Conclusions: Small aggregates of CD68 positive macrophages are more frequently present in CD than in UC and multiple MM are found exclusively in CD. Thus, the presence of multiple MM may aid in the distinction of CD from UC.

566 DNA Methylation Profiling of Anal Intraepithelial Lesions and Anal Squamous Cell Carcinoma

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Background: Anal intraepithelial neoplasia (AIN) is HPV-associated and may progress to invasive squamous cell carcinoma (SCC), which has recently been detected with increasing frequency in immunocompromised patients. Unfortunately, the biology of AIN is poorly understood and screening programs are not optimal. We hypothesize that AIN is associated with abnormal DNA methylation and that detection of these events may be utilized to improve screening programs.

Design: We identified 144 patients who underwent anal cytology screening and subsequent anoscopy and biopsy at our institution between 1999 and 2004 and correlated the cytologic and histologic diagnoses. A subset of these patient specimens were selected for DNA methylation analysis, including 184 anal biopsies (normal, n=57; AIN I (LSIL), n=74; AIN II-III (HSIL), n=41; and SCC, n=12) and 37 residual liquid-based anal cytology specimens (normal, n=11; LSIL, n=12; HSIL, n=14). DNA was extracted from each biopsy and cytology specimen and then bisulfite treated in preparation for real-time methylation-specific PCR (MSP) analysis of the following genes: HIC1, RASSF1, RARb, p16, p14, p73, APC, hMLH1, MGMT, DAPK1, and TSLC1.

Results: The histologic diagnoses on the biopsies included 19% normal mucosa, 47% AIN I, and 34% AIN II-III. Cytologic diagnoses on these cases included 5% negative, 30% ASC-US, 55% LSIL, and 10% HSIL. Referral of ASC-US, LSIL or HSIL cytology yielded a sensitivity for detection of biopsy-confirmed HSIL of 100%, but a specificity of 35%. Increasing the threshold of referral to HSIL increased the specificity to 85%, but reduced sensitivity to 25%. Real-time MSP analysis of biopsy samples revealed that aberrant DNA methylation was more common in SCC and HSIL than LSIL and normal mucosa. Specifically, methylation of TSLC1 and DAPK1 occurred at a high frequency in SCC (75% and 75% of cases, respectively) and HSIL (59% and 71%) but was absent in LSIL and normal biopsy samples. Methylation profiles of cytologic samples were similar to those found in the biopsy samples.

Conclusions: 1) Anal cytology is a highly sensitive for AIN II-III, but lacks specificity. 2) Aberrant DNA methylation is a frequent event in AIN II-III and anal SCC. 3) Methylation of TSLC1 and DAPK1 is unique to AIN II-III and SCC, and may serve as a useful molecular biomarker. 4) Aberrant DNA methylation can be detected in anal cytology specimens and the methylation profiles resemble those found in biopsies.

567 Absent or Low Expression of CK20 and CDX2 Associated with Microsatellite Instability in Poorly Differentiated Colorectal Carcinomas (PDCC)

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Background: The expression pattern of cytokeratin 7 (CK7) and cytokeratin 20 (CK20) has been routinely used in discriminating colorectal carcinomas (CK7-/CK20+) from other unknown primaries. Recently, CDX2 was identified as a sensitive and relatively specific marker for primary and metastatic colorectal carcinomas. Reduced expression of CK20 has been demonstrated in colorectal cancer associated with microsatellite instability (MSI). Moreover, MSI and loss of CDX2 expression have recently linked to minimally differentiated colorectal carcinomas. In this study we explore the relationship of CK20, CDX2, and MSI markers in PDCC.

Design: Nineteen cases of PDCC (defined as > 80% of the tumor with solid growth pattern with/without dense lymphocytic infiltration) were included in this study. This set of cases represented 1.5% of a large study group of colon cancers. Immunohistochemical stains were performed with monoclonal antibodies to AE1/3, CK7, CK20, CDX2, MSH2 and MLH1. The staining intensity is graded into weak, moderate and strong. The distribution is recorded into negative (<5%), 1+(6-25%), 2+(26-50%), and 3+ (>50%).

Results: Of 19 cases, all were strongly positive for AE1/3 and MSH2, and 18 were negative for CK7. Lack of expression of CK20 and CDX2 was present in 53% (n=10) and 58% (n=11) cases respectively. Additionally, low (1+) expression of CK20 and CDX2 was seen in 26% (n=5) and 21%% (n=4) cases respectively. Absence of MLH1 nuclear staining was noted in 68% (n=13) cases, all of which showed absent or low expression of CK20 and CDX2. Notably there were 4 cases that strongly positive for CK20 and also positive for CDX2 and MLH1.

Conclusions: This study shows that CK20 and CDX2 have little diagnostic value in differentiating PDCC from other unknown primaries. In addition, the high correlation of expression of CK20, CDX2 and MLH1 suggests a possible role of CDX2 and MLH1 involving the pathway of CK20 gene expression.

568 Occult *Helicobacter pylori* Infection Detected by PCR in Biopsies with Chronic Gastritis

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Background: *H. pylori* is the main cause of chronic gastritis and peptic ulcer disease and may lead to gastric carcinoma and lymphoma. *H. pylori* gastritis shows a characteristic pattern of inflammation, but only the detection of bacteria proves the disease. Histology is considered a sensitive method, but little is known about the presence of *H. pylori* in inflammed gastric tissue without histological proof of *H. pylori*. We developed two PCR methods to detect *H. pylori* in gastric biopsies and correlated the inflammatory changes with the presence of bacterial DNA.

Design: Archived gastric biopsies with at least minor inflammatory changes were included in the study. Biopsies were analyzed for the presence of *H. pylori* DNA by nested and quantitative PCR using primers of the SSA and urease C gene sequence. Biopsies were graded according to the revised Sydney classification.

Results: *H. pylori* was detected in 69 (54,8%) of 126 gastric biopsies; 54 (42,9%) by histology and/or PCR and 15 (11,9%) exclusively by PCR. *H. pylori* was found by nested PCR in all samples positive by histology but quantitative PCR failed to detect 10 (18,5%) of 54 biopsies positive by histology. The inflammatory score was significantly higher in biopsies positive only by PCR than in *H. pylori* negative biopsies (mean of neutrophils score 1.60 versus 0.90, p<0.006; mean of mononuclear cells score 2.27 versus 1.67, p<0.006) whereas the results were similar to biopsies positive for *H. pylori* by histology (mean of neutrophils score 1.60 versus 1.56, n.s.; mean of mononuclear cells score 2.27 versus 2.20, n.s.). Quantitative analysis finally showed weak correlation between inflammatory score and the amount of *H. pylori* assesed by histology, but no correlation was found between the inflammatory score and the amount of *H. pylori* DNA detected by quantitative PCR.

Conclusions: Our data show that PCR can detect *H. pylori* DNA in a considerable proportion of gastric biopsies with inflammatory changes but negative for *H. pylori* by histology. These biopsies, however, show a degree of inflammation similar to biopsies positive for *H. pylori* by histology, indicating the clinical relevance of *H. pylori* detection by PCR. The sensitivity of our detection systems seems to be depending on the gene region analyzed. However, quantitative analysis provides no additional information.

Genitourinary

569 Non-Hodgkins Lymphoma (NHL) of the Prostate: Emphasis on Useful Diagnostic Features

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Background: Hematological malignancies of the prostate are rare with a reported incidence of <1%. The majority of NHL of the prostate were reported using outdated nomenclature prior to the use of immunohistochemistry. We reviewed a series of primary and secondary NHL of the prostate with the aid of immunohistochemistry and used the WHO classification of NHL.

Design: The Nebraska Lymphoma Study Group database was searched (1984-2004) for cases of NHL involving the prostate. Cases were classified as primary (no extra-prostatic site identified within one month of diagnosis) or secondary. Clinical and histological material was reviewed to determine clinical presentation, type of specimen, zone of origin, presence of prostatic carcinoma (PCa) and histologic features of the NHL.

Results: Ten TURP specimens, 5 needle biopsies and 1 radical prostatectomy were retrieved for review. Clinical presentation was urinary retention and obstruction 7 cases, abnormal DRE 2 and weight loss/hematuria in 1 case each. In 5 cases clinical information was not available. In all cases of primary NHL (12/16) workup was directly related to symptoms of hyperplasia or elevated serum PSA level with only 2 cases having PCa. Zone of NHL orgin was predominantly transitional (10/15). In all cases an immunohistochemistry panel of PSA, PSAP, CD20, CD3, CD5, CD10 and cyclin D1 allowed diagnosis and classification.Types and features of the NHL are presented below:

WHO Type (n=16)	Pattern of Spread	Necrosis	Intraepithelial Lesions
Diffuse large B-cell (5)	Infiltrative 60%	Present 60%	Present 25%
MALT (4)	Nodular 75%	Absent	Present 25%
Small lymphocytic (3)	Infiltrative 66%	Absent	Present 66%
Intravascular large B-cell (1)	Angiotropic	Absent	Absent
Follicular (1)	Nodular	Absent	Present
Low grade B cell (1)	Infiltrative	Absent	Absent
Mantle cell (1)	Nodular	Absent	Present

Conclusions: NHL of the prostate frequently presents with symptoms of urinary retention (44%) and less often with an elevated serum PSA. In this series, primary NHL of the prostate was an incidental finding in 80% of the cases. Histologically diffuse large B cell was the most frequent (38%) characterized by a infiltrative growth pattern and necrosis (60%). Dense nodular lymphocytic infiltrates were characteristic of MALT and follicular lymphomas (80%). Small lymphocytic lymphoma was characterized by intraepithelial lesions (66%). The presence of nodular, infiltrative and cord like infiltrates and tumor necrosis should raise the possibility of NHL of the prostate and appropriate immunohistochemical markers should be performed.

570 Primary Testicular Lymphoma: Retrospective Clinicopathological Study of 34 Cases

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Background: Primary testicular lymphoma (PTL) is rare. Before the era of HIV infections it was estimated to comprise 5% of all testicular tumors, with tendency to occur in the elderly. However, more recent reports seem to indicate a higher incidence and a broader age spectrum. PTL are usually aggressive diseases, mostly of B cell immunophenotype. In this study, retrospective clinicopathological review of 34 cases was carried out with emphasis on clinical presentation, immunophenotypic features, and clinical behavior when available.

Design: Pathology reports and available material on primary testicular lymphomas seen in 3 medical centers were reviewed. Immunohistochemical stains were performed where these data were lacking and there were available paraffin blocks or unstained paraffin sections. Clinical, pathological and behavioral features were analyzed.

Results: 34 cases were found. Two referral cases had been initially misinterpreted as seminomas. The mean age at presentation was 54 years (range 4-87). In all the cases a testicular mass was the presentation. In 11 cases the size was recorded, averaging 7.4 cm (4-13 cm). Bilateral involvement was seen in 1 case and one tumor was reported in the epididymis. Diffuse large cell lymphoma (DLCL), NOS was the diagnosis in 15 older cases for which no material was available for further studies. The rest of DLCL cases (17) were all of B-cell type (DLBCL). One case was a solitary plasmacytoma; a second case a B-cell lymphoblastic lymphoma. Proliferative index using immunostaining for Ki-67 antibody revealed strong nuclear immunoreactivity (> 50%) in all DLBCL cases. Two cases were HIV+ve, with one having PTL as its initial presentation. One patient had multiple unusual skin and bone marrow metastases. In cases with available follow-up survival was in general poor.

Conclusions: Most cases of PTL fall into the broad category of DLBCL. However, our data show that they represent a subgroup of DLBCL associated with a particularly high proliferative activity and an aggressive clinical course. Misdiagnosis can occur especially in those cases where the presentation occurs at an age similar to the one in which germ cell tumors also occur. Therefore immunohistochemical confirmation is recommended in all cases. The similar pathological and behavioral characteristics seen in PTL argues for including these lymphomas into a separate clinico-pathological category.

571 Expression of Prostate Specific Membrane Antigen (PSMA) in Subtypes of Renal Cell Carcinoma

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Background: Renal cell carcinoma (RCC) is a heterogeneous group of tumors with distinct morphologic and genetic characteristics and clinical behaviors. Prostate specific membrane antigen (PSMA) is a type II membrane glycoprotein that is expressed in benign and neoplastic prostatic tissue. Recently, it has been shown that PSMA is also expressed in the neovasculature of various solid malignant tumors including renal cell carcinoma. As a result, this antigen has been the subject of several diagnostic and therapeutic trials. These studies however did not address the expression of PSMA in the different types of renal cell carcinoma (RCC).

Design: Formalin-fixed, paraffin-embedded archival material from 60 nephrectomies performed at our institution was reviewed. A representative section of each case was selected and stained by immunohistochemistry with antibody 16D3 directed against PSMA. The study included 30 conventional renal cell carcinoma (CRCC), and 15 of each of papillary and chromophobe RCC (PRCC and ChRCC). The staining pattern (diffuse: >50% of the tumor staining; focal: <50%) and intensity (scale of 1-3) were assessed in the tumor cells, tumor-associated vessels and adjacent kidney. A staining intensity of 2+ or 3+ was considered strong.

Results: The results are summarized in the table below. Immunoreactivity was detected in the neovasculature but not in tumor cells in all cases.

PSMA in neovasculature of RCC subtypes

CRCC	PRCC	ChRCC
29/30 (97%)	11/15 (73%)	13/15 (87%)

CRCC showed the most diffuse staining pattern (24/30 cases; 80%) followed by ChRCC (9/15; 60%). No diffuse staining was detected in any of the PRCC and only focal staining was detected in 11 cases (11/15; 73%). The staining intensity was the strongest in CRCC (25/30 cases showed strong staining intensity; 83%) followed by ChRCC (9/15; 60%), and PRCC (5/15; 33%).

Conclusions: Our study shows that PSMA is expressed in tumor associated neovasculature of the majority of RCCs. PSMA is most diffusely and intensely expressed in CRCC and least in PRCC. The differences in the expression of PSMA in different RCC subtypes may further be evidence of the diverse biologic behavior of these tumors. Therapeutic and diagnostic applications of PSMA can be expanded to include subtypes of RCC.

572 Prostate Specific Membrane Antigen (PSMA) Expression in Primary Prostatic Adenocarcinoma: Comparison of Expression by Gleason Grade, Hormonal Status and Metastatic Potential

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Background: Prostate specific membrane antigen (PSMA) is a type II membrane glycoprotein that is consistently expressed in benign and neoplastic prostatic tissue with its strongest and most diffuse expression being in prostatic adenocarcinoma, whether primary or metastatic. Its efficacy as a therapeutic agent is presently being studied in clinical trials. Detailed studies on the expression of PSMA in primary prostatic carcinoma (PCa) in different clinical and pathologic settings are lacking. We conducted our study to assess PSMA expression in primary PCa with and without metastasis (PCaM and PCaN), primary PCa following neoadjuvant therapy (PCaT) and "hormone-naive" primary PCa (PCaG).

Design: Tissue microarrays (TMA) from formalin-fixed, paraffin-embedded archival material from prostatectomies performed at Memorial Sloan-Kettering Cancer Center were stained by immunohistochemistry with antibody 3E2 directed against PSMA. The study included 136 PCaM, 48 PCaN, 72 PCaT and 147 PCaG. Normal prostatic tissue (NP) from 345 cases and 51 cases of non neoplastic prostatic tissue following neoadjuvant therapy (NT) were also included. Any immunoreactivity was recorded and considered positive.

Results: PSMA expression is listed in the two tables below.

PSMA expression in PCa and non neoplastic prostate

PCaM	PCaN	NP	NT
103/136=76%	15/48=31%	149/345=43%	18/51=35%

PSMA expression in PCa with different Gleason grade

G 3+3	G 3+4	G 4+3	G 4+4	G 4+5	G 5+4	G 5+5	Total
39/51	37/41	20/20	20/20	9/9	2/2	4+4	131/147
=76%	=90%	=100%	=100%	=100%	=100%	=100%	=89%

Conclusions: Our study shows that the majority of PCa express PSMA regardless of Gleason grade. The expression of PSMA is stronger with higher Gleason grade with 100% expression in grades 4+3 and above. PSMA is expressed in PCa after neoadjuvant therapy. Primary PCa with metastatic disease is more likely to express PSMA than PCaN. This study suggests that PSMA is a reasonable therapeutic target in patients with primary PCa independent of grade, stage and hormonal status.

573 Primary Retroperitoneal Lymph Node Dissection in Pathologic Stage II Testicular Germ Cell Tumors: A Clinicopathological Study

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Background: Testicular germ cell tumors (GCT) have a predictable pattern of metastatic spread with the retroperitoneum being the initial site of metastasis in the majority of cases. Primary retroperitoneal lymph node dissection (RPLND) is a treatment option in patients with clinical stage (CS) I and early CS II with normal serum tumor markers (STM). We studied a large series of RPLND performed at our institution and analyzed the pathologic findings, clinical data and outcome.

Design: Of 453 patients who underwent primary RPLND in our institution from 1988-2001, 139 with documented pathologic stage II disease with or without elevated STM were identified and constituted the material for our study. All slides from all 139 cases were reviewed and classified as to tumor type(s), size and extent of disease, and amount of tumor necrosis.

Results: The pathologic findings are listed in the table below. Total number of lymph nodes (LN) examined ranged from 5 to 80. The number of positive LN ranged from 1 to 40 (mean: 3.4). The mean number of positive LN in relapse cases was 4.3 compared to 3.2 in cases without relapse. Pure embryonal carcinoma (EC) was found in 73 patients (73/139=53%) 13 of whom relapsed (13/73=18%). Pure teratoma (TER) was found in 12 patients (12/139=9%) none of whom relapsed (0/12=0%). One patient with pure yolk sac tumor (YST) had multiple relapses and died of disease 2 years later. Combined EC and YST was seen in 35 cases (35/139=25%), of whom 6 relapsed (6/35=17%). Overall, 22 patients relapsed (22/139=16%) at least once and 3 of them died of disease (3/139=2.2%). Of 22 cases with relapse, 14 had extranodal extension (ENE). In comparing cases with and without ENE, no significant difference in relapse rate was found (14/93=15% vs 8/46=17%). The amount of tumor necrosis did not correlate with the outcome.

Pathologic findings in primary RPLND

EC	YST	TER	Size (mm)	ENE	Necrosis (%)
119/139=86%	36/139=26%	34/139=24%	0.2-60	93/139=67%	5-90

Conclusions: In this patient population, EC is the most common component of GCT followed by YST and TER. In mixed tumors, EC+YST is the most common combination. The presence of EC in RPLND, either in pure form or in combination with other components, is associated with the highest relapse rate while the presence of pure TER has the lowest relapse rate. Tumor necrosis and extranodal extension do not seem to influence the outcome of these patients.

574 EZH2 Protein Expression Correlates with High Tumor Grade in Renal Cell Carcinoma

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Background: EZH2 is a homologue of the *Drosophila Polycomb* group (*PcG*) gene enhancer of zeste, an essential regulator of homeotic gene expression in embryonic development. EZH2 is a transcriptional repressor involved in controlling cellular memory. Therefore, dysregulation of this gene silencing mechanism can lead to malignancy. Previous studies have demonstrated that the EZH2 gene is highly expressed in metastatic prostate cancer, lymphoma, and more recently in aggressive breast cancer. The clinical and pathologic significance of EZH2 expression in renal cell carcinoma (RCC) has not been previously characterized.

Design: Sections from 157 formalin-fixed, paraffin-embedded cases of RCC were immunostained with polyclonal rabbit anti-human EZH2 (ENX1) antibody (Zymed Laboratories Inc., San Francisco, CA) using an automated method (Ventana Medical Systems, Tucson, AZ). Immunoreactivity based on intensity and distribution of EZH2 were scored simultaneously by two pathologists, and the results were correlated against clinicopathologic variables.

Results: Nuclear expression of EZH2 was identified in 83 of 113 (73%) clear cell, 5 of 6 (83%) oncocytoma, 21 of 23 (91%) papillary, 9 of 9 (100%) chromophobe, and 4 of 5 (80%) sarcomatoid tumors. EZH2 protein expression correlated with high tumor grade in 38 of 40 (95%) of the high grade tumors compared to 84 of 116 (72%) in the low grade RCCs (p=0.003). EZH2 immunoreactivity did not correlate with histologic subtype, advanced stage, recurrence, or survival. On multivariate analysis, only recurrence (p=0.005) independently predicted disease-related death.

