGFR overexpression was considered (+) when staining intensity was 2+ or more in 50% or more of tumor cells. CISH amplification was present when >10 copies or large clusters where seen in >50% cancer cells; cases were considered as low amplification when 6-10 copies were present in >50% tumor cells.

Results: Eighteen hereditary RCC cases (82%) and 9 (69%) sporadic tumors showed EGFR overexpression by IHC with a predominant diffuse pattern present in both membrane and cytoplasm. No cases displayed gene amplification by CISH. Chr 7 trisomy was seen in 5 PRCC type I and one chromophobe

Conclusions: Although EGFR overexpression is seen by IHC in the majority of sporadic and hereditary RCC, especially in VHL cases, there was no evidence of EGFR gene amplification by CISH in any of the tumors. These findings suggest that IHC assay for EGFR does not correlate with EGFR gene amplification. EGFR status assessment by CISH may be important in order to evaluate clinical response to EGFR targeted therapies.

Type	Cases	+ Diffuse	+ Focal	Negative	CISH
Hereditary	22	15	3	4	NA
BHD	6	3	1	2	NA
HLRCC	6	5	-	1	NA
HPRCC	5	4	-	1	NA
VHL	5	3	2	-	NA
Sporadic	13	8	1	4	NA
Clear cell	7	7	-	-	NA
Chromophobe	1	1	-	-	NA
Oncocytoma	5	-	1	4	NA

NA: No amplification

708 Loss of Heterozygosity of *TSC2* and *TSC1* Gene in Perivascular Epithelioid Cell Tumor (PEComa)

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Background: Perivascular epithelioid cell tumor (PEComa) is a neoplasm composed chiefly of HMB-45-positive epithelioid cells with clear to granular cytoplasm and a perivascular distribution. Such tumors have been reported in different organs under a variety of designations. The cytogenetic features of these neoplasms have not been well studied.

Design: The cytogenetic features of these neoplasms have not been well studied. We collected 9 tumors (5 of kidney, 1 of prostate, 1 of urinary bladder, 1 of the pelvic cavity soft tissue, 1 of uterus) from 8 patients, including one patient with tuberous sclerosis complex (TSC). The paraffin blocks of tumor tissue were submitted for loss of

heterozygosity (LOH) analyses using microsatellite markers targeted on *TSC1* gene (D9S290, D9S164) and *TSC2* gene (D16S423, D16S404).
Results: Six tumors, including the 2 tumors of the patient with TSC, had LOH at *TSC2* locus. Two cases failed, and one was uninformative. One tumor showed miscrosatellite instability at *TSC2* locus. One tumor had LOH at *TSC1* locus.
Conclusions: The frequent LOH at *TSC2* gene indicates the oncogenetic relationship of PEComas with angiomyolipoma as a *TSC2*-linked neoplasm. From a molecular genetic perspective, the recurrent genetic alterations in both renal and extrarenal tumors further support the concept of PEComa as a distinctive tumor entity regardless of anatomic location.

709 Findings of Moderate and High Grade Carcinoma of the Prostate in a Sampling of the Elderly Population in New York: A Post-Mortem Analysis

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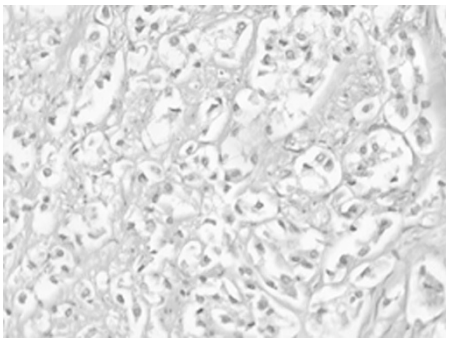
Background: Post-mortem studies indicate carcinoma of the prostate (CAP) occurs in close to 50% of patients over the age of 70. Out study sought to provide an update on these historic rates by conducting a post-mortem examination of a subset of the elderly population of New York. Our primary goal was to examine any chronological associations between CAP and age.
Design: 50 prostate glands were identified from cadavers obtained for medical school use from the greater New York area. The prostates were excised en bloc, bisected, cut into serial sections, stained with H & E, and mounted onto glass slides. Approximately 100 slides from each gland were examined by two independent pathologists. If discovered, the CAP was graded based upon the Gleason system of scoring by each pathologist. Frequencies and grades of CAP in the individuals were calculated based upon the subsets of 60-69, 70-79, and ≥ 80 years of age.
Results: CAP was discovered in 18/50 (36%) of the prostate glands. There were 0/5 cases in the 60-69 age group, 8/18 (44.4%) cases in the 70-79 age group, and 10/27 (37%) in the ≥ 80 age group. Eight individuals in the 70-79 subgroup yielded an average Gleason score of 6.6 ± 1.5 (means ± SEM). Ten individuals in the ≥ 80 subgroup yielded an average score of 6.1 ± 2.4 (means ± SEM). Moderate and high grade CAP, defined as a Gleason score of 6 or higher, was identified in 13/18 (72.2%) cases of carcinoma, with 53.8% in the 70-79 and 46.2% in the ≥ 80 subgroups.
Conclusions: Among this subset of the population of the greater New York area, incidences of CAP paralleled those found in previous studies where higher incidences were discovered in individuals over the age of 70. More importantly, To our knowledge this study is the first post-mortem analysis to show a trend towards higher grade CAP amongst a subset of the New York population examined.



710 Further Characterization of the Muscle Layers of the Urinary Bladder by Systematic Histologic Mapping: Implications for Pathologic Staging

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Background: The muscularis mucosae (MM) and muscularis propria (MP) are important landmarks for pT staging of urothelial cancer (UCa), which is the quintessential prognostic factor. In our routine practice, we have occasionally noted patterns of MM which do not always conform to the originally described configuration of thin slender bundles arranged in a single layer of interrupted, dispersed or continuous muscle.
Design: We evaluated the MM and MP characteristics in 30 cystectomies for UCa with systematic sampling of the dome, trigone, anterior, posterior, right and left lateral walls. Slides with tumor or previous biopsy sites features were excluded.
Results: MP muscle was typically in groups with distinct round thick compact muscle bundles each surrounded by perimysium. Most commonly (75-95%), the MP had a relatively smooth interface with the loose connective tissue of the lamina propria (LP). Regional variances included MP bundles extending variably into the mid LP (20-46%, mostly in the dome). The trigone (45%) was strikingly distinct with gradual dimunition of size of the muscle bundles as they extended to almost a suburothelial location. The MM was typically composed of individual or small groups of slender, wispy, and wavy fascicles arranged parallel to the surface (37-79%). MM was very rare in the trigone where there was difficulty in discriminating it from the superficially extending MP bundles. MM also had focal to rarely extensive hypertrophic appearance (52%, most common in dome [71%]) with two recognizable patterns: a) aggregates of hypertrophic MM with haphazard outlines distinct from that of MP (11-61%, mostly in dome), and b) hypertrophic MM muscle bundles arranged singly or in small groups of 2 -3 bundles resembling MP muscle but distinguishable from it based on the location in the LP (16-61%, mostly in dome).
Conclusions: 1) Awareness of the hypertrophic appearance of MM muscle, distinct from that which is traditionally described, is important to prevent overstaging of invasive UCa. 2) In transurethral resection specimens, lack of orientation may preclude distinction of the rare MP-like hypertrophic MM from MP. Appreciation of relation to the surface and comparison with more characteristic MP, if present, would be helpful; haphazard outlines favor MM over MP muscle. 3) The more superficial location of the MP and the rarity of MM in the trigone complicate the traditional pT stage evaluation of invasion in this region, including tumors with inverted growth.

711 A Novel Nuclear Grading Scheme for Chromophobe Renal Cell Carcinoma (ChRCC); Prognostic Utility and Comparison with Fuhrman's Nuclear Grade (FNG)

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Background: ChRCC is a histologic subtype of RCC which portends a favorable prognosis. It is controversial whether the FNG of ChRCC has prognostic utility. Irregular nuclei, prominent nucleoli and nuclear pleomorphism are inherently present in most ChRCC. Hence the FNG is innately higher even though the majority of tumors have a favorable outcome.
Design: The prognostic utility of a novel three-tiered nuclear grading system in which the constitutive nuclear atypia of ChRCC was discounted was compared with the FNG in ChRCC and other pathologic parameters. The ChRCC nuclear grade (CNG) categories were defined as: CNG 1: ChRCC with wide nuclear range without nuclear crowding or anaplasia (as defined in grade 2 and 3); CNG 2: Presence of nuclear pleomorphism (size variation > 3 times) and nuclear crowding (cellular clustering with high N/C ratios detectable at 10X objective and nuclear overlapping at 40X objective); and CNG 3: Presence of nuclear anaplasia including tumor giant cells, or sarcomatoid change.
Results: The FNG distribution between the tumors (n=142) was FNG 1: 1%, FNG 2: 20%, FNG 3: 75%, FNG 4: 5% and by the CNG (n=122) was CNG 1: 75%, CNG 2: 16%, CNG 3: 8%. All patients with available data contributed to statistical tests associating nuclear grading with other risk factors. For associating nuclear grading with clinical outcomes, only patients experiencing events within 36 months or followed event-free for a minimum of 36 months were included (n=59; 15 patients progressed [metastasis, recurrence or death]). The FNG did not correlate with pT stage, tumor size, tumor necrosis, vascular invasion or outcome. The new CNG correlated with pT stage ($p<0.001$) and outcome ($p=0.021$) but not with other parameters including tumor size, tumor necrosis and vascular invasion. In a multivariable logistic regression model, the new CNG independently predicted poor clinical outcome ($p=0.043$, adjusted odds ratio estimate=3.1).
Conclusions: This study provides objective data regarding the lack of clinical utility of the FNG in ChRCC. The novel ChRCC nuclear grading system proposed herewith has prognostic value and will potentially help stratify patients of ChRCC who need close surveillance.

712 Identification of an Aggressive Phenotype of Chromophobe Renal Cell Carcinoma (ChRCC) by Morphologic Parameters – Analysis of 143 Patients

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Background: The aggregate literature suggests that ChRCC is biologically a tumor of low malignant potential with reported 5 and 10-year survival rates of 87-100% and 83-90%, respectively. The parameters in the primary ChRCC that determine aggressive phenotype remains to be fully characterized.
Design: From a series of 143 patients, ChRCC cases exhibiting progression (recurrence, metastasis, and/or death; n=15) within 3 years of surgery were compared to patients without progression.
Results: The patients' ages ranged from 32-82 years (mean 59), male to female ratio 1:1, and tumor size 1-30 cm (mean 7.9 cm). The mean follow-up was 43 months (range 1-182). For statistical analysis, only patients progressing within 36 months or event-free over a minimum 36 months follow-up were included (n=59). Progression (recurrence 2, metastasis 12 and/or death 9, mean follow-up 60 months) was seen in 15 patients compared to no evidence of progression in 44 patients. Metastatic sites included liver (4), lung (4), brain (3), mediastinum (1), bone (1) and widespread (1). Pathologic parameters associated with progression included the pT stage ($p=0.023$), tumor necrosis ($p=0.042$) and sarcomatoid change ($p=0.013$) in univariable analysis (2-sided Fisher's exact tests). There was no association with specific histologic subsets of ChRCC (clear, eosinophilic, mixed or hybrid tumors), patient's age, gender, Fuhrman nuclear grade, or vascular invasion. Tumors in patients who progressed tended to be larger (mean 9.5 cm) compared to those without progression (mean 7 cm) although this finding was not statistically significant ($p=0.08$). A multivariable logistic regression model showed that pT stage ($p=0.07$, adjusted odds ratio 3.6) and sarcomatoid change ($p=0.07$, adjusted odds ratio 9.9) but not tumor necrosis, were independent predictors of prognosis.
Conclusions: Although the large majority of ChRCC have a favorable prognosis, a distinct subset of patients progress. pT stage of tumor, sarcomatoid change and tumor necrosis predict aggressive phenotype. The presence of these features in a nephrectomy specimen with ChRCC warrants active surveillance and these patients may be candidates for adjuvant therapies as they become available.

713 Predictive Values of Clinically Significant PCA after a Negative Prostatic Biopsy

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Background: The ten-core needle biopsy has been considered as the standard technique of prostatic biopsy due to its high rate of detection of prostatic adenocarcinoma (PCA). We investigated the predictive value (PV) for all PCA, and for clinically significant PCA undiagnosed after a ten-core protocol.

Design: One hundred-thirty-two consecutive radical prostatectomy (RP) specimens, along with corresponding ten-core biopsies were reviewed, with special attention to the cases with cores having unilateral involvement by the PCA. Morphometric analysis was conducted on the biopsy negative hemiprostate to determine the predictive value of the biopsy protocol, with respect to the size, position and the clinical significance of the lesion.

Results: There were a total of 70 biopsies with unilateral PCA involvement. In 32 of these cases, the RP specimens showed the absence of PCA in the biopsy negative side, while carcinoma was observed in the biopsy negative side in the remaining 38 cases. The latter group was categorized by morphometric criteria. Specifically, 23 cases showed one to six foci of PCA measuring from 0.3 to 16mm in the posterior peripheral zone (PPZ) (group 1); three cases showed two to six foci of PCA measuring from 0.5 to 12mm in both PPZ and anterior horn of the PZ (AH) / transitional zone (TZ) (group 2); and 12 cases showed one to four foci of PCA measuring from 0.5 to 20mm in the AH / TZ (group 3). The number of cases with clinically significant PCA was 0, 1 and 4 in the group 1, 2 and 3 respectively.

Conclusions: In the setting of this study, the PV of a five core biopsy protocol on a hemiprostate for all PCA, insignificant PCA and significant PCA are 47%, 40% and 7%, respectively. Most undiagnosed PCA were clinically insignificant, and all significant undiagnosed PCA, measuring up to 20 mm in diameter (1.3 cm^3), were in the AH and TZ of the prostate.

714 High Grade Urothelial Carcinoma of the Renal Pelvis: Clinicopathologic Study of 108 Cases with Emphasis on Unusual Morphologic Variants

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Background: Primary neoplasms of the renal pelvis account for only 5% of all renal tumors. The majority of them are of transitional cell type. Urothelium can display a wide range of metaplastic changes, and neoplasms arising from this epithelium can show several types of differentiation, especially in high-grade neoplasms.

Design: All cases of total nephrectomy specimens for carcinoma of the renal pelvis were retrieved from the surgical pathology files at the Ohio State University Medical Center between the years 1970 and 2000, and from the University Hospital in Plzen, Czech Republic between 1995 and 2001. Demographic features analyzed included age, sex, tumor location and clinical stage. The histologic features analyzed included: histologic grade, evidence of divergent differentiation, and unusual stromal features. Pathological staging was established using the current American Joint Committee on Cancer (AJCC/TNM) system. Selected cases were stained with a panel of immunohistochemical markers, including: CK7, CK20, cytokeratin AE1/AE3, vimentin, actin, CD31, CEA, CD10, and EBV latent membrane protein.

Results: A total of 157 cases were identified; from these, 108 (68.7%) were classified as high-grade neoplasms according to the WHO/ISUP consensus**.** Of these, 44 (40%) showed unusual morphologic features, such as micropapillary areas (4 cases); lymphoepithelioma-like (2); sarcomatoid (8), including pseudoangiosarcomatous and carcinosarcomatous types); squamous differentiation and squamous cell carcinoma (15); clear cells (2); glandular differentiation (2); rhabdoid, signet-ring or plasmacytoid cells (4); pseudosarcomatous stromal changes (4) and intratubular extension into the renal pelvis (3). Pathological staging was available in 62 cases; of these, 46 (74%) were in high stage (pT2-pT4) and 16 (26%) were in low stage (pTis, pTa, pT1). Clinical follow up ranging from 1-256 months (median: 50.2 months) was available in 42 patients; of these 26 (61%) died of tumor with a median survival of 31 months

Conclusions: Unlike urothelial carcinomas of the bladder, the majority of primary urothelial carcinomas of the renal pelvis are of high histologic grade and present in advanced stages. The possibility of a high-grade urothelial carcinoma should always be considered in the evaluation of a tumor displaying unusual morphologic features in the renal pelvis.

715 Prostate-Specific Membrane Antigen (PSMA) Expression as a Predictor of Prostate Cancer Progression

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Background: Randomized clinical trial comparing watchful waiting vs. radical prostatectomy demonstrated significant risk reduction in the development of metastatic disease and cancer specific death in clinically localized prostate cancer (PCa). However, this study also suggests that 15-19 men require surgical treatment to prevent one clinical event. Therefore with the limitation of clinical parameters to distinguishing aggressive from indolent PCa, there exists a significant need for molecular biomarkers. Expression of PSMA, a type II transmembrane glycoprotein, has previously been associated with the development of early disease recurrence in PCa and is the focus of this study.

Design: We analyzed PSMA expression in localized and metastatic prostate cancers in a total of 96 patients (mean follow up 4.5 yrs.) using tissue microarrays. PSMA expression was detected with immunohistochemistry (DAKO clone 3E6). Cytoplasmic immunoreactivity was scored for the mean stained area by an automted quantitative microscope system (Chromavision ACISII). Based on the area showing positive staining, we divided our cohort in specimens with high and low PSMA expression.

Results: At the univariate level, significant parameters for predicting PSA recurrence included lymph node positivity (LN+) (0-20% LN+: HR=2.5, 95%CI=1.0-6.2, p<0.05; >20% LN+: HR=5.5, 95%CI=2.3-13.3, p<0.001), extraprostatic extension (HR=6.2, 95%CI=1.5-25.9, p<0.01), seminal vesicle invasion (SVI) (HR=3.9, 95%CI=2.0-7.9, p<0.001), and Gleason score 8-10 (HR=2.71, 95%CI=1.0-7.3, p<0.05). High PSMA levels were associated with significant increase of PSA recurrence (HR=3.3; 95%CI=1.8-6.3, p<0.001). The best multivariate model to predict PSA recurrence included PSMA expression (HR=1.7, 95%CI=1.1-2.5, p<0.01) and extraprostatic extension (HR=10.4, 95%CI=1.4-77.0, p=0.02) after adjusting for Gleason score and SVI.

Conclusions: In our patient cohort, parameters known to predict PSA recurrence such as high Gleason score, pT3c stage and pN+ status were associated with earlier PSA recurrence. One of the strongest predictor for PSA recurrence was high PSMA expression. In this cohort of high-risk patients, PSMA expression may serve as predictor of PCa aggressiveness.

716 Expression of PDGFR-alpha in Sarcomatoid Differentiation of Renal Cell Carcinoma (SRCC)

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Background: Sarcomatoid differentiation is present in approximately 5% of Renal Cell Carcinomas (RCC) and is considered a high-grade transformation associated with poor prognosis because its local aggressiveness, high metastatic potential and no effective treatment currently available when recurrence or metastasis occur. PDGFR-alpha is a membrane tyrosine-kinase receptor targeted by Imatinib whose expression has not yet been characterized in SRCC. Moreover the presence of c-Kit has been described in SRCC and Imatinib has been proposed as a target therapy for this neoplasm. The aim of this study was to determine the expression of PDGR-alpha by immunohistochemistry in SRCC.

Design: One tissue microarray (TMA) composed of 14 cases of SRCC was used. Triplicate 1 mm cores of the epithelial and the sarcomatoid component of each case were included in the TMA. The epithelial part corresponded to Clear Cell RCC in 8 cases, Chromophobe RCC in 4 cases, Papillary RCC in 1 case and Non-classificable RCC in 1 case. Immunohistochemical study was performed using polyclonal rabbit antibody against PDGFR-alfa (C-20, sc-338, Santa Cruz; dilution 1/100). The percentage of positive stained cells was categorized as focal (1-25% of tumor cells), moderate (25-50%) and diffuse (>50%).The intensity of expression was evaluated on a three-grade scale and the staining pattern was also considered. Both components were assessed separately.

Results: PDGFR-alfa was expressed exhibiting a cytoplasmic staining pattern in both the epithelial and the sarcomatoid areas of 92% and 100% of SRCC respectively.The positivity in the epithelial component was diffuse in 55%, focal in 30% and moderate in 15% of cases being the weak intensity the most often found (60%). In contrast, the positivity in the sarcomatoid differentiation was diffuse with intermediate intensity in almost all cases (92%).

Conclusions: In most of the SRCC cases studied an overexpression of PDGFR-alfa in the sarcomatoid part was observed in relation to the corresponding epithelial part. Besides this, a more intense and diffuse pattern of staining was found in the sarcomatoid component. These observations pose a possible role of PDGFR-alfa in the pathogenesis of the sarcomatoid differentiation of RCC. Further studies are needed to establish whether this fact as well as the expression of c-Kit in SRCC could be the basis of a targeted therapy with Imatinib.

717 Primary Leiomyomas and Leiomyosarcomas of the Kidney: A Clinicopathologic Study of Twenty Cases

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Background: Primary smooth muscle tumors of the kidney are rare. The purpose of this study was to characterize the clinicopathologic features of 20 primary renal leiomyomas and leiomyosarcomas.

Design: The study cohort consisted of 8 renal leiomyomas and 12 renal leiomyosarcomas diagnosed at the Mayo Clinic and retrieved from the personal consultation files of two of the authors. The diagnoses were based on established histologic criteria, which included assessment of mitotic count, presence or absence of coagulative necrosis, and nuclear atypia/pleomorphism. Clinical features were reviewed in all instances. Myoid-rich angiomyolipomas and primary retroperitoneal smooth muscle tumors were excluded from this study. Immunohistochemical stains were performed for actin muscle specific, smooth muscle actin, desmin, cytokeratins AE1/AE3, cytokeratin CAM 5.2, wide-spectrum cytokeratin, KIT, calretinin, EMA, CD31, CD34, CD99, bcl-2, HMB-45 and S-100 protein.

Results:

	Clinical Features	
	Leiomyoma	Leiomyosarcoma
Male %	50	33
Female %	50	66
Age (years old)	52	64
Age Range (years old)	22 to 74	35 to 84
Available Follow-up	2/8	3/12
Dead of Disease	0/2	3/3*
Follow-up Range (mos)	10 to 115	1 to 89
Tumor Size (cm)	7.4	9.3
Tumor Size Range (cm)	2.2 to 19	5.5 to 23

*6/12 leiomyosarcomas were high-grade; 3/3 dead of disease had high-grade tumors

	Immunophenotypic Features	
% Immunoreactive	Leiomyoma	Leiomyosarcoma
muscle specific actin	75	83
smooth muscle -actin	88	83
desmin	63	50
MIC-2 (CD 99)	50	100
BCL-2 +	63	100

Conclusions: Primary smooth muscle tumors are rare. Leiomyosarcomas are more common in males and leiomyomas affect both sexes equally. Among the tumors with available follow-up information, tumors classified as leiomyomas had an uneventful clinical course. In contrast, all leiomyosarcomas behaved in an aggressive fashion, leading ultimately to patient death.

718 Decreased Expression of Desmin Is Associated with Extracapsular Extension and Survival in Prostate Cancer

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Background: We have previously reported that reactive stroma is a predictor of recurrence free survival in PCa. Desmin is normally expressed in late muscle differentiation but its expression is decreased in myofibroblast in reactive stroma adjacent to PCa. The clinicopathological importance of this finding has not been previously described. In this study we evaluated if reduced expression of desmin is of predictive value in PCa.

Design: Tissue microarrays (TMA) were built using 483 radical prostatectomies. All TMA slides were stained for desmin and digitalized. Morphologic parameters such as wispy, disorganized, short, thin and fibrillary stroma (reactive stroma) versus, regular, long, parallel oriented fascicles (benign stroma) were compared. Desmin was interpreted using a score system 0-3+ (intensity (I) and percentage (P)) only in the reactive stroma. An index for desmin was obtained (staining intensity x percentage of positive cell per array). Spearman's test was applied for correlation between desmin index and clinicopathological variables. Kaplan-Meier analysis and Cox proportional hazard regression were used to assess for prognosis variables.