Conclusions: The association of increased nuclear immunostaining of EZH2 in high grade renal cell carcinomas suggests that EZH2 may play a role in the tumor biology of RCC and warrants further study.

575 Perineural Involvement by Benign Prostatic Glands on Needle Biopsy

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Background: One of the pathognomonic features of prostate carcinoma is perineural invasion by cancerous acini. Although uncommonly benign prostatic glands can also be seen in the perineural space, this phenomenon has not been studied on needle biopsy.

Design: 26 prostate needle biopsies from 1988 to 2004 with nerve involvement by benign prostatic glands were collected. 21 (81%) cases were received in consultation to one of the authors out of a total of 47,125 cases, while 5 (9%) were in-house out of

a total of 7,721 cases. In 15 of 21 (71.4%) consult cases there was a question by the submitting pathologist regarding the focus; in the other 6 cases perineural benign glands were noted only upon expert review. Immunohistochemistry (IHC) with high molecular weight cytokeratin (HMWCK) was performed in 22 (84.6%) and with p63 in 18 (69.2%) cases. Five (19%) of the cases had Gleason score 6 carcinoma elsewhere in the case.

Results: The number of glands related to a nerve averaged 2 (range: 1-6). The following patterns were seen, some cases with >1 pattern. 13 (50%) cases had indentation of the nerve, by up to 3 glands. 8 (30.7%) cases had tracking along the length of the nerve, by up to 6 glands. 7 (26.9%) cases showed wrapping from ½ to ¾ around the nerve, by up to 3 glands, with one of these cases showing 95% wrapping around the nerve. 4 (15.4%) cases showed intraneural glands, by up to 3 glands. 2 (7.6%) cases had benign glands immediately adjacent to cross-sections of nerves, by up to 2 glands. In 10 (38.4%) cases the acini involving the nerve showed partial atrophy, while in 6 (23%) cases the glands had complete atrophy. Of 8 cases with the lesion still present on slides stained for IHC, HMWCK was positive in 6 (75%) cases and negative in 2 (25%) cases with partial atrophy, with the same staining pattern seen with p63. On H&E sections, basal cells were not identified in 12 (46%) cases, including 2 negative cases and 1 positive case stained for HMWCK.

Conclusions: In addition to perineural indentation which is well-recognized, other patterns of neural involvement by benign glands that are not widely recognized may also be seen. Patterns most closely mimicking cancer include intraneural and incomplete perineural involvement. In particular, neural involvement by benign atrophic glands causes diagnostic difficulty. Negative IHC for HMWCK in partially atrophic glands involving nerves further mimics cancer. Careful attention to H&E morphology and comparison of the perineural glands to adjacent and distant benign glands is necessary to rule out carcinoma.

576 Precursor of Prostate-Specific Antigen (ProPSA): Role of Immunohistochemical Staining in High Grade Prostate and Urothelial Carcinomas

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Background: Prostate specific antigen (PSA) is a serine protease that is normally secreted as a proenzyme (proPSA). We have reported earlier, immunohistochemical staining (IHS) with proPSA to be positive in both benign and malignant prostatic tissue. High grade urothelial carcinoma (UCC) and high grade prostate adenocarcinomas (PCa) can pose diagnostic problems on morphology alone. This study explores the role of proPSA IHS in such a setting.

Design: Forty five cancers (29 UCCs in 27 patients, 16 PCa [8 primary with Gleason scores 7-9 and 8 metastatic] in 14 patients) were collected from our files. IHS was performed for ProPSA, PSA, Prostate specific acid phosphatase (PAP) and thrombomodulin (TBM). The intensity of IHS was categorized as negative, weak, moderate or strong, and extent of staining as diffuse (>50% of cells), patchy (11-50% of cells) or focal (<10% of cells).

Results: **For PCa**:

All cases showed some degree of proPSA staining, with 13 (81.3%) being strong to moderate & diffuse. The majority of cases showed some staining with PSA and PAP, with strong to moderate & diffuse or patchy in 12 (75%) and 6 (37.5%), respectively. However 3 (18.8%) cases were negative for PSA, and 4 (25%) cases were negative for PAP. In the two (12.5%) cases that were negative for both PSA and PAP, proPSA showed weak to moderate staining. In the 7 (43.8%) cases where PSA or PAP showed weak staining, proPSA was strong & diffuse. All cases were negative for TBM.

For UCC:

ProPSA showed moderate & diffuse staining in one (3.4%) case, weak in 19 (65.5%) cases, and negative in 9 (31%) cases. The majority of cases were negative for PSA (27 or 93%) and PAP (21 or 72.1%), but weak staining was seen in 2 (6.8%), and 8 (27.6%) cases, respectively. Some degree of TBM staining was seen in 19 (65.5%) cases, with 16 (55.2%) strong to moderate, and 10 (34.5%) cases were negative.

Conclusions: ProPSA is more sensitive but less specific for distinguishing PCa from UCC compared to both PSA and PSAP. Staining for proPSA is more intense and diffuse in PCa compared to PSA and PSAP. TBM is a highly specific but weakly sensitive marker for UCC compared to PCa. ProPSA may be helpful as part of an immunohistochemical panel when the differential diagnosis includes high grade prostate cancer, as such tumors may be negative for both PSA and PAP.

577 Role of ProPSA Immunostaining in Fine Needle Aspiration of Metastatic Prostate Adenocarcinoma

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Background: ProPSA is one of the several molecular forms of prostate specific antigen (PSA). Earlier studies have shown that it may be a more sensitive immunomarker for prostate adenocarcinoma (PCA) compared to PSA and prostate specific acid phosphatase (PAP). Diagnostic role of ProPSA in cytologic material has never been studied.

Design: Twenty-four cytologic samples (23-fine needle aspirations [FNA], 1-pleural fluid) of metastatic PCA were identified. Specimens for immunoperoxidase staining (IPOX) consisted of cell block sections or smears. IPOX was performed with ProPSA (n=24), PSA (n=23), and PAP (n=20). The intensity of IPOX was categorized as negative, weak, moderate or strong, and extent of staining as diffuse (>50% of cells), patchy (11-50% of cells) and focal (<10% of cells).

Results: The patients' age range was 51-88 (mean 70) years. Sites of metastasis included: liver (11), lymph nodes (7) [retroperitoneal, right and left supraclavicular, pelvic and peri-aortic], lung (2), and adrenal, penis, pleural fluid and right shoulder soft tissue (1 each). ProPSA showed strong to moderate & diffuse or focal staining in 15 (62.5%), weak & diffuse or focal in 7 (29.2%), and 2 (8.3%) cases were negative. PSA showed strong to moderate & diffuse or focal staining in 13 (56.5%), weak & diffuse or focal in 4 (17.4%), and 6 (26.1%) cases were negative. PAP showed strong to moderate & diffuse or focal staining in 9 (45%), weak & diffuse in 1 (5%), and 10 (50%) cases were negative. ProPSA showed moderate to weak & diffuse or focal staining in the 4 (20%) cases that were negative for both PSA and PAP. The only two cases that were negative for proPSA were also negative for PSA and PAP. PSA or PAP was strong to moderate & diffuse in 4 (20%) cases, which showed weak & diffuse or focal proPSA staining.

Conclusions: Compared to PSA and PAP, ProPSA is a more sensitive marker for diagnosing metastatic PCA, showing more intense and diffuse immunoreaction. ProPSA can be extremely helpful in poorly-differentiated carcinomas where both PSA and PSAP might be negative. A panel of immunostains including proPSA should be performed when metastatic PCA is suspected. ProPSA IPOX staining can be adequately performed in cytologic material.

578 Secretory Urothelial Carcinoma of the Urinary Bladder. A Report of Seven Cases

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Background. Carcinomas producing mucins have been described at sites such as breast, cervix, ovary and bladder. We identified 7 tumors in the bladder which were histologically different from the recognized variants of urothelial carcinomas(UC) which were characterized by the presence of abundant extracellular and intracellular secretory material.

Design: Standard technique of mucin histochemistry, such as periodic acid_Shiff(PAS) was use to demonstrate mucoid deposits. The expression of apomucins as MUC1 and MUC5 were investigate in three tumors by immunohistochemistry. Patients's charts were reviewed for presentation, disease stage and clinical course.

Results: Microscopically, the tumors were characterized by multiple micro and macrocystic spaces containing abundant secretion, also, citologically, some tumor cells had a clear cytoplasm mimicking signet ring cells. In all cases, the secretion was positive with the PAS reaction. The expression of MUC1 and 5 was weak in one case and strongly positive in two cases. The tumors occurred in patients from 51 to 84 years of age and only two of them were deeply invasive; four were low grade and three high grade. Follow–up available in all cases(median 24 months) showed that 3 patients died of disease, 1 is alive with disease and 3 are alive without disease.

Conclusions: We report a series of rare primary carcinomas of the urinary bladder that closely resemble the secretory carcinoma of the breast and with a MUC expression patterns very similar to that of gastric and colonic carcinomas.

579 Pathologic Significance of the Invaginated Extraprostatic Space Involvement by Prostatic Carcinoma. A Study of 79 Cases of Stage T3b Prostatic Carcinomas

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Background: Invaginated extra-prostatic space (IES) is a true 'extra-prostatic' space around the ejaculatory ducts (EJ) and continues up to the verumontanum. It comprises of vascularized loose connective tissue that surrounds the EJ and invaginates the prostate during its embryologic development. Although well recognized in radiology literature, this entity has received little attention in the pathologic evaluation of prostatectomy specimens. Invasion of IES by prostatic carcinoma (PCA) has been suggested as one of the possible routes of direct involvement of seminal vesicle (SV) by PCA. This finding assumes significance in the new era of robotic and laparoscopic prostatectomies (RP/LP), where pathologists sometime receive fragmented and cauterized portions of SV.

Design: We retrospectively reviewed 79 radical retropubic prostatectomy specimens, pathologically staged at T3b (seminal vesicle invasion). Histologic features were evaluated with specific reference to zonal location of carcinoma, posterior capsular involvement, direct involvement of intra-prostatic seminal vesicles and the invasion of IES, in order to assess the possible route of SV involvement. Cases with PCA less than 1 mm from IES were also recorded.

Results: Of the 79 cases, 51 (64 %) cases showed IES involved by PCA, 14 (18 %) were less than 1 mm from the IES, and 14 cases (18 %) were negative for IES involvement (greater than 1 mm). Based on our evaluation, involvement of the IES as the only mode of PCA spread to SV was seen in 6 cases (8 %), which included cases with no or minimal posterior-peripheral zone involvement. Intraprostatic portions of seminal vesicles were identified in 28 cases (35%), and were directly involved by carcinoma in 21 of those cases. Extraprostatic extension appears to be the most common mechanism of SV involvement by PCA.

Conclusions: IES involvement by PCA is a definite route of direct tumor spread to the seminal vesicles. It accounts for the only mode of spread of PCA to the SV in a subset of cases, however, the presence of PCA close to, or within the IES in 82 % of our cases, suggests that it may additionally contribute to tumor spread in posterior-peripheral zone tumors. IES involvement may be a potential surrogate marker for SV involvement and may indicate a need to comprehensively evaluate SV for accurate pathologic staging.

580 The Utility of Pathology Parameters To Predict Prostate Cancer Specific Death: A Population-Based Study in Sweden

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Background: The Örebro Watchful Waiting Cohort is the largest population-based epidemiological study of watchful waiting with localized prostate cancer (CaP) at diagnosis who have been prospectively followed for a mean of 23 years. The goal of this study was to evaluate the utility of pathology parameters to predict CaP specific death.

Design: Transurethral resection of prostate (TURP) specimens for 240 cases were collected and evaluated by the study pathologist. Cases were diagnosed between 1977-91, before PSA-screening. Complete follow-up through Sept. 2003 was available. Tumor proliferation was determined by immunohistochemistry for Ki67 using an image analysis system (Chromavision, San Juan, CA.).

Results: 42 men have died of CaP and 160 of other causes. All men on this trial were T1, Nx, and M0 with 49% having pT1a disease (<5% involvement of the TURP sample). Only 5% of the cases had a Gleason score (GS) below 6. 57% had a GS of 6, 27% GS 7, and 12% > GS 8. The majority of tumors were WHO nuclear grade 1 (WHO grade 1= 74%; grade 2= 19%; grade 3=7%). The risk of CaP specific death significantly increased with higher nuclear grade with WHO grade 3 conferring an 11-fold increased risk compared to WHO grade 1 (HR=10.71 CI= 4.51-25.41). Larger tumor volume was associated with CaP specific death. A 17-fold increase in CaP mortality was observed among men with >50% tumor involving their TURP sample as compared to <5% (HR= 16.84 CI=5.97-47.50). A 9-fold significant difference was seen between men with GS 8-9 versus GS ≤6 tumors. The extent of Gleason pattern 4 (3+4 vs 4+3) was not significantly associated with CaP specific death. The best independent predictors of CaP specific death on multivariate analysis included GS, WHO nuclear grade and tumor volume. Ki67 expression was highly correlated with GS and higher Ki67 levels demonstrated a trend with CaP specific death but was not an independent predictor.

Conclusions: GS, WHO nuclear grade and tumor volume were independent significant predictors of CaP specific death in men followed for a mean of 23 years with clinically localized disease. However, given that >80% of all cases were classified as GS 6 or 7, the usefulness of this grading system may be limited. Molecular markers will be required to assist in following men on watchful waiting protocols.

581 Mixed Epithelial and Stromal Tumor of the Kidney vs Adult Cystic Nephroma – One or Two Entities? Reappraisal of 14 Lesions

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Background: Mixed epithelial and stromal tumor of the kidney (MESTK) is a rare, biphasic tumor composed of tubules and cysts lined by variable epithelium and spindle cell, ovarian-like stroma. Adult cystic nephroma (ACN) features cysts lined by hobnail or attenuated epithelium and thin septa containing loose stroma with fibroblasts and, focally, cells resembling smooth muscle. The histogenesis of both lesions is unclear.

Design: We sought to review and compare cystic renal lesions diagnosed as MESTK and ACN. We identified 14 such lesions. Routine histology and immunohistochemistry (IP) were performed in each tumor, electron microscopy (EM) was performed in 4 cases.

Results: On review, we classified 7 lesions as MESTK and 7 as ACN. MESTK occurred exclusively in women, 40-80 years old (mean 51.7); 5 were on long term estrogen therapy, 2 were obese. Seven tumors confirmed as ACN occurred in 3 females and 4 males, 29-71 years old (mean 48.5); 1 female was on longterm steroids. Grossly, tumors ranged in size from 0.5-10.0 cm, median 3.8. All tumors, except for two MESTK measuring <1cm, were cystic. However, while all ACN were composed of cysts with thin septa and no grossly discernible stroma, solid areas were seen grossly in MESTK. Microscopically, MESTK had irregular borders while ACN were well-demarcated. ACN contained a minimal amount of stroma, with spindle cells focally positive for smooth muscle actin (SMA). In MESTK, stroma was uniformly positive for SMA, ER and PR and focally for CD34 and CD117. In ACN, focal positivity for ER/PR was seen only in 2 women. Inhibin was negative in all 14 lesions. The epithelial lining in MESTK was morphologically variable with regard to shape and cytoplasmic appearance; by IP focal positivity for ck7, CD10 and high molecular weight keratin were seen in different areas. In ACN, the epithelial lining was more uniform and positive only for ck7. By EM, a range of epithelial lining corresponding to proximal and distal epithelium and loop of Henle was observed in 3 cases of MESTK, while ACN (1 case from a female) showed only features of distal tubule and loop of Henle.

Conclusions: All 14 tumors are benign. While MESTK has a strong association with the female sex and hormonal milieu, ACN can affect both sexes and, on occasion, may also have hormonal associations. Morphologically there is a considerable overlap between both lesions, which suggests that they may represent opposite ends of the spectrum of the same process.

582 Expression of GSK-3 Is Increased in Metasstatic Prostate Cancer

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Background: GSK-3 is involved in the Wnt/β-catenin signaling pathway which is implicated in cancer invasion and metastasis. Previously we demonstrated that high levels of GSK-3 were associated with positive lymph node status and other aggressive clinicopathologic features in PCa. This prompted us to examine the possible role of GSK-3 in development of metastasis of PCa.

Design: We used 499 PCa cases treated with radical prostatectomy to build 2mm tissue microarrays (TMA). Another 2mm TMA was built using 177 metastatic lesions (lymph nodes and other distant metastases). Antibodies to GSK-3 (non-phosphorylated form) were applied to TMA slides which were then digitized. Mann-Whitney test was used to test the difference between paired parameters.

Results: GSK-3 was expressed in cytoplasm and nucleus of both benign and cancerous epithelium. GSK-3 in primary PCa had an index of 2.05±2.88 (nuclear) and 4.55±3.06 (cytoplasmic), while GSK-3 in metastases had an index of 3.53±3.15 (nuclear) and 3.76±2.38 (cytoplasmic). The nuclear GSK-3 was significantly increased in metastases (p=0.0000), and cytoplasmic GSK-3 was slightly decreased in PCa metastases (p=0.0135) compared to GSK-3 expression in primary PCa.

Conclusions: Our data demonstrate that GSK-3 is probably associated with development of metastasis in PCa. Increased GSK-3 together with upregulation of other prosurvival molecules might facilitate tumor invasion and metastasis through Wnt signaling and/or other survival pathways.

583 Skip Areas with Absence of Basal Cells in Prostatic Intraepithelial Neoplasia and Atypical Adenomatous Hyperplasia: Diagnostic Problems in Prostatic Needle Biopsies

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Background: Skip areas without basal cells (SAWBC) in high grade prostatic intraepithelial neoplasia (PIN) in prostatic biopsies may cause problems in the diagnosis of prostatic carcinoma. We have investigated the maximum length of these SAWBCs.

Design: We reviewed 85 consecutive radical prostatectomies: 35 cases designated as atypical adenomatous hyperplasia (AAH) and 50 cases of biopsy harbouring PIN and eight cases of biopsy containing carcinoma measuring only up to 500 microns in diameter, which were subsequently confirmed on radical prostatectomy. One representative section from each case containing areas with either absent or inconspicuous basal cells were submitted for immunostaining with cytokeratin 34 beta E12 and p63 antibodies. The SAWBCs were measured in units of 50 microns and in areas containing acini under 150 um in maximum diameter.

Results: In large sections from the radical prostatectomies, the SAWBCs measured (in m m) 50-300 (139±104), 50-1000 (167±160), 50-200 (228±111), and 50-300 (134±68) for benign and non-PIN, non-transitional zone; benign prostatic hyperplasia; AAH; and PIN respectively. In addition, three foci of AAH had SAWBCs measuring from 1000 to 8000 m m. In biopsy specimens, the SAWBCs measured (in m m) 50-300 (115±61), 50-250 (152±70) and 400-500 (467±45) for benign and non-PIN, PIN, and carcinoma individually.

Conclusions: SAWBCs are frequently seen in prostatic tissue. The largest SAWBCs were associated with AAH and usually have small or inconspicuous nucleoli. On the other hand, PIN frequently displays conspicuous nucleoli and is associated with SAWBCs under 300 m m in length. We thereby conclude that areas of atypical small acini with conspicuous nucleoli with a length greater than 300 microns without basal cells (with or without adjacent PIN) are consistent with a diagnosis of carcinoma.

584 Gleason Grading Multifocal Adenocarcinoma in Prostatectomy Specimens: Implications for Grading Needle Biopsies

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Background: There is controversy as to the optimal way to Gleason grade (GG) multifocal prostate cancer when individual tumors have different grades. This study examines the variability of GG in multifocal prostate cancer and determines the optimal prediction of biochemical failure.

Design: 115 entirely submitted, organ confined, margin negative radical prostatectomy specimens (pT2) were reviewed and a GG assigned to each spatially separate tumor (greater than 2 mm separation in plane of section and non-overlapping on consecutive sections). The maximum dimension of each tumor nodule was recorded. An overall GG was also given considering the entire tumor in the specimen. Biochemical failure was defined as PSA>0.2 in 79 patients with available follow-up. Univariate Kaplan-Meier statistics (Gehan's Wilcoxon test) and multivariate regression analysis (Cox proportional hazard model) were performed using a cutpoint of greater than 3+4=7 (i.e. 4+3=7) to define adverse grade pathology. Worst, largest, and overall grade were analyzed to determine the best predictor of biochemical failure.

Results: Patients had a mean age of 62 +/- 7 with an average of 3.7 +/- 1.4 separate tumors per prostate. Mean available follow-up was 58 months. The largest tumor had a mean maximal dimension of 1.5 +/- 0.7 cm with an average size range of tumors in a given prostate of 1.0 +/- 0.8 cm. 23% of cases were significantly upgraded using worst grade over the largest or overall categories. Largest, worst, and overall grades with a cutoff for adverse grade pathology of greater than 3+4=7 were statistically significant predictors of biochemical failure with all p values < 0.015. Multivariate regression analysis had significant Cox proportional hazard models for adverse grade pathology p=0.00065. Highest adverse grade pathology was the only significant parameter p=0.03 in this model for predicting biochemical failure.

Conclusions: Highest GG of any spatially separate tumor nodule found in a radical prostatectomy specimen appears to provide the best prediction of post operative biochemical failure using a cutpoint of greater than 3+4=7. This finding should aid in the understanding of grading in extended biopsy strategies.

585 Minimally Invasive Prostatectomy (Laproscopic and Robot-Assisted): Clinicopathological Characteristics of 82 Cases from a Single Institution

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Background: Minimally invasive prostatectomy (MIP) including, laproscopic and robot-assisted radical prostatectomy is an increasingly used alternate surgical modality for organ confined prostate cancer (PCa). The current study is a review of our initial experience with MIP with emphasis on the clinical and pathological characteristics.

Design: 82 consecutive cases of MIP performed at our institution by experienced urologists between 2000 and 2004 were studied. Hematoxylin and eosin stained slides were studied for the largest tumor size, Gleason score (GS), margin status, extraprostatic extension (EPE), pathological stage (pT), and for presence of ischemic changes in the tumor and non-tumor tissues. Morphological areas, demonstrating ischemic necrosis were quantitated on incremental scale of 10% of the total tissue involved. Other clinical parameters analyzed included patient age, pre-biopsy PSA, total number of positive cores and GS on needle core biopsies.

Results: In 81 of 82 cases diagnosis of PCa was confirmed, whereas in one case no residual PCa was detected. Median age was 60 years. Median pre-biopsy PSA was 5.1 and number of biopsy core involved by PCa was 2. In 61.3%, 37.1% and 1.6% of biopsy PCa were of GS 6, 7, and 8 respectively. For the MIP specimens, the tumor size ranged form 0.3 to 4.4 cm (median 1.4). The GS distribution was: 3+3=6 (26%), 3+4=7 (52%), 4+3=7 (18.5%) and 4+4=8 (3.5%). Majority (92.6%) were confined to prostate (pT2) while 7.4% had EPE (pT3a). Tumor extended to surgical resection margins in 17% (11 focal, 3 extensive) cases. Ischemic necrosis in > 10% of the non-tumor prostate glands were observed in a total of 26 (32%) cases. In 7 cases (8.6%) the ischemic changes comprised between 40 to 50% of the entire tissue. The tumor area itself appeared to be less frequently affected, with >10% ischemic changes identified in only 3 (3.7%) cases. In some instances ischemic changes posed difficulties in morphological interpretation, especially in separating benign glands from PCa, particularly in the vicinity of tumor or close to margin.

Conclusions: MIP offers the benefits of minimally invasive surgery without compromising pathological outcomes in general. Ischemic necrosis present in some cases, presumably related to longer operative time might occasionally create difficulties in the interpretation and also potentially impact the quality of procured tissues for future molecular studies.

586 Expression of Renal Cell Carcinoma Antigen (RCC) in Renal Epithelial and Non-Renal Tumors: Diagnostic Implications

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Background: Antibody to Renal Cell Carcinoma Antigen (RCC), a normal human proximal brush border antigen, has recently become commercially available and has been reported to be a highly specific and relatively sensitive marker for renal cell carcinoma. Of the non-renal tumors occasional carcinomas have been reported to express RCC, notably breast carcinoma. Using tissue microarrays, we investigated the utility of this antibody on a large number of renal epithelial neoplasms (REN) and non-renal tumors, especially those potentially confused with REN because of location or histological resemblance.

Design: Three tissue microarrays containing 261 REN samples, 192 samples of a wide variety of neoplasms (multi tumor array) and 198 adrenal tumor samples (normal and tumor) were stained with a monoclonal (clone 66.4.C2) antibody specific for RCC (Vector Laboratories, Burlingame, CA). RCC expression was scored for staining intensity (0=negative, 1=weak, 2=moderate, and 3=strong staining) and percentage expression (Negative, focal (<25%) and diffuse (>25%).

Results: 187 out of 261 of the REN were RCC positive (Sensitivity 71.5 %): clear cell 72%, papillary 95%, chromophobe 91%, sarcomatoid 20%, unclassified 85% and metastatic RCC 40%. Consistently, the overall immunostaining intensity was much higher in papillary and clear cell RCC (3 to 4) than in other tumors. Eighty two out of 362 of non-renal tumor samples demonstrated either focal or diffuse expression for RCC (Specificity 77.3%). These included: adrenocortical neoplasms 37/170 (22%), colonic 11/29 (37.5%), breast 3/9 (33%), 5/18 (27.7%) prostate, 2/17 (11.7%) ovary, 3/18 (16.6%) melanoma, 1/7 (14.2%) lung, and 2/2 (100%) parathyroid. RCC expression was seen equally among adrenal adenoma and carcinoma group. 8 out of 28 (28.5%) normal adrenal cores also stained for RCC.

Conclusions: This study supports the RCC as a useful marker in the work up of differential diagnosis of REN albeit with significant limitations. Cautious use of this marker in a panel with other positive and negative markers may only aid in the accurate diagnosis of REN. RCC does not reliably differentiate REN, especially classic clear type from adrenocortical neoplasms, which are frequently confused due to its close anatomical proximity.

587 Follow-Up of Patients with Initial Benign Ten-Core Biopsy

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Background: The reported prostate cancer (PCa) rates following a benign biopsy range from 11%-24.5%. We have recently found PCa on repeat ten-core biopsy in 40% of patients after atypical biopsy and in 27% after high-grade prostatic intraepithelial neoplasia (PIN). The follow-up of patients with initial benign ten-core biopsy who undergo repeat biopsy due to elevated PSA or suspicious rectal exam has not been studied previously.

Design: We identified 125 consecutive patients in our institutional database during one year (01/01-12/01) who had benign initial needle biopsy and who had a subsequent prostate sampling procedure in our institution. Repeat procedures included needle biopsies in 102 patients and transurethral resection of prostate (TURP) in 23 patients. The total number of needle biopsies performed during the same period was 1824 and 703 (38.5%) were benign. All needle biopsies were performed using ten-core sampling.

Results: In patients with repeat needle biopsies, PCa, PIN, and benign findings were identified in 14 (13.7%), 10 (9.8%), and 78 (76.5%) patients, respectively. In patients with repeat TURP, 1 (4.3%) patient had PCa and 22 (95.7%) had benign findings. Repeat prostate procedures (both needle biopsies and TURP) revealed PCa, PIN, and benign findings in 15 (12%), 10 (8%) and 100 (80%) patients, respectively. PCa was diagnosed on the first repeat biopsy in 13 (92.9%) patients and one patient had atypical PIN prior to the PCa diagnosis. Mean time interval between the initial benign biopsy and the repeat biopsy with PCa was 18.2 months (range 3.4 to 31.5). The mean age of patients with PCa vs. patients with benign repeat biopsy was similar (60.5 and 59 years) and they had similar mean PSA (6.1 vs. 7.7 ng/ml) and mean PSA density (0.16 vs. 0.14). Seven (50%) patients had PCa in only one core (range 1-5). Biopsy Gleason score was 6 (in 8 patients) or 7 (in 6 patients). Prostatectomy was performed in 9 patients and final Gleason score was either 6 (in 6 patients) or 7 (in 3 patients). Organ confined PCa was found in 8 patients and 1 patient had extraprostatic PCa. Two patients had positive resection margins. All patients had negative seminal vesicles and lymph nodes.

Conclusions: 1.) PCa is found on repeat ten-core biopsy after initial benign biopsy approximately two and three times less in comparison with PCa found after initial biopsy with PIN and initial biopsy with atypia, respectively. 2.) Biopsy and prostatectomy findings in men with PCa after benign biopsy show that some men harbour PCa of Gleason score 7 and advanced stage PCa.

588 Immunohistochemical Expression of Calretinin, CD99, and α-Inhibin in Sertoli and Leydig Cells and their Lesions, Emphasizing Large Cell Calcifying Sertoli Cell Tumor

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Background: Calretinin has been suggested as useful in distinguishing neoplastic and non-neoplastic Leydig cells (LC), with Sertoli cells (SC) showing less frequent staining. In contrast, CD99 has been reported to stain SC more consistently. Inhibin is said to label both LC and SC, the former more strongly. We studied Leydig cell tumors (LCT), typical Sertoli cell tumors (SCT), and Large cell calcifying Sertoli cell tumors (LCCSCT), as well as non-neoplastic testis, with antibodies to calretinin, CD99, and α-inhibin, to evaluate their utility in distinguishing SC, LC, and their tumors, and to further characterize LCCSCT.

Design: Archival tissue was obtained from 31 testicular lesions and normal controls including 3 LCT, 5 SCT, 9 LCCSCT, 5 Leydig cell hyperplasia (LCH), 1 Sertoli cell hyperplasia (SCH), and 8 normal testes. Immunohistochemical staining was performed with antibodies to calretinin, CD99, and α-inhibin. Staining intensity and the percentage of cells labelling were assessed semi-quantitatively as follows: intensity: 0=none, 1+=weak, 2+=moderate, and 3+=strong; % cells staining: focal=1-25%, patchy=26-50%, multifocal=51-75%, and diffuse=76-100%.

Results: LC in all 8 controls stained strongly for calretinin and inhibin; 3/8 stained weakly for CD99. SC in controls labelled strongly for CD99 (8/8), weakly for calretinin (1/8), and focally for inhibin (4/8). The 6 LCH/SCH exhibited strong calretinin staining in LC, variable inhibin staining, LC greater than SC, and strong CD99 staining in SC, with rare, weak LC labelling. All 3 LCT showed strong calretinin and variable inhibin staining. CD99 was focally weakly positive in 3/3 LCT. All 5 SCT were negative for calretinin. Inhibin focally, weakly labelled 2/5 SCT. CD99 showed at least focal staining in 3/5 SCT. Eight of 9 LCCSCT exhibited strong calretinin staining, and 9/9 labelled for inhibin. One stained focally, weakly for CD99.

Conclusions: In normal testis, LC/SC hyperplasia, LCT, and SCT, calretinin and α-inhibin are sensitive and relatively specific markers of LC. CD99 is a useful marker of SC, especially non-neoplastic cells. Although tubule formation, intratubular growth, and Charcot-Bottcher crystalloids suggest that LCCSCT have Sertoli cell features, their immunohistochemical profile is more Leydig cell-like. LCCSCT may be a unique tumor with a mixed phenotype.

589 Immunoreactivity for Oct-3/4 Using a Monoclonal Antibody Distinguishes Embryonal Carcinomas and Seminomas from Other Epithelioid and Round Cell Malignant Neoplasms

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Background: Oct-3/4 (Oct-4; POU5f1) is a homeobox transcription factor expressed in embryonic stem cells and germ cells that is important for maintaining pluripotency. Recent studies using a polyclonal antibody have shown that staining for Oct-3/4 is limited to embryonal carcinoma (EC) and seminoma (SEM) among germ cell tumors (GCT). However, expression of Oct-3/4 in other tumor types has not been extensively examined. Recently, a monoclonal antibody (MAb) to Oct-3/4 has become available. The purpose of this study was to evaluate the specificity of this MAb to Oct-3/4 for GCT in comparison to other epithelioid and round cell malignant neoplasms.

Design: 251 tumors were studied: 45 GCT in tissue microarrays (22 SEM, 13 EC, 10 mixed GCT) and 206 tumors in conventional sections: 34 GCT (11 SEM, 4 EC, 18 mixed GCT, 1 teratoma); 54 carcinomas (12 stomach, 11 colon, 11 prostate, 10 breast, 10 lung); 46 sarcomas (13 epithelioid angiosarcoma, 12 proximal-type epithelioid sarcoma, 11 Ewing's sarcoma/PNET, 10 alveolar rhabdomyosarcoma); 45 lymphomas (10 each diffuse large B-cell lymphoma, anaplastic large cell lymphoma (ALCL), classical Hodgkin lymphoma, Burkitt lymphoma; 5 precursor T-lymphoblastic lymphoma); 10 epithelial-type mesotheliomas; 10 metastatic melanomas; and 7 thymomas. Immunohistochemical studies were performed following heat-induced epitope retrieval (0.01 M citrate buffer, pH 6.0; pressure cooker) using a

MAb to Oct-3/4 (dilution 1:2000; clone C10; Santa Cruz Biotechnology) and processed using an indirect immunoperoxidase technique. The extent of immunoreactivity was graded according to the percentage of positive tumor cell nuclei: 0, no staining; 1+, <5%; 2+, 5-25%; 3+, 26-50%; 4+, >50%.
Results: All cases of EC and SEM showed 4+ nuclear staining for Oct-3/4, as did foci of intratubular germ cell neoplasia (ITGCN). In contrast, all areas of teratoma, yolk sac tumor, and choriocarcinoma were negative. Among non-GCT, only 1/10 ALCL showed 2+ nuclear reactivity. All other tumors were negative, except for rare cases with weak, equivocal nuclear staining.
Conclusions: Oct-3/4 is a sensitive marker for EC, SEM, and ITGCN with extensive nuclear staining in all cases using MAb clone C10. Immunoreactivity for Oct-3/4 is rarely observed in other epithelioid and round cell malignant neoplasms. Staining for Oct-3/4 may be useful to support germ cell origin in the differential diagnosis of poorly differentiated malignant tumors.

590 E-Cadherin Pattern of Expression Differentiates Chromophobe Renal Cell Carcinoma from Oncocytoma: An Immunohistochemical Study of 137 Renal Neoplasms

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Background: Cadherins are a family of transmembrane glycoproteins responsible for calcium-dependent intercellular adhesion and are involved in development and maintenance of tissue architecture. E-cadherin is expressed by the normal distal renal tubule and collecting duct epithelium and is not present in the proximal renal tubules. Although E-cadherin has been previously reported to be expressed in oncocytomas and chromophobe renal cell carcinomas (RCC), we have observed a difference in the staining pattern. The purpose of this study was to evaluate a large number of renal neoplasms to validate our observations.
Design: 137 renal neoplasms, including chromophobe RCC (n=58), oncocytoma (n=54) and conventional (clear cell) RCC (n=25) were analyzed by immunohistochemistry using a monoclonal antibody against E-cadherin (Zymed, San Francisco, CA). The results were interpreted as negative (<10% of cells staining), 1+ (10-30%), 2+ (30-75%) and 3+ (>75% of cells staining). Two staining patterns were recorded: a pure membranous pattern and a mixed cytoplasmic and membranous pattern.
Results: All 58 chromophobe RCCs were positive for E-cadherin with a pure membranous staining pattern. The intensity of staining was variable, with 3+ staining in 44 of 58 cases (75.8%), and 2+ in the remainder. All six eosinophilic variant chromophobe RCCs had a pure membranous staining pattern (3+). Two sarcomatoid chromophobe RCCs were negative for E-cadherin in the sarcomatoid component. All 54 oncocytomas were positive (3+) for E-cadherin with a mixed, granular cytoplasmic and partially membranous staining pattern. Seventeen of 25 conventional RCCs (68%) were negative for E-cadherin. Eight positive cases (32%) showed a very focal, membranous staining pattern (1+).
Conclusions: E-cadherin has two patterns of staining that are helpful in distinguishing chromophobe RCC from oncocytoma and conventional RCC. All chromophobe RCC cases in our study, including the eosinophilic variant, had an intense, pure membranous staining pattern. In contrast, all oncocytomas had a mixed granular cytoplasmic and focally membranous staining pattern. Conventional RCCs are usually negative for E-cadherin, but rare cases may be focally and weakly positive.

591 A Functional Polymorphism in an RGS Gene Modulates the Risk of Bladder Cancer

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Background: RGS proteins negatively regulate heterotrimeric G protein signaling. Recent reports have shown that RGS proteins modulate neuronal, cardiovascular and lymphocytic activity; yet their role in carcinogenesis has not been explored. In an ongoing hospital-based case-control study, we explored the association between 12 non-coding single nucleotide polymorphisms (SNPs) in five RGS genes identified in the NCBI dbSNP database and the risk of transitional cell carcinoma (TCC) of the bladder, a cause of over 12,000 US deaths per annum.
Design: In an epidemiological study of 477 TCC patients and 446 matched controls, three non-coding single nucleotide polymorphisms (SNPs) in RGS2 and RGS6 were each associated with a statistically significant reduction in bladder cancer risk. Using laser capture microscopy and cultured cell lines, we explored the expression of RGS2 and RGS6 in TCC and lympocytes. To validate the biologic significance of these SNPs, we also sought to identify functional changes in transcript levels, alternative splicing events and protein translation efficiency that may result from the presence of the variant alleles.
Results: The risk of TCC was reduced by 74% in those individuals with the variant genotype at all three SNPs (O.R. 0.26; 95% CI= 0.09-0.71). When the SNPs were analyzed separately, the RGS6-rs2074647 (C/T) polymorphism conferred the greatest overall reduction in risk of bladder cancer (O.R. 0.66; 95% CI= 0.46-0.95). These reductions in risk were more pronounced in ever smokers, suggesting a gene-environment interaction. In transfection assays, the RGS6-rs2074647 (C/T) polymorphism increased the activity of a luciferase-RGS fusion protein by 2.9 fold, suggesting that this SNP is functionally significant. Finally, we demonstrate that RGS2 transcripts and several splice variants of RGS6 are expressed in bladder cancer cells.
Conclusions: These data provide the first evidence that RGS proteins may be important modulators of cancer risk. The presence of variant SNP alleles in RGS2 and RGS6 are associated with a significant reduction in the risk of TCC and may be used to screen those individuals at risk.

592 Tissue Microarray of the British Testicular Tumour Panel Series: Mining a Historical Archive

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Background: The British Testicular Tumour Panel (BTTP) reviewed difficult testicular cases from the1950s to 1980s, examining thousands of testicular tumours. The BTTP archive includes both human and animal tumours, detailed case summaries, macroscopic photographs, slides and some paraffin embedded material. Although the original diagnoses have been used in some previous case series, detailed examination and computerisation of this archive had not been performed in the era of immunohistochemistry. This project was an investigation of the remaining archival material to see if sufficient material remained for further research, whether the material was suitable for immunohistochemistry, and to compare diagnoses from the pre-immunohistochemistry era with modern techniques and diagnoses.
Design: 4000 archive cases were reviewed for cases where paraffin blocks were available resulting in 758 cases in 2795 paraffin blocks. The list of these cases was computerised with the original diagnoses. Disagreements in the panel's diagnoses were also listed. As an initial assessment, 200 cases diagnosed as classical seminoma were examined. These were then distilled using tissue micro-array (TMA) into 6 donor blocks. Three cores were taken from each tumour. H and E sections were cut and immunohistochemistry was performed for placental alkaline phosphatase (PLAP) and inhibin.
Results: Immunohistochemistry for PLAP and inhibin revealed strong membranous staining for PLAP in 193/200 tumours (96.5%). Weak or focal staining was seen in 6 cases. There was one entirely negative case. This tumour, was strongly positive for inhibin, and examination of the morphology revealed a sertoli cell tumour with a sparse lymphoid infiltrate and some spindled tumour cells and cytolplasmic clearing similar to seminoma.
Conclusions: The accuracy of the BTTP has been confirmed. Immunohistochemical staining was no different from modern series, and no loss of antigenicity was seen on this initial assessment. The diagnosis of seminoma, before the era of immunochemistry revealed only one erroneous diagnosis by the panel. TMA is an excellent technique for distilling ancient archival collections, allowing for fast and cheap identification of rare tumours. The TMA work may prove of great use in testing novel antibodies, discovering rare cases for future series, and for education .