Results: Desmin expression was variable but in general decreased in compared to the native smooth muscle bundles of the prostate. Decreased desmin stain is negative correlated with extracapsular extension and survival (rho= -0.105, p= 0.0208). Lack of desmin expression was associated with a higher risk of biochemical recurrence on univariate but not multivariate analysis (118 moths versus 71 moths, p=0.0433).

Conclusions: Desmin stain is decreased is reactive stroma. The morphologic recognition of reactive stroma and its grading could provide additional valuable information to be documented in pathology reports in the future.

719 Expression of Claudin 1 and Claudin 7 in Prostatic Adenocarcinoma Whole Mount Sections

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Background: Claudin family members are associated with tight junctions of epithelial cells and interact with each other and regulate the paracellular permeability barrier between epithelial cells. Although changes in claudin pattern and their role in cancer development have been reported, their expression and localization in prostatic adenocarcinoma (PAC) has not been clearly defined. In this study, claudin 1 and claudin 7 expression in PAC is examined and compared with that in benign prostatic tissue.

Design: Formalin-fixed paraffin-embedded whole-mount radical prostatectomy specimens from 172 patients with PAC were evaluated for claudin 1 and claudin 7 expression by immunohistochemistry. Slides were stained by automated methods on the Xmatrx (BioGenex, San Ramon, CA) using monoclonal antibodies (Zymed, San Francisco CA). Hematoxylin and Eosin stained slides were reviewed and tumors graded based on the Gleason grading system. Benign prostatic tissue and tumors were scored for membraneous claudin 1 and claudin 7 expression semi quantitatively based on the distribution. These results were compared with Gleason grade and clinical stage.

Results: 43% (30/75) of low grade tumors with Gleason score of 6 or less showed increased expression of claudin 1 while 39% (29/75) of showed no increase or decrease in expression. 39% (38/97) of high grade tumors with Gleason score of 7 or higher, showed decreased expression of claudin 1 while 22% (21/97) showed no increase or decrease. 36% (27/75) of low grade showed increased expression of claudin 7 while 41% (31/75) showed no increase or decrease in expression. 39% (38/97) of high grade tumors showed decreased expression of claudin 7 while 45% (44/97) showed no increase or decrease. Loss of caudin 1 and claudin 7 expression in high grade tumors were significant (p=0.011 and p=0.004 respectively). Finally, when the results of staining distribution were compared with stage, neither of the claudins showed a statistically significant relationship with stage (p=NS).

Conclusions: Increased expression of claudin 1 and claudin 7 in low-grade PAC and the loss expression in high grade PAC suggest their role in prostate cancer progression. Given this inverse correlation of claudin 1 and claudin 7 expression with Gleason grade, known predictor of disease outcome, claudin 1 and caudin 7 can be used as potential prognostic factors in PAC.

720 Focal and Non-Focal Extraprostatic Extension. A Proposal for an Objective Evaluation

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Background: The International Consultation Committee organized by the WHO recommended to subdivide pT3a disease into $pT3a_1$ (focal extraprostatic extension, EPE) and $pT3a_2$ (non-focal EPE) due to the fact that prognosis differs considerably depending on whether there is focal or non-focal EPE. However, the best method to stratify the degree of EPE remains the subjective designation of focal *versus* non-focal. The aim of this study is to propose an objective evaluation of EPE according to the number of quadrants showing EPE in whole mount surgical specimens.

Design: From a total of 230 consecutive patients submitted to radical prostatectomy, 54 had EPE in surgical specimens histologically evaluated by complete embedding and whole mount processing. EPE 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. Focal EPE was evaluated by two methods: a) present in only one quadrant; or, b) in two quadrants of the specimen. Diffuse EPE: a) present in more than one quadrant; or, b) in more than two quadrants. These two methods of evaluation were compared according to clinicopathological findings. Tumor extent was evaluated by a point count semiquantitative method. The data were analyzed using the Mann-Whitney test and the Fisher's exact test. Time to PSA progression was compared using a log-rank survivorship analysis.

Results: There was no statistically significant difference between focal and non-focal EPE for the variables preoperative PSA (p=0.68), Gleason score (p=0.14), tumor extent (p=0.15), positive margins (PM) (p=0.40), seminal vesicle invasion (SVI) (p=0.15), and time to biochemical progression (p=0.16) when EPE was considered focal whenever present in only one quadrant. For EPE considered focal whenever present in two quadrants, there was statistically significant difference for Gleason score (p=0.04), tumor extent (p=0.01), and SVI (p<0.01). There was a trend for a shorter time to PSA progression in patients with non-focal EPE (p=0.09) and no difference for preoperative PSA (p=0.86) and PM (p=0.58).

Conclusions: This proposal for objective evaluation of focal (≤ 2 quadrants) and diffuse (> 2 quadrants) EPE in whole mount surgical specimens significantly stratified patients according to Gleason score, tumor extent and SVI. There was also a trend for shorter time to PSA progression in cases with diffuse EPE.

721 Nucleolar Pattern Telomerase Catalytic Subunit Staining as a Selective Aid in Prostate Biopsy Diagnosis

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Background: High levels of telomerase activity are associated with prostate cancer (PCa) but is low to undetectable in benign prostatic tissue. The majority of these studies have been performed using in-situ hybridization or telomerase activity assays. Recently a human telomerase catalytic subunit (hTERT) antibody, which is stated to preferentially stain malignant nuclei highlighting prominent nucleoli, has been described for the use in formalin fixed paraffin embedded tissues (NCL hTERT; Novacastra). Since the sine qua non for the diagnosis of PCa is the presence of prominent nucleoli in the absence of basal cell layer, we hypothesized that an antibody with selective localization for the nucleolus could be an adjunctive diagnostic marker in small and atypical foci of PCa.

Design: 40 needle core biopsies encompassing a wide range of prostatic histopathology were immunostained using monoclonal antibody specific for hTERT. The nucleolar and nuclear staining characteristics in benign, premalignant, and neoplastic acini were evaluated.

Results: 20/20 PCa foci (10, Gleason score 6; 8, Gleason score 7; 2, Gleason score 8) showed distinct nucleolar hTERT staining characterized by the presence of a single intense large prominent nucleolus. The staining was observed in most acini, irrespective of their prominence at the H & E level. Very frequently PCa also had multiple nucleoli often with peripheral margination of the nucleoli. The staining pattern of HGPIN (4/4) significantly overlapped with PCa staining pattern. One case of atypical adenomatous hyperplasia lacked the nucleolar staining pattern. Background lymphocytes showed pan-nuclear staining whereas basal cell hyperplasia nuclei showed a speckled nucleolar staining pattern similar to PCa and HGPIN. Benign prostate acini were typically negative although occasionally small inconspicuous nucleoli could be discerned at intermediate to high power magnification.

Conclusions: 1) Immunohistochemical staining for hTERT has potential but limited utility as a diagnostic adjunct for small atypical foci being considered for carcinoma. In the appropriate light microscopic context and in conjunction with a basal cell specific marker, the intense nucleolar staining for telomerase may be helpful in confirming carcinoma. 2) Contrary to molecular data, at the protein level telomerase activity is not restricted to malignant prostatic epithelium. Its expression in basal cells particularly those that are proliferating, is keeping with the totipotential reserve cell nature of the basal cells.

722 An Inverse Relationship between E-cadherin and COX-2 Expression Correlates with Aggressive Histologic Features in Prostate Cancer

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Background: The identification of biomarkers in prostatic carcinoma has yielded important data regarding prognosis and has aided in increasing diagnostic accuracy. Additionally, this approach has yielded important insights into the biology of prostatic carcinoma. A marker of interest is E-cadherin, which is an important protein involved in cell-extracellular matrix adhesion, and therefore a potential target in tumorigenesis. A second marker is COX-2, the inducible cyocloxygenase enzyme, known to be involved in many epithelial carcinomas.

Design: Retrospective identification of primary radical prostatectomy specimens from patients with extensive clinical followup was performed with the assistance of the Department of Urology. Following identification of these patients, cases were reviewed in their entirity, and tumor and normal specimens selected for construction of the tissue microarray. 3 tumor spots and 3 tissue spots were arrayed from 179 patients utilizing previously described techniques. Microarrays were cut and stained for E-cadherin and COX-2 expression by immunohistochemistry.

Results: The expression of the cyclooxygenase isoenzyme, COX-2 is significantly increased in prostatic carcinoma, while that of the cell adhesion molecule, E-cadherin, is decreased (p<0.001 for all comparisons). The expression of COX-2 was positively

correlated with higher tumor stage, perineural invasion, and the presence of carcinoma in surgical margins at prostatectomy ($p<0.001$ for all comparisons). Conversely, the expression of E-cadherin was inversely related to these prognostic indicators. Lastly, the expression of COX-2 and E-cadherin were very strongly and inversely correlated ($p<0.001$).

Conclusions: These results provide important insights into the biologic underpinnings of prostate carcinoma; and further studies into COX-2 expression in prostate core biopsies may show utility in pre-prostatectomy prognostication. Furthermore, these results may provide a rational basis for therapeutic intervention with COX-2 inhibitor therapy in prostate carcinoma.

723 E-Cadherin Protein Expression of Prostate Adenocarcinoma Independently Predicts Salvage Radiotherapy Outcomes

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Background: Pelvic or prostate bed radiotherapy (RT) is commonly utilized as attempted salvage treatment for patients who develop biochemical evidence of recurrent prostate cancer (PCA) after radical prostatectomy (RP). Although 4-year progression free survival is achievable in approximately 40% of patients, the majority of patients ultimately develop recurrent disease despite RT. Molecular biomarkers may improve predictive and prognostic models in patients unlikely to benefit from salvage RT. The purpose of this study was to determine if E-Cadherin expression was associated with outcomes after salvage RT among patients with biochemical evidence of failure after RP.

Design: A tissue micro array (TMA) was constructed from RP of 62 patients who underwent salvage RT for biochemical failure after RP. TMA was stained with antibody to E-Cadherin, a cell adhesion marker and scored for membranous and cytoplasmic staining intensity and percentage of tumor and normal staining. Normal E-Cadherin tumor staining was defined when all cores demonstrated => 70% strong membranous with <= 10% cytoplamic staining. The effect of E-Cadherin expression and other clinical and pathologic risk factors on a time to biochemical failure (post -RT nadir PSA plus 0.2 ng/ml, confirmed by second increase for patients receiving adjuvant androgen suppression therapy) were analyzed using Kaplan-Meier methods.

Results: Total 37 patients with long follow-up were available for E-Cadherin analysis. Twenty five patients had aberrant and twelve had normal E-Cadherin staining pattern. Of the various variables analyzed, only aberrant E-Cadherin expression was significantly associated with decreased failure-free survival (FFS) on univariate ($p=0.02$) and multivariate ($p=0.05$) analysis. There was an association between aberrant E-Cadherin expression and seminal vesicle invasion. Two year FFS was 55% for patients with aberrant E-Cadherin expression compared with 92% for patients with normal expression.

Conclusions: Aberrant E-Cadherin staining predicts for inferior outcomes after attempted salvage RT for biochemical failure after surgery and likely indicates subclinical disseminated disease in these patients. Early systemic therapy may be warranted in these patients. Molecular biomarkers may improve risk stratification models allowing improved tailoring of therapy recommendations and providing insights that will enhance efficacy of prostate RT.

724 Chromophobe Renal Cell Carcinoma and Oncocytoma Show Distinctive Expression Profiles by DNA Microarray and by Reverse Transcription-PCR Analysis

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Background: Morphological distinction between chromophobe renal cell carcinoma (ChrRCC) and oncocytoma (Onc) can be difficult in some cases. Although recent studies have shown that different subtypes of renal cell carcinoma can be distinguished by DNA microarray analysis, current literature suggests that ChrRCC carcinoma and Onc cannot be separated based on this gene expression profiling technique, possibly reflecting their common origin from the distal tubules.

Design: Oligonucleotide microarray analysis was performed on 6 cases of ChrRCC and 6 cases of Onc using Affymetrix U133plus 2.0 gene chip. Genes found to be differentially expressed were selected for validation with conventional and quantitative reverse transcription (RT)-PCR, using RNA extracted from both fresh-frozen and formalin-fixed tissues. A total of 27 ChrRCC and 22 oncocytoma were analyzed.

Results: Hierarchical clustering segregated six cases of ChrRCC from six cases of Onc based on the expression profiles of differentially expressed gene sets. Of >47,000 transcripts evaluated, 964 genes showed significant differences at expression level between ChrRCC and Onc (>2 fold changes and 1-way ANOVA $p<0.01$). Of six ChrRCC cases, two cases of eosinophilic variant were indistinguishable from the other four cases. Among the 964 genes, 14 genes were selected for validation by conventional RT-PCR using a panel of 14 fresh-frozen samples (8 Onc, 6 ChrRCC). Six of the 14 genes showed most consistent differential expression between ChrRCC and Onc. Three—IGFBP1, NBL1, and KCNG3—showed higher expression in Onc, whereas the other three—PROM2, PRSS8, and GATA3—showed higher expression in ChrRCC. Quantitative RT (qRT)-PCR was then performed, and these 14 fresh-frozen specimens were accurately classified based on the calculated mRNA expression ratios among these six genes. To further validate these findings and test whether they can be used in routine practice, RNA prepared from 35 paraffin-embedded specimen (14 Onc and 21 ChrRCC) were analyzed. Thirty of 35 cases (86%, including 14/14 Onc and 16/21 ChrRCC) were accurately predicted, indicating the potential of this molecular diagnostic approach.

Conclusions: ChrRCC and Onc are distinguishable by DNA microarray analysis. Furthermore, this difference in gene expression can be detected by qRT-PCR analysis of a small panel of genes using either fresh or formalin-fixed tissues, suggesting its potential in diagnostic pathology.

725 Classification of Renal Neoplasms on Needle Biopsies Based on Histology and a Molecular Diagnostic Algorithm

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Background: Ablative therapy (cryoablation or radio frequency ablation) and observation protocols are emerging as potential alternatives to nephrectomy in the management of benign renal neoplasms and non-clear cell renal cell carcinomas (RCC). To select patients for such protocols, it is essential to establish a pre-operative diagnosis in patients presenting with renal masses.

Design: Fifty-eight total or partial nephrectomy specimens were evaluated. Immediately after resection, the renal mass was localized by palpation and core biopsies (CBx) were performed on the specimen, simulating a pre-operative biopsy. Two cores were submitted for routine histology, and two were snap-frozen for RNA extraction. mRNA levels of carbonic anhydrase IX (CA9), racemase (AMACR), parvalbumin (PVALB), and kidney-specific chloride channel (CLCNKB) were analyzed by quantitative RT-PCR. The mRNA expression ratios among these genes were used to classify RCC based on a molecular diagnostic algorithm (MDA) that we previously established. A diagnosis on the CBx was rendered by combining histological and MDA results, which was then compared to the final diagnosis on the resection specimen.

Results: By combining histology of the CBx and the MDA, the correct diagnosis was established in 52 (90%) of 58 renal masses, including 43 of 46 RCC (29/31 clear cell, 9/10 papillary, 4/4 chromophobe, 1/1 unclassified), 4 of 4 oncocytoma, and 5 other diagnoses. The remaining 6 cases included 2 clear cell RCC, 1 papillary RCC (diagnosed as RCC-unclassified on CBx), 1 urothelial carcinoma, 1 gastrointestinal stromal tumor and 1 non-neoplastic lesion. Almost all core biopsies in these 6 cases were non-diagnostic due to sampling error, either no tumor or necrotic tissue only in the cores. Of the 52 cases correctly diagnosed and classified, histology alone was sufficient in 46 cases, with supporting MDA results seen in 41 cases. Of the 6 cases in which the histology on the core was either non-diagnostic or insufficient for RCC classification, MDA correctly diagnosed them as 4 clear cell, 1 papillary, and 1 chromophobe RCC.

Conclusions: Accurate diagnostic classification can be achieved by core biopsy in the majority of renal masses. Evaluation of gene expression profiles helps confirm histological diagnoses and is particularly useful in helping establish correct classification of RCC.

726 Comparision of Melanocytic Markers in Renal Angiomyolipomas

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Background: Renal angiomyolipoma (AML) is composed of varying amount of adipose tissue, smooth muscle and vessels. Characteristically, tumor cells express melanocytic markers, HMB-45 and Melan A being best characterized. Recently, several other melanocytic markers have been described to have excellent diagnostic sensitivity in cutaneous melanocytic lesions. In this study, we compared the sensitivity of 5 melanocytic markers in the diagnosis of renal angiomyolipoma. In addition, we also studied the expression of different melanocytic markers in 3 different components of AML.

Design: A tissue microarray (TMA) of 20 renal AML were constructed. For each case, 3 cores from fatty, vessel and smooth muscle predominant tumor areas were taken to construct the TMA. The TMA was then stained for 5 different melanocytic markers, including HMB-45, Melan-A, tyrosinase, NK1/C3 and CD117. Each core was graded as negative (no or weak staining), or positive (moderate or strong staining). A case was graded as positive for a marker if any one of the 9 cores from 3 components was positive.

Results: HMB-45, Melan-A, tyrosinase, NK1/C3 and CD117 were positive in 95% (19/20), 85% (3/20), 50% (10/20), 70% (14/20) and 40% (8/20) of cases, respectively. HMB-45 or Melan-A was positive in 100% (20/20) of cases. However, these 5 markers had different sensitivity in the fatty, vessel or smooth muscle predominant tumor areas (table). 95% (19/20) of fatty or smooth muscle predominant areas, and 85% (17/20) of vessel predominant areas, were positive for any one of the 5 markers. A combination of HMB-45, Melan-A and NK1/C3 improved the positive staining in smooth muscle predominant areas to 95% from 90% when only HMB-45 and Melan-A were used. Such triple markers did not improve the positive staining in fatty and vessel predominant areas.

Conclusions: HMB-45 or Melan-A is positive in 100% of renal AML and we recommend the use of these two markers in the work-up of AML. Other markers are of limited use. Since different melanocytic markers have different sensitivity in different components of AML, a tissue block containing of all 3, or fatty and smooth muscle components, should be used when performing staining for melanocytic markers.

	HMB-45	Melan-A	NK1/C3	tyrosinase	CD117
Fatty area	90%	70%	55%	30%	20%
Smooth muscle area	85%	60%	55%	40%	20%
Vessel area	80%	40%	45%	10%	10%

727 Does Prostate Central Zone Cancer Exist?

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Background: The prostate contains three anatomic zones, central (CZ), peripheral (PZ) and transition (TZ) with different susceptibilities to prostate cancer (PCa) and benign prostatic hyperplasia. Embryologically, most of the prostate gland derives from the urogenital sinus, but CZ is presumed to derive from the Wolffian duct, same as the seminal vesicles (SV). The characteristic histologic appearance and the reported lower proliferative and apoptotic index of the CZ confirms the unique biology of this region. This study documented the incidence and pathological characteristics of CZ PCa.

Design: For 250 consecutive radical prostatectomy (RP) for PCa, we mapped the tumor outline, determined the tumor volume (TV), the location relative to TZ, PZ and CZ, the Gleason score (GS), extraprostatic extension (EPE), SV and ejaculatory ducts (ED) involvement, lymphovascular invasion (LVI), nodal and resection margin status. Considering that the ducts of the CZ arise on the verumontanum and arborize proximally

surrounding the ED to make up most of the base of the prostate, only tumors involving the base and the ED region and tapering in volume towards the apex were considered as CZ PCa.
Results: We identified 10 PCa (4%) that appeared to lie entirely (n=3), or almost entirely (n=7) within the CZ. The age of the patients ranged from 46 to 66 years (mean 58.6) and the preoperative PSA (iPSA) ranged from 4.3 to 29.0 mg/ml (mean 11.7). The GS was 7 (n=5), 8 (n=1) and 9 (n=3). One patient received androgen deprivation therapy. The TV ranged from 200 mm2 to 640 mm2 (mean 416.5): the mean TV and iPSA for the PCa entirely within the CZ was 233.3 mm2 and 4.6 mg/ml, and 495 mm2 and 15.2 mg/ml for PCa spreading into adjacent zones. All tumors involved both lobes of the prostate and established EPE was present in all cases. The margins of resection were positive in 8 cases. The loose mesenchyme of the ED apparatus, and SV were involved by tumor in 8 (80%) cases, and in one there was peri-SV involvement. LVI was detected in 5 (50%) cases. Node dissection was performed in 1 case showing metastatic PCa in 1/15 nodes.
Conclusions: CZ PCa is uncommon and accounts for 4% of PCa. It frequently extends into adjacent prostate parenchyma, making the identification of the probable zone of origin challenging. It seems to be associated with adverse pathological features.

728 Does Quantitative Gene Expression Analysis of Androgen Recepter in Benign and Neoplastic Prostate Cancer Cells Predict PSA Recurrence?

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Background: Androgen signaling pathway is critical for growth and differentiation of prostate gland and its alterations may contribute to prostate cancer. The androgen pathway is also a major target of pharmaceuticals used in treatment of prostate cancer. Alterations of androgen receptor (AR) including mutation, amplification and over expression have been reported in advanced prostate cancer especially in the hormone refractory disease. We hypothesize that AR over expression may have the potential to define CaP with an aggressive clinical behavior in newly diagnosed hormone naïve patients.
Design: 105 hormone naïve patients with prostate cancer who underwent a radical prostatectomy (RP) at a singe institution were selected. Benign and neoplastic prostate epithelial cells were collected with LCM from frozen tissue slides obtained from the RP specimens. The expression of AR and a house-keeping gene, GAPDH were measured by duplex quantitative real-time RT-PCR (TaqMan) in 210 specimens. Fold change of AR expression in tumor (T) versus benign epithelial cells (N) was correlated with clinico-pathological features, such as Gleason score, pathological stage, age, race and PSA recurrence. Mean follow up was 38.9 months.
Results: The average age was 60 years and mean follow up was 38.9 months. 25.7% were African American, 54.5 % were pT3 or greater, 65.4% had a Gleason score of 7-9. 20% had a PSA recurrence. Paired t-test was used to compare AR expression normalized to GAPDH in tumor and benign cells. It demonstrated that over all AR expression is 50 times lower in the tumor than in normal tissue (p=0.0037). Stepwise Logistic Regression model based on age, race, PSA at time of diagnosis, pT stage, Gleason score, follow up time and AR expression (fold change between T and N) showed that increased AR fold change is an independent factor predicting PSA recurrence (p=0.0205), for the same pT stage and Gleason score. For each 2 fold increase in AR, the odds of PSA recurrence increased by 36.4%.
Conclusions: Quantitative comparison of AR gene expression in LCM-derived malignant and benign epithelial cells may define CaP with an aggressive behavior and may predict PSA recurrence. Although overall AR gene expression was lower in the tumor LCM-derived CaP epithelial cells, if AR fold change (tumor versus benign) was higher, there was an increased risk of PSA recurrence.