593 Intraoperative Evaluation of Pelvic Lymph Nodes during Radical Prostatectomy: Is 'Gross Evaluation Only' Enough?

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Background: The incidence of pelvic lymph node (PLN) metastases in patients with prostatic carcinoma (PCa) has declined. Although, the utility of performing PLN dissection in patients with PCa remains controversial in the surgical literature, intra-operative evaluation of PLN are still being requested at the time of the surgery. Frozen section evaluation of PLN can be technically difficult and time consuming due to either the large number and size of the nodes or due to extensive fatty or fibrous replacement of the lymphoid tissue. Can 'gross evaluation only' provide reliable information for appropriate surgical management? The aim of this study was to compare the utility of gross evaluation (GE) only versus frozen section (FS) in the intraoperative management of prostate surgeries.
Design: We retrospectively reviewed surgical pathology reports and histology slides of 1046 consecutive radical prostatectomies performed at our institute over a 10-year period. The results of intraoperative procedures performed in each case (GE, FS, both or neither) was recorded and compared to the final surgical pathology results.
Results: Pelvic lymph node dissection was performed in 806 (77%) cases. Of these, Intraoperative consultation was requested in only 523 (65%) cases. Of these cases with IOC, GE only was performed in 413 cases, whereas FS was performed in remaining 110 cases. Only one case showed metastatic carcinoma involvement (0.9%) on frozen section. There was one false negative case where metastatic carcinoma was identified on permanent sections. In the 413 cases where GE only was performed (no FS), there were 9 false negative cases (2.2%). Seven of these cases had micrometastases ($\leq$ 2.0 mm); whereas metastatic foci (3.0 mm and 8.0 mm, respectively) were identified on the permanent sections in the remaining two cases. Thus, overall only two cases (0.5%) had significant metastases that were missed by GE only.
Conclusions: Sampling of lymph nodes from any site for IOC can be a technically complex issue. The incidence of nodal positivity in PCa is extremely low, majority being micrometastases that can be easily missed or lost during frozen section trimming. 'Gross evaluation only' of nodes appears sufficient for intraoperative management in most cases. Frozen sections can be reserved for limited cases with a high index of suspicion.

594 Relationship of Age to Outcome and Clinicopathologic Findings in Men Submitted to Radical Prostatectomy

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Background: It is controversial whether age is associated with higher grade and worse outcome with increasing age. Some studies have not found age to be related to outcome or found a younger age to be associated with better response to therapy.
Design: The study population consisted of 27 patients age 55 years or younger and 173 patients 56 years or older submitted to radical prostatectomy. The clinicopathological variables studied were: preoperative PSA, Gleason score, Gleason predominant grade, tumor extent, positive surgical margins, extraprostatic extension (pT3a) and seminal vesicle invasion (pT3b). Tumor extent was evaluated by a point count semiquantitative method. The data were analyzed using the Mann-Whitney test

for comparison of independent samples and Fisher's exact test for evaluating differences between proportions. Time to PSA recurrence was compared between the groups using a log-rank survivorship analysis.

Results: The mean and median follow-up periods among men without biochemical recurrence were 3.5 and 3.2 years, respectively. During this time, 44 patients (22%) developed a biochemical recurrence. Comparing patients age 55 years or younger and 56 years or older, there was no statistically significant difference for all variables studied: preoperative PSA (p=.4417), Gleason score (p=.3934), Gleason predominant grade (p=.2653), tumor extent (p=.1190), extraprostatic extension (p=.3447) and seminal vesicle invasion (p=1). Log-rank analysis revealed no difference in PSA recurrence between men age 55 years or younger and 56 years or older (log-rank analysis: p=.2990).

Conclusions: The findings of our study showed that younger and older men do not have significant differences as related to preoperative PSA, pathologic findings in the surgical specimen and outcome following radical prostatectomy. Our findings suggest that age alone do not influence the biological aggressiveness of prostate cancer.

595 Prostate Cancer: Are Clinical Stages T1c and T2 Similar?

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Background: A recent study have found that PSA recurrence rate for clinical T1c tumors is similar to T2 tumors, indicating a need for further refinement of clinical staging system. To test this finding we compared clinicopathologic characteristics and the outcome of patients with clinical T1c tumors to those with T2 tumors.

Design: From a total of 186 consecutive patients submitted to radical prostatectomy, 59 (31.72%) had clinical T1c tumors and 127 (68.27%) T2 tumors. The variables studied were: age, preoperative PSA, prostate weight, Gleason score, tumor extent, positive surgical margins, extraprostatic extension (pT3a), seminal vesicle invasion (pT3b), and biochemical recurrence. Tumor extent was evaluated by a point count semiquantitative method. The data were statistically analyzed using the Mann-Whitney test for comparison of independent samples and Fisher's exact test for evaluating differences between proportions.Time to PSA recurrence was compared between the groups using a log-rank survivorship analysis.

Results: Patients with clinical T2 tumors were more likely to have higher Gleason score (p=.0212) to those with T1c tumors. There was no significant difference in preoperative PSA (p=.3791), prostate weight (p=.6301), tumor extension (p=.1857), positive surgical margins (p=.3163), extraprostatic extension (p=.1020) and seminal vesicle invasion (p=.2481). There was a tendency for a higher age in patients with clinical T2 tumors (p=.0788). The mean and median follow-up periods among men without biochemical recurrence were 3.3 and 3.2 years, respectively. During this time, 41 patients (22.04%) developed a biochemical recurrence. Log-rank analysis revealed no difference in PSA recurrence between men with clinical T1c and T2 tumors (p=.3105).

Conclusions: Patients with clinical T2 tumors have higher Gleason score and a tendency for higher age. However, the PSA recurrence for T1c tumors is similar to T2 tumors. Our findings suggest a need for further refinement of clinical staging system.

596 Defining Prostate Cancer Progression Using a Multiplex Panel of 13 Tissue Biomarkers

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Background: Expression array technology has lead to the development of discrete molecular signatures to predict cancer progression. This study explores the utility of a focused biomarker panel developed from a proteomic study to distinguish indolent from aggressive Prostate Cancer (PCA).

Design: A high throughput proteomic screen of prostate tissue extracts identified a panel of 50 differentially expressed proteins from over 1500. 41 Candidate proteins that best distinguished between benign prostate tissue, localized PCA, hormone naïve and hormone metastatic PCA were selected for further analysis. This list included PSA, AMACR, E-Cadherin, p27, Fatty Acid Synthase (FAS), Ki67 and the Androgen Receptor (AR). This list also includes genes that have been recently associated with PCA such as E2F, EZH2, JAGGED1, MTA1, p63, ZAG, MUC1, XIAP, and TPD52. Immunohistochemistry (IHC) was performed on a PCA progression tissue microarray (TMA). The IHC staining intensity was scored using a semi-automated analysis system. Linear Discriminant Analysis (LDA) was applied on the dataset of 41 genes. The biomarker panel was validated at the transcriptional level using expression ar

Results: LDA determined a set of 13 genes from the original 41 proteins that could accurately distinguish the prostate samples. Previous work suggested a good correlation between protein expression and transcription level. We therefore tested this multi-gene model on a set of 200 localized PCA. The expression of this set of genes separated the localized PCA cases into two clusters with non-random distribution with respect to PSA failure (group 1 failure=41% (40/97) vs group 2=60%(62/103), p =0.01). Kaplan-Meier analysis taking time into account demonstrated a significant difference in time to PSA failure between the two groups (log rank p=0.042).

Conclusions: A discrete set of 13 proteins was determined to distinguish between benign prostate tissue and stages of PCA progression. This model was independently validated at the level of gene expression using PSA failure as the clinical outcome on 200 PCA cases. This study also highlights that the mode of discovery of a biomarker does not restrict the final testing modality of the biomarker.

597 Solid Muscle-Invasive Transitional Cell Carcinomas of the Bladder Reveal Microsatellite Instability and Somatic Down-Regulation of Mismatch Repair

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Background: Histologic patterns have been demonstrated prognostically useful in transitional cell carcinomas (TCC) of the bladder, but no information is available on the prognostic significance and genetic profile of muscle-invasive TCC by infiltration patterns. The contribution of DNA mismatch repair abnormalities to this profile remains unknown.

Design: The predominant infiltration pattern of 72 muscle-invasive TCC was assessed in the deep compartment and classified as 'solid' (diffuse infiltration effacing the muscle fibers, 45 cases) or 'single-file' (tumor infiltration dissecting the muscle and inducing an intense stromal reaction, 27 cases). Tumors were studied by compartments (superficial and deep to muscularis mucosa), DNA being extracted from both compartments to analyze the microsatellite pattern of TP53, RB1, WT1, and NF1 by PCR/denaturing gradient gel electrophoresis. Mitotic index, Ki-67 index, in situ end labeling, and DNA ploidy analysis were evaluated in the same areas. Statistical differences were tested using ANOVA and Fisher's exact test.

Mismatch repair was assessed by MLH1 and MSH2 sequencing and immunostaining in TCC with ≥2 abnormal microsatellite loci.

Results: Single-file TCC showed lower cell turnover (Ki-67 index 14.94±4.28, ISEL 14.1±10.0), lower incidence of aneuploid DNA content, and shorter mean survival (20 months) than solid TCC (Ki-67 index 20.65±4.94, ISEL 20.2±22.7, 37-month survival, respectively).

The genetic profile was demonstrated significantly different for RB1 (p=0.0003) and NF1 (p=0.0023) only. Single-file TCC showed a low incidence of genetic deletion/single necleotide polymorphism(s), not involving RB1 locus, and very occasionally NF1 locus (2 cases, 13%). A significant decrease of MLH1 or MSH2 protein expression (absence of any of these proteins) with no gene mutations were identified in TCC with high microsatellite instability and solid growth pattern.

Conclusions: 1. A somatic down-regulation of mismatch repair proteins (MLH1/MSH2) in solid muscle-invasive TCC results in microsatellite instability characterized by deletion/single nucleotide polymorphism(s) in RB1 and NF1.

2. This microsatellite instability profile correlates with the higher cellular turnover (both proliferation and apoptosis) and longer survival observed in patients with solid TCC (as compared with those of single-file TCC).

598 Clear Cell Renal Cell Carcinoma: T2 Tumors Are Extremely Rare

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Background: Tumor stage and grade are the most important prognostic parameters in renal cell carcinoma. The 2002 TNM classification included involvement of renal sinus fat and renal sinus veins as staging parameters, T3a and T3b, respectively, and added substaging to the T1 category. This study prospectively examines the relationship between and size and grade, and renal sinus involvement.

Design: A prospective study of 106 clear cell renal cell carcinomas (CC) was designed to identify the frequency and nature of involvement of the renal sinus. The interface between the tumor and the renal sinus was carefully examined and a minimum of 5 blocks submitted. Sections were examined for sinus fat (T3a) and sinus vein (T3b) involvement.

Results: 57 of 106 tumors involved the sinus fat and/or veins (54%) and 27 invaded the capsule (26%). All capsular invasive tumors also involved the sinus. 15% of grade 1 and 2 tumors involved the renal sinus compared to 74% of grade 3 and 4 tumors. Sinus vein involvement T3b +/- fat involvement was far more common (80%) than isolated sinus fat involvement (20%). The relationship between size and sinus involvement is shown in the table. Sinus positive tumors <4 cm were infrequent (13%), but increased markedly in the >4-7 cm range (77%). Only 1 T2 tumor was identified (1/32=3%).

Conclusions: (1) Renal sinus involvement is much more common than capsule involvement in CC (54% vs.26 %). (2) No tumor invaded the capsule that did not also involve the sinus. (3) Renal sinus vein involvement is far more common (80%) than invasion of sinus fat without vein involvement (20%). (4) Renal sinus invasion markedly increases once tumors exceed 4 cm in size. (5) T2 CC is an extremely rare event (3% tumors >7cm), negating the value of this category as currently defined.

Relationship between size and sinus invovlement

Tumor Size	0-2 cm	>2-3 cm	>3-4 cm	>4-5 cm	>5-6 cm	>6-7 cm	>7 cm
# Sinus Negative	20	15	7	4	0	2	1
# Sinus Positive	2	2	2	7	6	7	31
% Sinus Positive	10%	12%	22%	64%	100%	78%	97%

599 The Renal Sinus in Partial Nephrectomies: A Study of 50 Cases

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Background: The 2002 TNM classification of renal tumors includes involvement of renal sinus fat and renal sinus veins as new staging parameters, T3a and T3b, respectively. The incidence of renal sinus tissue in partial nephrectomies, and the nature and frequency of sinus involvement by renal tumors has not been evaluated. This prospective study examines the renal sinusinvolvement by renal tumors in 50 partial nephrectomy specimens.

Design: The entire interface between the renal tumor and the surgical resection margin, or the entire specimen was examined in 50 partial nephrectomy specimens. The frequency of renal sinus tissue and its relationship to tumor size and tumor type, and the incidence of renal sinus involvement was determined. This included 32 clear cell renal cell carcinomas (CC), 2 multilocular cystic clear cell carcinomas (MLCC), 6 papillary renal cell carcinomas (PAP), 3 chromophobe cell renal cell carcinomas (Chr), 6 oncocytomas (Onco), and 1 cystic nephroma.

Results: Twenty of 50 specimens included renal sinus (13 CC, 1 MLCC, 1 Pap, 2 Chr, 2 Onco, and 1 CN). The relationship between size of tumor and presence of renal sinus is as follows: 5/21 tumors < 2 cm, 15/24 tumors 2-4 m, 0/3 tumors 4-6 cm and 0/1 tumors 6-7cm contained renal sinus. No tumor invaded the renal capsule. Only 1 tumor (3 cm CC) demonstrated renal sinus invasion; it invaded a sinus vein (T3b). The remaining cases consisted of 45 T1a and 4 T1b tumors. In 4/50 cases the surgical margin was positive, three on frozen section leading to additional tissue removal with a negative margin. In the 4th case no frozen section was performed.

Conclusions: 1) Renal sinus tissue was present in 40% of partial nephrectomy cases 2) There was no relationship between size or tumor type, and presence of renal sinus tissue 3) Involvement of the renal sinus was rare 1/50 cases (2%), indicating excellent patient selection.

Relationship Between Size, Tumor Type, and % of Resections That Include Renal Sinus Tissue

	0-2 cm	2-3 cm	3-4 cm	4-5 cm	5-6 cm	6-7 cm
Clear Cell Ca	27 %	46 %	100 %	0 %	—	0 %
Papillary Ca	0 %	50 %	0 %	—	0 %	—
Chromophobe Ca	100 %	—	50 %	—	—	—

600 Indepentant Evaluation of the NCI Sponsored Cooperative Prostate Cancer Tissue Resource (CPCTR) Microarray

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Background: The NCI established the CPCTR (RFA 1999CA-99-012, $2.4 Mil/Yr) to create tissue banks to provide large numbers of clinically annotated prostate cancer (PCA) specimens to investigators. The goal of the current study is to perform an independent evaluation of their publicly available PCA tissue microarray (TMA).

Design: 3 pairs of TMAs were obtained through the CPCTR public website (http://cpctr.cancer.gov/) after completing the on-line application process. The TMAs are composed of PCA from radical prostatectomy specimens from 299 patients and control tissues. An H&E slide (slide 1) was prepared. Immunohistochemical staining for AMACR (p504s) and the basal cell cocktail (p63/34βE12) were performed on slides 2 and 3, respectively. The study pathologist reviewed each TMA core to confirm the target diagnosis. Image analysis to determine staining intensity (AMACR) and percentage of positive cells (BCC) was performed.

Results: TMA pairs contained 600 (0.6 mm) tissue cores labeled as PCA and 64 benign prostate. An Excel spreadsheet was provided with a physical map of the TMA and detailed information in a format compliant with the Common Data Element XML standard. Information included age, race, PSA, tumor size, TNM stage, Gleason score, and vital status. Cores designated as PCA by the CPCTR contained PCA in 66.7% (399/598), 64.5% (387/600), and 57.7% (346/600) of the TMA pairs 1, 2, and 3, respectively. Tissue was missing on 12.7% (76/598), 13.8% (83/600), 26.7% (160/600) for slides 1, 2, and 3, respectively. Benign tissue was observed in cores designated as PCA in 15.7% (94/598), 10.5% (63/600), and 9.5% (57/600) for slides 1, 2, and 3, respectively. 44.5% of the PCA samples were available on all three levels TMA-pairs. AMACR intensity for benign and PCA were significantly distinct with mean intensity scores of -1.36 and 0.18, respectively ($p < 0.0001$). BCC expression for benign and PCA were significantly different with mean intensity scores of 2.19 and -0.298, respectively ($p<0.0001$). These differences are consistent with previous work. Evaluation for clinical outcome was not possible as the cases were not collected in a uniform manner.

Conclusions: The CPCTR TMAs provide high quality tissue samples for biomarker screening. However, significant tissue loss was observed, which may be due to samples processed without the tape system as originally described (Nat Med 4, 844, 1998). Although the samples are well annotated, the cohort of 299 cases cannot be used for outcome assessment.

601 Gleason Grading in Clinical Trials: The Need for Centralized Pathology Review

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Background: The Physicians Health Study (PHS) were two randomized placebo-controlled trials in the primary prevention of cardiovascular disease and cancer among 29,000 US male physicians. Between 1982-2003, 2,320 incident prostate cancer (CaP) cases were diagnosed among 29,000 participants. This study reports on the central pathology review of these cases from the PHS.

Design: We re-evaluated the Gleason scores from the pathology reports of 134 randomly selected CaP cases and compared those to the Gleason scores of all CaP cases from the PHS. Two study pathologists reviewed and documented the Gleason score in all the cases, in a blinded manner by light microscopy.

Results: The results are presented in the table. In those cases with histologic central review there were no cases of Gleason score 2-4, and only 3 cases of Gleason score 5. The Gleason scoring was consistent between reported Gleason and standardized review for only 25 (19%) of 134 cases. For 92 cases (69%), the Gleason score increased by at least one unit. Indeed, 34 of the cases increased their total score by two units, and 22 cases increased by 3 or more units; in the 17 cases in which the reported Gleason score was higher than the standardized review, the majority (N=13) differed by only one unit. There appears to be evidence of time trends in the quality of Gleason grading. Among cases diagnosed between 1982-1994, Gleason scores for 63% of cases differed by two or more points compared to 39% of those diagnosed more recently (1995-2003).

Distribution Of Gleason Score For The Physicians' Health Study (1982-2003)

	PHS Prostate Cancer Cohort (N=1,850)		Randomly Selected Cases (N=134)		Central Pathology Review (N=134)	
	Based on Pathology Review				Central Review	
Gleason score	N	%	N	%	N	%
2-4	355	19.2	18	13.4	0	0
5	401	21.7	21	15.7	3	2.2
6	451	24.3	51	38.1	28	21.0
7(3+4)	432*	23.4*	21	15.7	52	38.8
7(4+3)			10	7.5	29	21.6
8	118	6.4	8	6.0	17	12.7
9-10	93	5.0	5	3.7	5	3.7

*No separate reporting of 3+4 versus 4+3

Conclusions: This study demonstrates that there appears to be a tendency to under grade prostate cancer. The reporting of Gleason scores should therefore undergo central review in the setting of large multicenter trials as there is considerable variation amongst pathologists

602 Chromosome 1 Analysis in Chromophobe Renal Cell Carcinoma with Tissue Microarray (TMA) Facilitated Fluorescence In-Situ Hybridization (FISH)

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Background: The morphologic overlap between chromophobe renal cell carcinoma (ChRCC) and oncocytoma has been widely recognized. However, whether these tumors are genetically related and represent a spectrum of benign to malignant tumor progression remains an open question. We previously showed that the most common chromosomal abnormality in renal oncocytomas is loss of chromosome 1 or 1p (J. Urol., 171:602-604, 2004, Mod Pathol., 17:suppl 1, 171A, 2004). In this study we evaluated chromosome 1 in ChRCC using the same set of probes and number of tumors.

Design: A total of 21 ChRCCs from 13 males and 8 females from 2 institutions were studied. Formalin-fixed paraffin-embedded tissue blocks from ChRCCs, 7 normal adjacent kidneys and 2 oncocytomas were used to construct TMA. Representative tumor and normal adjacent kidney tissue was selected and a minimum of 2 tissue cylinders (2 or 5 mm in diameter) were obtained and arrayed manually or using tissue microarray (Beecher Instruments, Silver Spring, MD). The recipient blocks were cut into 4-um thick sections for immuno/histochemistry and FISH analyses. FISH studies were performed using the 1p36.3 probe (29MB from the end of the chromosome, containing TP73 and EGFL3) in tandem with 1q25 (Vysis,Inc.). At least 30 cells/tumor section were counted.