729 Positive Predictive Value of an Isolated HGPIN in Needle Biopsy Cores for Prostatic Adenocarcinoma – A Study with Complete Sampling of Hemi-Prostates with Correlating Negative Biopsies

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Background: High-grade prostatic intraepithelial neoplasia (HGPIN) is a putative premalignant lesion of prostate adenocarcinoma (PCa) and is closely associated with PCa in resected prostate (RP) specimens. The significance of isolated HGPIN in initial biopsies as a marker of PCa in repeat biopsies has been extensively investigated, but little is known of the actual rate of cancer in the whole prostate in this setting since repeat biopsies may miss the area of cancer. In this study we aim to define a more precise positive predictive value (PPV) of isolated HGPIN in initial biopsies in predicting cancer in the prostate gland.
Design: From 123 consecutive RP specimens we thoroughly examine the hemi-prostates for which all 5 biopsy cores are negative for PCa.
Results: Seventy RPs were associated with cores positive for PCa in only one side of the gland. The hemi-prostates corresponding to the sides negative for PCa on biopsy were grouped into : a) hemi-prostates with cancer (n= 38, including 7 clinically significant PCas). A total of 78 foci of HGPIN were identified in these hemi-prostates, and these were associated with eleven corresponding biopsies containing foci of HGPIN ; b) hemi-prostates without cancer (n= 32). A total of 46 foci of HGPIN were identified in these hemi-prostates, and these were associated with six corresponding biopsies containing foci of HGPIN. In the group of hemi-prostates with cancer, HGPIN was more often plurifocal and of micropapillary/flat type than in the group without cancer. Clinically significant PCas were associated with 4 out of 11 biopsies positive for HGPIN as compared with 3 out of 21 biopsies negative for HGPIN. The PPV of HGPIN was 64%, with a sensitivity of 28% and a specificity of 81%.
Conclusions: Despite a low sensitivity, HGPIN is associated with a high PPV and specificity for PCa in subsequent repeat biopsies and has a definite role in the indication for repeat biopsies. PCas associated with isolated HGPIN in initial biopsies were also more likely to be clinically significant.

730 Angiomyolipoma, Fat-Poor Variant – A Distinct Clinico-Pathologic Entity?

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Background: Angiomyolipoma (AML) is a triphasic neoplasm composed of irregular thick-walled blood vessels, smooth muscle and adipose tissue. Occasional AMLs have been shown to contain minimal amounts of fat, i.e. fat-poor (FP). As more radiologic studies are performed, such lesions appear to be more frequently discovered. We sought to evaluate their pathology and correlate with clinical picture.
Design: We identified 22 AMLs from archived specimens:13 females, 3 males, age range 28-83 and 44-50 respectively. The lesions were categorized based on the relative amount of adipose tissue as follows; FP, fat-average and fat-rich (<25%, 25-75%, >75% of fat respectively). IHC stains for SMA, HMB45, estrogen receptor (ER), and progesterone receptor (PR) were performed using standard methods. Patient histories were reviewed for pertinent information
Results: Only 1 woman had a history of tuberous sclerosis (TS) and lymphangioleiomyomatosis, all other lesions were sporadic. Nineteen AMLs were from females, 3 from males. Sixteen lesions were FP (73%), 4 fat average (18%) and 2 fat-rich (9%). Of the 2 fat-rich AMLs, 1 was found incidentally in a kidney donor and the other occurred concurrently with a FP-AML in a female with TS. The fat content did not correlate with a tumor size: largest FP and smallest fat-rich lesions were >6cm and <2cm, respectively. All lesions stained with SMA and HMB45. Nine lesions (40%) were positive for ER (8 females, 1 male) of which 7 (32%) were also positive for PR (6 females,1 male). ER/PR positive FP-AML in a male patient was diagnosed as epithelioid AML. Four females had >1 lesion and 4 also had uterine leiomyomata. Males had only single lesions. There is no evidence of metastases in any of the patients (follow-up 1 month-9 years).
Conclusions: In our series, fat content in AML was independent of tumor size and most cases were sporadic. FP-AML affected predominantly women (F:M 4:1) The majority of AMLs in this series were FP indicating that AMLs with a small component of adipose tissue radiologically mimic renal malignancy. FP-AML belongs to a spectrum of smooth muscle proliferations which encompases also mixed epithelial and stromal tumor of the kidney, leiomyoma and lymphangioleiomyomatosis. Thus, FP-AML should be included in the differential diagnosis of all renal spindle lesions. FP-AML may represent a distict entity characterized by smooth muscle proliferation which may be hormonally driven. FP-AMLs may be biologically different from “conventional” AMLs

731 Age and Race Distribution and Pathologic Characteristics of Radical Prostatectomy Specimens (RPS) of Patients Diagnosed with Prostate Cancer (Pca) with a Serum PSA <= 2ng/ml

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Background: There are increasing data to indicate that previously suggested “cut off “ values for serum PSA to help predict Pca detection on needle biopsy are significantly inconsistent. The findings of a recent prostate cancer prevention trial (PCPT) showed that 15% of men with normal rectal exam and a serum PSA < 4 ng/ml proved to have Pca on biopsy with 22% of these “unexpected” tumors having a GS of >=7. The objective of this study was to analyze the clinical profile of patients and the pathological features of their RPS performed for Pca diagnosis with a serum PSA <=2ng/ml.
Design: All patients treated with radical prostatectomy at our institutor between 1990 -2005 who had a preoperative serum PSA of <=2 ng/ml and have not received radiation or hormonal treatment prior to surgery were included in this study. The age, race, clinical stage, pathological stage, tumor volume and GS of the RPS were available on all patients.
Results: Ninety nine patients met the study criteria representing 3.7% of our RP cohort. 37, 56 and 6 (37%,56% and 6%) were African American (AA) Caucasians © and of other race/ethnic groups respectively. Mean age for AA 59 years (42-75) compared to 60, (41-78) for C, p=NS. Of the AA patients, 32 (86%), 2 (5%), 1 (3%), 1 (3%) and 1 (1%) had organ confined disease, Pca with positive margins, with extraprostatic extension, seminal vesicle invasion and with regional lymph node metastases respectively. Correlates with figures for C men were 47 (84%), 4 (7%), 2 (4%), 3 (5%) and 0 respectively. AA patients had a mean tumor volume of 3.12cc (0.01-72.24) compared to 1.19cc (0.02-5.76) in C men. Five (14%), 25 (68%), 4 (11%) and 3 (8%) of AA patients had an RPS Gleason score of 5, 6, 7 and 8or higher compared to 12 (21%), 27 (48%), 13 (23%) and 4 (7%) for C patients.
Conclusions: The mean age of Pca patients diagnosed with serum PSA of <=2ng/ml of 59.6 years is lower than that of the rest of our radical prostatectomy population (63.2 years) p<0.05. There is no significant difference in the racial representation in this cohort relative to the racial proportion of our surgical treatment population. While the majority of the study cohort (85%) had an organ confined disease and 74% have an RPS Gleason score of <=6, an important subset of these patients exhibited unfavorable pathological features in their RPS raising further questions about the correlation of serum PSA values with important Pca prognosticators.

732 The Significance of Finding a Small Focus of Prostatic Adenocarcinoma on Needle Biopsy

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Background: The significance of finding a minute focus of prostatic adenocarcinoma on needle biopsy in current practice in Australia is unknown.
Design: Surgical pathology files of 3 years from 2002 to 2005 were searched for patients with a minute focus (equal to or <0.5 mm) of Gleason score 6 prostatic adenocarcinoma

diagnosed on needle biopsy and treated by radical prostatectomy. Six to 20 biopsies were performed in each case with an average of 11 biopsies. Significant tumors were defined as cancers with a volume greater than 0.5cc with a Gleason score of 6 or cancers of any size with a Gleason score >6 in the radical prostatectomy.
Results: There were 46 patients from a total of 4104 prostate cancers diagnosed on needle biopsy during this period (1.1%) The mean age was 58 years (range 44 -68 years) and mean PSA, 6.5ng/ml. All patients were in clinical stage T1c. Average tumor volume was 2.3cc. Thirty-nine patients (84.7%) had clinically significant tumor with 15.2% showing stage 3 disease. Mean PSA and PSA density in patients with significant tumor were 6.3 (range 0.7 to 14.2) and 0.13 (range 0.04 to 0.37) compared with 7.4 (range 4.1-9.9) and 0.11 (range 0.04 to 0.16) in those with potentially insignificant carcinoma**.** Ten patients with clinically significant carcinoma (26%) had a PSA density of >0.16. The median time to radical prostatectomy in patients with and without significant cancer was 18 weeks and 15 weeks respectively. In eight patients with significant tumor, the predominant location of carcinoma in the radical prostatectomy was anterior or antero-lateral.
Conclusions: Finding a minute focus of prostate cancer on needle biopsy, even with the extended biopsy technique, does not mean clinically insignificant carcinoma in most cases. Given these findings, it appears inadvisable to adopt a watchful waiting approach in younger patients with minute foci of carcinoma on needle biopsy.

733 Analysis of PTEN Deletions in Clear Cell Renal Cell Carcinomas (CCRCC) Showing Intratumoral Grade Heterogeneity

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Background: CCRCC is the most common primary malignant kidney tumor in adults. Alterations in the VHL gene are the most common genetic change in CCRCC and are thought to play a pivotal role in early tumorigenesis. Loss of function of PTEN, a tumor suppressor gene on chromosome 10, leads to dysregulation of the AKT and MAP-kinase pathways. Recent evidence suggests that deletion of PTEN may be associated with CCRCC tumors of higher grade and stage. Intratumoral grade heterogeneity (ITGH) has been described in CCRCC, such that a given tumor may harbor clones of low and high-grade cells. The molecular events, including any role for PTEN deletion, associated with ITGH have not been described. The purpose of this study was to determine whether PTEN deletions are present in all tumor cells in CCRCC with ITGH, or only in high-grade cells as a part of a clonal progression event.
Design: We identified four cases of CCRCC in which there was striking ITGH characterized by a solid proliferation of low grade cells (Fuhrman grade 1-2/4) immediately adjacent to aggregates of high grade cells (Fuhrman grade 3-4/4), such that the two populations were easily discernible at low magnification. Archived formalin-fixed, paraffin embedded tissues from these cases were analyzed by standard dual-color FISH using commercially available DNA probes for the PTEN locus (band 10q23) and the centromere for chromosome 10 (band 10p11.1-q11.1). At least 200 non-overlapped intact interphase nuclei were scored for both low and high-grade areas in each tumor. These signal counts were compared to those obtained from benign proximal tubule epithelium in two control sections of normal renal parenchyma using standard statistical methods.
Results: Loss of PTEN signal relative to normal tubular epithelium was identified in 4/4 tumors. No homozygous PTEN deletions were observed with our FISH-based method. In 3/4 cases, loss of PTEN signaling was detected in both low and high-grade tumor cells. Apparently selective deletion of PTEN in high-grade cells was only identified in 1/4 cases showing ITGH.
Conclusions: Based on the assumption that ITGH in is indicative of clonal progression in CCRCC, we conclude that PTEN deletions are an early event in tumorigenesis and are not likely associated with evolution to phenotypically more aggressive disease.

734 Beta-Tubulin Immunoreactivity in Prostatic Intraepithelial Neoplasia (PIN) and Prostatic Adenocarcinoma

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Background: Beta-tubulin is a 55-kDa protein that heterodimerizes with alpha-tubulin; together they polymerize to form microtubules, a major cytoskeleton component and crucial element for mitosis. Anti-microtubule agents such as the taxanes and vinca alkaloids have been widely used in cancer chemotherapy. It was recently reported that beta-tubulin was one of the top genes overexpressed in prostate cancer by microarray analysis (Stuart RO et al. Proc Natl Acad Sci U S A. 2004). However, gene expression patterns for potential cancer biomarkers must be validated using other techniques such as immunohistochemistry. In the current study we evaluated beta-tubulin immunoreactivity in prostatic adenocarcinoma and high-grade PIN.
Design: Archival formalin-fixed, paraffin-embedded prostatic tissue comprising cases of high-grade PIN (n=30), Gleason pattern 3 (n=29) and Gleason pattern 4 (n=31) prostatic adenocarcinoma on tissue microarrays, were subjected to immunohistochemistry with a monoclonal antibody to beta-tubulin (1:1000). Immunoreactivity was scored semiquantitatively (0-3+).
Results: Typically, benign prostatic glands showed negative to minimal staining, while 29 of 30 cases of high-grade PIN (97%) showed strong immunoreactivity (mean 2.32), as did 28 of 29 cases of Gleason pattern 3 (97%; mean 2.17) and 26 of 31 cases of Gleason pattern 4 prostatic adenocarcinoma (84%; mean 1.94).
Conclusions: Using immunohistochemistry, we confirmed elevated expression of beta-tubulin in prostatic adenocarcinoma and high-grade PIN. The finding of beta-tubulin expression in PIN suggests that its overexpression may be an early event in prostate carcinogenesis. Our study also provides a rationale for potential medical therapy by inhibiting prostatic adenocarcinoma using anti-microtubule drugs.

735 Ceruloplasmin Immunoreactivity in Renal Cell Carcinoma

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Background: cDNA microarray technology is a powerful tool for identifying potential cancer biomarkers based on gene expression patterns. Using subtractive hybridization, a less sophisticated method, other investigators have shown that the gene encoding ceruloplasmin, the major serum copper-binding protein, is overexpressed in renal cell carcinoma (RCC) compared to noncancerous tissue. We found that ceruloplasmin mRNA was 16.9-fold higher than that of normal kidney tissue in a cDNA microarray analysis of 30,000 genes in RCC. In the current study we evaluated ceruloplasmin immunoreactivity in a set of renal epithelial tumors.
Design: Archival formalin-fixed, paraffin-embedded renal tumors and uninvolved parenchyma were used to construct tissue microarrays comprising 30 clear cell RCCs, 23 papillary RCCs, 11 chromophobe RCCs, 16 oncocytomas and 5 cores of noncancerous renal tissue. The tissue microarrays were stained with a monoclonal antibody to ceruloplasmin (1:50) and immunoreactivity was scored semiquantitatively (0-3+).
Results: Twenty-seven of 30 clear cell RCCs (90%) showed immunoreactivity (mean 2.17), as did 19 of 23 papillary RCCs (83%; mean 1.52), 11 of 11 chromophobe RCCs (100%; mean 2.36), and 12 of 16 oncocytomas (75%; mean 1.31). Noncancerous renal tissue typically showed negative or weak staining (mean 0.8).
Conclusions: Ceruloplasmin is overexpressed in renal epithelial tumors (especially chromophobe and clear cell RCC) relative to noncancerous kidney tissue. Further studies are necessary to evaluate whether ceruloplasmin can be used as a serum biomarker for RCC, considering that ceruloplasmin serum testing is readily available in clinical chemistry laboratories.

736 Renal Cell Carcinoma in Patients under 40

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Background: Renal cell carcinoma (RCC) types, as defined by the 2004 WHO classification, and clinical outcome for young patients with renal cell carcinoma are not well-characterized.
Design: Using a computer database search for nephrectomies for RCC (partial and radical) performed at Barnes-Jewish and St. Louis Children's Hospitals during the years 1989 to 2005, 63 samples from 57 patients younger than 40 years were identified. The RCCs were typed according to the 2004 WHO classification, and data on nuclear grade, number of masses, size, involvement of the ureter or renal vessels, lymphvascular space invasion and extrarenal extension were captured. Immunohistochemical staining for TFE3 (overexpressed in Xp11.2 translocation RCC) was performed in 54 cases.
Results: The study group included 36 men and 21 women. 48 patients (84%) were 30-39 years of age, 7 patients (12%) were 20-29, and 2 (3.5%) were younger than 10. 43 of 57 (75%) tumors were RCC of clear cell type, 5 were chromophobe, and 3 were papillary. The remaining cases were as follows: 2 translocation carcinomas, 1 tubulocystic, 1 multilocular cystic, 1 mixed tumor with clear cell and papillary features, and 1 sarcomatoid carcinoma. Mean tumor size was 4.7 cm, with a median of 3.6 cm (range 1 to 13 cm). In 43 (75%) cases there was a single mass. 8 (14%) tumors had Fuhrman nuclear grade 1, 34 (60%) grade 2, 13 (23%) grade 3, and 2 tumors (2.5%) grade 4. Renal vein involvement and lymphvascular space invasion were documented in a single case. 40 tumors (70%) were stage pT1, 10 (17%) stage pT2, 5 (8.8%) stage pT3. TFE3 immunostain was strongly and diffusely positive in the carcinoma nuclei of 2 cases; both were patients under 10. Currently, 43 of 55 (78%) patients have no evidence of recurrence. 4 of 55 (7%) patients died of cancer, at a mean of 155 +/- 7 months. These patients were 31, 32, 39 and 39 years old at the time of diagnosis. 3 of these patients had clear cell carcinoma and the fourth sarcomatoid carcinoma.
Conclusions: Most RCCs in patients younger than 40 years are clear cell type and confined to the kidney. TFE3-positive RCCs are rare and in this series were found exclusively in patients less than 10 years of age. The outcome for young patients with RCC appears favorable.

737 M2A Antigen (Podoplanin) Distribution in Prepubertal and Adult Testes and Testicular Tumors

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Background: The oncofetal antigen M2A is probably identical to podoplanin, which can be demonstrated by the monoclonal antibody D2-40. M2A has been reported in human germ cell tumors of the testis and ovary. The purpose of this study is to evaluate the distribution of D2-40 in the testis and testicular tumors of adults and prepubertal children.
Design: Sections of testicular tumor of 7 prepubertal children and 27 adult patients were utilized. Following deparaffinization the sections were incubated in D2-40 (Biocare Medical Concord, CA) at a dilution of 1:20 for 1 hour. Followed by 30 minutes in biotinylated horse antimouse (Vector Burlingham, CA) 1:400 and ABC (Vector, Burlingham, CA). Vector VIP was used as a chromogen . Slides were counterstained in hematoxylin dehydrated and mounted.
Results: In the adult and prepubertal testis, D2-40 was identified in endothelial cells of lymphatics, the epithelium of the rete and in the mesothelial cells of the tunica. Epididymal cells were negative. In the prepubertal testis, Sertoli cells were consistently positive. The benign germ cells in the prepubertal and adult testis were negative. Intratubular germ cell neoplasia, unclassified type and all 7 seminomas were consistently strongly positive. Eleven of thirteen embryonal carcinomas were positive, usually in less than 25% of cells. In prepubertal children all 3 yolk sac tumors were negative, and 3 of 6 yolk sac tumors in the adult patients were positive. Of eight teratomas in infants and adult patients, four were positive in rare glandular epithelial cells and stromal cells. The choriocarcinoma was negative. Isolated syncytiotrophoblasts occurring in other germ cell tumor types were only rarely positive. All 4 spermatocytic seminomas

were negative. One Leydig cell tumor was focally positive (25% of cells) and a juvenile granulosa cell tumor was negative.
Conclusions: D2-40 is a good marker for intratubular germ cell neoplasia of the unclassified type. Of the germ cell tumors, only seminoma is consistently positive. In addition to germ cell tumors stromal tumors can be positive.

738 Podoplanin Expression in Prostate

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Background: Podoplanin is a marker of lymphatic endothelial cells. It has also been identified in a number of other cell types including myoepithelial cells of glandular organs. The purpose of this study was to determine the distribution of podoplanin in the prostate.
Design: Sections of 23 prostates with carcinoma and sections of 9 basal cell hyperplasias were utilized. Seven of the carcinomas were whole mounts and 16 were conventionally processed. Following deparaffinization the sections were incubated in D2-40 (Biocare Medical Concord, CA) at a dilution of 1:20 for 1 hour. Followed by 30 minutes in biotinylated horse antimouse (Vector Burlingham, CA) 1:400 and ABC (Vector, Burlingham, CA). Vector VIP was used as a chromogen . Slides were counterstained in hematoxylin dehydrated and mounted.
Results: The endothelial cells of lymphatics were positive for D2-40. Only a subset of basal cells in benign and hyperplastic glands were positive. Secretory cells were consistently negative. Urothelial cells in the prostatic urethra and prostatic ducts were negative. Basal cells associated with prostatic intraepithelial neoplasia were sometimes positive. Ejaculatory ducts and seminal vesicles were negative. Five cases of basal cell hyperplasia were focally positive. All prostatic adenocarcinoma irrespective of grade were negative. In 3 of the 23 patients with prostate cancer lymphatic invasion by tumor could be identified. All of these were whole mounts and the lymphatic invasion was very focal and usually at the advancing edge of the tumor. In this small number of cases we were unable to identify lymphangiogensis in the tumors.
Conclusions: D2-40 is helpful identifying lymphatic invasion, which in the H&E stained sections may be either missed or misinterpreted as artifactual space due to shrinkage following fixation. The reason for the focal positivity in basal cells is not clear.

739 High Carbonic Anhydrase (CA) IX Protein Tissue Expression Predicts Response to Interleukin (IL)-2 Based Therapy for Advanced Renal Cell Carcinoma Patients

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Background: Recent understanding of the molecular changes associated with renal cell carcinoma (RCC) may lead to more specific predictors of prognosis and response to therapy. High dose interleukin-2 (IL-2) remains only approved therapy for metastatic RCC. However, only small subset of patients respond to such therapy and treatment is associated with high degree of toxicity. We investigated the histological parameters and potential biologic markers associated with prediction of response to IL-2 therapy.
Design: A 426 core tissue micro array (TMA) of 27 patients with advanced RCC, treated with IL-2 based therapy was stained with polyclonal anti-CA IX antibody (1:200, NOVUS BIOLOGICALS). Membranous staining intensity on the scale of 1(none), 2 (weak), 3 (moderate) to 4 (strong) and percentage expression on a scale of 1-100% were evaluated in each core. Overall expression was evaluated by multiplying the stain intensity by 100 and adding percentage expression. The significance of CA IX expression, histological type and prediction of response to IL-2 therapy was determined.
Results: Of 27 patients treated with IL-2 therapy, 11 patients were responders, 13 non-responders and 3 were not tested. Within responders 10 had clear cell RCC, and 1 with papillary RCC morphology, while within non-responders 9 had clear cell RCC and 4 with other than clear cell RCC morphology. The difference between median expression of CA IX between responders (median 490, range 220-500, standard error (SE) 90) and non responders (median 470, range 100-490, SE 123) was significant using Wilcoxon Sum Rank test (p=0.01). CA IX was also associated with response more often in patients without clear cell RCC (p<0.01, Kruskal-Wallis test).
Conclusions: High expression of CA IX is associated with positive response to IL-2 therapy. Patients with clear cell RCC are more responsive to treatment than with non clear cell morphology, however in patients with non clear cell RCC; high CA IX expression was also associated with response to treatment. Our results support that CA IX staining is a valuable adjunct to morphological assessment in prediction to the response to therapy.

740 Neurovascular Tissue Amount on Prostatectomy Specimens Is Less Predictive of Quality of Life Outcomes Than Surgeon's Description of Nerve Sparing Procedure

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Background: Preservation of neurovascular (NV) tissue at the time of prostatectomy resection has been correlated to the patient's subsequent erectile function. During surgery NV tissue may be bilaterally or unilaterally spared or resected. We evaluated whether the amount of NV tissue remaining on the prostatectomy specimen could predict surgeon's intention of nerve sparing and quality of life outcomes.
Design: Sixty patients with various degrees of nerve sparing procedures were evaluated for NV tissue amount. Patients were matched by age, pre-operative PSA, Gleason score, and clinical stage. NV tissue thickness as 1 (none), 2 (few nerve twigs), 3 (frequent nerve bundles/small ganglion but not associated with big vessels), and 4 (thick nerve/ganglion associated with big vessels) were bilaterally measured at the apex, base and seminal vesicles in 48 patients with available pathology. Forty-two patients had completed the Extended Prostate Cancer Index Composite (EPIC), a validated quality of life questionnaire. The differences in NV tissue amount and surgeon's description of nerve sparing procedure were analyzed using Fisher's exact test, while Kruskal-Wallis test was used to determine potency rate differences between groups.
Results: Surgeon's intent of NV preservation was associated with post-operative potency (P=0.05). However, amount of NV tissue (4 versus 1-3 group) compared to surgeon's intent at NV preservation did not correlate, particularly at the base of the prostate but it does approach significance at the left apex and seminal vesicle (P=0.082 and 0.056, respectively). The majority of patients had thick NV tissue (4) at the base of prostate, regardless of intent at NV preservation. No difference existed in baseline adjusted sexual function domain scores and NV tissue thickness although patients appeared to return to their baseline when less tissue was taken at the base from either side.
Conclusions: The amount of NV tissue thickness is less predictive of post-operative potency rates than surgeon's description of nerve sparing procedure. The significant amount of thick NV tissue (4) at the base could be related to nerve innervations at that spot and NV tissue associated with potency may be present at the apex. The fact that surgeon's intent does predict post-operative potency may demonstrate that how the tissue is handled intra-operatively and not the amount of NV tissue is what impacts functional outcome.