Results: The patients ranged from 34 to 82 years old (mean=62, median=61). All ChRCCs were unilateral. In 8/21 tumors, the Fuhrman grade was 2/4, and in 13, 3/4. Colloidal iron stain was positive in all tumors on TMA. FISH analysis showed an abnormal chromosome 1 in 20/21 (95%) ChRCCs as follows: 18 tumors (90%) had loss of the entire chromosome 1, 2 (10%) had loss of 1p36.3 only; one tumor from a 34yo male was apparently normal diploid for chromosome 1. Normal cortex, medulla and 2 oncocytomas were diploid. We previously showed that 72% of oncocytomas have abnormalities of chromosome 1 by cytogenetics and/or FISH. It is believed that a putative tumor suppressor gene resides on the short arm of chromosome 1. This study shows that 95% of ChRCC show similar abnormality.

Conclusions: In this study, 95% of ChRCCs showed abnormalities of chromosome 1 by FISH. This result provides further evidence to support a genetic link between ChRCC and oncocytoma. TMA facilitated FISH is a powerful approach in the analysis of chromosome abnormalities in renal tumors.

603 Detection of Small Focal Prostate Cancer by α-Methylacyl Co-A Racemase/P504S Immunohistochemistry in Previously Negative Biopsies

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Background: The recognition of small foci of prostate cancer on needle biopsy is one of the major diagnostic challenges in surgical pathology. α-Methylacyl Co-A Racemase (AMACR)/ P504S has been shown to be a good prostate cancer marker that can distinguish cancer from benign glands with high sensitivity and specificity. In this study, we investigate the possibility of detecting small focal prostate cancer on needle biopsies previously interpreted as negative for carcinoma on routine H&E stained sections.

Design: Five hundred twenty two prostate biopsies previously interpreted as benign prostate tissue by conventional morphology from 410 patients with prostate cancer diagnosed in other biopsy cores taken at the same session were stained with monoclonal antibody (P504S) on a DAKO automated immunostainer using an EnVision staining procedure. If a biopsy stained positively for P504S, two GU pathologists independently reviewed the original corresponding H&E stained sections (five levels of glass slides with more than ten levels of the tissue sections). The diagnosis of prostate cancer or atypia was established by an agreement of two GU pathologists from original H&E stained slides and further confirmed by the lack of basal cells as shown by a 34bE12 and p63 cocktail immunostaining. This study was approved by the Institutional Review Board.

Results: 59/522 (11.3%) biopsies demonstrated positive AMACR immunoreactivity. Seven of these AMACR positive biopsies (7/522, 1.3%) contained prostate carcinoma. All of these were small focal cancer (≤1mm) with Gleason grade 3 patterns. 3 of these 7 were foamy gland or atrophic type. Two other cases with positive AMACR staining that did not meet criteria for prostate cancer on the original H&E slides were considered as atypia. In addition, small focal cancer (2/522, 0.3%) or atypia (1/522,

0.2%) was found in the deeper immunostained sections but the lesions were not present in the original H&E slides. Thirty-two cases (6.1%) contained high-grade intraepithelial neoplasia (PIN). Fifteen benign prostates (2.9%) showed weak expression of AMACR. All other benign prostate biopsies (88.7%) were negative for AMACR.
Conclusions: AMACR/P504S immunohistochemical staining has shown the ability to improve detection of small focal prostatic carcinoma, especially unusual types of prostate cancer, and high grade PIN that could be missed by conventional histologic examination.

604 Immunohistochemical Expression of Alpha-Methylacyl-CoA Racemase (AMACR) in Ductal Adenocarcinoma of the Prostate

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Background: Prostatic ductal adenocarcinoma accounts for about 0.8% of all prostate cancers. Alpha-methylacyl-CoA racemase (AMACR) is a recently described immunomarker that is highly expressed in prostate cancer. Recent studies have shown AMACR expression in some subtypes of prostatic adenocarcinoma. However, there is limited information on AMACR expression within the ductal adenocarinoma subtype.
Design: Thirteen cases of prostatic ductal adenocarcinoma (including 9 cases with an associated acinar adenocarcinoma component) were retrieved from the surgical pathology files of 2 institutions. Clinical-pathologic characteristics for each case, including PSA, histologic/pathologic grade and clinical outcome, were recorded for each case. The index tissues were subjected to immunohistochemistry using an MAb to the AMACR gene product (P504S, 1:500 dilution, Dako, Carpinteria, CA, USA). For each case, the ductal and acinar adenocarcinoma components were evaluated separately and a composite score (ranging from 0 to 3) was assigned to the resultant AMACR immunostaining.
Results: The patients ranged in age from 51 to 72 years old (mean = 64 yrs). Ductal adenocarcinoma was the primary component in 8 of the cases (62%). Characteristic histological features including complex papillae, acini or cribriform structures were seen. Composite AMACR immunostaining scores in the 13 cases with respect to the ductal adenocarcinoma component ranged from 0.5 to 2.8 (mean = 1.9). The majority of the cases showed intense "granular" type cytoplasmic staining that has been previously described in acinar carcinomas. In addition to the ductal component, comparable intensity with similar composite scores were noted in the adjacent acinar carcinoma components (n=9; composite scores ranging from 1.1 to 3.5; mean = 2.3). When present, foci of prostatic intraepithelial neoplasia were also highlighted by the AMACR immuostain.
Conclusions: Our study illustrates the value of AMACR as a sensitive and specific "positive" immunohistochemical marker for the ductal adenocarcinoma morphologic variant of prostate cancer. To our knowledge, this is the first series of resection specimens with prostatic ductal adenocarcinoma evaluated for the immunohistochemical expression of AMACR. Our results indicate that AMACR immunostaining in the ductal subtype was similiar to that of the acinar component.

605 Papillary Squamous Cell Carcinoma of the Penis. A Report of 18 Cases

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Background: There is a group of low-grade papillomatous squamous cell carcinomas of the penis, collectively designated as "verruciform", that are difficult to subclassify. A proposal of classification grouped these tumors in condylomatous (warty), verrucous and papillary NOS. We are presenting clinicopathologic features of 18 papillary carcinomas.
Design: From 110 cases of "verruciform" carcinomas 18 cases, distinct from verrucous or warty carcinomas, were selected. Clinical and pathological information was: age, tumor site, size, grade, thickness, levels of invasion, associated lesion and inguinal node status. All specimens were penectomies. Papillary carcinomas were exophytic low-grade squamous neoplasms with hyperkeratosis and papillomatosis. Papillae were variable in length and shape. The tip was either straight, undulated, spiky or blunt. There were no koilocytosis. The interphase between tumor and stroma was jagged.
Results: Age was 61 years. Tumors were large (5.4 cm) and involved multiple (12 cases) or one site, the glans (6 cases). Tumors were well or moderately differentiated (16 cases). Focal poorly differentiated areas were present in 2 cases. Tumor thickness was of 12 mm; the most commonly involved anatomical levels were the corpus spongiosum and dartos (16 cases). Corpus cavernosum was affected in 2 cases. Vascular and perineural invasion were unusual. Associated lesions were squamous hyperplasia and low grade SIL (18 and 13 cases). High grade SIL was unusual (3 cases). Lichen sclerosus was identified in 9 cases. Nodal metastasis was identified in one of 4 patients in which an inguinal dissection was performed.
Conclusions: Papillary squamous cell carcinoma is the third distinctive type of penile low-grade verruciform neoplasm. Differential features from verrucous and warty carcinomas are based on the heterogeneity of the papillae, the lack of koilocytosis and the jagged irregular interphase between tumor and stroma.

606 Alteration of Prognostic Value of COX-2 in Prostate Cancer by Maspin

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Background: Studies have shown that over-expression of COX-2 in prostate cancer is associated with tumor progression. Maspin, a tumor suppressor gene, has been recently examined in human prostate cancer in a few studies. Its role in prostate cancer progression has not been well established. The objective of this study is to investigate COX-2 and Maspin expression and their interaction in prostate cancer.
Design: Tissue blocks from 78 radical prostatectomy specimens were collected from the department of pathology. Of those 78 cases, 52 had pT1/pT2 disease and 26 had pT3/pT4 disease. Gleason scores were ≤ 6 in 25 patients and ≥7 in 45 patients. One representative tissue block from each case was used for constructing tissue microarray (TMA) blocks. Each case was sampled in duplicate. Immunohistochemical studies for COX-2 and Maspin were performed according to standard protocol. Expressions of COX-2 and Maspin were determined by semiquantitatively assessing the percentage of stained tumor cells and the staining intensity.
Results: COX-2 expression was significantly associated with pT. Of 21 cases with pT3/pT4, 12 (57.1%) over-expressed COX-2. In contrast, only 13 of 47 (27.7%) cases with pT1/pT2 over-expressed COX-2 (p=0.020). Maspin was expressed in 19 of 69 (27.5 %) cases. No correlation between Maspin expression and pT was observed. However, when the cases were divided into subgroups according to Maspin expression status, the association between COX-2 expression and pT was only observed in the Maspin-positive subgroup, with 3 of 14 (21.4 %) cases with pT1/pT2 in contrast to 4 of 5 (80 %) cases with pT3/pT4 over-expressed COX-2 (p=0.02). In contrary, no correlation between COX-2 expression and pT was observed in the Maspin-negative subgroup (p=0.180). COX-2 expression was also significantly associated with surgical margin status (SM), with 11 of 25 (44 %) in cases with positive SM and 8 of 43 (18.6 %) in cases with negative SM over-expressed COX-2. Similarly, This relationship was only observed in the Maspin-positive subgroup (p=0.02).
Conclusions: COX-2 over-expression is associated with higher pT and positive SM. However, the prognostic value of COX-2 expression is only significant in the Maspin-positive prostate cancer. These results indicate that Maspin may define two distinct molecular pathways in which COX-2 may play different roles in prostate cancer progression.

607 Characteristics of Metastatic Prostate Cancer

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Background: Excluding superficial skin cancers, prostate cancer (Pca) is the most common malignancy afflicting American men. It is the second most common cause of cancer death, after lung cancer, in American men. Among the pts died of Pca, 85% has hematogenous metastases primarily affect the bones. The objective of this study is to characterize the features of pts with metastatic Pca.
Design: Surveillance Epidemiology and End Results (SEER) data for men diagnosed with metastatic Pca at our institution during 1990 and 2003 were used in this study. During this period, a total of 5453 pts were diagnosed with Pca. 1329 of those (24.4%) developed metastatic Pca. Africans Americans (AA) and whites consisted of 54% and 44% of the pts with metastatic Pca respectively. Median follow-up was 52 months (66 for alive pts). At last follow-up, 807 and 522 pts were alive and deceased respectively.
Results: Of the 1329 pts with metastatic Pca, 32% had multiple metastases. Bone involvement was present in 21%, 23%, 32% and 48% of pts with 1st, 2nd, 3rd and 4th metastasis respectively. In overall, 23.93% pts with metastatic Pca had bone involvement. Median age was 69 and 64 years for pts with bone and non-bone metastasis respectively (p<0.01). Median survival was 24 and 56 months for pts with bone and non-bone metastasis correspondingly (p<0.01). Multiple metastases were associated with poor survival only in pts with bone involvement (22.5 vs. 34 months, p<0.001). In contrast, in pts with non-bone metastasis, no significant survival difference was found between pts with single or those with multiple metastases (69.5 vs. 61 months, p>0.05). AA had significantly higher rates of bone metastasis (28.1% vs. 18.9%, p<0.01) and multiple metastases (31.1% vs. 16.5%, p<0.01). Compared to Whites, AA had significant shorter survival in pts with non-bone metastasis (56 vs. 70 months, p<0.01). In contrast, there was no statistically significant racial difference in survival in pts with bone metastasis (24 vs. 30 months, p=0.19).
Conclusions: In overall, 23.9% pts with metastatic Pca has bone involvement. Multiple metastases are associated with poor survival only in pts with bone involvement. AA has higher rates of bone metastasis and multiple metastases. There is more significant racial difference in survival in pts with non-bone metastasis.

608 Intraprostatic Spermatozoa (IS): Zonal Distribution and Association with Atrophy

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Background: The role of IS in the development of atrophy has not been investigated. Prostatic atrophy has been considered as a potential precursor of prostatic carcinoma (PC). We studied the presence of IS and its association to atrophy.
Design: Whole mount sagittal sections of 69 consecutive entirely submitted radical prostatectomy cases for PC were used. A central slice including the seminal vesicle ejaculatory duct urethra complex (SVEDU) from each case was stained with Berg's stain to identify spermatozoa (S) and their locations. Percentage of atrophy was obtained by using a grid method. The atrophy type [simple atrophy (SA) and postatrophic hyperplasia (PAH)] was recorded.
Results: Eighteen cases (26.1%) revealed S in the SVEDU and in the prostate (Group 1). Twenty-two cases (31.9%) showed S in the SVEDU but not in the prostate (Group 2). Twenty-nine cases (42.0%) had no S in either site (Group 3). In no cases were S seen in the prostate alone. In Group 1, IS were located 72% in the peripheral zone, 22% in the central zone and 6% in the transitional zone. The percentage of atrophy was greater in IS cases (Group 1) than in non-IS cases (Groups 2 and 3) (25.7% vs. 18.1%; p=0.039) and was greater in Group 1 than in Group 2 (25.7% vs. 15.3%; p=0.009). No significant difference was found between Groups 1 and 2 for age, prostate weight, tumor size, Gleason score, and pathologic stage. All cases in Groups 1 and 2 showed SA. However, more cases in Group 1 showed PAH than in Group 2 (72.2% vs. 40.9%; p=0.048).

Conclusions: 1) The percentage of IS in PC is 26.1%, more than previously reported (10%). 2) IS are predominantly (72%) located in the peripheral zone similar to atrophy and PC. 3) Prostates with IS have larger atrophic areas and increased frequency of PAH. 4) The role of IS in the development of atrophy and PC should be further investigated.

609 mRNA Expression Ratios among Four Genes Accurately Predict Subtypes of Renal Cell Carcinoma

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Background: Although classification of renal cell carcinoma (RCC) into clear cell, papillary, and chromophobe types is usually straightforward, diagnostic dilemmas do occur. In addition, the distinction between chromophobe carcinoma and oncocytoma can be problematic. Recent microarray studies identified genes that were differentially expressed in various renal neoplasms. These findings, together with technical advances that allow extraction of RNA from paraffin-embedded tissues, suggest that gene expression analysis could be useful in classifying difficult cases of renal cancer.

Design: Microarray data were evaluated and 5 genes that showed differential mRNA expression in various types of RCC were selected: carbonic anhydrase IX (CA9), racemase (AMACR), defensin-beta (DEFB1), parvalbumin-beta (PVALB), and chloride-channel KB (CLCNKB). Quantitative RT-PCR (qRT-PCR) for these genes were performed using RNA extracted from fresh-frozen samples of clear cell (9), papillary (5), and chromophobe (5) carcinoma, as well as oncocytoma (6). The data were analyzed and molecular criteria that would distinguish these tumor types were set. The criteria were tested against 18 unknown renal tumors, using RNA extracted from paraffin-embedded tissues.

Results: qRT-PCR data showed high expression of CA9 in clear cell, AMACR in papillary, and DEFB1 and CLCNKB genes in chromophobe/oncocytomas. PVALB expression was low in clear cell and papillary types, high in chromophobe, but variable in oncocytoma. The following diagnostic algorithm was established:

1. High CA9/CLCNKB ratio (>8) is diagnostic of clear cell carcinoma.
2. Low CA9/CLCNKB ratio (<0.004) is diagnostic of chromophobe carcinoma/ oncocytoma.
3. Intermediate CA9/CLCNKB ratio with high AMACR expression is diagnostic of papillary carcinoma.
4. Intermediate CA9/CLCNKB ratio with low AMACR expression is diagnostic of clear cell if CA9/CLCNKB>1, and chromophobe/oncocytoma if CA9/CLCNKB<1.
5. Among chromophobe/oncocytoma group, high PVALB [PVALB/GAPDH (control gene)>4] favors chromophobe carcinoma, whereas low PVALB favors oncocytoma.

Based on these criteria, the 18 unknown cases were corrected classified as 4 clear cell, 4 papillary, and 10 oncocytoma/chromophobe types. Of the 10 oncocytoma/ chromophobe, 5/6 chromophobe had high PVALB, whereas 4/4 oncocytoma (and 1 chromophobe) had low PVALB expression.

Conclusions: Comparison of RNA expression ratios based on the proposed four-gene panel can accurately predict the subtypes of renal carcinoma and may help distinguishing oncocytoma from chromophobe carcinoma.

610 Clonal Origin of Multiple Lymph Node Metastases in Patients with Solitary Urothelial Carcinoma of the Bladder

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Background: High-grade urothelial carcinoma of the bladder frequently metastasizes to multiple regional lymph nodes in the pelvis. Whether or not these multiple lymph node metastases originate from the same tumor clone is uncertain.

Design: We examined 19 patients who underwent radical cystectomy for urothelial carcinoma. All patients had multiple lymph node metastases. Loss of heterozygosity (LOH) assays for 3 microsatellite polymorphic markers on chromosome 9p21 (D9S171, region of putative tumor suppressor gene *p16*), 9q32 (D9S177, putative tumor suppressor gene involved in urothelial carcinoma tumorigenesis), and 17p13 (TP53, the *p53* locus) were performed. In addition, X-chromosome inactivation analysis was performed.

Results: The overall frequency of allelic loss was 63% (12/19) in the primary urothelial carcinomas and 79% (15/19) in the metastatic cancers. The primary urothelial carcinoma showed LOH at the D9S171, D9S177, and TP53 loci in 39% (7/18), 31% (5/16), and 26% (5/19) of informative cases, respectively. LOH in one or more lymph node metastases was seen at the D9S171, D9S177, and TP53 loci in 33% (6/18), 50% (8/16), and 47% (9/19) of informative cases, respectively. Seven cases showed identical allelic loss patterns at all DNA loci in both the primary tumor and all of their corresponding lymph node metastases. Three cases showed allelic loss in the metastatic tumor but not in its matched primary tumor. Seven cases showed a different LOH pattern in each of its lymph node metastases. Clonality analysis showed the same pattern of non-random X-chromosome inactivation in both the primary urothelial carcinoma and all of the lymph node metastases in 4 of 5 cases studied.

Conclusions: LOH and X-chromosome inactivation assays show that multiple lymph node metastases and matched primary urothelial carcinomas of the bladder have the same clonal origin, suggesting that acquisitions of the capability for metastasis may be an infrequent phenomenon which often arises in only a single clonal population in the primary tumor which then spreads to regional lymph nodes.

611 Thyroid Transcription Factor-1 Expression in Small Cell Carcinoma of the Urinary Bladder: An Immunohistochemical Profile of 44 Cases

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Background: Small cell carcinoma of the urinary bladder is a rare and aggressive tumor resembling small cell carcinoma of the lung. Thyroid transcription factor-1 (TTF-1) expression is common in small cell carcinomas arising in the lung. However, studies of its expression in extrapulmonary small cell carcinomas have yielded variable results. Because information concerning the immunohistochemical profile of small cell carcinoma of the urinary bladder is limited, we investigated the immunoreactivity of this tumor to a battery of antibodies in a series of 44 cases.

Design: Using 5-micrometer sections cut from paraffin embedded tissue blocks, immunohistochemistry was performed to detect TTF-1, cytokeratin 7 (CK7), cytokeratin 20 (CK20), and uroplakin antigenicity in 44 cases of small cell carcinoma of the urinary bladder. None had primary lung tumors. The control slides stained appropriately.

Results: The TTF-1 immunohistochemical stain showed nuclear positivity in 17 cases (39%). Positive immunostaining for cytokeratin 7 was observed in 22 cases (50%). There was no positive staining with either cytokeratin 20 or uroplakin (0 of 44 cases, 0%).

Conclusions: TTF-1 expression in small cell carcinoma of the urinary bladder was found in 39% of the cases studied, demonstrating that this marker is expressed in small cell carcinomas other than those of pulmonary origin. Small cell carcinoma of the urinary bladder is positive for cytokeratin 7 immunostaining in one-half of cases (50%) consistent with a proposed origin from urothelium. Unlike urothelial carcinoma, immunohistochemical expression of cytokeratin 20 and uroplakin in small cell carcinoma of the urinary bladder is consistently negative, and thus these stains do not appear to be useful in the diagnosis of this neoplasm.