741 Topographical Distribution of Prostatic Urothelial Carcinoma in Patients with Bladder Cancer: Implication for Transurethral Biopsy and Pathologic Examination

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Background: Prostatic involvement by urothelial carcinoma (UCa) is a frequent finding in patients with bladder UCa. Accurate assessment of prostatic involvement by UCa provides important information regarding tumor staging, prognosis, choice of urinary diversion, the risk of recurrence in the retained urethra, and overall treatment strategies. Very few studies have determined the location and frequency of prostatic UCa within the entire prostate.
Design: Entire prostate were examined by whole-mount sections for UCa in 214 cystoprostatectomy specimens from the files at the Methodist Hospital, Houston, Texas between 1988 and 2003. Specifically, we evaluated: 1) Location of UCa: bladder neck, prostate base, mid, and apex portion of prostate; 2) Location of CIS: prostatic urethra; prostatic duct, or prostatic acini; and 3) Types of invasive UCa: transurethral invasion versus penetration from bladder cancer.
Results: Prostatic involvement of UCa was detected in 69 of 214 (32%) patients with bladder UCa. The UCa involved all levels of prostate in 17 (25%), bladder neck margin or prostate base in 35 (51%), mid prostate in 12 (17%), and prostatic apex in 5 cases (7%). Thirty cases (44%) were CIS only and 39 (56%) were invasive UCa. CIS occurred either in prostatic urethral (20%), in prostatic ducts and acini (47%) and or in both prostatic urethra and ducts/acini (33%). Ten of the 39 (26%) invasive UCa represented direct penetration from the primary tumor in the bladder and the remaining 29 (74%) cases arose from prostatic urethra or ducts.
Conclusions: Prostatic UCa was identified predominantly proximal to the verumontanum extending to the base of prostate and bladder neck. Prostatic urothelial CIS was found more often in prostatic ducts and acini than the prostatic urethra, highlighting the importance of thorough pathologic examination of the prostate for accurate assessment of prostatic UCa involvement. The pathway of prostatic invasive UCa, either via transurethral invasion or direct penetration from the primary bladder cancer should be carefully evaluated and noted in the pathology report.

742 Mucinous Tubular and Spindle Cell Carcinoma of Kidney: Histologic and Immunohistochemical Features Favoring Proximal Nephron Differentiation

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Background: The new 2004 WHO classification on renal tumors includes a new and rare morphologic variant of renal carcinoma under the term of mucinous tubular and spindle cell carcinoma (MTSCC). A number of studies have described this rare morphologic entity, however, its histogenetic origin or differentiation remains unclear. In this study, we described in detail the clinicopathologic features and immunohistochemical profiles of 11 new cases of MTSCC.
Design: Eleven cases of MTSCC with characteristic morphologic features were retrieved from the files of three institutions. Slides and pathology reports were reviewed. Immunohistochemical stains were performed for markers of proximal tubules (RCC marker, CD15, P504), distal tubules (Ksp-cadherin) and others.
Results: The age range of the patients was 35 to 73 with a median of 56 years. The male to female ratio was 9:2. All the tumors were confined to the kidney without perirenal fat, pelvic, or renal vein invasion. Two patients had nodal metastasis, one at the time of nephrectomy and the other had nodal metastasis 5 years after nephrectomy. None of the patients died with follow-up between 4 months to 6 years. The mean tumor size was 6.9 cm (range 2-17 cm). All tumors were composed of tubular and spindle cell areas with focal to prominent myxoid stroma. Foamy macrophages were seen in 10 of 11 cases, and were prominent in 4 cases. Focal compressed tubulo-papillary growth pattern was seen in 9 of 11 cases. The tumor cells in the tubular areas were uniformly cuboidal to ovoid and had inconspicuous nucleoli (Furhman nuclear grade 2 or 3). Focal necrosis was seen in 2 cases. Immunostains were positive for RCC marker (11/11), CD15 (9/11), P504 (10/11), Ksp-cadherin (1/11), vimentin (7/7), CD10 (3/7), EMA (4/5), CK20 (0/4), chromogranin (2/11), and synaptophysin (2/11).

Conclusions: We report the histologic features and immunohistochemical profiles on 11 cases of MTSCC of kidney. Its morphologic features such as presence of myxoid-mucinous stroma, foamy macrophages, focal compressed tubulo-papillary growth pattern, and preferential strong expression of proximal tubule markers support that this tumor is of a RCC with proximal tubular differentiation and appears closely related to or an actual morphologic variant of papillary RCC.

743 Metastatic Renal Cell Carcinoma: Patterns, Histology and Comparison to Primary Tumor

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Background: Approximately 30% of patients with renal cell carcinoma (RCC) initially present with metastasis. Metastatectomy for metastatic RCC is not infrequently performed for therapeutic purposes or confirmatory diagnosis before initiation of systemic therapy. However, only a few studies have addressed the metastatic patterns and routes of RCC by histologic subtypes and correlated with the primary kidney tumors.

Design: A total of 257 metastatic RCCs were identified from a review of the pathology database (1990-2005) at a single institution. The histopathologic features of metastatic tumors were reviewed and were correlated with those of primary tumor treated by nephrectomy if available. Comparisons were made between different histologic subtypes and time to metastasis from nephrectomy.

Results: The patients' mean age was 59 years (range 19-92). The male to female ratio was 188:69 (2.7:1). The histologic subtypes and frequency of metastasis were as follows: clear cell 199 (77%), papillary 22 (9%), chromophobe 4 (2%), collecting duct carcinoma 4 (2%), unclassified 7 (3%), and unspecified 14 (6%). The most frequent sites of metastasis were bone & soft tissue (79), lung (75), lymph node (25), brain (21), liver (12), pancreas (8), and GI tract (7). The lung and bone & soft tissue metastasis accounted for 62% of metastatic clear cell RCC; however, lymph node metastasis account for 41% of metastatic papillary RCC. 25% patients had at least 2 or more metastasis. The most common sites with multiple metastases were lung (32), bone and soft tissue (29), brain (14) and lymph node (10). Pathology material from 137 nephrectomies was available for review. All patients underwent radical nephrectomy. Seventy-three percent of the tumors were clear cell types, 11% were papillary and 7% were unclassified RCCs. Seventy-four percent of the tumors were of grade 3 or 4, mean tumor size was 9.2 cm, and 68% were stage T3 or T4 tumors. For the time from nephrectomy to metastasis (n=132), 17%, 23%, 26%, and 26% were identified before, during, within 2 years of, and longer than 2 year after nephrectomy, respectively.

Conclusions: Metastatic RCC phenotypes closely recapitulate the primary histology. Clear cell RCC has a strong predilection for hemotogenous spread and frequently metastasizes to the lung, bone and soft tissue, brain, and liver, whereas papillary RCC often metastasizes to regional lymph nodes. RCC metastases occur evenly before, during and many years after nephrectomy.

744 The Role of P501S and PSA in the Diagnosis of Metastatic Adenocarcinoma of the Prostate

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Background: A small subset of metastatic prostatic adenocarcinoma is negative for widely used prostatic markers (PSA and PSAP) and can present as metastatic carcinoma of unknown origin. P501S is a novel prostate-specific marker that originally was identified by cDNA library subtraction in conjunction with high-throughput microarray screening of prostate carcinomas. It is a 553-amino acid protein (also known as prostein), that is localized to the Golgi complex and is expressed by both benign and neoplastic prostate tissue, but has not been detected in any other normal or malignant tissue examined.

Design: Five micron sections of a previously constructed tissue microarray (TMA) containing 78 cases of metastatic prostatic adenocarcinoma (14 distant sites, 64 pelvic lymph nodes), 20 cases of primary prostatic adenocarcinoma, 20 cases of benign prostate tissue from the peripheral zone of the prostate, as well as samples of benign brain, pancreas, kidney, thyroid, testis, skeletal muscle, fibroconnective tissue were subjected to immunohistochemistry with a monoclonal mouse anti-p501s (clone 10E3, DakoCytomation, Carpintera, CA) antibody and a monoclonal mouse anti-PSA (clone ER-PR8, DakoCytomation, Carpintera, CA) antibody.

Results: Similar staining pattern (intensity and distribution) was identified for both markers in 63 metastatic tumors (10 distant sites, 53 pelvic lymph nodes), in all 20 primary tumors and 20 benign prostate tissue. Two distant metastases were negative for PSA and focally positive (weakly) for P501S, and two other distant metastases were only weakly PSA positive, but strongly P501S positive. Metastases in the pelvic lymph nodes showed similar staining pattern in 53 cases for both markers, 7 showed stronger PSA positivity and 4 showed stronger P501S positivity. None of the tumors were negative for both markers and all non-prostate tissue was negative for both markers. In summary, 2 of 78 cases (2.6%) of metastatic prostatic carcinomas were PSA negative, whereas all cases showed at least focal weak reactivity for P501S (100%).

Conclusions: Immunohistochemistry for P501S is a sensitive and highly specific method for identifying prostate tissue (benign or malignant). Immunohistochemistry for P501S can be useful for identifying prostatic origin in metastatic tumors of uncertain origin alone or in combination with other prostate specific markers (i.e.: PSA) resulting in increased specificity.

745 Chronic Orchialgia: Histologic Findings in 23 Cases

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Background: Chronic testicular pain is a difficult clinical problem. Treatment may result in epididymectomy or orchioepididymectomy, after excluding known causes of pain such as hernia, tumor, torsion, infection, varicocele, hydrocele, and spermatocele. In the few published series describing the histologic features in epididymectomy specimens performed for post-vasectomy chronic orchialgia, dilated epididymal tubules with inspissated spermatozoa and sperm granulomas were the most frequent findings. The goal of this study was to expand the current knowledge about these uncommon pathological specimens.

Design: Computerized surgical pathology files were searched for orchiectomies and epidymectmies performed for chronic testicular pain over a 5-year period (1999-2004). For a control group, a similar search was performed for orchiectomies and/or epididymectomies performed for causes other than pain and in autopsy archival tissues of testis and epididymis over a one-year period. The hematoxylin and eosin stained slides were reviewed and pathologic features noted. Identified histological abnormalities in each group were tabulated and the extent of histologic changes was graded on a scale of 1 to 3 (representing limited to diffuse histologic abnormalities). Features that were graded 2 or 3 were considered significant. The pathologic abnormalities observed in the study group were compared to the findings in the control group.

Results: A total of 72 orchiectomy and epididymectomies were performed in the four-year period. 23 specimens were reviewed representing 23 patients with history of chronic orchialgia, (12 orchioepididymectomy, 10 epididymectomy and one orchiectomy). The mean age was 47.8 years and 47.7 years for the study and control groups respectively. In the study group, the most frequently identified histological abnormalities of at least moderate severity were, testicular atrophy (n=12), dilatation of epididymal tubules with inspissated spermatozoa (n=9), interstitial chronic inflammation (n=8) and sperm granuloma formation (n=3). Two testes were completely atrophic and were clinically associated with undescended testis. In contrast, the control group showed only one case each of significant testicular atrophy and of cystically dilated epididymal tubules.

Conclusions: In our study atrophy, dilatation of epididymal tubules with spermatozoa retention, and chronic interstitial inflammation showed a positive association with chronic testicular pain leading to surgical intervention. The histologic abnormalities suggest obstruction as a possible pathogenesis of chronic orchialgia.

746 Minichromosome Maintenance Protein 6 Expression in the Prostate: A Tissue Microarray Analysis

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Background: Minichromosome maintenance proteins are DNA binding proteins that have been found to be good proliferation markers. They have not been well studied in prostate carcinomas, which are slow-growing neoplasms. Previous studies using proliferation markers such as, Ki-67 and proliferating cell nuclear antigen, do not show clinical utility in differentiating benign prostatic tissue from prostatic intraepithelial neoplasia (PIN) and prostatic carcinoma (PCa). Here we describe an immunohistochemical (IHC) study using tissue microarrays to compare the expression of MCM6 and Ki-67 in benign prostate, PIN and PCa.

Design: Two prostate tissue microarrays were constructed from 80 prostatectomy cases containing normal prostate (N), BPH, PIN in one, low grade (Gleason pattern 1-3, LG) and high grade (Gleason pattern 4-5, HG) PCa in the other. They were stained with MCM6 (goat polyclonal, Santa Cruz) and Ki-67 (MIB-1, Dako). Staining intensity (0 to 3+) and percentage of positive epithelial cells (nuclear staining) were scored, and the product of the two was used to produce a proliferation index (PI). The location of the positive cells was also noted. PI was used to separate cases into two groups: Low ($PI < 10$) and High ($PI \geq 10$). Fisher Exact test was used to generate p values for statistical testing.

Results: Nuclear staining was seen mostly within the basal layer in benign prostatic tissue. Compared to Ki-67, MCM 6 had more cases with a high PI in both benign and malignant prostatic tissue. Ki-67 did not discriminate between benign prostate, PIN and PCa. There were statistically significant differences in MCM 6 expression between benign prostate and PIN ($p = 0.012$), between benign prostate and PCa ($p<0.01$), and between PIN and PCa ($p = 0.009$).

	Results			
	Ki-67 (% cases with High PI)	P value	MCM6 (%cases with High PI)	P value
Benign	0.85%		4.81%	
PCa	6.03%	0.032	54.46%	<0.001

p value compared to Benign

Conclusions: MCM6 is a good marker for proliferation in prostatic carcinoma and has increased expression in PIN and PCa compared to benign tissue. It is also more sensitive than Ki-67, which can facilitate analysis of small prostate tissue samples (needle biopsy and TMA core). MCM6 is a potentially useful proliferation marker to distinguish between benign prostate, PIN and PCa. Further studies regarding the utility of MCM6 and other members of the MCM protein family in predicting PCa prognosis, using prognostic tissue microarrays, should be pursued.

747 Mirror Image Tissue Banking of Radical Prostatectomy Specimens Enables Isolation of Epithelial Plasma Membranes for Proteomics While Preserving Pathologic Quality of Retrieved Tissues

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Background: Proteomic studies on human prostate cancer are limited by the fact that this malignancy cannot be reliably detected by gross examination. We sought to devise and test a protocol for processing radical prostatectomies(RP) which permits epithelial plasma membrane isolation for proteomics while allowing for tissue retrieval with retention of good histologic quality.

Design: RP from 84 patients(median age 61; median PSA 5.9; 66% nonpalpable, T1c) were processed with alternate slices submitted for histology and tissue banking. Benign and malignant foci were macrodissected from the banked sections using the pathologically mapped, mirror image histology sections as a guide. Epithelial plasma membranes were isolated and their purity was assessed by Western blot probing for epithelial(PSMA, ESA) and non-epithelial(CD45(lymphocytes), CD90(fibroblasts)) cells and other intracellular organelles(P62(nucleus), calnexin(ER), Tom20(mitochondria)). Tissue homogenates were also probed for malignant (AMACR) and benign (P63) markers to test the accuracy of this protocol. 37 banked slices from 17 patients were retrieved, thawed and compared histologically(score: 1=fair, 2=good, 3=excellent) and immunohistochemically(antibodies: CKN 34βE12, P63, AMACR, PSA) to their corresponding routinely processed alternate slices.

Results: Plasma membrane preparations showed enrichment of epithelial plasma membrane markers (PSMA and ESA) with minimal marker expression of plasma membrane from non-epithelial cells(CD45, CD90) or intracellular organelles(P62, calnexin, Tom20). Cancer tissue homogenates showed AMACR/P63 upregulation whereas AMACR/P63 was downregulated in benign tissue homogenates. There was 30% benign (P63+) contamination in macrodissected cancer tissue and 5% cancer (AMACR+) contamination in benign tissue. Retrieved, thawed tissue showed retention of immunoreactivity while its histology was always adequate for diagnosis though slightly inferior to routinely processed tissue(mean score: 2.65 vs 2.84, p=0.0016).

Conclusions: Mirror image banking of prostatectomies is relatively accurate, permits plasma membrane isolation for proteomics and enables retrieval of banked tissue for clinical purposes with retention of good histologic and immunohistochemical quality.

748 Genetic Heterogeneity of androgen Sensitive and Androgen Independent Prostate Cancer: An Analysis of Androgen Receptor CAG Repeat Lengths in Germline, Somatic and Hormone Refractory Prostate Cancer Tissues

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Background: Shorter CAG repeat lengths(CRL) of the androgen receptor(*AR*) gene have been related to elevated *AR* transactivational activity in prostate cancer(Pca) cell lines and associated with increased risk of prostate cancer in studies done on peripheral blood leukocytes. Recent data on microdissected prostate cancer tissues has shown genetically heterogeneous CRL. Whether clones with shortened or deleted CRL are selected for in an androgen deprived milieu is not known as no data exist on CRL in human androgen independent Pca tissues. As well, no direct CRL comparison has been reported on blood and somatic tissues. We therefore investigated *AR* CAG repeat lengths in androgen independent Pca tissues as well as in matched samples of germline blood leukocytes and somatic androgen sensitive prostate tissues.

Design: Androgen independent Pca tissues were obtained from transurethral resectates from 4 patients with hormone refractory Pca(HRPca). Androgen sensitive Pca and matched germline DNA were obtained from prostatectomies and pre-operative blood samples, respectively, in 3 patients. Lesional tissue(average 2500 cells per sample) was collected using laser capture microdissection with DNA isolated and amplified by nested PCR. PCR products were separated on a polyacrylamide gel and directly sequenced. Immunohistochemistry to *AR* and PSA was performed.

Results:

CAG repeat lengths in hormone refractory and androgen sensitive prostate cancer and in blood leukocytes

Hormone refractory Pca		Androgen sensitive Pca		Blood leukocytes	
Patient	CRL	Patient	CRL	Patient	CRL
A	23	E	15	E	22
	24		16		
			17		
B	18		20		
	19		22		
C	24	F	19	F	21
	25		20		
	26		22		
D	0	G	0	G	22
	23		19		

CRL- CAG repeat lengths; n=0 denotes deleted CRL

All cases of androgen sensitive Pca were immunoreactive for both PSA and *AR*. 2/4 cases of HRPca were immunoreactive for PSA and *AR*.

Conclusions: Our results confirm a unique germline CAG repeat length(CRL) per patient in leukocyte DNA and show that polyclonal cell populations heterogeneous for CRL develop in somatic, androgen sensitive prostatic neoplasia. This genetic heterogeneity is *maintained* in hormone refractory Pca(HRPca) which showed normal sized, shortened and deleted CAG repeats. This suggests that clonal selection of cells with shortened or deleted CRL may be one factor in the pathogenesis of HRPca though it is not necessary for transition to an androgen independent state.

749 Spondin-2 (DD-P108) Expression in Carcinoma of the Prostate: A Study of 121 Patients

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Background: Tissue and serum expression of Spondin-2 (DD-P108), a member of the F-Spondin superfamily of secreted extracellular matrix proteins, has previously not been evaluated in carcinoma of the prostate.

Design: Formalin fixed, paraffin embedded archival radical prostatectomy specimens from 121 patients were evaluated for Spondin-2 expression using a monoclonal antibody (mAb) specific for Spondin-2. Stain intensity (0-3+) and percentage of Spondin-2 expression (0, 1=1-20%, 2=21-60% 3=≥60%) were incorporated into a combined index score (ranging from 0 to 6) that was compared to Gleason grade and stage for each tumor. Serum levels of Spondin-2 were measured by a sandwich ELISA utilizing two anti-Spondin-2 mAbs, in a subgroup of 11 patients. Positive staining was defined as moderate to strong stain intensity (≥2) in greater than 20% of tumor cells.

Results: 154 carcinomas were evaluated from 121 patients. 85 (55%) carcinomas had positive staining while 69 (45%) had negative staining for Spondin-2. 46 of 59 (78%) high grade carcinomas (Gleason grade ≥ 7) were positive while only 39 of 95 (41%) low grade carcinomas (Gleason grade ≤ 6) were positive [p<0.0001, odds ratio 5.08 (95% CI from 2.43 to 10.64)]. The median index score was 2 for low grade carcinomas and 5 for high grade carcinomas. 24 of the 52 (46%) organ-confined lesions (stage II or less) and 46 of the 69 (67%) extra-capsular lesions (stage III) had positive staining. The correlation between extra-capsular extension and positive tissue staining for Spondin-2 was statistically significant [p=0.024, odds ratio 2.33 (95% CI from 1.11 to 4.89)]. 5 of the 11 (55%) patients from whom serum samples were obtained had positive tissue staining for Spondin-2. All 5 patients with positive tissue staining (100%) also had elevated Spondin-2 serum levels (>40 ng/ml) that were significantly higher than the normal control values. Using a 40 ng/ml cutoff, the correlation between positive tissue staining and elevated serum levels of Spondin-2 was statistically significant (p=0.015).

Conclusions: Spondin-2 is over-expressed in prostate cancer tissues and the expression correlates with higher Gleason grade carcinomas, more advanced stage diseases, and elevated serum levels of Spondin-2. Further studies are required to determine the biological role of Spondin-2 in the development of prostate carcinoma and its potential use as a serum prostate cancer marker.

750 CD24 Is a Biomarker for Prognosis and Therapeutic Target in Bladder Cancer

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Background: CD24 is a mucin-like GPI-linked cell surface protein that has gained attention as a marker for cancer and cancer progression in many tumor types. Recent reports identify CD24 as a prognostic marker for several common malignancies. We aimed to characterize the expression of CD24 in urothelial carcinoma (UC) and determine correlations of expression with patient outcome.

Design: We investigated the expression of CD24 mRNA in a microarray series of 20 UCs and 6 normal urothelia. Additionally, we stained a small tissue microarray (TMA) cohort of 51 human UC samples as well as formalin fixed UC cell lines. To investigate a role for CD24 in cancer cell growth, we selected CD24-positive UC cell lines for treatment with siRNA targeting CD24 and characterized growth *in vitro* and on soft agar.

Results: We compared expression of CD24 mRNA in UC and normal urothelium and found significant overexpression of CD24 in UC (p=.01). By IHC, overexpression of CD24 was an independent prognostic factor for patient disease-free survival, approaching significance in multivariate analyses (p=.07), even in our small cohort. Finding positivity for CD24 in 13 of 24 cell lines stained, we selected positive UC cell lines for treatment with siRNA targeting CD24. Depletion of CD24 resulted in abrogated growth *in vitro* and anchorage-independent proliferation on soft agar.

Conclusions: In conclusion, we believe that CD24 is an important mediator, prognostic biomarker, and therapeutic target for human UC. This report extends the prognostic utility of CD24 to all five of the most commonly diagnosed solid malignancies in the United States.

751 Expression of MMP-2 and -9 Is Different According the Pattern of Growth/Invasion in Penile Carcinoma

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Background: Few papers have contributed to understand the pathogenesis and tumor progression in squamous cell carcinoma of the penis (SCCP). The present study examined whether the expression of MMP-2 and MMP-9 is correlated with the pattern of tumor growth in SCCP.

Design: MMP-2 and -9 immunoexpression were examined in samples of 115 SCCP patients. Cases were further divided in three groups according the pattern of growth and invasion: G1 (n=28): well differentiated SCCP, exophytic pattern of growth (PG) and pushing pattern of invasion (PI); G2 (n=50): keratinizing SCCP, endophytic PG and PI in large sheets; G3 (n=37): non-keratinizing SCCP with endophytic PG and PI in small group of cells. The pattern of MMP-2 and MMP-9 expression was also evaluated on the pre-invasive lesions.