612 Differential Expression and Quantitation of 5-Alpha Reductase (5αR) in Prostate Cancer

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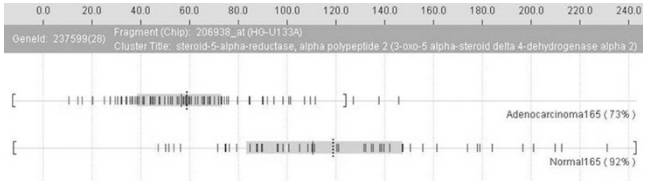
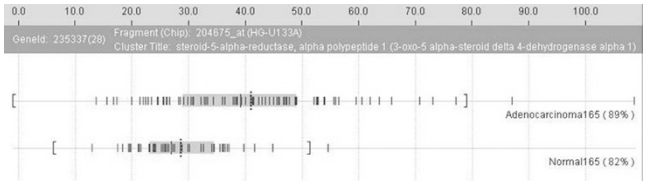
Background: Finasteride, a 5αR2 inhibitor shrinks the size of the prostate in benign prostatic hyperplasia, decreases PSA levels and reduces the incidence of prostate cancer with long term treatment. The therapeutic response to finasteride is unpredictable and might be due to variable endogenous expression levels of 5αR in different individuals.

Design: Microarray gene expression of 5αR in extracts of tissue from normal and prostate cancer(PC) areas of 89 radical prostatectomies was examined using Affymetrix (Santa Clara, CA) and GeneExpress Software Tools (Gene Logic Inc., Gaithersburg, MD). Quantitative PCR (Q-PCR) was used to quantitate and validate the microarray data using real time Taqman RT-PCR assay on the ABI PRISM 7900 Sequence Detection System (ABI, Foster City, CA).

Results: Consistently decreased expression of 5αR2 was observed in all PC samples when compared to matched normal tissue.



A small increase in 5αR1 gene expression was noted in PC.



The normal tissue samples showed a nearly 5-fold difference in expression between the highest and lowest expression levels. In contrast to PC, there is no tight clustering seen in normals. Examination of these samples by Q-PCR confirmed the approximately 5-fold range in expression in normal prostate.

Conclusions: Results from this study demonstrated the differential expression of 5αR in prostate cancer by microarray techniques. This variation in 5αR2 expression in normal tissue may have implications for future studies that target this enzyme in prevention or treatment of PC.

613 Glandular Neoplasms of the Urachus: Clinicopathologic and Immunohistochemical Analysis of 43 Cases with Special Emphasis on Low-Grade Mucinous Cystic Tumors

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Background: Most published literature on glandular neoplasms of the urachus deals with invasive adenocarcinomas. We report our experience with a wider histologic spectrum including mucinous cystic neoplasms which have histologic similarities with the better known ovarian, appendiceal and pancreatic counterparts.

Design: 43 glandular urachal tumors from the authors' consultation and institutional files were classified into mucinous cystic neoplasms [cystadenoma (1), tumor of low malignant potential (15), and invasive cystadenocarcinoma - intraepithelial (1), with microinvasion (5) or frankly invasive (2)] and invasive adenocarcinoma [enteric (6), mucinous (5), not otherwise specified (2) and mixed including with signet ring cell (6)]. 17 cases were studied by immunohistochemistry (IHC) [ß-catenin, CDX2, a-methylacyl-CoA racemase (AMACR) - colon cancer-related gene products and enzymes; CK7, CK20, estrogen and progesterone receptors (ER, PR)].

Results: Mucinous cystic tumors were more common in women (1.9:1, F:M), with mean age 47.6 yrs. Follow-up (mean 49.6, median 53 mos, n=7) showed no evidence of disease progression in 100% of cases including 3 cases with microinvasion (54, 65 and 69 mos follow-up). Invasive non-cystic tumors occurred equally in men and women, mean age 50.7 yrs at presentation with progression (recurrence, metastasis and/or death) in 55% (mean 45.5, median 42.9 mos follow-up, n=10). Mucinous cystic tumors were [CK7 (±) (0:67%, +:33%), CK20 (+) (0:0%, +:100%), CDX2 (±) (0:33%, +:67%); AMACR, ER, PR and ß-catenin: all negative]. Control group of metastatic colon cancer cases were CDX2 (100%), AMACR (42.8%) and ß-catenin (nuclear) (76.2%) positive.

Conclusions: Our experience indicates that invasive urachal adenocarcinomas are often associated with poor prognosis. There is a distinct group, however, of cystic tumors classifiable in a manner similar to ovarian or pancreatic mucinous neoplasms with a favorable prognosis after complete excision. IHC evaluation suggests partial protein profile homology with colorectal carcinoma with expression of enteric carcinogenesis gene products and enzymes. A panel of CK7, CK20, CDX2, AMACR and ß-catenin has potential practical utility in distinction of a primary urachal tumor from metastatic colonic carcinoma.

614 Increased Expression of Insulin-Like Growth Factor Binding Protein-3 in Urothelial Carcinoma of the Renal Pelvis

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Background: Insulin-like growth factor binding protein-3 (IGFBP-3) plays an important role in regulating cell growth. The expression and role of IGFBP-3 in renal neoplasia has not been studied. Recently, we have found a significant increase of IGFBP-3 gene expression in the majority of conventional renal cell carcinomas. In addition, two of three urothelial carcinomas of the renal pelvis also showed IGFBP-3 mRNA increase by cDNA microarrays. The purpose of this study was to investigate the IGFBP-3 expression in urothelial carcinoma of the renal pelvis by immunohistochemistry.

Design: Sixteen cases of urothelial carcinoma of the renal pelvis were identified for evaluation. Diagnoses were confirmed and tumor grades recorded (11 low grade, 6 high grade). Immunohistochemistry was performed on formalin-fixed paraffin embedded tissue samples using a monoclonal antibody specific for IGFBP-3. Immunoreactivity was first analyzed with conventional scoring. Additionally, a subset of cases was subjected to quantitative imaging analysis using ChromaVision Automated Cellular Imaging System II (ACISII) to determine the intensity and percentage of IGFBP-3 staining.

Results: Benign urothelium and renal parenchyma showed minimal or negative IGFBP-3 staining. Ninety four percent (15/16) of urothelial carcinomas were reactive for IGFBP-3. In the majority of cases, IGFBP-3 reactivity was diffuse and found in more than 50% of tumor cells, however, a subset of tumors showed superficial or apical staining. High grade tumor cells tended to be more intensely positive. Using ACISII analysis, the average intensity and percent of IGFBP-3 staining were 147, 59% in low grade tumors.

Conclusions: This study confirms our previous observation of elevated IGFBP-3 mRNA expression in urothelial carcinoma of the renal pelvis. Furthermore, the level of IGFBP-3 immunoreactivity is correlated with tumor grades. Future studies including a larger number of cases, particularly urothelial carcinoma of the bladder, are necessary. Since tests for IGFBP-3 in serum and body fluids are readily available, the presence of IGFBP-3 in urothelial carcinoma is promising for developing a urine test for urothelial cancer.

615 High Level of Pim-1 Expression in Prostatic Intraepithelial Neoplasia and Adenocarcinoma

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Background: Pim-1 is a serine/threonine kinase that has been shown to play an integral role in the development of a number of human cancers, such as hematolymphoid malignancies. In order to further our understanding of its role in prostate cancer, we investigated the immunohistochemical Pim-1 expression in normal, premalignant, and malignant prostate tissue.

Design: Using immunohistochemistry, Pim-1 expression was analyzed in prostate tissue from 120 radical prostatectomy specimens. In each case, Pim-1 staining was evaluated in benign prostatic epithelium, high-grade prostatic intraepithelial neoplasia (PIN), and prostatic adenocarcinoma.

Results: Pim-1 immunoreactivity was identified in 120 cases (100%) of adenocarcinoma, 120 cases (100%) of high-grade PIN, and 62 cases (52%) of benign glands. The number of cells staining in benign epithelium (mean, 34%) was much lower than that in high-grade PIN (mean, 80%; P<0.0001) or adenocarcinoma (mean, 84%; P< 0.0001) (Table 2). There was no significant difference in the percentage of cells staining positively for Pim-1 between high-grade prostatic intraepithelial neoplasia (PIN) and adenocarcinoma (P = 0.34). The staining intensity for Pim-1 was significantly lower in benign prostatic tissue compared with PIN and adenocarcinoma (P<0.001). </INS>There was no statistically significant correlation between the level of Pim-1 expression and Gleason score, patient age, tumor stage, lymph node metastasis, perineural invasion, vascular invasion, surgical margin status, extraprostatic extension, or seminal vesicle invasion.

Conclusions: Pim-1 expression is elevated in neoplastic prostatic tissue compared to benign prostatic epithelium. This finding suggests that the dysregulation of Pim-1 may play a role in prostatic carcinogenesis.

616 Expression of Epidermal Growth Factor Receptor in Renal Neoplasms

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Background: Epidermal growth factor receptor (EGFR) has been found to play important role in tumor cell proliferation, apoptosis, angiogenesis, metastasis, and response to treatment in many cancers. Overexpression of EGFR has been described in renal cell carcinoma (RCC) with increased expression linked to poor prognosis. Little is known about its expression in other types of renal neoplasms. Many anti-EGFR compounds are currently available; therefore identification of tumors that overexpress EGFR can facilitate the selection of candidates for anti-EGFR therapy. This study describes the expression of EGFR in a variety of renal neoplasms.

Design: A tissue microarrays (TMA) was constructed from 60 normal kidneys, 23 clear cell-renal cell carcinomas (CCRCC), 20 papillary renal cell carcinomas (PRCC), 17 chromophobe renal cell carcinomas (ChRCC), 19 oncocytomas (ONC), 14 transitional cell carcinomas (TCC), and 19 angiomyolipomas (AML). The TMA was immunostained for EGFR protein. EGFR expression was scored according to the staining intensity and the percentage of positive cells. Strong circumferential membranous staining was scored 3, weak circumferential membranous staining 2, weak partial membranous staining 1, and absence of staining 0. The composite staining score was computed by multiplying the staining intensity by the percentage of positive cells, with a range of 0-300. EGFR overexpression was defined as composite score of 20 or greater.

Results: Normal kidney was negative for EGFR. EGFR overexpression was found in 60.9% (14/23) of CCRCC, 80% (16/20) of PRCC, 70.6% (12/17) of ChRCC, 21.5% (4/19) of ONC, 50.0% (7/14) of TCC, and 36.8% (7/19) of AML. The highest expression level was seen in PRCC and ChRCC (composite staining score=86 and 93, respectively) compared to CCRCC, ONC, TCC and AML (composite staining score=43, 13, 55 and 23, respectively, p<0.05). For CCRCC, PRCC and ChRCC, the EGFR composite staining score did not correlate with tumor size, Furhman nuclear grade and stage.

Conclusions: EGFR overexpression is observed in a variety of renal neoplasms. PRCC is the most common renal neoplasm to overexpress EGFR. PRCC and ChRCC have highest EGFR expression.

617 Activation of the ATM-p53 Cell Cycle Checkpoint by Prostate Cancer Radiotherapy: An Immunohistochemical Study

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Background: Radical prostatectomy (RP) and radiation therapy (RT) serve as the main treatment options for men with localized prostate cancer (PCa). Little is known about the effects of RT on PCa cell cycle markers. Phosphorylated ATM (P-ATM) is induced by DNA damage from RT. P-ATM activates p53 by phosphorylating serine 15 (ser15 p53). Activated p53 causes cell cycle arrest at the G1/S checkpoint via p21. A better understanding of RT biology is essential for future development of pathological predictors and indicators of treatment success or failure.

Design: Paired PCa tissue samples were collected from 12 patients (N=12) at Princess Margaret Hospital or Sunnybrook and Women's College Health Sciences Centre in Toronto prior to pre-operative RT (25Gys, 5 fractions) and one-week post-RT at the time of RP. REB approval was obtained for all samples. Wild type p53 was confirmed by direct DNA sequencing (N=12). All markers were assessed using IHC on formalin fixed and paraffin embedded tissue. P-ATM expression was examined on randomly selected cases (n=4) and p21, p53, ser15 p53, and MIB1 were examined on all cases. In situ end-labeling (ISEL) was used to assess apoptosis (n=10). MIB1 and ISEL were analyzed quantitatively using ImagePro Plus (3 fields, 400X). The remaining markers were assessed qualitatively. Single variable ANOVA was used for data analysis.

Results: The mean expression (ME) of P-ATM increased post-RT (p=0.293). P21 was negative pre-RT and positive in 7 post-RT cases. The ME of p21 increased from 0 to 50% (p=0.001). P53 expression increased in 7 cases post-RT. The ME of p53 increased from 41.1 to 51.8% (p=0.623). Ser15 p53 expression decreased in 6 cases post-RT. The ME of ser15 p53 decreased from 50.8 to 10% (p=0.025). MIB1 expression decreased in 11 cases post-RT. The ME of MIB1 decreased from 11.7 to 3.8% (p=0.001). ISEL decreased in 8 cases post-RT from a ME of 5.7 to 3.3% (p=0.334).

Conclusions: This is the first study to assess biomarkers of ATM and p53 radio-response in PCa immediately post RT. Our observations of increased p21, decreased ser15 p53, and a decrease in MIB1 expression are consistent with decreased PCa proliferation via p21-mediated cell cycle arrest at the G1/S checkpoint. Our results do not support apoptosis as a dominant mode of cell kill.

618 Any Proportion of Histological Grade 3 Is Related to Metastasis in SCC of Penis. An Electronically Quantified Study of Proportions of Grades

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Background: High histological grade predicts metastasis in penile SCCs. Many tumors are heterogeneous with more than one grade. Focal presence of high grade (grade 3) in a predominantly low grade tumor is problematic to report and studies claim that 50% of anaplastic cells are necessary to classify a tumor as grade 3. This study electronically quantified proportions of grade 3 to determine the effect of the presence and proportions of this higher grade areas on lymph node status.

Design: 115 penectomies with lymph node dissections were evaluated. 55 tumors were homogeneous (one grade) and 60 were heterogeneous (more than one grade). 53 of these heterogeneous lesions showed various proportions of grade 3. The other 7 cases were tumors with a mixture of grade 1 and 2 areas. **Grade 1:** totally differentiated, similar to normal squamous cells. **Grade 3:** anaplastic cells with high nucleus/cytoplasm ratio, irregular nuclei with prominent nucleoli, atypical mitosis and scant to none keratin formation. **Grade 2**, the remainder cases. On each HE stained slide (3 per case) with more than a grade, using a Stabilo F universal marker, a circle of variable color given to each grade was drawn around the surface (blue, green, and red for grades 1, 2 and 3 respectively). The slides were scanned using a SmartScan 2700 (SmartDisk Corporation, Florida, USA); the encircled areas were measured on a Sony Vaio PCG-GRT250 with the software ImageJ 1.32j (NIH) and converted in % and mm^2. The proportions of grade were related to metastasis.

Results:

Grades and metastasis in 115 tumors

Grades	Total	Positive nodes	Negative nodes	% metastases	p-value
1-2	31	4	27	13%	p<0.0001
3	84	49	35	58%	

Proportions of grade 3 and metastasis in 53 heterogeneous tumors

Grade 3	Total	Positive nodes	Negative nodes	% of metastases	p-value
1-5%	4	2	2	50%	p=0.4
6-10%	5	2	3	40%	
11-20%	6	2	4	33%	
21-99%	38	25	13	66%	

Conclusions: Metastasis was significantly more frequent in patients with high grade tumors. Focal and extensive proportions of grade 3 were equally related to a high incidence of metastasis. The finding of any foci of grade 3 should be sufficient to classify the tumor as high grade with a major risk for regional metastasis.

619 Intuitive vs Electronic Quantification of Proportions of Grades in Squamous Cell Carcinomas of the Penis

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Background: More than one histological grade is found in half of penile SCCs complicating the subjectivity of grading systems. To evaluate a grading model we compared the "naked-eye" intuitive estimations of proportions of grades with an electronic measurement method in 60 heterogeneous SCCs.

Design: Intuitive estimation consisted in a pathologist giving percentages to grade components of each case after microscopic examination of HE stained slides with an Olympus BX40 at 10 and 40x magnifications. The electronic measurement was performed after encircling in the slide the surface corresponding to each grade with a marker of various colors (blue, green and red for grs 1,2 and 3).Then the slides were scanned using a SmartScan 2700 (SmartDisk Corporation, Fla). The areas were measured in square mm on a Sony Vaio PCG-GRT250 using the software ImageJ 1.32j (NIH) and converted to %s. Criteria for grades were: Grade 1. extreme differentiation with squamous cells undistinguishable from normal cells. Grade 3: anaplastic cells with scant cytoplasm and keratin, pleomorphic nuclei, mitosis and prominent nucleoli and grade 2: the remainder cases. Statistical analysis were student s t-test.

Results:

Grade	Number of cases*	Cases with perfect concordance	Average of variance	Range of variance	p-value
1	20	2 (10%)	5.1%	1 to 17%	0.2
2	58	7 (12%)	5.2 %	1 to 18%	0.8
3	53	9 (17%)	4.1%	1 to 18%	0.3

* all cases had more than one grade

Conclusions: There was no significant difference in the estimation of proportions of grades by naked-eye or by electronic media quantification. The simplicity and reproducibility of the proposed grading system emphasizing both ends of the spectrum, total differentiation for grade 1 and total anaplasia for grade 3 is supported by the striking correspondence found in the intuitive vs electronic estimations of the proportions of grades.

620 Increased Pim-2 Is an Adverse Indicator of Aggressive Disease and Is Increased in Perineural Invasion

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Background: The serine/threonine kinase Pim-2 acts as a transcriptionally regulated apoptotic inhibitor and is implicated in promoting cell-autonomous survival. However, its clinical and pathological significance remain largely unknown.

Design: We used 640 PCa cases treated with radical prostatectomy to build tissue microarrays. Normal prostate tissue, BPH and index tumor were cored in triplicate (0.6 mm). Another two arrays (2mm) were built which had 177 of metastatic PCa and 226 of perineural invasion (PNI), respectively. Slides were immunostained with an antibody to Pim-2 and then digitized. Mann-Whitney test was used for the difference between paired parameters. Spearman test was conducted for the correlations between Pim-2 expression and clinicopathological variables. Kaplan-Meier analysis and Cox proportional hazard regression were used for possible prognostic value of Pim-2.

Results: Pim-2 was expressed in both the cytoplasm and/or nucleus or both of benign and cancerous epithelium. Nuclear Pim-2 was more strongly expressed in normal tissue as compared with PCa and BPH. However, increased nuclear Pim-2 in PCa was associated with more adverse clinical and pathological features including clinical stages (rho=0.159 , p=0.007), positive lymph node status (rho= 0.114, p=0.0141), extracapsular extension (rho= 0.117 , p=0.0121), and seminal vesicle invasion (rho=0.097 , p=0.0377). Of note, both nuclear and cytoplasmic Pim-2 was increased in PNI cancer compared to primary PCa (p=0.000). High level of nuclear Pim-2 in the PCa was associated with higher risk of biochemical recurrence (HR: 1.021-2.419, p=0.0399) in PCa.

Conclusions: Our data suggested that Pim-2 is an adverse indicator of aggressive disease in PCa. These findings provide support that increased Pim-2 might result in activation of enhanced anti-apoptotic pathway, leading to a more aggressive PCa and development of perineural invasion.

621 Malignant Mesotheliomas of Tunica Vaginalis

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Background: It has been reported that asbestos exposure is as frequent in intrascrotal mesotheliomas as it is in the pleural ones. It has been our impression that these sites may show multifocal disease more often than the literature reflects.

Design: Malignant mesotheliomas of tunica vaginalis/albuginea were retrieved from the files. 31 were judged to be unequivocally malignant and follow up was obtained.

Results: Over a period of 5 months to 4 years 10 of the 31 developed proven or suspected mesothelioma of the peritoneum. Of these 10, four patients developed proven or suspected pleural disease. One patient had lung lesions interpreted as metastatic. At last follow-up (4 years) 7 of the 31 were dead or terminal from disease.

Conclusions: Metastatic disease to mesothelial surfaces is obviously a possibility but we believe most of these likely represent multifocal mesotheliomas initially manifested within the scrotum.

622 Interobserver Reproducibility of a Proposed Classification of Focal Prostate Atrophy Lesions

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Background: Focal prostate atrophy is common in prostate needle biopsies, radical prostatectomies and trans-urethral resections, yet a classification system for the types of atrophy has not been tested.