Results: In normal epithelium, MMP-2/9 positive cells are restricted to basal layer. In dysplastic epithelium, intermediate and superficial layers overexpressed MMP-2 (membrane) and MMP-9 (cytoplasm). Intraepithelial carcinoma showed a diffuse and strong expression of MMP-2/9. Expression of MMP-2/9 in SCCP can be appreciated in tables 1 and figs.1/2. Tumors with MMP-2 and MMP-9 overexpression are less differentiated and more deeply invasive. Also, expression of MMP-2 changed from

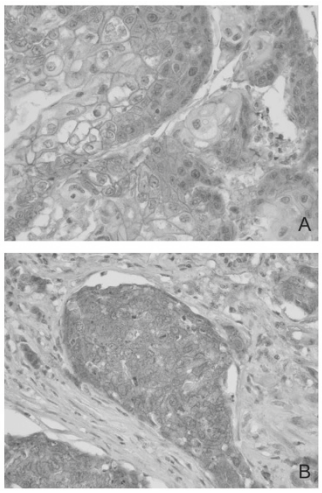
membrane to cytoplasmatic pattern in invasive tumors, maybe representing the activation of MMP-2.

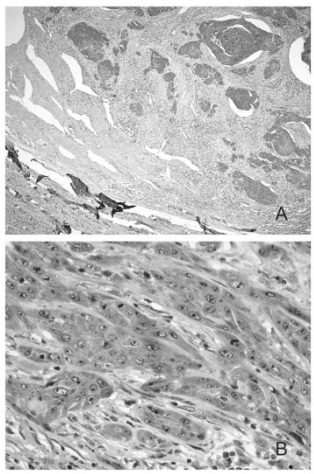
Conclusions: These findings aloud us to conclude that invasive pattern is associated with overexpression of MMPs involved in basal membrane degradation, and maybe justify the morphological subtypes of SCCP.

Table 1: Expression of MMP-2 and -9 according growth and invasion pattern

Groups	MMP-9			MMP-2		
	N	WP	WP	N	WP	P
G1 (n=28)	24 (86%)	4 (14%)	0 (0%)	3 (11%)	23 (82%)	2 (7%)
G2 (n=50)	19 (38%)	16 (32%)	15 (30%)	3 (6%)	6 (12%)	41 (82%)
G3 (n=37)	4 (11%)	9 (24%)	24 (65%)	1 (3%)	33 (89%)	33 (89%)

N: negative; WP: weakly positive; P: positive





752 Diagnostic Utility of Racemase (P504S)/34βE12/p63 Antibody Cocktail in the Assessment of Routine Prostate Needle Biopsies

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Background: Distinguishing prostate cancer from benign mimics in contemporary needle biopsies based solely on morphology is often challenging. The difficulty arises from the small amount of tissue provided by needle biopsy and from the limited number of small atypical glands in many foci. To establish a definitive diagnosis in difficult cases, the racemase (P504S)/34βE12/p63 cocktail is used routinely in the authors' laboratory. The aims of this study were to determine how often the cocktail is used, and how useful is the cocktail in differentiating prostate cancer from its mimics in biopsies.

Design: A total of 2315 consecutive prostate needle biopsies were examined over a 3-month period at the Bostwick Laboratories. Diagnosis was established in the majority of specimens based on the evaluation of H&E sections alone. Immunohistochemistry using a cocktail of racemase (P504S)/34βE12/p63 antibodies on a single additional slide was performed on 200 (8.6%) of the biopsy specimens as an ancillary tool for the final diagnosis.

Results: Of 200 biopsies stained with the cocktail, 102 contained prostate cancer. Of these, 90 (88%) showed moderate to strong racemase staining in cancer cells and absence of basal cells (100%). The incidence of atypical small acinar proliferation suspicious for but not diagnostic for malignancy (ASAP) was 1.6% (38/2315) after cocktail staining. Four of 98 (4%) benign biopsies showed weak staining for racemase; however, no benign glands were simultaneously positive for racemase and negative for basal cell markers. High-grade prostatic intraepithelial neoplasia was positive for all markers with fragmented basal cell layer. The sensitivity, specificity, and positive predictive value of this cocktail for the cancer detection were 88%, 96%, and 95%, respectively.

Conclusions: Triple-antibody (racemase/34βE12/p63) cocktail is an assay with high sensitivity and specificity for the detection of small prostate cancer. This immunohistochemical test also preserves prostatic tissue morphology and, by simultaneously performing all three tests, shortens turn-around time.

753 Sarcomas and Pseudosarcomas of the Urinary Bladder

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Background: Pseudosarcomatous fibromyxoid tumor (PFT), sarcoma, and sarcomatoid carcinoma of the bladder are frequently difficult to distinguish histopathologically with significant differences in disease-related outcomes.

Design: A retrospective review of our pathology registry over the last 25 years identified a total of 54 cases, 17 pseudosarcomatous fibromyxoid tumors, 21 primary bladder sarcomas, and 16 sarcomatoid carcinomas. The available histopathological slides with accompanying immunohistochemical stains were reevaluated. The clinical presentation, treatment, and disease-related outcomes of these patients were reviewed and contrasted.

Results: Most patients with PFT, sarcoma, and sarcomatoid carcinoma presented between the ages of 50 to 60 years with PFT most commonly detected in women. Of the patients with bladder sarcoma, different subtypes included leiomyosarcoma (N=11), high-grade sarcoma (N=4), angiosarcoma (N=3), rhabdomyosarcoma (N=2), and osteosarcoma (N=1). Sarcomas and sarcomatoid carcinomas were characterized by cellular atypia, high mitotic activity, and the presence of necrosis. In contrast PFT was characterized by myofibroblastic proliferation with associated inflammation and infrequent mitoses. A previous history of urological instrumentation was present in 59% of patients with PFT but rarely in other tumor types. Although the majority of patients in all 3 groups were treated surgically, transurethral resection and partial cystectomy was used in all patients with PFT whereas radical cystectomy was used in 33% and 56% of patients with sarcoma and sarcomatoid carcinoma, respectively. Local recurrences and distant metastases frequently occurred in patients with sarcoma and sarcomatoid carcinoma with 52% and 63% of patients dying of disease, respectively. In contrast, PFT had a benign course with no recurrences or disease-related deaths.

Conclusions: PFT is often suggested by a prior history of urological instrumentation as well as exhibiting unique histopathological features which must be considered in distinguishing this entity from primary bladder sarcoma and sarcomatoid carcinoma.

754 Annexin II Immunoreactivity in Prostate Tissue: A Tissue Microarray (TMA) Study

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Background: Annexins (ANX) are calcium-binding proteins that bind phospholipids. ANXII is implicated in migration inhibition, extracellular matrix alteration and regulation of inflammation. By immunohistochemistry (IHC), ANXII is expressed in prostatic basal cell (BC) cytoplasm and on the cytoplasmic membrane of benign epithelium. However, expression is lost in prostate cancer (PCa) and is poorly defined in high grade prostatic intraepithelial neoplasia (HGPIN). In this study we use a HGPIN and a PCa TMA to investigate ANXII expression.

Design: Tissue from seventy (N=70) radical prostatectomies (RP) was used for construction of two TMAs; one HGPIN (n=35) and one Gleason grade 6(3+3) PCa (n=35). Patients had no therapy prior to RP. Each case was represented by three 1mm tissue cores for a total of 105 cores per array. IHC was used to assess p63 and ANXII expression on serial sections of the TMAs. Digital images of TMA slides were scanned using the T3 ScanScope by Aperio Technologies (0.5µm/pixel resolution). H&E stained sections of each core were compared to digital images of serial sections stained with p63, a nuclear BC marker, and ANXII. All glands were visually scored for the percentage of cells positive for membranous expression of ANXII.

Results: ANXII expression in BC cytoplasm coincided with nuclear p63. This demonstrated lack of a BC layer in PCa and an interrupted BC layer in HGPIN. 96% of PCa was negative or weak for ANXII expression. Moderate to strong expression for ANXII was observed in 100% of benign (BG) glands. 76% of HGPIN epithelium demonstrated negative to weak immunoreactivity, reaching statistical significance ($p<0.001$). A distinct subset of glands were borderline for a diagnosis of HGPIN. These glands showed occasional loss of BC, increased nuclear to cytoplasmic ratio and frequent pin-point nucleoli, with rare cells containing a prominent nucleolus. ANXII expression was moderate-strong in 36% of these glands and negative-weak in 64%.

Conclusions: ANXII is a positive marker of benign glands, a negative marker of PCa and has the potential to futher define the diagnosis of HGPIN in prostate biopsies. Since ANXII stains BC, it is helpful in the distinction of HGPIN from PCa. Differential expression in HGPIN may add value to this diagnosis with respect to clinical outcome. ANXII negative HGPIN may be a predictor of coexistent malignancy. Loss of ANXII may be an early event in prostatic neoplasia. The clinical significance of ANXII expression in HGPIN warrants further investigation.

755 OCT4 Is Superior to CD30 in the Diagnosis of Metastatic Embryonal Carcinomas after Chemotherapy

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Background: Correctly diagnosing a metastatic germ cell tumor may be challenging due to diverse morphologic manifestations. Thus, a sensitive and specific marker for certain germ cell components is invaluable. Both OCT4 and CD30 are sensitive markers for the identification of primary embryonal carcinomas; however, the loss of CD30 expression has been reported in metastatic embryonal carcinomas after chemotherapy. The present study was conducted to evaluate the expression patterns of OCT4 and

CD30 in post-chemotherapy metastatic embryonal carcinomas and compare their values as diagnostic tools.
Design: Twenty-five cases of post-chemotherapy mixed germ cell tumors metastatic to retroperitoneal lymph nodes, each containing an unequivocal embryonal carcinoma component, were immunohistochemically analyzed for CD30 and OCT4. The staining intensity (0: negative, 1: weak, 2: moderate, 3: strong) and percentage of positively staining cells were compared.
Results: Eight cases (32%) revealed diffuse (> 50%), moderate to strong cytoplasmic CD30 and nuclear OCT4 staining in embryonal carcinoma components. Eleven cases (44%) demonstrated similarly strong OCT4 staining but markedly diminished CD30 expression, four of which were completely negative for CD30. The remaining six cases (24%) were negative for both CD30 and OCT4.
Conclusions: OCT4 is a useful diagnostic marker to identify metastatic embryonal carcinomas after chemotherapy with a better sensitivity and readability than CD30.

756 Trends of Prostatic Core Needle Biopsies in the Japanese Patients

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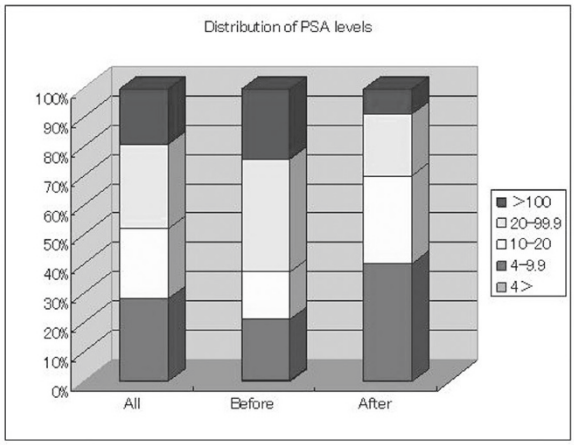
Background: Previously, Japan was one of the countries in which the incidence of prostate cancer (PCa) as lowest, although it has been increasing rapidly in recent years. The Current Japanese Emperor was diagnosed as PCa in January, 2003. Since then, the PCa has been widely noticed in Japan and a campaign against the disease such as a recommendation of the annual PSA testing has been accelerated.
Design: To know the trend of the PCa in the Japanese patients and relationship to the Emperor's incidence, 633 core needle biopsy cases, taken between January 2000 and June 2005, were analyzed. The cases were collected from multiple institutions including private clinics and community-based hospitals in multiple areas of Japan. Data of 633 cases ("All" cases), the first 200 cases before the Emperor's incidents ("Before" cases), and recent 200 cases after the incidents ("After" cases) were analyzed.
Results: Distribution of the Gleason scores (G. S.) and PSA levels in "All", the "Before", and the "After" cases were shown on Fig. 1 and 2, respectively. In the "After" cases, the ratio of G. S. 9 and 10 decreased and that of G. S. 7 increased than the "Before" cases. Similarly, ratio of the PSA level >20 ng/mL decreased and that of 4.0-9.9 increased in the "After" cases. Still, 12.0 % and 27.5% of the patients showed G. S. =9 or 10, and the PSA levels >20 in the "After" cases, respectively. The ratio of the cases showing extraprostatic fat invasion were 16.9%, 20.0%, and 7.0%, in the "All", "Before", and "After" cases, respectively.
Conclusions: The cases such as high G. S., high PSA, showing extraprostatic invasion has been decreasing in the Japanese patients. One of the reasons of these trends is associated with the Emperor's cancer diagnosis. Thus, a proper campaign and the general attentions to the Pca should make an important role for an earlier detection of the disease in other countries.



757 Prognostic Significance of Paneth Cell-Like Neuroendocrine Cells (PNEC) in Adenocarcinoma of the Prostate

EF Tamas, JI Epstein. The Johns Hopkins Hopspital, Baltimore, MD.
Background: PNEC are occasionally seen in prostate cancer with unknown prognostic significance.
Design: We studied 36 cases of prostate adenoca. with PNEC. Follow up was obtained in 94% of cases with a mean follow-up of 29 mos.
Results: Mean age was 65.5 years (48-76). Gleason score was: 6 in 11 cases (30.6%); 7 in 8 cases (22.2%); 8 in 12 cases (33.3%); 9 in 3 cases(8.3%); and 10 in 2 cases (5.6%). Stage was T1 in 3 cases (8.3%); pT2 in 13 cases (36.1%); pT3 in 14 cases (38. 9%); pT4 in one case (2.8%); and unknown in 5 cases (13.9%). Histology: Tumor was conventional in 30 cases (83.3%), pure ductal adenocarcinoma in 2 cases (5.6%) and conventional with ductal features in 4 cases (11.1%). PNEC were observed within glands as isolated (58.3%), clustered (22.2%), or diffuse growth of cells (19.4%). PNEC were also seen as single cells, cords, or nests of cells with no lumina, which were graded as Gleason pattern 5, although their bland cytology resembling a carcinoid tumor raised questions as to whether this unique histology should not be diagnosed as high grade. Percentage of glands/nests/cords with PNEC averaged 41% (1-100%). PNEC were uniformly positive for both chromogranin and synaptophysin in 19/19 cases studied. Radical Prostatectomy (RP): 13/36 (36.1%) underwent RP. Tumor was organ confined in 8/13 (61.5%) cases, with extra-prostatic extension and positive margins in 5/13 (38.5%) cases. Lymph nodes were negative in all cases. At 5 years follow-up, all patients but one were progression free. At 7 years, approximately 80% were progression free. Patients who failed had pT3 stage disease with positive surgical margins. Of 5 cases that were assigned a Gleason pattern 5, yet resembled a carcinoid tumor, they were organ confined in 4/5 cases and had no evidence of disease (NED) at 1,31,46,48,61 months. Nonsurgical Therapy: 1 patient had metastatic disease at time of the diagnosis. Comparable prognostic results were obtained with non-surgical therapies. Of the 6 cases that were assigned a Gleason pattern 5, yet resembled a carcinoid tumor, all were NED at 14,24, 44,45,48,77 months.
Conclusions: PNEC do not appear to adversely impact the prognosis of men with prostate adenocarcinoma. Although some patterns with PNEC simulate a high grade pattern, their bland cytology, carcinoid appearance, favorable pathological findings at RP, and good prognosis suggests that assigning them a high grade may not be accurate. We recommend not grading tumors with this pattern, but rather assigning the grade based on areas of adenocarcinoma without PNEC.

758 Needle Tract Seeding of Perinephric Adipose Tissue Following Imaging-Guided Biopsy of Renal Tumors

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Background: In recent years there has been an increase in the incidence of adult renal tumors, partly based on increased use of radiographic scans. Accompanying this rise in incidence has been an increase in the use of fine needle aspiration biopsy (FNA) and needle core biopsy (NBx) for the diagnosis of incidentally detected renal tumors in adults. The purpose of this study was to examine the incidence and pathologic features of tumor seeding of the perinephric adipose tissue in patients undergoing imaging-guided biopsies of renal masses.
Design: Patients undergoing image-guided biopsy (FNA and/or NBx) of a kidney mass at a single institution (between 10/1996 and 8/2005) were evaluated for this study.
Results: 130 patients underwent an imaging-guided biopsy, of which 47 (36.15%) had a subsequent nephrectomy (27 radical nephrectomy, 20 partial nephrectomy). Three of the 47 (6.38%) patients who underwent a resection showed tumor seeding of the perinephric adipose tissue along the needle tract. All 3 patients were men (ages 64, 54, and 54 years), who underwent a partial nephrectomy. Intervals between biopsy and nephrectomy were 64, 238 and 256 days; as, all three patients were treated first for other tumors that were considered more life threatening (malignant melanoma, pancreatic adenocarcinoma and oral squamous cell carcinoma). The tumor sizes were: 2.1 cm, 3.0 cm and 4.0 cm. Two were in the right kidney and one in the left. All 3 tumors were type 1 papillary renal cell carcinomas (RCC), with Fuhrman nuclear grades 2, 3, and 2. All 3 tumors would be confined to the kidney (pT1a), if tumor seeding were not present. None of these biopsy tracts (in the perinephric fat) was grossly visible. One of the patients died secondary to his other malignancy 31 months after nephrectomy. However, there was no clinical evidence of tumor recurrence along the biopsy tract in those 31 months. The other two patients have a follow-up of less than one month.
Conclusions: Of these 47 patients who underwent a nephrectomy subsequent to imaging-guided biopsy, 3 (6.38%) patients with papillary RCC had seeding of tumor along the biopsy tract in the perinephric fat. None of the other RCC types seeded the biopsy tract. As these foci of seeding were incidentally detected, the true incidence of perinephric fat seeding may not be readily evident. The true clinical significance of this finding also remains to be determined, as only one of our patients had prolonged followup.

759 Papillary Adenoma(s) in Renal Resection Specimens: Incidence and Relationship to Renal Neoplasia

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Background: Papillary (renal) adenoma (PA) is a benign tumor, most often associated with papillary renal cell carcinoma (RCC). Most information regarding PA is derived either from autopsy studies or based on the older surgical pathology literature. The aim of our study was to evaluate the incidence and pathologic features of this benign tumor in a contemporary surgical pathology series, and examine its relationship to other adult renal epithelial neoplasia.

Design: Kidneys resected at a single institution between 4/1994 and 8/2005 were evaluated for this study. All specimens were grossed using similar technique, with submission of a minimum of 1 section/cm of maximum tumor dimension and minimum of 1 section of grossly normal renal parenchyma.
Results: PA were detected in 3.07% (75/2446) of kidneys resected (39/1609 radical nephrectomy, 31/593 partial nephrectomy, 1/182 nephroureterectomy, 3/62 assorted resections). These were from 69 patients; age range: 45 to 84; 10 women and 59 men (5 with bilateral staged nephrectomies, 1 with bilateral partial nephrectomy). PA size ranged from <1.0 mm to 5.0 mm. A single PA was detected in 61% (46/75) of kidneys on routine sections. Multiple PA (range 2-9) were present in 39% (29/75) of kidneys. Distribution of PA was almost similar on both sides (35/75 on the right kidney, 40/75 left side). All PA were tubulo-papillary with a Fuhrman nuclear grade of 1-2. PA were associated with a variety of renal tumors including: renal oncocytoma (12%, 9/75), clear RCC (29.33 %, 22/75), papillary RCC (38.67%, 29/75), papillary urothelial carcinoma of the renal pelvis (2.67%, 2/75), cystic nephroma (1.33%, 1/75), and renal cortical cyst (1.33%, 1/75). 8% (6/75) were present in kidneys with multiple renal tumors. 6.67% (5/75) were detected in patients undergoing surgery for non-renal tumors (retroperitoneal sarcoma, etc). PA were present in 15.59% (29/186) of papillary RCC resected in this time frame, compared to 1.54% (22/1427) clear RCC and 6.92% (9/130) renal oncocytomas.
Conclusions: In our surgical series papillary adenoma were present in 3.07% of kidneys resected between 4/1994 and 8/2005, compared to up to 22 % reported in autopsy series and earlier surgical series. The highest incidence of PA was in papillary RCC (15.59%), compared to 1.54% in clear RCC and 6.92% in renal oncocytoma. PA were in kidneys with renal oncocytoma, however, none of the chromophobe RCC in our series had an associated PA.

760 Are Some Collecting Duct Carcinomas Indeed HLRCC Tumors?

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Background: Collecting duct carcinomas (CDC) represent a somewhat controversial category in the renal cell carcinoma (RCC) classification. These highly aggressive and rare tumors display a variegated morphology that includes papillary and tubular patterns. Their immunohistochemical profile suggests a distal (collecting duct) origin. On the other hand, renal tumors of the hereditary leiomyomatosis and renal cell carcinoma (HLRCC) syndrome are also biologically aggressive neoplasms with tubulo-papillary configuration. It is not surprising, therefore, that some genetically-proved HLRCC tumors had been diagnosed as CDC. We herein compare morphological, immunohistochemical, genetic, and molecular markers postulated as characteristic of each of these two entities, in an effort to segregate one from the other.
Design: Formalin-fixed, paraffin embedded tissue sections from 12 HLRCC tumors and 5 CDC were examined using morphologic (H&E and mucin stains), immunohistochemical (*Ulex europaeus* agglutinin, HMWK, CK7, CK20, CD10, HER2), genetic (CISH™ for *HER2/c-erbB2*), and molecular (LOH for 1q42-44 and 1q32.2) parameters postulated as useful for defining one or the other entity. DNA extracted from microdissected tissue was used for molecular analysis.
Results: Morphologic overlapping was evident between the two entities; however, HLRCC tumors tended to display characteristic nuclear features (inclusion-like nucleoli) and to be more cystic than CDC. CDC showed more definite stromal desmoplastic reaction. Both neoplasms were negative for "proximal nephron markers" (CD10, CK7); the positivity for "distal markers" (HMWK, UEA, CK20) was not a constant finding in any of the categories. Neither over expression nor gene amplification of HER2 was found in the two tumor groups. LOH in 1q42 (fumarate hydratase gene) was found in 85% of HLRCC tumors; additionally, a subset of HLRCC tumors (57%) showed also LOH in 1q32.2 (reported as a region of minimal deletion in CDC).
Conclusions: Our study suggests that at least some of the tumors categorized as CDC are indeed part of the newly described HLRCC syndrome. Morphological, immunohistochemical, genetic, and molecular overlapping between HLRCC and CDC point to the distal portions of the nephron as the putative sites of origin of both entities. Our findings emphasize the necessity to rule out HLRCC whenever a diagnosis of CDC is entertained in the differential diagnosis.

761 Is Perineural Invasion (PNI) by Cancer on Biopsy a Predictor of Adverse Outcomes on Radical Prostatectomy (RP)?

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Background: Prognostic significance of PNI on needle biopsy remains uncertain. In previous studies, PNI has been found to be a predictor of RP stage in univariate analyses, but not in multivariate analyses. The significance of PNI presence on extended ten-core biopsy has not been studied previously.
Design: We investigated the significance of PNI in a cohort of 1150 matched ten-core biopsies with follow-up RP, performed in our institution between 07/00 and 04/05. Biopsy, RP, and clinical data were retrieved from our institutional database. We used univariate and multivariate logistic regression analyses to investigate whether presence of PNI on biopsy predicts adverse RP outcomes: a.) stage pT3, b.) positive margins, c.) positive seminal vesicles (SV) or lymph nodes (LN), and d.) tumor volume of >10% gland involvement (by semi-quantitative assessment).
Results: PNI was found in 20% of biopsies. There was a strong correlation between PNI and abnormal rectal exam (p=.0014), ultrasound findings (p<.0001, both Fisher's exact test), and PSA density (p<.0001, Wilcoxon), but not with PSA. Interestingly, the frequency of PNI decreased as the gland size increased (p=.0065, Wilcoxon), which may be due to lower tumor volumes in bigger glands (Spearman rho = -0.311; p<.0001). PNI demonstrated strong relationship with the biopsy variables such as: Gleason Score (GS), total percent cancer, cancer length, and number of positive cores (all p <.0001; Wilcoxon). On univariate analysis, PNI strongly correlated with stage pT3 (odds ratio 2.33), positive margins (odds ratio 1.97), >10% cancer on RP (odds ratio 2.54; all p<.0001), and SV or LN invasion (odds ratio 2.72; p=.0004). However, in multivariate logistic regression models that included multiple biopsy variables, PNI was an independent predictor only for the positive margins (p=.0485), but not for the other outcomes. PNI showed low sensitivity and PPV, but higher specificity and NPV for tested outcomes.

	Sensitivity %	Specificity %	PPV %	NPV %
Stage pT3	36.2	82	20.4	90.9
Positive margins	29.6	83.7	40.9	75.7
Positive SV or LN	39.3	81	9.6	96.3
TU volume >10%	28.5	86.8	62.6	60.9

Conclusions: 1.) PNI frequency of 20% on ten-core biopsy is in the previously reported range of 11% to 38%. 2.) PNI was strongly related to the cancer extent on biopsy and the biopsy GS. 3.) Although PNI correlated with all adverse RP outcomes by univariate analysis, on multivariate analysis it was an independent predictor only for positive margins, but not for other adverse outcomes.

762 Findings in 1150 Radical Prostatectomies after Ten-Core Biopsies

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Background: Extended core biopsy sampling and prostate specific antigen (PSA)-driven biopsies have become routine practices in many centers. Radical prostatectomy (RP) findings following routine ten-core biopsies have not been explored in a larger contemporary study.
Design: We reviewed the clinical and the pathology findings in 1150 consecutive RP performed in our institution between 07/2000 and 04/2005. The biopsies were performed without organized PSA screening, using ten-core sampling that included additional bilateral cores from the base and the mid-zone. All RP were completely sampled and the clinical data were retrieved from our institutional database.
Results: Patient mean age was 60.1 years (60.6 median). Mean prostate specific antigen (PSA) value was 6.9 ng/ml (5.8 ng/ml median) and PSA density was 0.2 (0.16 median). In 69% of patients preoperative PSA was between 4 and 10 ng/ml (18% had PSA <4 ng/ml and 13% had PSA >10 ng/ml). Mean gland volume measured 41.8 ml (35.5 ml median). Digital rectal examination and ultrasound findings were reported abnormal in 24% and 23% of cases, respectively. On RP, prostate cancer demonstrated mean Gleason score (GS) of 6.5 (primary GS 3.1 and secondary GS 3.4). GS≤6 was found in 47%, GS 7 in 48%, while GS≥8 was found in only 5% of RP. Stage pT3 disease was identified in 11% of patients, and 89% had organ-confined disease (pT2). Surgical positive margins were found in 28% of RP. When the margin extent was documented, they were reported established in 60% and focal in 40% of cases. Seminal vesicle invasion was identified in 4.2% and regional lymph node invasion was documented in 1% of RP. Lymphatic/vascular invasion was seen in 7% of RP. Mean tumor volume was measured by semiquantitative assessment and involved 13.9% (10% median) of the prostate gland. In 37% of RP there was ≤5% of gland involvement with cancer. Cancer involvement was >5% to ≤10% of the gland in 19% of RP and cancer involvement >10% was found in 44% of cases.
Conclusions: 1.) Patients with prostate cancer that currently undergo RP after routine ten-core biopsy are typically detected at an earlier stage with organ-confined disease (pT2) and GS <8. 2.) Stage pT3 disease is identified in only 11% of patients after ten-core biopsy, which further highlights the stage shift of the disease in the current practice. 3.) Adverse findings on RP, including GS≥8, invasion of seminal vesicles and lymph nodes, and lymphatic/vascular invasion are uncommon findings in patients diagnosed currently with cancer on routine ten-core biopsy.

763 Pathology of Prostate Cancer Treated with Neoadjuvant Chemotherapy: Features Predictive of Clinical Outcome

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Background: Neoadjuvant chemotherapy protocols are being used to treat patients with organ-confined prostate cancer that is at high risk of progression. The pathologic findings and correlations with clinical outcome of neoadjuvant prostate cancer therapy have not been widely described. We characterized the pathology of prostate cancer subjected to a specific chemotherapy regimen and evaluated the predictive power of pathologic features of the treated cancers.
Design: Fifty patients with clinically localized, high risk prostate carcinoma (serum PSA ≥ 15 ng/ml, Gleason score ≥ 4+3, or stage >cT2a) were treated with four cycles of Mitoxantrone plus Docetaxel, followed by radical prostatectomy. The pathology of the cancer in totally embedded prostates was evaluated and the histologic findings were correlated with clinical outcome parameters (pathologic stage, radiological evidence of metastases, and post-op serum PSA). The median follow-up time was 18 months.
Results: The tumors in a subset of patients (20%) exhibited combinations of the following features, evaluated by the degree of histologic effect (on a 0 to 3+ scale): intraductal carcinoma (21%), collapse of tumor glands (44%), cribriform cancer glands (12%), single cell vacuolization (23%), and inconspicuous tumor cells (28%). None of these histologic features were present in the pre-chemotherapy needle biopsy specimens. Correlation with outcome was evaluated. Of these histologic features, only intraductal carcinoma correlated with the following clinical outcome parameters in a univariate analysis: radiological evidence of surgical failure (relative risk 44% vs 14%; $p < 0.04$) and post-op rising serum PSA (relative risk 37% vs. 11%; $P < 0.09$).
Conclusions: The histologic features of chemotherapy-treated prostate carcinoma may provide evidence of likelihood of tumor progression. Although these changes did not predict likelihood of progression independent of traditional prognostic markers (pre-treatment serum PSA, clinical and pathologic stage, status of surgical margin, and Gleason score) in a multivariate analysis, a longer follow-up of this patient cohort and/or of a larger patient cohort may reveal independent prognostic significance of these parameters.

764 Lymphovascular Invasion in Urothelial Carcinoma of the Bladder: Diagnostic Utilities and Biologic Implications of the Lymphovascular Markers CD34, CD31, and D2-40

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Background: Lymphovascular invasion (LVI) by tumors cells is an important prognostic and therapeutic parameter in urothelial carcinoma (UC) of the urinary bladder. The diagnosis of LVI in routine sections may be problematic. CD34, CD31 and D2-40 were recently introduced as sensitive and specific markers for lymphovascular endothelial cells. A comprehensive study to evaluate the diagnostic utilities of these markers in LVI of urinary bladder UC is not available.

Design: Consecutive tissue sections of 116 urinary bladder specimens (normal 6, chronic cystitis 26, low grade dysplasia 6, high grade dysplasia 4, noninvasive UC 12, and invasive UC 62) were submitted to hematoxylin & eosin (H&E) stain and immunostain for CD34, CD31, and D2-40. The morphology and frequency of the lymphovascular channels and their invasion by tumor were compared among these stains

Results: The endothelial cell staining was as follow

	CD34	CD31	D2-40
Blood Vessels	3+ Diffuse	1-3+ Focal	0
Lymphatics	0-3+ Focal	0-2+ Focal	3+ Diffuse

These patterns were similar for channels with or without tumor invasion. The frequency of LVI identified by H&E, CD34, CD31, and D2-40 stains in the 62 invasive UC was 16, 32, 22, and 32%, respectively. H&E stain failed to detect LVI in 10 cases, in each of which the invasive foci were within the tumor and completely occupied the affected vascular lumens. In contrast, foci suspicious for LVI by H&E stain within or outside the tumor were not confirmed by lymphovascular markers in 12 cases. CD34 and D2-40 helped identify LVI equally well, and were superior to CD31. Whether the invasion was lymphatic or vascular or both could be determined in at least 6 cases, due to the differential staining of lymphatic *vs* vascular endothelial cells. More lymphovascular channels were observed within the tumor than in adjacent tissue, suggesting tumor-mediated angio- and lymphangiogenesis.

Conclusions: In the context of UC of the urinary bladder**,** identification of and further differentiation between vascular and lymphatic channels are possible. H&E stain is problematic or even misleading in evaluating LVI. Lymphatic *vs* vascular *invasion* can be differentiated. LVI is much more frequent than being suggested by the H&E stain. Tumor-mediated angio- and lymphangiogenesis may play a role in LVI.

765 Expression of Galectin 3 in Renal Cell Carcinoma (RCC): A Tissue Microarray and Immunohistochemical Study

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Background: Galectin-3 (Gal 3) is a protein member of the lectin family that binds b-galactosides. It plays an important role in tumor cell adhesion, proliferation, differentiation, angiogenesis, and metastasis. A preliminary gene expression study showed Gal 3 overexpression in chromophobe RCC, but the overall diagnostic or prognostic significance of Gal 3 in renal epithelial neoplasms is unknown.

Design: Fifteen normal kidneys and 216 renal neoplasms [oncocytoma (23), clear cell (134), papillary (33), chromophobe (20), collecting duct (2) and unclassified RCC (2)] were constructed into four tissue microarray blocks. They were immunostained using a monoclonal Ab against Gal 3. Tumors were considered positive when there is strong staining of at least 30% of the tumor cells.

Results: In normal kidney, Gal 3 expression was observed in distal tubules and collecting duct, with a strong staining for intercalated cells. Very weak or no expression was seen in proximal tubules, glomeruli or stromal cells. Strong Gal 3 expression was observed in 74 of 214 (35%) of renal neoplasms, with a distinctly different pattern and frequency for different histologic types. While 22 of 23 (96%) oncocytomas and 19 of 20 (87%) chromophobe RCC expressed Gal 3, only 4 of 29 (12%) papillary RCC and 29 of 134 (25%) clear cell RCC expressed Gal 3, suggesting a possible role for this marker in the differential diagnosis of renal neoplasms. In clear cell RCCs, expression was seen in 39% of high grade vs 12 % of low grade tumors ($p<0.001$); and the 5-year survival for patients with Gal 3-negative tumor was 65% vs. 49% for those with Gal 3-positive tumor ($p=0.15$).

Conclusions: Gal 3 is a specific marker for distal tubular differentiation. Its strong expression in oncocytoma and chromophobe RCC confirms the distal tubular origin of these tumor types and suggests a role in the differential diagnosis of renal neoplasms with oncocytic or granular cells. The strong association of Gal 3 overexpression and high nuclear grade and the decreased survival of Gal 3-positive clear cell RCC imply that Gal 3 may serve as a novel prognostic marker and therapeutic target for renal neoplasms.

766 Unusual Cytoplasmic Staining of p63 in Benign Epithelial, High Grade PIN, and Cancer Cells of Prostate

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Background: Recently, there were some reports that revealed the diagnostic utility of p63/alpha-methylacyl-CoA racemase (AMACR) cocktail immunostaining in prostatic carcinoma, especially in minute foci of prostatic carcinoma. Since p63 is a nuclear protein and AMACR is a cytoplasmic protein, some of groups used dual staining by the same chromogen. We report unusual cases showing cytoplasmic and membranous staining of p63 for benign epithelial cells, high grade PIN (HGPIN) cells, and prostatic carcinoma cells, which could be misled to interpret when p63/AMACR cocktail staining with the same chromogen.

Design: From October 1, 2003 to April 28, 2004, 219 needle biopsy cases (total core number: 1378, cancer core number: 258, HGPIN core number: 55, benign core number: 1065) were stained by p63 (DakoCytomation), prospectively. They were also stained by AMACR (Zieta corporation), separately.

Results: Eight cores of five cases (four cores of two cancer cases, three cores of two benign cases, and one core of one HGPIN case) showed cytoplasmic and focal membranous staining pattern for p63. Their cytoplasmic staining pattern was dusty with apical predominance, which staining pattern was mimicking AMACR staining pattern. The benign glands showed no AMACR expression, the HGPIN glands showed weak AMACR expression, and the cancer glands showed strong cytoplasmic AMACR expression, respectively. Although p63 showed finer granular staining pattern than those of AMACR, it was difficult to discriminate them precisely.

Conclusions: Although it is very rare, we have to remember that p63 can show cytoplasmic staining pattern and should be cautious to interpret in p63/AMACR cocktail staining using the same chromogen.

767 Endogenous Biotin Activity in Renal Oncocytoma (RO) and Chromophobe Renal Cell Carcinoma (ChRCC): A Significant Diagnostic Problem

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Background: Endogenous biotin (EB) activity in pink cell tumors of the kidney may cause problems in diagnostic pathology by giving rise to false positive immunohistochemistry (IHC) results. Blockage of EB may lead to more reliable results. We have seen several cases in our consultation practice where this did result in diagnostic confusion. To evaluate the effect of EB in IHC, we studied different IHC methods on ChRCC and RO, using CD10, a marker that is expressed in only a small percentage of these tumors.

Design: The study included 15 ChRCC and 15 Onc. The IHC methods performed were: 1) a standard ABC method (S-ABC) that includes an endogenous peroxidase block and a normal (horse serum) blocking solution; 2) a standard ABC method that includes an additional EB blocking step that utilizes free biotin and egg white avidin (S-ABC-BB); and 3) the Ventana-polymer method (VP) that does not utilize the ABC technique. Intensity of the CD10 expression of the tumor cells was graded as 0, no staining; 1, very weak; 2, weak; 3, moderate; 4, strong; 5, very strong. The area stained for the antigen was rated as 0, none; 1, <1%; 2, 1-5%; 3, 6-60%; and 4, >60%. Staining indices were obtained by multiplication of the intensity and area of the staining (0-20).

Results: A significant statistical difference between the IHC results of the three methods ($p<0.001$) was demonstrated. EB activity was found to be the highest with the S-ABC method with significant non-specific reactivity seen in 14 cases (47%), mean score 6.47±5.0. The S-ABC-BB method eliminated some of the EB activity and improved the immunostaining (significant non-specific reactivity in 8 cases [27%], mean score 5.7±5.0), the difference between these methods was not statistically significant ($p=0.09$). EB interference was significantly lower with the VP method than both other methods with significant reactivity seen in 3 cases (10%), mean score 2.30±4.76 ($p<0.001$ and $p<0.002$, respectively). Only the VP method showed a significant staining difference between ChRCC and RO ($p=0.043$).

Conclusions: This study confirms the importance of the blockage of EB in IHC studies in the setting of renal tumors, in particular ChRCC and RO. The known IHC pattern of RO and ChRCC - non-reactivity or seldom reactivity for CD10, was only achieved with the VP method in which EB interference is eliminated.

768 Prognostic Utility of Ki-67, EGFR, Cyclin D1, IGF-I, IGFBP-3 and L1-CAM in Clear Cell Renal Cell Carcinoma (CRCC)

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Background: CRCC is the most common malignancy involving the adult kidney. Molecular markers have the potential to accurately characterize the biological behavior of these tumors and could serve as therapeutic targets.

Design: Immunohistochemistry (IHC) was performed on 101 cases of CRCC (60 from Turkey; 41 from USA), using antibodies against EGFR, cyclin D1, IGF-I, IGFBP-3, L1-CAM and Ki-67. Tumors were grouped by nuclear grade as low-grade (LNG; grades 1,2) or high-grade (HNG; grades 3,4), and by stage as localized (LS; pT1,pT2), or locally invasive (IS; pT3,pT4). IHC analysis was based on intensity and distribution of the markers. Marker status and established predictors of prognosis (tumor stage, nuclear grade) were considered when developing a prognostic model for recurrence/metastasis (RM) and disease-specific survival (DSS).

Results: High proliferation (Ki-67) correlated with HNG ($p<0.001$), IS ($p<0.001$), increased risk of RM ($p<0.0001$) and poor DSS ($p<0.002$). Strong EGFR expression was frequent (76/101, 75.2%), with high EGFR expression associated with HNG ($p<0.008$), IS ($p<0.017$) and increased RM ($p=0.046$). IGF-I and IGFBP-3 over-expression correlated with each other ($p<0.001$), and with high L1-CAM ($p<0.001$) level; low expression of both IGF-1 and IGFBP-3 predicted poor DSS ($p<0.002$) and IS ($p<0.05$). There was a close correlation between cyclin D1 and IGF-I levels ($p=0.057$). L1-CAM over-expression correlated with decreased DSS ($p<0.05$). HNG correlated with larger tumor size ($p<0.02$), high proliferative activity ($p<0.001$), EGFR over-expression ($p<0.001$), IS ($p<0.001$), and high RM ($p<0.013$). IS correlated with larger tumor size ($p<0.001$), high proliferative activity ($p<0.001$), EGFR over-expression ($p<0.004$), and worse DSS ($p<0.031$). Females were more likely to have small tumor size ($p<0.03$), low expression of EGFR ($p=0.047$) and IGFBP-3 ($p=0.022$).

Conclusions: A combination of markers (low-reactivity for both IGF-I and IGBP-3 and high reactivity for EGFR, with a high proliferation index) identifies a group of patients with CRCC at high risk for RM and decreased DSS. The frequent high expression of EGFR indicates a potential therapeutic target in CRCC. Similarly, IGF-I and IGFBP-3 may be potential candidates for therapeutic manipulation in patients with advanced CRCC.

769 The Genetic Alterations in Papillary Renal Cell Carcinoma (PRCC) Evaluated by DASL (cDNA-Mediated Annealing, Selection, Extension and Ligation) Assay System

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Background: PRCC, accounting for 10 to 15% of RCCs is currently divided into 2 types (Type 1 and Type 2) on morphologic grounds. Available data indicate that molecular and genetic, as well as, prognostic differences may exist between these subtypes, however this remains an area of controversy. Molecular cytogenetic approaches might be an efficient way to address this question. In this study formalin-fixed paraffin-embedded (FFPE) tumor samples were processed by using the DASL Assay System to identify the genes that might distinguish types 1 and 2 PRCC.

Design: We identified 36 cases of PRCC tissues from two institutions (Ankara University and Wayne State University). Patient demographic and pathology information was collected. Tumors were classified into Types 1 and 2 based on the published histopathological features. The Illumina DASL has been recently developed for expression profiling of RNA obtained from FFPE tissues and other sources of partly degraded RNA. For total RNA isolation from FFPE tissues, three 20um thick sections were cut from each tissue block. The High Pure RNA Paraffin Kit (Roche) was used for RNA extraction. 200 ng of total RNA was converted to cDNA. Specimens were analyzed by the Illumina DASL Cancer Panel of 512 cancer related genes using a Sentrix® Universal Array Matrix and scanned by an Illumina Bead Station.

Results: Analyzable gene expression data was obtained in 23 cases (8 Type 1 and 15 Type 2). The preliminary DASL analysis identified a number of differentially expressed genes in the PRCCs with significant differences between Type 1 and 2 tumors. Among the more than 20 such genes identified were *IGFBP2, BCR, MMP14, FGFR1, GLI3, RARB, CCND2, CCND3, CRK, LIF, MAF, MLLT6, AHR, DAPK1, ERBB2, MET* and *KIT*. Ongoing analysis shows that we will be able to build a classifier to distinguish Type 1 and Type 2 tumors with DASL analysis.

Conclusions: Cytogenetic information from gene expression data will be useful in developing a classification of PRCC that incorporates pathological, cytogenetic and gene expression-based components. This study indicates that the DASL Assay System can be run successfully with FFPE tissues to evaluate the cytogenetics of RCCs. By increasing the numbers of cases evaluated it will be possible to construct a model to successfully subclassify PRCC and correlate this with the morphological subtyping.

770 A Preoperative Model Evaluating the Tissue Effects of Chemopreventive Agents in Clinically Localized Prostate Cancer

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Background: A model exploiting the time between histologic diagnosis of prostate cancer (Pca) and prostatectomy provides a framework for evaluating biomarkers modulated by chemopreventive agents. The presence of multiple tumor foci and zones create challenges to meaningful comparison of biomarkers across various interventions. In a study complementary to the ongoing Selenium and Vitamin E Cancer Prevention Trial, we evaluated the effects of L-selenomethionine (SeMET) and vitamin E (VE) in modulation of selenoprotein expression, through which their chemopreventive effects may be mediated.

Design: 48 patients (pts) were enrolled in a single-institution, randomized, double-blind trial of VE and SeMET. Eligible pts [T1c/T2 disease, PSA <10 ng/mL, GS ≤7, and prostatectomy (RPS) scheduled 3–6 wks after treatment initiation] were randomized to receive daily VE (400 IU), SeMET (200 mg), the VE+SeMET combination or placebo. The number of tumor foci and GS, zonal origin (peripheral zone [PZ], transition zone [TZ], or central zone [CZ]) and tumor volume (TV) of each focus were recorded in 36/39 evaluable pts. Using immunohistochemistry, glutathione peroxidase (GPX) and thioredoxin reductase (TR) expression were evaluated semiquantitatively in each tumor focus and in the different zones. Either Fisher's exact test or exact McNemar's test was used to compare changes between treatment groups.

Results: The number of tumor foci in RPSs varied from 1 to 7: 1 (6), 2 (7), 3 (11), 4 (9), 5 (2), and 7 (1). The dominant tumor focus was in the PZ (30—GS 6 [6]; GS 7 [23]; GS 9 [1]), followed by the TZ (5—GS 6 [4]; GS 7 [1]) and CZ (1—GS 6 [1]). There was no significant difference in total TVor expression of GPX or TR between treatment groups. In cancer cells, higher levels of GPX were detected in the TZ than in the PZ ($p = .04$), whereas in normal cells the levels were higher in the CZ than in the PZ ($p = .03$). The levels of TR in normal cells were higher in the CZ than in either the PZ ($p = .008$) or TZ ($p = .03$).

Conclusions: Using detailed, systematic pathologic evaluation, we observed that there may be differential zonal expression of selenoproteins. A clinicopathologic model, such as the one proposed herein, which takes into consideration the multifocality and multizonal heterogeneity of Pca, would expedite evaluation and cross comparison of tissue effects of chemopreventive agents in Pca.

771 Utility of Polyclonal Anti-PSA and High-Molecular Weight Cytokeratin Immunohistochemistry in the Differentiation of Poorly Differentiated Prostatic Carcinoma from Urothelial Carcinoma

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Background: Immunohistochemistry using the prostatic marker, PSA and the urothelial carcinoma (UCa) associated marker high-molecular weight cytokeratin (HMWCK) has been shown to be useful in differentiating high-grade prostate cancer (PCa) from poorly differentiated UCa. Polyclonal anti-PSA has been reported to be more sensitive than monoclonal anti-PSA in high-grade PCa. However, previously published studies have almost exclusively evaluated morphologically unequivocal cases of PCa and UCa. We retrospectively evaluated the utility of the above two markers in a series of cases of poorly differentiated carcinoma with an unresolved differential diagnosis of PCa versus UCa.

Design: 16 cases of poorly differentiated carcinoma (14 in transurethral resections, 2 in metastatic sites) that had been signed out during a 5-year period as poorly differentiated carcinoma - ?PCa, ?UCa after immunostaining with monoclonal antibodies to PSA, PSAP and/or CEA at the time of diagnosis, were identified. The study immunohistochemistry was performed using polyclonal anti-PSA (DAKO corp.) and HMWCK antibody clone 34βE12 (DAKO corp.) and the cases were classified as PCa (PSA+, HMWCK-), UCa (PSA-, HMWCK+) or unclassified poorly differentiated carcinoma (PSA-, HMWCK- or PSA+, HMWCK+).