Design: Pathologists with expertise in genitourinary pathology were invited to view a set of "training" images (phase I) and descriptions over the Internet. Atrophy was categorized into 4 subtypes as: simple atrophy, simple atrophy with cyst formation, post atrophic hyperplasia (PAH) and partial atrophy. Each participant then classified over the Internet 140 separate "test" images (phase II) consisting of the 4 subtypes of atrophy as well as images of normal epithelium, high grade PIN, and carcinoma. The diagnoses for each image from each pathologist were compared to a "target diagnosis" for each image.

Results: 28 pathologists completed the study. The inter-rater reliability (median Kappa) for type of lesion (normal, cancer, PIN, focal atrophy) was 0.97. The median sensitivity and specificity of the diagnosis of atrophy were 98.8% and 100% respectively. The overall median Kappa for subtype of atrophy was 0.81. The median sensitivity and specificity for the atrophy subtypes were: simple 60.6% and 96.4%; simple with cyst formation 100%, 93.1%; PAH 92.4%, 99.2%; partial atrophy 93.8%, 100%. The lower sensitivity for simple atrophy reflected a propensity to diagnose some of these as simple atrophy with cyst formation.

Conclusions: Interobserver reliability for the diagnosis of focal prostate atrophy lesions and the proposed subtypes was excellent. This scheme should facilitate studies of the role of various patterns of prostate atrophy in future investigations of the etiology of prostate cancer.

623 Utility of a Panel of Immunohistochemical Markers in Differentiating Prostate Carcinoma from Benign Seminal Vesicle Epithelium: A Study of 31 Radical Prostatectomy Cases with Seminal Vesicle Invasion

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Background: Differentiation of prostatic carcinoma (PCa) from native benign seminal vesicle (SV) epithelium can sometimes be difficult on histology. Cases with small tumor foci in the SV, presence of cautery artifact and fragmentation of SV (recently increasingly encountered in laparoscopic and robotic assisted prostatectomies) add to this difficulty. We assessed the utility of a panel of immunohistochemical markers in differentiating PCa from SV epithelium.

Design: Paraffin blocks containing both SV and PCa were selected from 31 cases of radical prostatectomy, pathologically staged at pT3b. Immunohistochemical evaluation was performed using a panel of antibodies to cytokeratin 7 (CK 7), cytokeratin 20 (CK20), high molecular weight cytokeratin (HMW-CK; clone 34E12), p63, p504S, prostate specific antigen and prostatic acid phosphatase.

Results: The SV epithelium showed diffuse intense membranous staining with CK 7 in both luminal and basal cells. There was diffuse staining of the basal cells with p63 and HWM-CK in all cases. PSA and PAP were negative in SV epithelium, except in 3 cases which showed faint focal cytoplasmic staining adjacent to intensely staining PCa cells. In contrast, foci of PCa were CK7 negative in 22 cases (71%) and 9 cases(29%) showed rare isolated positive cells. PCa foci were consistently negative for p63 and positive for PSA/PAP. HMW-CK showed focal patchy staining in 15 (50%) of PCa and diffuse weak to moderate staining in 2 cases (6%). p504s stained all cases of PCa with moderate to strong intensity, however, rare isolated positive cells were seen in SV in 3 cases (10%). CK 20 was consistently negative in both SV and PCa.

Conclusions: Overall CK 7 and p63 immunostains were most sensitive for SV epithelium, whereas PSA, PAP and p504s stained PCa cells consistently. Focal positive HMW-CK staining seen in some cases of PCa involving SV restricts its utility. The coarse variably sized refractile golden brown granules of lipofuchsin pigment within the SV epithelium should not be mistaken as positive immunoreactivity with any antibody. A combination of these commercially available markers would be valuable for evaluating histologically challenging cases of SV with questionable PCa involvement.

624 Expression of Oxidative Complex Enzymes Assist in the Differential Diagnosis of Renal Tumors

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Background: Mitochondria are the main source of energy for eukaryotic cells through the production of ATP. Referred to as oxidative phosphorylation (OXPHOS) or the respiratory transport chain, generation of ATP is carried out by a series of enzymatic complexes embedded in the inner mitochondrial membrane. Previous studies have evaluated levels of OXPHOS complexes in limited numbers of kidney neoplasms, with decreased complex production being associated with tumor aggressiveness and progression. Additionally, decreased production of succinate-ubiquinone oxidoreductase has been shown to correlate with a similar reduction of cytochrome c oxidase, which could be associated with neoplasms of the kidney.

Design: Twenty-eight cases of renal cell tumors were evaluated. These included nine cases of clear cell RCC, 5 oncocytomas, 5 chromophobe RCC, 6 cases of BHD hybrid tumors, 2 cases of papillary type I, and one case of papillary type 2 RCC. Immunohistochemistry (IHC) for these enzymes was performed utilizing antibodies to; succinate-ubiquinone oxidoreductase (70-kDa subunit of Complex II) a nuclear encoded enzyme that functions in the tricarboxcylic acid cycle (TCA) and OXPHOS and to Cytochrome c oxidase (subunit II of COX IV), the last complex of the respiratory transport chain, which has mitochondrial and nuclear origins.

Results: High expression levels of both complexes were seen in the cytoplasm of all oncocytomas. BHD/Hybrid cases had mild/nonhomogeneous levels of expression of complexes II and IV in the cytoplasm representing the two cell types (oncocytic and clear) seen in these tumors. Chromophobe RCC and the papillary type 2 tumor showed low staining only in those cells in which eosinophilic cytoplasm was noted. Clear cell RCC and papillary type I tumors showed negative staining with both complexes. As expected, high levels of expression of both complexes were seen in the distal tubules of normal kidney.

Conclusions: We demonstrate for the first time, the utility of immunohistochemical staining with two complexes of OXPHOS, which may have use in distinguishing between malignant and benign renal tumors. Complex II and IV were shown to be consistently highly expressed in oncocytomas and normal kidney. Tumors such as BHD and chromophobe RCC showed low expression only in the eosinophilic cells, meanwhile all cases of clear cell RCC and papillary tumors were negative. Our study suggests that IHC determination of these enzymes may play an important role in diagnosis.

625 Benign Prostatic Hyperplasia: Towards the Development of a Clinically Relevant Molecular Signature

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Background: Benign prostatic hyperplasia (BPH) is characterized by a hyperplastic growth of epithelial and stromal cells in the prostate. Despite the high prevalence of the disease, little is known regarding the molecular etiology of BPH. The goal of the current study was to develop a clinically relevant molecular signature of BPH that would identify men who may not benefit from the 5-alpha reductase inhibitor therapy, finasteride.

Design: DNA was extracted from 7 BPH specimens and hybridized to Affymetrix HG-100K single nucleotide polymorphism (SNP) arrays (Affymetrix, Inc). A novel informatics platform, dChip SNP, was used to detect DNA copy number alterations and to distinguish loss of heterozygosity (LOH) events. Quantitative polymerase chain reaction (Q-PCR) was used for validation. Total RNA was extracted from 71 BPH specimens from men with extensive hyperplasia and a set of 12 histologically normal prostate tissues and expression array analysis was performed.

Results: A genome-wide interrogation failed to identify significant LOH or homogygous deletions using the SNP arrays. However, we observed a few regions of amplifications containing genes found to be over expressed at the transcript level by cDNA analysis. EDG3 was overexpressed at the transcript level(fold change = 3.2) and had a significant copy number increase by SNP array analysis (2 fold change, t-test $p<0.001$) and Q PCR (1.5 fold change, $p = 0.001$). We found 197 genes significantly differentially expressed in BPH compared to control samples, with a fold change of 3.0 at a 10% false discovery rate. Finally, 11 genes were found related to finasteride response prior to surgery including Androgen Receptor, IGFBP4, IGFPB5, and Lumican (expected number of false significant is 0.8, minimal fold change is 1.35).

Conclusions: This is the first BPH study to assess genome wide DNA and transcript alterations in combination. No large scale genomic alterations were detected. However, areas of amplification were identified which also corresponded to over expression at the transcript level. A molecular signature associated with finasteride treatment was identified. Validation of this signature would be clinically useful to determine which men are most amenable to therapy.

626 Changes in Tumor and HGPIN Characteristics in Radical Prostatectomy Specimens from 1990 to 2004: Results from the NCI Cooperative Prostate Cancer Tissue Resource (CPCTR)

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Background: Starting about 1990, PSA testing and radical prostatectomy became common practice for the diagnosis and treatment of prostate cancer. We asked whether the pathological characteristics of cancers and HGPIN in the prostate resection specimens changed over time.

Design: We have examined 4,702 radical prostatectomy cases performed between 1990 and 2004 using two-tailed Chi squared and ANOVA tests. These cases were collected through the NCI-funded Cooperative Prostate Cancer Tissue Resource (http://www.prostatetissues.org).

Results: Between 1990-1994 and 1995-1999 the mean size of dominant tumor nodules dropped from 1.60 to 1.40 cm, ($p<0.0001$) while there was increased identification of mutifocal tumor (72% to 81% of cases, $p<0.0001$). There was no significant difference for size of dominant tumor or multifocality between 1995-1999 and 2000-2004. While unchanged before 2000, tumor volume decreased in the period 2000-2004 as compared to the 1990-1999 ($p<0.0001$); cases with less than 5% of the prostate involved by tumor increased from 26 to 33% while cases with tumor involving >25% of the prostate dropped from 23 to 13%. From 1990-1994 to 2000-2004 the rate of cases with HGPIN increased (81-91%, $p<0.0001$). In a subset of 3,858 cases the amount of HGPIN was further evaluated. Multifocal HGPIN (as opposed to 1 or 2 foci) increased from 55% to 70% between 1990 and 2004 ($p<0.0001$). Between 1990-1994 and 2000-2004 the mean patient age dropped from 64 to 60 years ($p<0.0001$). When grouped by age, the described changes in pathologic findings were consistently present for all age groups except for men less than 50 years.

Conclusions: The histological examination presents a changing picture of the tumors identified in radical prostatectomies, with the early removal of large tumors, and the subsequent removal of multifocal tumor occurring in large volumes. More early cancers and precancerous changes (HGPIN) were identified in recently performed radical prostatectomies. This may be due in part to an increase in younger men who have undergone surgery in recent years.

627 MUC1 Expression in Acinar Prostatic Adenocarcinomas

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Background: MUC1 is a high molecular weight trans-membrane glycoprotein, usually expressed on the luminal surface of glandular epithelia. MUC1 is abundantly over-expressed in a hypo-glycosylated form in some human adenocarcinomas (lung, colon, pancreas and others). There has been recent interest in MUC1 expression in prostatic adenocarcinomas (PRCA), with some recent publications showing a correlation between poor outcomes and decreased expression of glycosylated Muc1. This study was designed to further explore this issue.

Design: Paraffin Tissue Micro-Arrays (TMA) were used to evaluate Muc1 expression in PRCA. Two different antibodies were used; one directed against hypo-glycosylated Muc1 (clone 4H5: commercial source), and the other against glycosylated Muc1 (clone 3C6: gift from Olivera Finn, Univ. of Pittsburgh). A four-slide tissue microarray set was used to stain 800 tissue samples from 146 patients. The TMA set had 352 samples of PRCA, 64 metastatic prostate cancer (28 lymph node (LN) metastases and 36 non-LN metastases), 92 foci of high grade prostatic intra-epithelial neoplasia (PIN), 88 benign prostatic hypertrophy (BPH), 96 normal adjacent to tumor (NAT) and 64 samples of "true normal" donor prostate. Cytoplasmic staining of glandular epithelium was considered positive. A single observer graded staining intensity on a scale of 0-3 (0: no staining, 1, 2 and 3: cytoplasmic staining seen at 20 X, 10 X, and 4 X respectively).

Results: Assessment of staining for hypo-glycosylated Muc1 (4H5) demonstrated minimal staining in the foci of high grade PIN and PRCA. Occasional foci of NAT did show Muc1 staining with this clone. Staining for 3C6 (glycosylated Muc1) showed an increase in expression in foci of PRCA (mean intensity of 1.5), as compared to NAT ($p = 0.001$), PIN ($p= 0.003$), BPH ($p < 0.001$) and Donor ($p = 0.002$). Metastatic PRCA demonstrated increased expression, similar to that seen with PRCA.
Conclusions: 1. There seems to be differential expression of glycosylated and hypo-glycosylated Muc1 in prostate carcinogenesis. 2. The current literature supports a down regulation of glycosylated Muc1. Our results support that finding. In addition, a concomitant up regulation of non-glycosylated Muc1 is also seen. 3. The data is preliminary and needs to be analyzed further for relationship with outcomes. In addition, we plan to assess regional changes in distribution (field effect) and attempt dual staining, to evaluate discordance in expression of glycosylated and non-glycosylated Muc1.

628 Increased Expression of Ephrin A2 (Eph A2) Tyrosine Kinase in Metastatic Prostate Cancer

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Background: Eph A2 is a transmembrane receptor tyrosine kinase that has been shown to be overexpressed in epithelial malignancies from several sites including colon, esophagus, breast, ovarian and the prostate. It is considered a tumor-associated antigen. Increased levels of Eph A2 have been shown to be associated with higher stage/ metastatic disease. The role of EphA2 in prostate carcinogenesis has not been well characterized. The aim of this study was to characterize the immunohistochemical (IHC) expression of Eph A2 in different histologic types of prostatic tissue.
Design: Paraffin Tissue Micro-Arrays (TMA) were used to evaluate Eph A2 expression in prostate adenocarcinoma (PRCA) and other prostate tissues. A four-slide tissue microarray set was used and it consisted of 800 tissue samples from 146 patients. The TMA set had 352 samples of PRCA, 64 metastatic prostate cancer samples (28 lymph node (LN) metastases and 36 non-LN metastases), 92 samples with high grade prostatic intra-epithelial neoplasia (PIN), 88 samples of benign prostatic hypertrophy (BPH), 96 foci of normal adjacent to tumor (NAT) and 64 samples of "true normal" donor prostate (OD). IHC was performed using monoclonal antibodies against Eph A2 (gift from Walter Storkus, Ph.D., Univ. of Pittsburgh).
Results: The foci of OD, NAT and BPH showed mean intensity levels of Eph A2 of 1.08, 1.2 and 0.25 respectively. Eph A2 was overexpressed in prostatic adenocarcinoma, prostatic intraepithelial neoplasia and metastatic carcinoma with mean staining intensity scores of 1.35, 1.66 and 1.80, respectively. Intensity scores for Stage II, Stage III and Stage IV cases were 1.51, 1.28 and 1.18, respectively ($P=0.084$ Kruskal-Wallis rank sum test). The mean intensity scores for Gleason grade <7 and 7-10 were 1.01 and 1.36 respectively ($P=0.0011$ Kruskal-Wallis rank sum test).
Conclusions: 1. EphA2 was overexpressed in cases of metastatic adenocarcinoma. 2. Over-expression of EphA2 was also seen in high grade PIN, suggesting a potential role in early oncogenesis. 3. There were differences in Eph A2 expression related to Stage and Gleason score. Increased expression of Eph A2 was seen with a lower T stage and lower Gleason score.
Due to its increased expression in metastatic prostate cancer, EphA2 may have a potential role as a therapeutic target and as an important tumor marker. Further studies are needed to fully characterize the role of EphA2 in prostatic cancer development and progression.

629 The 2003 pN Classification for Renal Cell Carcinoma: Can Its Prognostic Accuracy Be Improved?

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Background: The 2003 AJCC pN classification for renal cell carcinoma (RCC) is based on the number of positive regional lymph nodes (LNs) identified during lymphadenectomy. Our objective was to examine the associations of pathologic features of the LN metastases with patient outcome to improve the prognostic accuracy of the current classification.
Design: We studied 2,076 patients treated with radical nephrectomy for unilateral, sporadic pM0 RCC between 1970 and 2000. There were 36 patients with metastasis in a single regional LN (pN1) and 36 with metastases in more than one LN (pN2). The pathologic features of the LN metastases, including number and percentage of positive LNs, total number of LNs removed, grade, necrosis, extranodal extension and the largest dimension and surface area of the metastases, were determined by two pathologists (HHD and JCC). Cancer-specific survival was estimated using the Kaplan-Meier method.
Results: There was not a statistically significant association between the 2003 pN classification and death from RCC (risk ratio for pN2 versus pN1 of 1.01; 95% CI 0.60 – 1.69; p=0.980). However, patients with extranodal extension were twice as likely to die from RCC compared with patients whose metastases did not extend outside the capsule of the LN (risk ratio 2.02; 95% CI 1.18 – 3.45; p=0.010). Cancer-specific survival rates at 5 years following nephrectomy were 18% and 35% for patients with and without extranodal extension, respectively.
Conclusions: The determination of pN status in RCC provides important prognostic information. However, our results indicate that further information can be gained by examining the pathologic features of the LN metastases. We believe a pN classification based on the presence or absence of LN metastases with a notation regarding the presence or absence of extranodal extension represents a significant improvement in the prognostic accuracy of the current pN classification.

630 Prostate Biopsies for Abnormal Digital Rectal Examinations

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Background: Digital rectal examination (DRE) is a simple and routine procedure in urological practice. Abnormal DREs of the prostate are described as palpable nodules, firmness, irregularity, induration, or asymmetry and are often biopsied. The purpose of this study is to investigate the frequency in which cancer and other pathologic entities are encountered with an abnormal DRE.
Design: A total of 749 patients who underwent prostate biopsies for abnormal DREs were included this study. These cases represented 33.4% of the 2245 consecutive prostate biopsies received 25 urologists from January 2002 to August 2004. The final diagnoses were determined by a urological pathologist performing microscopic examination of hematoxilin and eosin stained slides and immunostained slides, if applied.
Results: One third of all 749 cases were malignant (33.2%, 249/749). Of 306 men with PSA > 4 ng/ml, 51.3% (157 cases) were malignant, while the cancer detection rate was 21.4% (95/443 cases) when the PSA was ≤ 4 ng/ml. The malignancies encountered include adenocarcinoma, small cell (neuroendocrine) carcinoma, transitional (urothelial) carcinoma, and lymphoma. Other pathologic findings of prostates with abnormal DREs include high grade prostatic intraepithelial neoplasia and/or atypical small acinar proliferation (13.1%, 98 cases), acute and/or prostatitis with or without lymphoid aggregates including granulomatous prostatitis (7.3%, 55 cases), basal cell hyperplasia/ adenoma (6%, 45 cases), transitional/squamous metaplasia (5.2%, 39 cases), stromal nodules (2%, 15 cases), and 2% others such as adenosis, calculi, infarct, and amyloid nodule. The remaining 233 (31.1%) cases were classified as benign (normal) prostate tissue.
Conclusions: One third of the prostates with abnormal DREs were malignant. When the PSA was > 4 ng/ml, the cancer detection rate was 51.3%, 2.4 times greater than the 21.4% rate seen with PSA ≤ 4ng/ml in the situation of abnormal DREs.

631 Immunohistochemical Detection of VEGF in Prostate Cancer

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Background: Vascular endothelial growth factor (VEGF) has been implicated in tumor angiogenesis and is a potential therapeutic target in prostatic carcinoma (Pca). Immunohistochemical (IHC) analysis has been used to demonstrate VEGF expression in Pca, as well as in various other tumors including breast carcinoma, renal cell carcinoma, hepatocellular carcinoma, and gliomas. Prior studies have reported markedly varied VEGF expression in benign prostatic hyperplasia (0-100%) and Pca (30-100%). The objective of this study was to measure VEGF expression in Pca specimens, using IHC analysis with antibodies from different manufacturers as well as different antigen retrieval techniques.
Design: Cases were identified from an ongoing study analyzing 1316 cases of Pca diagnosed at various Veterans Health Administration hospitals in the New England region during 1991 to 1995. From this study population, (20 cases including 10 biopsies, 5 transurethral resections of prostate (TURPs), and 5 radical resections) were selected. In all cases tissues were fixed in 10% formalin and embedded in paraffin. Four different antibodies were used for IHC using indirect peroxidase method (Table). For each antibody different dilutions and antigen retrieval methods (steam with EDTA, Steam with low pH, waterbath with Target Unmasking Fluid, trypsin, proteinase K) were tested. EnVision+ system was used to overcome non-specific staining. Appropriate positive and negative controls were used.
Results: Using different antibodies, positive staining of varying intensity was seen in benign glands, malignant glands, endothelial cells and fibromuscular stroma (Table). Some cases showed cytoplasmic and granular staining in prostatic glands. Except for focal and minimal staining in the endothelial cells, however, the staining disappeared in all cases when EnVision+ system was used.
Conclusions: Our results show that when non-specific staining is blocked, no staining is found for VEGF within the prostate, in either benign or malignant glands. Our study may help to explain variable results reported in previous studies, and suggests caution in interpreting VEGF expression in studies of Pca and benign glands.