Results: Using polyclonal PSA and HMWCK antibodies, a definite diagnosis could be established in 15 (93.8%) of the originally equivocal cases. The study diagnosis was PCa in 9 cases and UCa in 6 cases. In 2 of these cases the possibility of synchronous PCa and UCa had originally been considered in view of patchy immunoreactivity with monoclonal anti-PSA, but polyclonal anti-PSA showed diffuse positivity establishing a diagnosis of PCa. The remaining case was negative for PSA and HMWCK, and an extended immunohistochemical panel was also non-diagnostic. Clinical findings in this case favored UCa but no follow-up was available.

Conclusions: Histopathologic distinction between UCa and PCa is critical due to markedly differing therapeutic modalities for the two malignancies. Our findings confirm the clinical utility of a simple immunohistochemical panel composed of polyclonal anti-PSA and HMWCK for this differential diagnostic dilemma.

772 Anatomical Levels of Invasion and Tumor Thickness in Invasive Penile Squamous Cell Carcinoma (SCC) of the Glans. A Proposal of Modification of the TNM System

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Background: There is a correlation of depth of invasion and regional metastasis in SCC of the penis. The T assessment in the UICC TNM system is based on the invasion of lamina propria (T1) or corpora spongiosa and cavernosa (T2). We had previously observed that tumors invading the superficial portion of the corpus spongiosum seemed to have a better prognosis than those invading deeper portions of the corpus spongiosum; however no formal study had been done to confirm this hypothesis. The aim of this study was to compare the presence of regional metastasis according to tumor involvement of anatomical levels and tumor thickness and to assess possible different prognosis in tumors involving superficial and deep corpus spongiosum

Design: Eighty seven penectomies and node dissections for SCCs of the glans were evaluated. Anatomical levels were lamina propria (LP), corpus spongiosum (CS) and corpus cavernosum(CC). Thickness (mm) was from tumor surface to deepest point of invasion. Superficial CS was defined as the erectile tissue to a depth not superior to 4.9 mm.

Results:

Level of invasion, tumor thickness and presence of nodal metastasis (87 cases)

Level of invasion	# Negative nodes	# Positive nodes	% Positive nodes	p value
LP and Sup CS	8	0	0	0.008
Deep CS	20	20	50	0.9
CC	18	21	54	
Tumor Thickness (mm)				
0.1-4.9	8	0	0	0.008
5-10	17	18	51	0.9
> 10	21	23	52	

Sup CS: superficial corpus spongiosum

Conclusions: Superficial tumors invading LP or CS to a depth not superior to 4.9 mm were not associated with metastasis. Tumors invading deep CS (5 mm or more from the surface) and CC showed more frequent metastasis. Since tumors invading the superficial CS behave more like those invading LP and and they have significantly better prognosis than deeper lesions, we believe it would be more accurate to separate them from tumors invading deep CS and CC. Based on these findings we propose the modification of the TNM system as follow: T1, invasion of lamina propria and superficial CS and T2, invasion of deep CS and CC.

773 Distinct DNA Methylation Profiles between Papillary and Non-Papillary Bladder Urothelial Carcinoma

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Background: Urine cytology is the first line clinical screening for bladder cancer. Although high grade flat carcinoma in situ (CIS) can be readily detected in urine cytology, detection of papillary urothelial carcinoma (TCC) in urine is notoriously challenging due to the overlapping morphologic features between benign and malignancy. Recent studies have revealed that papillary and non-papillary TCC are two distinct biologic processes involving different cellular signaling pathways. However, epigenetic alterations of these two types of TCC have not yet been studied. We have studied the promoter methylation profiles of 14 tumor suppressor genes (TSG) in 35 urothelial carcinomas in urine.

Design: Thirty-five urine samples consist of 12 low grade papillary urothelial carcinoma (LGTCC), 13 high grade papillary urothelial carcinoma (HGTCC) and 10 flat high grade carcinoma in situ (CIS). All cases had subsequent surgical follow up. Genomic DNA from urine was extracted and chemically converted by bisulfite treatment. Promoter methylation of 14 candidate TSGs (*APC, RAR-beta, p14, p15, p16, p73, RASSF1a, hMLH1, DAPK, MGMT, BRCA-1, SOCS-1, GSTP* and *FHIT*) was analyzed by methylation-specific PCR.

Results: The most frequently and significantly methylated TSGs in flat CIS were *RASSF1a* (80%), *RAR-beta* (70%), *p15* (60%) and *APC* (50%). The most frequently methylated TSGs in HGTCC were *BRCA-1* (69%), *FHIT* (62%) and *SOCS-1*(54%). Methylation of *DAPK* (75%) and *RAR-beta* (59%) was more frequently seen in LGTCC. Concurrent methylation of multiple TSG promoters was seen in all three types of TCC. Methylation of five or more TSG promoters was seen in 33% LGTCC, 54% HGTCC and 70% CIS. In correlation of cytologic with histology, 54% (19/35) cases were cytologically diagnosed as either negative (4) or atypical (15), including 11/12 LGTCC, 5/13 HGTCC and 3/10 CIS. Based on multiple methylation profiling, 85% of cytologically "negative" or "atypical" cases can be re-classified as "positive" urine. Methylation of *p14, p16, GSTP, MGMT*, and *p73* showed no significant difference among three groups.

Conclusions: Our study indicates that different DNA methylation profiles exist between papillary and non-papillary TCC. Progressive accumulation of multiple epigenetic changes from LGTCC to HGTCC to CIS supports current concept of concerted genetic alterations during carcinogenesis. Our study suggests that application of methylation profiling may be helpful in facilitating early and accurate detection of bladder urothelial carcinoma in urine cytology.

774 Urothelial Carcinomas with Mixed Histology: Incidence, Clinicopathological Spectrum, and Biological Significance

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Background: Urothelial carcinomas (UC) have a propensity for divergent histological differentiation, where a non-urothelial variant may be seen accompanying otherwise typical UC. We investigated the incidence, clinicopathological spectrum, and biological significance of UC with mixed histology in comparison to pure UC.

Design: A retrospective review of 506 consecutive transurethral resections of bladder tumor (TURBT) and 256 cystectomies was performed. UC containing mixed histology were classified according to histologic type and percentage as focal (<15%), moderate (15-75%) and extensive (>75%). Pure non-UC cases were excluded from analysis. The pathologic stage, presence of carcinoma in situ (CIS) and lymphovascular invasion were recorded for each case if available. Pure UC with similar histologic grade were compared with UC containing mixed histology for comparison.

Results: Mixed histology was identified in 18% (91 of 506) of TURBT. Mixed histology was uniformly (100%) associated with high grade UC and with invasive carcinoma in all but 1 case (99%). Pathological spectrum of mixed histology was as follows in order of frequency: squamous (42%), glandular (15%), sarcomatoid (12%), micropapillary (10%), small cell (9%), and lymphoepithelial (1%). Eleven percent of cases had multiple types of mixed histology. Thirty-three percent of cases had focal, 24 % moderate and 43 % extensive component of mixed histology. Associations between type (p=1.14, Fischer's exact test) and percentage (p=0.51) of mixed histology with pathologic stage were not statistically significant. The difference between pathologic stages in cystectomies between UC with mixed histology versus pure high-grade UC was statistically significant (p=0.0001, Chi-squared test of association). Specifically, UC with mixed histology were more likely to be associated with higher stage compared to pure UC. Presence of mixed histology was also commonly associated with presence of CIS and lymphovascular invasion.

Conclusions: Mixed histology is common (18%) with wide pathological spectrum and typically seen in higher grade and advance stage UC. Presence of mixed histology in a TURBT should be reported, as it is associated with a higher stage of tumor. The biological significance of UC containing mixed histology in relation to therapy response and outcome is currently underway.

775 Iatrogenic and Non-Iatrogenic Positive Margins (PM): Incidence, Site, Factors Involved, and Time to PSA Progression Following Radical Prostatectomy (RP)

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Background: There are conflicting data regarding the incidence and prognostic significance of PM resulting from iatrogenic incision into the prostate (pT2+) or non-iatrogenic inability to excise extraprostatic extension (EPE) of tumor.

Design: The surgical specimens were whole mount processed. The bladder neck and the apical margins were amputated and processed through perpendicular sections. PM was defined as cancer cells touching the inked surface of the prostate. EPE 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 perirprostatic tissue was seen. Site of PM was considered as being in the bladder neck, apex, or circumferential (anterolateral, posterolateral or both according to the quadrants involved). Nerve-sparing, tumor extension and Gleasons score were considered possible factors involved in PM. Tumor extent was evaluated with a point count semiquantitative method. The data were analyzed using the Mann-Whitney test, Fisher's exact test and the log-rank survivorship for comparing the time to PSA progression between the groups.

Results: From a total of 230 consecutive patients submitted to retropubic RP, PM resulted from iatrogenic incision (pT2+) in 61/230 (26.52%) prostates and from non-iatrogenic inability to excise EPE in 34/230 (14.78%) prostates. The site involved in pT2+ prostates was bladder neck (9.84%), apex (22.95%), and circumferential (85.25%) (anterolateral 29.51%, posterolateral 40.98%, both 14.75%). In cases with EPE the site involved was bladder neck (35.29%), apex (26.47%), and circumferential (67.65%) (anterolateral 17.65%, posterorateral 23.53%, both 67.65%). PM occurred equaly in patients with and without nerve-sparing in both groups (p>0.99). Tumors were significantly more extensive (p<0.01) and with higher Gleasons score (p<0.01) in patients with EPE. Time to PSA progression equaly occurred in patients with pT2+ or EPE.

Conclusions: In our study, iatrogenic incision as a cause of PM was more frequent than non-iatrogenic. PM occurred equaly in patients with or without nerve-sparing. Tumors were significantly more extensive and with higher Gleason score in patients with PM and EPE. The most frequent site with PM was circumferential (posterolateral quadrants in whole mount processing). Time to PSA progression occurred equaly in both groups.

776 MLH1 Immunoreactivity in Prostatic Adenocarcinoma

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Background: MLH1 is one of the major DNA mismatch repair genes. Alteration of MLH1 gene has been found in colon adenocarcinoma and is believed to play a major role in microsatellite instability. Increased MLH1 immunoreactivity has also been observed in aggressive cancers such as glioblastoma, melanoma, high-grade lymphoma and metastatic renal cell carcinoma. Recently, genetic analyses have shown that MLH1 is altered in prostatic adenocarcinoma. However, the information about MLH1 protein expression in human prostatic tissues is limited.

Design: Immunohistochemistry using a monoclonal antibody specific for MLH1 protein was performed in prostatic adenocarcinoma (n=65), high grade prostatic intraepithelial neoplasia (n=26) and compared with benign prostatic tissues (n=71) on tissue microarrays. The MLH1 nuclear immunoreativity was graded as negative (0), weakly positive (+1), positive (+2) and strongly positive (+3). We also tested a new double staining cocktail containing the MLH1 antibody labeled with DAB Betazoid as chromogen and a 34βE12 antibody labeled with Vulcan Fast Red a chromogen.

Results: The normal prostatic epithelium typically showed weak MLH1 reactivity and prostatic stroma showed no staining. The majority (75.3%) of prostatic adenocarcinomas demonstrated MLH1 immunoreacitiy compared to benign prostatic tissue (5.6%). Nearly one half of HGPIN were positive for MLH1. The mean intensity was 2.09 for prostatic adenocarcinoma, 1.31 for high grade PIN and 0.69 for benign prostatic epithelium. Double immunostaining showed distinct brown MLH1 nuclear staining in prostatic adenocarcinoma in contrast to red cytoplasmic 34βE12 staining of the basal cells in benign glands.

Conclusions: The significantly increased MLH1 immunoreactivity in the majority of prostatic adenocarcinomas could be associated with genetic alterations. Our findings suggest a possible role of MLH1 in prostatic carcinogenesis. Although the mechanism remains to be investigated, MLH1 may function like p53 in regulating prostatic cell growth and differentiation. Furthermore, MLH1 may serve a diagnostic marker and therapy target for patients with prostate cancer. Because of its strong nuclear staining in prostatic adenocarcinoma, the MLH1 antibody may be potentially used in a newly improved antibody cocktail detecting prostate cancer.

	MLH-1 Immunoreactivity		
	Benign Glands	High Grade PIN	Prostatic Adenocaricnoma
Positivity	4/71	12/26	49/65
Percentage	5.6 %	46.2%	75.3%
Intensity	0.69	1.31	2.09

777 Refinement of Classification of Papillary Renal Cell Carcinoma

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Background: Papillary renal cell carcinoma (PRCC) is the second most common subtype of kidney cancer. Despite its moderate incidence, the genetic background of PRCC is disproportionately poorly understood. A morphologic classification of PRCC into tumor types 1 and type 2 has been recently proposed, but its biological relevance and histological and immunohistochemical definition remain uncertain.

Design: We studied the gene expression profiles of 34 cases of PRCC with Affymetrix HGU133 Plus 2.0 arrays (54,675 probe sets) using both unsupervised and supervised analyses. Comparative genomic microarray analysis (CGMA) was also used to infer the cytogenetic profile for each tumor. Based on the gene expression profiles, the expression of keratin 7 and topoisomerase IIα was further evaluated by using immunohistochemistry in 78 and 58 cases, respectively.

Results: Two distinct molecular PRCC subclasses were identified with defined morphologic and biological correlation. The first class (n=23), with excellent survival, corresponded to 3 histological subtypes: type 1, low-grade type 2 (2A) and mixed type 1/2A tumors. The second class (n=11), with poor survival, corresponded to high-grade type 2 (2B) tumors. Characteristic chromosomal aberrations were generated for class 1 and class 2 tumors using CGMA. Immunohistochemical analysis confirmed the high expression of keratin 7 in 54/57 (95%) class 1 tumors, while only 3/21 (14%) class 2 tumors showed a high level of keratin 7 expression. In contrast, the topoisomerase IIα expression was high (12/16) or moderate (4/16) in class 2 tumors, compared to moderate (4/42) or low (38/42) expression in class 1 tumors. The key features of class 1 and class 2 papillary renal cell carcinomas are summarized in the Table.

Conclusions: We define two subclasses of papillary renal cell carcinoma with molecular characteristics, which correlate with the current histological subclassification. Application of this biologically distinct and clinically relevant classification may be beneficial for diagnosis and management of the patients.

Classification of Papillary Renal Cell Carcinoma

Class	Molecular Feature	Histology	Grade	TopIIα	CK7	Behavior
Class 1	Profile 1	Type 1	1-2	Low	Positive	Indolent
	Profile 1	Type 2A	1-2	Low	Positive	Indolent
	Profile 1	Mixed Type 1 & 2A	1-2	Low	Positive	Indolent
Class 2	Profile 2	Type 2B	3-4	High	Neg. / Weak Pos.	Aggressive

778 Bilateral Testicular Germ Cell Tumors

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Background: Germ cell tumors are the most common type of testicular tumors and the most common malignancies in men between the ages of 15 to 34 years. It has been well recognized that men who have had one testicular germ cell tumor have an increased risk of developing a second germ cell tumor in the contralateral testis.

Design: We reviewed 317 testicular germ cell tumors in patients treated at our institution between 1995 and 2005 and identified nine patients with BTGCT. All patients underwent radical orchiectomy. We examined the clinical and the histological characteristics, the treatment and the clinical outcomes of these patients.

Results: BTGCT were seen in 2.8% of our patients with testicular germ cell tumors. The mean age at diagnosis of the first tumor was 31 years (range 18-46) and the mean age at diagnosis of the second tumor was 38 years (range 23-56). Eight out of nine patients had metachronous tumors and one presented with synchronous tumors. Among the patients with metachronous tumors, the time interval between the diagnoses ranged from 17 to 202 months (median 57). In two patients the diagnosis of the second tumors was established 16.7 and 16.8 years after the initial diagnosis in the opposite testis. These patients were 56 and 54 years old at the time of the second diagnosis. Only 2 out of 11 patients had tumors with identical histologic type, which was pure seminoma in both testes. The patient with synchronous tumors revealed different histopathology in each testis (see table). After a mean follow-up period of 132 months (range 32-289) eight patients were alive with no evidence of disease and one was alive with metastatic disease to the lung.

Patient	Histology (1st tumor)	TNM Stage (1st tumor)	Histology (2nd tumor)	TNM Stage (2nd tumor)	Interval between tumors (months)
1	Embryonal	I	Mixed GCT	I	90
2	Mixed GCT	I	Mixed GCT	I	57
3	Seminoma	I	Seminoma	I	201
4	Yolk Sac	II	Mixed GCT	II	Synchronous
5	Teratoma	I	Mixed GCT	II	55
6	Seminoma	I	Seminoma	II	110
7	Seminoma	I	Mixed GCT	I, Relapsed to II	46
8	Seminoma	III	No viable tumor	III	17
9	Mixed GCT	II	Seminoma	I	202

Conclusions: Patients with a history of testicular germ cell tumor require careful long term follow-up of the contralateral testicle due to the risk of bilateral disease which may occur after a long time interval. The majority of the patients with BTGCT have a good clinical outcome.

779 Pure Teratoma of the Testis in Adults

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Background: Post pubertal testicular teratomas are malignant tumors with metastatic ability. Testicular teratoma is most commonly seen as a component of mixed germ cell tumors and it presents uncommonly as a pure histologic type in adults.

Design: We studied retrospectively testicular germ cell tumors in 204 patients who underwent radical orchiectomy for testicular germ cell tumor in our institution between October 1999 and May 2005. Of these, 11 patients had pure testicular teratomas. We reviewed the histology with particular attention to the presence of intratubular germ cell neoplasia of the unclassified type (ITGNU).

Results: Overall, 5.3% (11/204) of patients with germ cell tumors demonstrated pure teratoma, which represent 20% (11/54) of all mixed germ cell tumors in our study. The mean age at diagnosis was 28 years (range 22-40). Median tumor size was 1.5 cm (range 1-6.5 cm). Both mature and immature components were present in five patients. Six patients exhibited only mature elements. Parenchymal scars, which possibly represent partially burned-out lesions, were identified in two cases. ITGNU was identified in 81% (9/11) of the cases. In two patients, where no ITGNU was identified, only mature elements were present and no parenchymal scars were seen. Both tumors were of small size (1 and 1.5 cm) and demonstrated non-cutaneous teratomatous elements in addition to a squamous epithelium-lined cyst, which raised the possibility of dermoid cyst in the differential diagnosis. However, one of the two patients developed retroperitoneal lymph node metastasis after one year, which was composed only of mature cystic teratoma. Mean clinical follow-up for all patients was 33 months (range 7-68). Four out of 11 patients (36%) developed retroperitoneal metastases. One of the patients with metastasis also developed metachronous mixed germ cell tumor in the contralateral testis.

Conclusions: Pure testicular teratoma is an uncommon malignant testicular tumor in adults. ITGNU may not be identified in some cases of pure testicular teratomas. Despite the lack of ITGNU and bland histological features which may overlap with a morphology of a dermoid cyst, pure testicular teratomas may demonstrate metastatic potential.

780 Prevalence of Incidental Prostate Cancer in the General Population

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Background: Prostate cancer detection has surged dramatically in recent years due to improved cancer screening. There has been a lot of interest focused on early detection, with studies suggesting presence of cancer in younger individuals. We analyzed our data set of incidental prostate cancer, derived from a project accruing prostate tissues for research from "normal" organ donors.

Design: The Tissue Bank of the UPMC harvested 240 prostates for research from people who died suddenly in the time span 1994-2005. Stroke, motor vehicle accidence, homicidal and suicidal gunshot wound to head, cardio-respiratory arrest, and trauma accounted for over 90% of the causes of death of the donors. None of donors was known to have prostate cancer ante-mortem. Serum levels of prostatic specific antigen were not available. Donors age ranged from 13 to 78 years (mean = 40 17). The donors consisted of 229 Caucasians, 10 African-Americans and 1 Hispanic. Serially-sectioned prostate tissues were evaluated were genitourinary pathologists.±´

Results: The mean age of donors with high-grade prostatic intraepithelial neoplasia (HGPIN) was 56 years, and was 60 years for those with adenocarcinoma. The table 1 below details the presence of prostatic neoplastic lesions in different age groups.

Conclusions: This study provided an insight into the prevalence of prostatic HGPIN and adenocarcinoma in the general population. There was an age-dependent increase in HGPIN starting from the 4th decade of life. Prostatic adenocarcinoma escalated from the 5th decade onwards, with one in three chance of carrying incidental cancer by the age of 60 years.

Prostatic neoplastic lesions in different age groups.

	Total	≤; 39 year-old	40-49 year-old	50-59 year-old	60-78 year-old
Number of donors	240	115	37	52	36
HGPIN only	23 (9.6%)	0 (0%)	6 (16.2%)	9 (17.3%)	8 (24.2%)
Adenocarcinoma	20 (8.3%)	1 (0.9%)	0 (0%)	7 (13.5%)	12 (33.3%)
Mean Gleason scores	6.3	8	N/A	6	6.5

781 Apoptosis Regulators in Superficial Urothelial Carcinoma: An Analysis of Eleven Proteins of the Caspase Family and the Inhibitor of Apoptosis Protein (IAP) Family

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Background: The caspase family proteases are key proteins in the apoptotic signalling pathways while the IAP proteins effectively antagonize the caspases. Limited studies of a few of these molecules in urothelial carcinomas suggested their deregulation might contribute to urothelial neoplasia. However, the overall expression status of the caspase and IAP family proteins in urothelial neoplasms is not clear. We evaluated the expression of these molecules in superficial (pTa or pT1) urothelial carcinomas and non-neoplastic urothelium.

Design: The expression of caspase-3, 6, 7, 8, 9, and 10, of the caspase family, and c-IAP1, c-IAP2, XIAP, survivin, and livin (ML-IAP, KIAP), of the IAP family, was examined by immunohistochemistry in twenty-five low-grade and nineteen high-grade superficial (pTa or pT1) urothelial carcinomas, and eleven non-neoplastic, apparently normal urinary bladder mucosal samples. All samples were formalin-fixed and paraffin-embedded. Standard labeled streptavidin-biotin immunohistochemical staining was performed. Immunostaining was scored integrating staining extent and intensity.

Results: (1) Caspases and IAPs were observed to be expressed in both non-neoplastic, apparently normal urothelium and the superficial urothelial carcinomas. (2) Expression of the caspases was generally weak to moderate in normal urothelium, but varied to a greater extent in the urothelial carcinomas. Interestingly, moderate to strong expression of both initiator and executioner caspases was observed in a subset of both low- and high-grade urothelial carcinomas. (3) Expression of the IAP family proteins were stronger in urothelial carcinomas than normal urothelium. Among the IAP family members, livin appeared to be less strongly expressed in urothelial carcinomas than other IAPs. (4) A difference in expression of the caspase and IAP proteins was not apparent between low- and high-grade superficial urothelial carcinomas.

Conclusions: The expression pattern of the caspases and IAPs in superficial urothelial carcinomas as compared to normal urothelium suggested their participation in deregulated cell survival and proliferation, although a difference between the low- and high-grade superficial urothelial carcinomas was not apparent.

782 Immunohistochemical Analysis of the Micropapillary Variant of Urothelial Carcinoma of the Urinary Bladder

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Background: Micropapillary urothelial carcinoma (MUC) is a rare and distinct variant of urothelial carcinoma of the urinary bladder. MUC is usually associated with high grade and high stage of disease, with a high propensity for lymphovascular invasion and metastasis ("metastasizing phenotype"). The molecular mechanism behind the aggressive nature of MUC is yet to be elucidated. In this study, we investigated the expression of tumor prognostic markers in MUC by immunohistochemistry.

Design: Eleven MUC cases were identified from the archived radical cystectomy specimens at the Department of Pathology of Columbia University Medical Center, between 2002-2005. Immunohistochemical staining was performed on formalin-fixed and paraffin embedded tissue sections, using the following monoclonal antibodies: fascin (clone 55K-2), CD44 (clone DF1485), E-cadherin (clone NCH-38), β-catenin (clone β-catenin-1), and uroplakin III (clone AU1). The staining was scored semi-quantitatively. The percentage of stained cells (PSC) was graded from a scale of 0 to 3: 0 (non/rare cells staining), 1 (<5%), 2 (5-50%), and 3 (>50%), while intensity was scored from 1 to 3: 1 (weak), 2 (moderate), and 3 (strong). Staining with PSC ≥ 1 was considered positive.

Results: Consistent with reported aggressive pathological and clinical features of MUC, most cases presented with high grade and high stage disease: 11/11 (100%) cases had high nuclear grade (G3-4), 10/11 (91%) with lymphovascular invasion, 8/11 (73%) with regional lymph node metastasis, and 10/11 (91%) at T2-T4 stages. Most cases (8/11, 73%) were either negative (6 cases) or only focally and weakly (2 cases) positive for fascin. CD44 was focally to diffusely positive in the majority of cases (8/11, 73%). All eleven cases show focal to diffuse, membranous positivity for E-cadherin and β-catenin. Uroplakin was focally and diffusely positive (cytoplasmic/ membranous) in 4/11 (36%) cases; additionally, 2/11 (18%) cases show aberrant, nuclear staining.

Conclusions: Over-expression of fascin, and decreased expression of CD44, E-cadherin, and β-catenin have been reported to be associated with aggressive behavior and poor prognosis of conventional urothelial carcinomas of the urinary bladder. In contrast, the absence or low expression of fascin and increased expression of CD44, E-cadherin, and β-catenin, shown in MUC cases in our study, suggest a distinct molecular mechanism(s) for the aggressive behavior of MUC. Further study of the molecular mechanism of invasion and metastasis of MUC is warranted.

783 BNIP3 Appeared To Be Increased in Prostate Adenocarcinoma Cells and Tissues

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Background: BNIP3 is a pro-cell death protein involved in the control of mitochondrial permeability transition pore. BNIP3 is regulated by hypoxia-inducible factor 1 alpha, and has been shown to be down regulated in several cancers, such as gastrointestinal and pancreatic carcinomas, via methylation of its promoter. In a survey of apoptosis-related genes using cDNA microarrays, however, we observed apparent high level of BNIP3 mRNA in prostate adenocarcinoma cells. We further examined its expression in prostate cancer cell lines and tissues using multiple assays.

Design: An apoptosis pathway-specific cDNA array (HS-002, SuperArray, Frederick, Maryland) was used to evaluate the expression status of genes involved in apoptosis regulation in three prostate adenocarcinoma cell lines, LNCaP, DU145, and PC-3, as compared with non-neoplastic prostatic epithelial cells collected by laser capture microdissection from fresh surgical specimens. BNIP3 mRNA level was further analyzed using reverse transcription polymerase chain reaction (RT-PCR). A monoclonal anti-BNIP3 antibody (clone ANa40, Sigma, Saint Louis, Missouri) was used for standard immunostaining of formalin-fixed, paraffin-embedded prostate adenocarcinoma tissues (40 cases) and non-neoplastic tissues (15 cases).

Results: cDNA array analysis showed that BNIP3 in the prostate adenocarcinoma cell lines was over 3-fold higher than that in the non-neoplastic prostatic epithelial cells. RT-PCR results were consistent with the array analysis. Moderate to strong positivity of BNIP3 immunohistochemical staining was observed in 65% (26/40) of prostate adenocarcinomas, but only in 40% (6/15) of non-neoplastic prostatic glandular tissues.

Conclusions: BNIP3 appeared to be increased in prostate adenocarcinoma as compared with normal prostate epithelial cells. More analyses are under way to further examine the phenomenon.

784 Sarcoma Arising in Cystic Nephroma and Mixed Epithelial and Stromal Tumor of the Kidney

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Background: Cystic nephroma (CN) and mixed epithelial and stromal tumor of the kidney (MESTK) are rare benign renal tumors with epithelial and stromal elements. Malignant transformation, or sarcoma arising in these tumors, is exceedingly rare, with only 5 cases reported in the literature so far. We report 3 such cases that we have encountered during a 23-year period at authors' institution.

Design: CN or MESTK with malignant or sarcomatous components were retrieved from surgical pathology archives and reviewed. Clinical and follow up information were collected through IRB-approved chart review and patient contact. In all cases, fluorescent in situ hybridization (FISH) for translocation involving SYT (18q11) was performed.

Results: Between 1982 and 2005, 31 diagnosis of CN or MESTK were made, 3 of these had sarcoma (2 in CN; 1 in MESTK). The Patients demographics are shown in the Table. In all 3 cases, the sarcomatous components were malignant non-descript spindle cells with nuclear pleomorphism and frequent mitosis. No lineage differentiation was apparent histologically, although the tumor in the 14-year old resembled pediatric clear cell sarcoma. Sarcomatous components were intimately associated with benign-appearing epithelial elements, and often entrapped them. The sarcomatous components were positive for vimentin, but negative for cytokeratins, EMA, actin, desmin, or S-100. They were negative for SYT chromosomal translocation associated with synovial sarcoma. One patient developed lung and bone metastasis and died of the disease 25.6 months after surgery. The 2nd patient experienced local recurrence along with bone and brain metastasis, culminating in death 84.8 months after the initial diagnosis. The 3rd patient was well 10 months after surgery.

Conclusions: Sarcomas can arise in CN and MESTK. Although uncommon, they have a poor prognosis. Cystic lesions of the kidney should be extensively sampled.

	Case 1	Case 2	Case 3
Age/ Gender	36/Female	14/Male	67/Female
Recurrence	Lung, bone	Local, bone, brain	None
Follow up time (months)/outcome	25.6/ dead	84.8/dead	9.7/ alive
Tumor location	Left upper pole	Right lower pole	Right upper pole
Tumor size (cm)	12x9x5	10x8x7	2.5x2x2
Gross	Cystic mass with white-pink friable solid area	Cystic mass with white-pink friable solid area	Cystic mass
SYT translocation	Negative	Negative	Negative

785 Loss of Expression of the Tumor Suppressor 14-3-3 Sigma Protein in Chromophobe Renal Cell Carcinoma Is Associates with Promoter Hypermethylation

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Background: Tumor suppressor gene 14-3-3 sigma is a p53-regulated G2/M inhibitor, and regulates numerous cellular signaling pathways involved in cell cycle control, DNA repair and apoptosis. Recent studies have indicated that the 14-3-3 sigma gene is inactivated mainly through promoter hypermethylation in breast and hepatocellular carcinomas. Our previous study found that 14-3-3 sigma expression was retained in most of oncocytoma, but reduced in renal cell carcinoma (RCC), especially chromophobe RCC (ChRCC). This study was designed to test whether decreased 14-3-3 sigma expression in ChRCC resulted from promoter hypermethylation.

Design: Two tissue microarrays that consisted of 20 ChRCC and 67 oncocytomas were immunohistochemically evaluated for the expression of the 14-3-3 sigma protein. Genomic DNA from 20 ChRCC and 24 oncocytomas were extracted and the promoter methylation of 14-3-3 sigma was assessed using methylation-specific PCR.

Results: Diffuse moderate to strong expression of 14-3-3 sigma was found in 83.6% (56/67) of oncocytomas. Expression of 14-3-3 sigma was dramatically decreased in ChRCC, present only in 10% (2/20) cases. The difference between oncocytoma and ChRCC was statistical significant (P<0.001). Promoter methylation of 14-3-3 Sigma was detected in 13/20 ChRCC, among them 92% (12/13) showed no 14-3-3 sigma protein expression by immunohistochemistry. Among 11 oncocytomas with negative 14-3-3 sigma immunostain, 81.8% (9/11) cases showed hypermethylation of the 14-3-3 sigma promoter. Eight cases (6 of ChRCC and 2 of oncocytoma) showed neither promoter methylation nor protein expression by IHC, in which inactivation of 14-3-3 sigma gene could be due to mechanisms other than promoter hypermethylation in these tumors.

Conclusions: We have demonstrated for the first time the loss of expression of 14-3-3 sigma protein in majority of ChRCC. Frequent methylation of 14-3-3 sigma promoter in ChRCC with no 14-3-3 sigma protein expression suggests that promoter methylation is the mainstay mechanism in silencing 13-3-3 gene in ChRCC. As overexpression of 14-3-3 sigma protein was seen in majority of oncocytoma, immunohistochemical stain of 14-3-3 sigma could be utilized in differentiating these two entities.

786 Pathological Characteristics of Prostate Cancer Following Initial Diagnosis of "Atypical Small Acinar Proliferation"

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Background: A diagnosis of "atypical small acinar proliferation (ASAP)" in prostate needle biopsy is associated with approximately a 50% of risk of detecting prostate cancer (PCa) in subsequent biopsies. The pathological characteristics of pCa following initial diagnosis of ASAP are not known. Specifically, is such PCa likely to be pathologically insignificant?

Design: Surgical pathology archives were queried for prostate biopsies with diagnoses of ASAP. Biopsies with concomitant cancer diagnosis were excluded. Number of ASAP foci, presence of concomitant high grade PIN (HGPIN), date and diagnosis of follow up biopsies were recorded. If a radical prostatectomy was performed for confirmed PCa, Gleason grade (GG), tumor volume (TV), extraprostatic extension (EPE), seminal vesicle invasion, pelvic lymph node metastasis, surgical margin status were also recorded.

Results: Between 1992 and August 2005, 337 of 10,500 (3.2%) of prostate biopsies carried a diagnosis of ASAP. 99 patients (29.4%) had follow up biopsies. The time interval between the first biopsy and last follow up biopsy was 15.1 months (range 0.5 to 74.5 months), and the average number of follow up biopsies was 1.4 (range 1 to 3). The majority of cases had single focus of ASAP, 9 cases (9.1%) had ASAP involving more than 1 biopsy core. 49 (49.5%) had concomitant HGPIN. Follow up biopsies showed benign prostatic tissue in 27 (27.3%), HGPIN in 10 (10.1%), ASAP in 5 (5.1%) and PCa in 57 (57.6%). The number of ASAP foci and presence of HGPIN did not correlate with detection of PCa in follow up biopsy. Of patients with PCa detected in follow up biopsies, 19 underwent radical prostatectomy and 1 underwent pelvic lymph node dissection alone that revealed metastatic PCa. 9 (31.6%) had potentially pathologically insignificant PCa (GS<=6, TV< 0.5 ml, organ confined), and 11 (57.9%) had pathologically significant PCa. GS was <=6 in 9 (47.3%), 7 in 5 (26.3%), 8 in 2 910.5%), and non-applicable due to hormonal ablation in 3 (15.8%). EPE was present in 5 (26.3%). TV was >0.5 ml in 6 (37.5%). Positive surgical margins were present in 2 (10.5%).

Conclusions: The majority of PCa detected following initial diagnosis of ASAP is pathologically significant. Patients with a diagnosis of ASAP should be followed up aggressively.

787 Constitutive Activation of Mammalian Target of Rapamycin (mTOR) and p70S6K in Prostate Cancer

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Background: A variety of preclinical studies have implicated the mTOR signaling pathway as an important component of prostate cancer. Almost all of this research has been based in cell lines and xenograft models utilizing rapidly growing, androgen-independent and highly metastatic clones that do not necessarily reflect the wide and heterogeneous spectrum of prostate cancer. In addition, the state of expression and activation of the mTOR pathway in a well characterized series of cases of prostatic adenocarcinoma has not been fully established and that is the focus of this study.

Design: A tissue microarray was constructed from archival paraffin-embedded material on sixty-four (64) cases obtained from radical prostatectomy specimens. These cases comprised organ-confined and previously untreated adenocarcinomas of the prostate with Gleason scores ranging from 5 to 10. Immunohistochemical procedures were carried out to detect the expression of activated mTOR (phosphorylated at Ser 2448) and one of its downstream substrates, p70S6K (phosphorylated at Thr 389). The sections were evaluated by bright-field microscopy to assess both intensity of the chromogenic signal on a 0-3+ scale and cellular compartmentalization.

Results: Sixty-one (61) out of 64 cases demonstrated good quality, reproducible staining for p-mTOR, and sixty-two (62) for p-p70S6K. Fifty-seven (57) out of 61 tumors (93%) exhibited moderate to strong (2+ to 3+) cytoplasmic/plasmalemmal expression of p-mTOR and fifty-three (53) out of 62 (85%) showed similar moderate to strong intranuclear expression of p-p70S6K. There was no statistically significant difference in expression of p-mTOR between those with Gleason scores of 5-6 versus 7-10.

Conclusions: Constitutive activation of the mTOR pathway, independent of Gleason score, is evident in the vast majority of cases of prostatic adenocarcinoma. This is evidenced by the cytoplasmic/plasmalemmal expression of p-mTOR(Ser 2448) and of its downstream substrate, p-p70S6K (Thr 389) with nuclear translocation. These findings coincide with preclinical studies in supporting a role for the mTOR pathway in the biology of prostate cancer and in suggesting a potential target for its treatment.

788 Glypican 3: A Novel Marker in Testicular Germ Cell Tumors

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Background: Glypican-3 (GPC3) is a membrane-bound heparin sulfate proteoglycan which is postulated to regulate cell division in certain tissues. GPC3 is mutated in Simpson Golabi-Behmel syndrome, an overgrowth syndrome characterized by an increased risk of embryonal tumors, including Wilm's tumor, neuroblastoma, hepatoblastoma, and testicular germ cell tumors. Recently, GPC3 was reported as one of the overexpressed genes in testicular yolk sac tumors by gene expression microarray analysis. The purpose of the study was to elucidate the expression of GPC3 in the different histological components of testicular germ cell tumors by immunohistochemistry.

Design: Specimens from 71 patients with testicular germ cell tumors were subjected to immunohistochemical study using monoclonal antibody specific for GPC3. The histological components were analyzed independently (42 seminomas, 36 embryonal carcinomas, 23 yolk sac tumors, 17 mature teratomas, 17 immature teratomas, and 6 choriocarcinomas). Cytoplasmic and membranous immunoreactivity was seminquantitatively evaluated as negative (< 5% of cells stained), focally positive (5-10% of cells stained), or positive (>10% of cells stained).

Results: All yolk sac tumor and choriocarcinoma components were immunoreactive for GPC3 while there was no immunoreactivity in residual benign testicular tissues (see table). The majority of embryonal carcinomas as well as all seminomas and mature teratomas did not express GPC3. Approximately 70% of immature teratomas were also negative for this marker.

Conclusions: We report a novel immunohistochemical marker, GPC3, which is differentially expressed in the histological components of testicular germ cell tumors, suggesting its possible role in tumor cell differentiation. GPC3 was expressed in all yolk sac tumors and choriocarcinomas, but was infrequently identified in embryonal carcinomas, teratomas and seminomas. Based on our data, GPC3 may be a useful marker in pathological diagnosis.

	YST	CC	Imm TER	EC	SEM	Mat TER
Positive	22 (96%)	5 (83%)	5 (29%)	2 (6%)	0	0
Focally Positive	1 (4%)	1 (17%)	0	3 (8%)	0	0
Negative	0	0	12 (71%)	31 (86%)	42 (100%)	17 (100%)
Total	23	6	17	36	42	17

Gynecologic

789 Volume of Borderline Change in Ovarian Neoplasms: Do Cystadenomas with Focal Borderline Change Warrant a Full Operative Staging?

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Background: Intra-operative evaluation of ovarian neoplasms helps determine the need for a full staging procedure at the time of surgery. However, the volume of borderline change in an otherwise benign cystadenoma can be quite variable. We sought to determine what volume of borderline change would justify a full surgical staging.

Design: Primary ovarian neoplasms with at least focal borderline change in the final diagnosis were identified in the University of Washington pathology database between 1995 and 2003 and available pathology reviewed. Estimates of the amount of borderline change were recorded based on histologic examination and the gross description (scored as 1-4 microscopic foci (40X field), 1-2cc, 2-4 cc or >4cc as well as percent of the total tumor volume). Comparison of the frozen section and final diagnoses were made. Staging data and clinical follow-up were reviewed.

Results: 59 cases with a final diagnosis of at least focal borderline change between 1995 and 2003 were identified in the pathology database and were available for review. Patient ages ranged from 18 to 84 years (median = 45). Ovarian masses ranged in size from 0.4 – 33 cm (median = 10) and were 67% serous (40/59), 29% mucinous (17/59), 2% endometrioid (1/59) and 2% mixed (1/59). 72% were stage 1 and 28% stage 2-3. 19% (11/59) had only 1-4 microscopic foci of borderline change, 13% (8/59) had 1-2cc, 24% (14/59) had 2-4 cc and 44% (26/59) had > 4cc. Of 19 cases with <2cc of borderline change, only one case (5%) was upstaged by a positive wash (all cases with <2cc were stage 1a-c). In contrast, 21 of 39 cases (54%) with >2cc of borderline change had positive cytology (13% stage 1c) or extra-ovarian disease (41% stage 2-3). Thus, cases with more than 2cc of borderline change in the ovary were significantly more likely to have extra-ovarian disease (p=0.0001, two-tailed, Fishers Exact). Clinical follow-up was available on 34 cases with a range of 6-108 months (median=28.5). There were no recurrences in the <2cc group. The >2cc group recurred in three cases, all of which had stage 3 disease. There were no deaths due to disease.

Conclusions: The volume of borderline change in an otherwise benign ovarian neoplasm significantly predicts final surgical stage. If intra-operative examination reveals less than 2cc of a papillary or solid component to a borderline neoplasm, then surgical staging appears unnecessary.

790 p16 Staining Profile of Biopsy Negative High Risk HPV Positive Women

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Background: p16(INK4A) is a well recognized biomarker of high-grade squamous intraepithelial lesion (HSIL). p16 protein levels increase with viral DNA integration and binding of the E7 protein to the retinoblastoma (Rb) gene and gene product. The staining pattern of HSIL shows high intensity, full thickness staining of the cell nucleus and cytoplasm while low-grade lesions shows cytoplasmic and nuclear staining within the basal layers. To the best of our knowledge, no p16(INK4A) staining studies have been reported for women who have had negative coloposcopically obtained biopsies with a positive high-risk HPV status. Our study attempts to determine the p16(INK4A) staining pattern in high-risk HPV infected women with morphologically benign biopsies.

Design: 31 women, aged 18 to 63 years (mean age 32), were identified in a four month period following a diagnosis of ASC-US on Pap test and a positive high-risk HPV result by hybrid capture. Each patient had a subsequent morphologically negative, colposcopically obtained, cervical biopsy and/or endocervical curettage. The negative control group consisted of 36 age-matched women who underwent hysterectomy between the dates of 05/04 to 06/04 for benign disease and who had no history of SIL on Pap testing. The samples were stained with the p16(INK4A) protein (CINtec histology kit by Dako at a 1:25 dilution, modified by use of Dako high pH 9.0 retrieval solution) to determine the amount of protein present in morphologically negative biopsies in women with known high-risk HPV infection. p16(INK4A) staining was considered positive with moderate to strong staining of squamous nuclei and cytoplasm.

Results: Four of thirty-one morphologically benign specimens showed basal cytoplasmic and nuclear staining, suggesting HPV infection in a LSIL pattern. No biopsies showed intense, full thickness staining, as would be seen in HSIL ($p = 0.03$). None of the thirty-six control specimens showed significant p16(INK4A) staining.

Conclusions: The increased level of p16(INK4A) protein in the morphologically benign biopsy specimens would suggest active HPV infection. The lack of morphologic changes has two possible explanations. First, this could represent an evolving infection with viral DNA integration and little morphologic change. The second possibility is a resolving infection with clearing of the virus and normalization of morphologic features. Both conditions would explain increased p16(INK4A) protein levels with little or no morphologic changes to the epithelium.

791 Biphasic Tumors of the Uterus and Breast with Malignant Mesenchymal Component: A Comparative Immunohistochemical Study

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Background: Biphasic tumors of the uterus and breast composed by benign epithelial and malignant mesenchymal components are very rare neoplasms which share similar histologic features. Our aim was to compare hormonal receptors and HER2 status, differentiation, proliferative activity, and tumor suppressor genes expression of biphasic tumors of the breast and uterus with malignant mesenchymal component.

Design: Representative samples (3 cores of each tumor) of 9 adenosarcomas of the uterus and 11 cases of malignant phyllodes tumors, with both mesenchymal and epithelial components, were used in a tissue microarray construction. Three cores of normal breast and endometrial tissue were used as controls. An immunohistochemical staining for ER, PR, HER2, p53, Ki-67, beta catenin, bcl2, CD10, CD34, CD99, CD117, collagen IV, HHF35, calponin (Dako), and laminin γ2 (Chemicon) was performed.

Results: Benign epithelial component shows the same immunophenotype of normal epithelium. Table 1 and 2 show the immunoprofile of the sarcomatous component. CD117, CD10, laminin γ2, HER2, and CD99 were negative in all the tumors.

Conclusions: 1) Biphasic tumors of the uterus and breast (adenosarcoma and malignant phyllodes tumor) beside morphology also share similar immunophenotype. 2) Different expression of estrogen and progesterone receptors in malignant mesenchymal components of adenosarcomas and malignant phyllodes tumors may be related to the expression of these hormonal receptors in the normal stroma of the uterus and breast.

Table 1

Tumor type	ER	PR	Ki-67 >10%and<50%	Ki-67 ≥50%	p53	βcatenin cytoplasmatic	βcatenin nuclear
Phyllodes Tumors	0/11	0/12	6/11	1/11	7/11	5/11	1/11
Adeno-sarcoma	9/9	8/9	3/9	1/11	4/9	4/11	1/9

Table 2

Tumor type	CD 34	Collagen IV	HHF 35	Calponin
Phyllodes Tumor	1/11	3/11	6/11	0/11
Adenosarcoma	1/9	3/11	7/9	1/9

792 Identification of Cell Cycle Regulatory Molecules as Biomarkers in Cervical Cancer Using Gene Expression Profiling

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Background: Biomarker discovery has enhanced diagnostic accuracy in many diseases including cervical cancer. Immunohistochemistry has demonstrated that disease biomarkers can also be used to aid in diagnosis of premalignant disease conditions such as cervical intraepithelial neoplasia. Gene expression profiling has revolutionised the process of biomarker discovery, allowing assessment of thousands of potential biomarkers simultaneously. In addition, it facilitates identification of interactions between genes, thus providing new insight into pathways of disease development.

Design: CaSki, C33A and HeLa cell lines, obtained from the ATCC, were grown in recommended media with 10% fetal bovine serum and underwent serial passages. Cells were harvested for RNA extraction at each passage. Total RNA was extracted using the RNeasy Minikit from Qiagen, with on-column DNA digestion. RNA quality was confirmed by gel electrophoresis. Normal cervical total RNA was obtained from BioChain. 5µg total RNA from 3 passages of each cell line and 5µg total normal cervix RNA in triplicate was labelled with digoxigenin using a 2 step RT-IVT approach and hybridised to Applied Biosystems human genome survey microarrays as per the manufacturer's protocol. Gene expression profiles were validated by RT and quantitative real time PCR using ABI Gene Expression TaqMan assays.

Results: Data analysis was performed using Spotfire software. All samples were normalised and p values were calculated using t tests. 6325 genes were found to be significantly differentially expressed on comparison of malignant (CaSki, C33A, HeLa)