Antibody	Without EnVision+			With EnVision+		
	Stroma	Benign Glands	Pca	Stroma	Benign Glands	Pca
Oncogen, MA	5	10	5	0	0	0
Santa Cruz, CA	6	12	5	0	0	0
Zymed, CA	0	5	6	0	0	0
Neomarker, CA	2	8	10	0	0	0

632 Current Practice of Diagnosis and Reporting of Prostate Cancer among Genitourinary (GU) Pathologists

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Background: As there is a lack of hard data in the literature about many of the issues relating to diagnosing and reporting prostate cancer, we sought to survey GU pathologists as to their practice.
Design: A questionnaire was sent to 93 GU pathologists in countries around the world with the purpose to survey current practices of diagnosing and reporting prostate cancer.
Results: The response rate was 69% including 40 North American pathologists and 24 from other continents. Almost all respondents (95%) used formalin as fixative for needle biopsies (NBX). Three levels of NBX were used routinely by 63%. Unstained intervening sections were retained by 47%. For verification of cancer, high-molecular weight cytokeratin was still the most commonly used immunohistochemical marker (91%), followed by p63 (58%) and AMACR (50%). Features considered pathognomonic for cancer were circumferential perineural invasion (84%), collagenous micronodules

(64%), glomeruloid bodies (58%) and growth in fat (36%). With none of these present, 39% required a minimum of 2 - 10 glands (median 3) to diagnose cancer, while the others would not define such a lower limit. The majority (83%) claimed that their diagnosis was not influenced by patient age. A Gleason score (GS) was given even to minute cancer foci by 86% and typically a GS 6 would then be assigned (77%). Perineural invasion was mentioned by 86%. The extent of cancer on NBX was quantified by all respondents with number of involved cores (80%) being the most commonly used measure. Linear extent was estimated by almost all, either as a percentage of total length (80%), mm cancer length (41%) or both (22%). Measuring cancer from end to end or subtracting intervening benign tissue were almost equally common.

Conclusions: Our survey data provide information to general pathologists about the most common practices among GU pathologists in the diagnosis and reporting of prostate cancer.

633 Current Practice of Diagnosis and Reporting of PIN and Glandular Atypia among Genitourinary (GU) Pathologists

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Background: Although there is a sizable body of literature relating to PIN and atypical glands suspicious for cancer, many areas remain unresolved and practice patterns are varied.

Design: A questionnaire was sent to 93 GU pathologists in countries around the world with the purpose to survey current practices of diagnosing and reporting prostate needle biopsies with PIN and "atypia".

Results: The response rate was 69%. The term PIN was universally acknowledged for preneoplastic lesions. However, if cytological or architectural atypia were pronounced, 44% would use "intraductal carcinoma". PIN was graded by 83%, usually as low/high grade PIN (LGPIN/HGPIN) or, more commonly, as HGPIN only. Lesions that may qualify for LGPIN were never mentioned (58%) or only rarely mentioned in the descriptive part of the report (25%). Architectural patterns of PIN were usually not specified (81%) and those who specified never commented on their significance. The majority (75%) did not comment that HGPIN is premalignant and 63% would not recommend a repeat biopsy. With invasive cancer also present, 69% would still mention HGPIN. Basal cell stains were used in <5% of HGPIN cases (67%). HGPIN would be diagnosed by 56% in the absence of prominent nucleoli, most commonly based on prominent pleomorphism (53%), marked hyperchromasia (47%) or mitotic figures (28%). Among diagnostic criteria for HGPIN were different degrees of nucleolar prominence (52%), or nucleoli seen in at least 10% of cells (33%). Number of cores involved with HGPIN were specified by half of the respondents.

Lesions suspicious for but not diagnostic of carcinoma were reported as ASAP (47%) or atypia/atypical glands/suspicious (48%). Degree of suspicion of cancer in atypical acinar lesions was defined by 41%. Only 34% always recommended repeat biopsy, while 30% would do it depending on referring doctor and 13% depending on patient age.

Conclusions: For controversial areas relating to PIN and atypical glands, our survey provides information to general pathologists about how GU pathologists deals with these issues.

634 Molecular Analysis of Inverted Tumors of the Urinary Bladder

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Background: Inverted papilloma of the urinary tract (IP) is thought to be benign, but some urothelial carcinomas (UC) show a prominent inverted growth pattern which may pose a diagnostic dilemma to the pathologist and clinician. Ancillary markers may help to resolve diagnostically challenging cases and may contribute to the ongoing discussion of a possible malignant potential of some IP.

Design: Seventy-two inverted urothelial lesions of the urinary tract (inverted papilloma n=63, non-invasive UC with prominent inverted growth pattern n=9) were studied immunohistochemically (CK20, Mib-1, MSH2, MLH1, MSH6). Microsatellite status and loss of heterozygosity was assessed utilizing the Bethesda microsatellite panel and markers on chromosomes 17p, 9p and 9q. Mutation analysis of the Fibroblast Growth Factor Receptor gene (FGFR3) was performed as well.

Results: Immunohistochemical analysis of MSH2, MLH1, MSH6 and CK20 revealed no differences between IP and UC with inverted growth pattern. IP did not show molecular changes typically seen in early stages of UC of the urinary bladder. The Mib-1 proliferation index correlated strongly with the histologic impression ($p<0.0001$). FGFR3-mutations were more often seen in UC with inverted growth, compared to IP ($p=0.05$).

Conclusions: Inverted papilloma of the urinary tract does not show molecular changes associated with a malignant urothelial phenotype. The presence of FGFR3 mutations and a Mib-1 proliferation index >10% favor a diagnosis of UC with inverted growth pattern, which could help resolve histologically ambiguous cases.

635 Low-Volume Prostate Cancer Is Not Necessarely Pathologically and Clinically Insignificant

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Background: Tumor volume is one of the most powerful predictors of patient outcome in prostatic adenocarcinoma. Previous studies suggest that prostate cancer (PCa) with a volume of 0.5 cc. or less and a radical prostatectomy (RP) Gleason score (GS) of less than 7 may be clinically insignificant and may be managed with watchful waiting. We assessed the pathologic features and clinical outcome of low-volume PCa.

Design: The surgical pathology database at the authors' institution was queried for RP with low-volume (<0.5 cc or <65 sq mm) PCa performed between January 1999 and December 2003. Patients who received neoadjuvant antiandrogen treatment were excluded. Clinical and pathologic features evaluated included pretreatment serum prostate specific antigen (PSA), prostate volume, PSA density (PSAD), GS of the RP, presence of extraprostatic extension (EPE), surgical margins status, bladder neck involvement, and serum PSA followups.

Results: A total of 323 RP were evaluated. The mean PSA was 6.3 ng/ml (range 0.1 to 39.5, median 5.5). The percentage of low-volume PCa increased over time from 10.3% in 1999 to 15.4% in 2003 of the total RP performed at our institution. Two hundred-ten cases (65%) were GS 6 or less - 12 were 3+2, 198 were 3+3. One hundred-thirteen cases (35%) were GS 7 or higher - 95 were 3+4, 16 were 4+3, and 2 were 4+4. EPE was present in 12 cases (3.7%), 4 of which were GS 6. Margins were positive in 26 cases (8%), 2 of which in multiple sites: apex (8 cases), postero-lateral (16 cases), anterior (3 cases), and bladder neck (1 case). PCa was present at the bladder neck in 4 cases (1.2%), two of which were GS 6. Of the 323 patients, 136 (42.1%) had PSAD>0.15 ng/ml/cc (range 0.152-0.550) - 77 of which were GS 6. Serum PSA follow-up was available for 284 (88%) patients (median: 20 months; range: 1-66 months). Seven (2.5%) men had biochemical failure, defined as 2 consecutive PSA levels of 0.2 ng/ml or one PSA of > 0.3 ng/ml.

Conclusions: Thirty-seven percent of the men having insignificant volume of cancer has a clinically significant PCa as evidenced by GS>6, presence of EPE, or bladder neck involvement. Biochemical failure occurred in 2.5% of the patients. One-fourth of the GS 6 PCa had PSAD>0.15 ng/ml/cc. Low-volume tumor in RP specimens does not guarantee clinical insignificance.

636 Significance of High Grade Prostatic Intraepithelial Neoplasia in the Era of Extended Prostate Needle Biopsies

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Background: The clinical significance of finding high grade PIN in the era of extended prostate biopsy strategies is uncertain. In this study we examine the cancer detection rate on follow-up biopsies after a PIN diagnosis in the sextant (98-00) and extended (01-03) biopsy eras at our institution.

Design: Approximately 10% of 5851 prostate needle biopsies from 1998-2003 were found to have high grade prostatic intraepithelial neoplasia (PIN) on at least one core, with no adenocarcinoma on any other core, and were used for this study. The database at our institution was searched for subsequent biopsy history. Information regarding diagnosis on first follow-up biopsy and all subsequent follow-up biopsies was recorded. The percent of all PIN biopsies with at least one follow-up biopsy was determined, and the cancer detection rate on these follow-up biopsies was calculated. Many patients had numerous follow-up biopsies, and the cancer detection rate for all follow-up biopsies was calculated. Extended prostate biopsies gained popularity at our institution at the turn of the century, and this was used as the cutoff for statistical comparison. Student t-test (two tail) was used to compare annual rates for each of the 3 year periods.

Results: Prostate cancer was detected in 33.5% of patients who underwent repeat biopsies after a diagnosis of PIN. 25.4% had cancer on their first repeat biopsy. Only 42.4% of cases had follow-up biopsies.

Prostate Cancer Detection After PIN Biopsy

	% CA 1st bx	% 1 followup bx	% CA (all bxs)	% with >1 bx	PIN rate
1998	26%	42%	42%	7%	9%
1999	38%	48%	43%	2%	11%
2000	17%	50%	34%	9%	15%
2001	28%	44%	33%	2%	13%
2002	28%	35%	33%	2%	9%
2003	19%	37%	19%	0%	7%
p=	0.76	0.09	0.10	0.09	0.47
mean 98-00	27%	47%	40%	6%	12%
mean 01-03	25%	39%	28%	1%	10%

p value comparing means for 3 yr intervals

Conclusions: No statistically significant difference ($p<0.05$) was observed for cancer detection rates on first, or all subsequent follow-up biopsies. A trend was observed for decreasing overall cancer detection rate over time, but this correlated with a trend for decreased number of follow-up prostate biopsies. This is likely due to the shorter follow-up time with recent biopsies. Larger series and longer follow-up will be required to determine if these trends are significant.

637 Endoglin (CD105) and Vascular Endothelial Growth Factor (VEGF) as Prognostic Markers in Prostatic Adenocarcinoma

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Background: Angiogenesis is an essential requirement for the development, progression and metastasis of malignant neoplasms. Endoglin, a membrane protein and member of the transforming growth factor β-1 receptor complex, has been shown to be a more sensitive marker for tumor angiogenesis than other pan-endothelial markers such as CD31 and CD34. Previous studies have indicated that increased expression of endoglin and VEGF is associated with a poorer prognosis in a number of carcinomas. However, the expression of endoglin as a prognostic factor in prostatic adenocarcinoma has not been studied.

Design: 50 radical prostatectomy specimens for prostatic adenocarcinoma were retrieved. Cases were further categorized according to the Gleason grading system into: grade 8 to 10 (9 cases), grade 7=4+3 (9 cases), grade 7=3+4 (14 cases), grade 6 (13 cases), and grade 4 to 5 (5 cases). All cases were immunostained for endoglin (CD105), VEGF, and CD31. Positively stained microvessels (MV) were counted in

densely vascular foci (hotspots) at 400X field in each specimen. For VEGF, intensity of staining was scored on 3 tiered scale. Results were correlated with other prognostic parameters, including Gleason grade, perineural invasion, capsular invasion, lymphovascular invasion, lymph node metastasis, seminal vesicle extension, and stage of the tumor using Spearman correlation.

Results: IHC staining for endoglin demonstrated significantly more vessels than with CD31 (mean 37±15 versus 22±17, p<0.001). Low VEGF expression was seen in 21(42%) and high expression was seen in 29 cases (58%). There was a positive correlation of endoglin with Gleason grade, lymph node metastases, and stage of the tumor (p<0.05), while CD31 was insignificant (p=0.08). VEGF showed significant correlation with lymphovascular invasion and Gleason grade (p<0.05).

Conclusions: Endoglin, by staining the proliferating vessels in prostatic adenocarcinomas, is a more specific and sensitive marker for tumor angiogenesis than the commonly used panendothelial marker CD31. Endoglin staining showed prognostic significance with positive correlation with Gleason grade, lymph node metastases, and stage of the tumor. Both endoglin and VEGF may serve as prognostic markers in the prostatic adenocarcinoma and could possibly lead to future clinical trials with antiangiogenic therapy.

638 Quantitative Analysis of Alpha-Methylacyl CoA Racemase (AMACR) and Fatty Acid Synthase (FAS) Co-Expression in Localized Prostate Cancer

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Background: Both alpha-methylacyl CoA racemase (AMACR) and fatty acid synthase (FAS) are biomarkers identified by expression array analysis and confirmed by tissue microarray (TMA) to be over expressed in prostate carcinoma (PCA) compared to normal prostatic glands. Under normal conditions FAS is an androgen-regulated enzyme required for de novo lipogenesis. FAS expression increases from normal to prostatic intraepithelial neoplasia to low grade to high grade PCA, and is expressed at highest levels in androgen-independent bone metastases. AMACR is a peroxisomal and mitochondrial enzyme important for beta-oxidation of branched-chain fatty acids. AMACR expression is high in localized PCA and decreases in metastatic PCA. It is not regulated by the androgen receptor. Herein we explore the relationship between AMACR and FAS protein expression levels and the use of their ratio as a biomarker for PCA outcome.

Design: AMACR and FAS protein expression was detected by immunohistochemistry in tumor and benign prostate tissue from 161 men with clinically localized prostate cancer at time of diagnosis and treated with radical prostatectomy. Patients were followed for up to 8 yrs or until detection of serum PSA of > 0.2 ng/ml. Protein expression was quantitated using a semi automated image analysis system (ACIS II System, Chromavision, San Juan Capistrano, CA) using a scale between 0 and 255 chromogen intensity units.

Results: A two-tail Spearman correlation analysis demonstrates a strong correlation between AMACR and FAS expression within localized prostate carcinoma (Spearman's rho correlation coefficient = 0.67, p<0.001). There was a weak association of the FAS/AMACR ratio with PSA recurrence at the univariate level, which does not reach statistical significance. No association between FAS or AMACR and the Gleason score was identified.

Conclusions: Despite its prevalence, the etiology of PCA is still poorly understood. The correlation of AMACR and FAS expression implicate fatty acid metabolism in the growth and survival of PCA.

639 Evaluation of *p63* Expression in Testicular Germ Cell Tumors

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Background: The tumorigenesis of germ cell neoplasia is incompletely understood. *p63* is a novel transcription factor and epithelial stem-cell regulatory protein. *p63* has not been previously studied in a large series of testicular germ cell tumors.

Design: Thirty four cases of testicular germ cell tumors (19 seminomas (7 had intratubular germ cell neoplasia), 1 pure teratoma, 3 pure embryonal (2 with intratubular germ cell neoplasia), 1 pure yolk sac, 10 mixed tumors: Embryonal/seminoma/immature teratoma /yolk sac (n = 3), embryonal/teratoma/seminoma (n = 2) teratoma/seminoma (n = 2) embryonal/seminoma (n = 2), yolk sac/embryonal/choriocarcinoma (n = 1) . Four µ sections from archival formalin-fixed parraffin-embedded sections were incubated with anti-p63 monoclonal 4A4 (Santa Cruz; 1: 800) then stained with EnVision-Plus(Dako), and counterstained with hematoxylin.

Results: *p63* was consistently positive in teratomas with squamous epithelia, and in basal cells of respiratory/endodermal differentiation. *p63* staining was focally positive in embryonal carcinoma (4/10), yolk sac tumor (2/5), and cytotrophoblasts of choriocarcinoma (1/1). Seminomas and in intratubular germ cell neoplasias and non-neoplastic seminiferous tubules were consistently negative. We also identified consistent positivity in the basal cells of epididymi while efferent ductules were negative (10).

Conclusions: Teratomas consistently expressed *p63* similar to their non-gonadal epithelial counterparts. A minority of embryonal and yolk sac tumors and cytotrophoblasts of choriocarcinoma had a population of *p63* positive cells. Seminomas and intratubular germ cell neoplasia were consistently negative for *p63*. These findings may suggest the presence of potentially pleuripotent stem-cell-like nests in yolk sac, embryonal carcinomas and cytotrophoblasts of choriocarcinoma, or may represent areas of teratoma which are undetectable on H - E sections. Whether *p63* positivity identifies a subset of embryonal, yolk sac, or choriocarcinoma with altered clinical behaviour or aggressiveness is unknown. Basal cell epididymi positivity, and efferent ductule negativity may reflect different embryogenesis of these structures.

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640 Prostatic Central Zone Carcinoma

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Background: The central zone (CZ) of the prostate is embryologically, anatomically and histologically distinct. CZ prostatic adenocarcinoma (PAC) have not been well studied

Design: We reviewed consecutive 200 prostatectomies for PAC to identify CZ with aid by the immunostaining with Ulex Europeus-I which displays positive reactivity for CZ epithelia and seminal vesicles, and often negative reactivity for normal non-CZ epithelia

Results: There were seven PAC arising primarily in the CZ and presenting as prominent tumor nodules and 193 non-CZPAC having satellite tumor nodules (19 cases), and secondary contiguous spread to the CZ (80 cases). CZPAC tended to have higher Gleason'scores (7.1±0.6 vs 6.3±0.7, p<0.05) and higher rate of seminal vesicle spread (2/7 vs 7/193, p<0.05) than the non-CZPAC. There were no statistical significant difference in patient age, tumor diameter, pre-operative serum PSA level and rate of capsular penetration between CZ and non-CZ PAC. In addition, HGPIN was often seen in CZ but less frequent (155 versus 190 cases) than in non-CZ and smaller extent than in PZ.

Conclusions: Prostatic CZ tends to be associated with lower potential of development of PAC with lower frequency and smaller extent of PIN and lower frequency of CZPAC than the other zones. However CZPAC has a higher Gleason's score and is more prone to invade the seminal vesicles.

641 An Immunohistochemical (IHC) Study of Osteoclast-Like Giant Cell Neoplasms of the Urinary Tract: Extraskeletal Giant Cell Tumor of Bone or Osteoclast-Type Giant Cell Carcinoma

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Background: Osteoclast-like giant cell neoplasms of the urinary tract are extremely rare, with only a few cases reported. They are composed of ovoid or spindle-shaped mononuclear cells with evenly spaced osteoclast-like giant cells. Terminology, histogenesis, and biologic behavior of these tumors remain controversial.

Design: 6 cases were identified from the consultation files of two of the authors.

Results: Patients were all male and elderly (range 67-82), with the exception of one 39 year-old. 3/6 tumors developed in the bladder and 3/6 in the renal pelvis. Size ranged from 5 cm to 11 cm. 1 bladder and 3 renal pelvis tumors were high stage (pT3) at time of presentation. Adjacent to the osteoclast-like giant cell neoplasm in the same specimen, all patients had CIS and/or high grade papillary urothelial carcinoma. IHC studies with mesenchymal markers were comparable to giant cell tumors of bone and soft parts. Varying % of mononuclear cells expressed α-Actin (smooth muscle) (5/6), desmin (1/6), S-100 (4/6), LCA (2/6) and CD68 (6/6). However, mononuclear cells were also positive for epithelial markers in 4/6 tumors (cytokeratins AE-1/AE-3, Cam 5.2, CK7 and/or EMA). Multinucleated cells had identical morphological and IHC properties of osteoclasts; positive for CD-68, LCA and CD51 in all cases, and negative for cytokeratins and EMA. Studies of additional markers of osteoclasts and osteoclastogenesis (CD54, RANK and RANK-L) are in progress. p53 stained mononuclear tumor cells in 3 cases, paralleling the accompanying urothelial carcinoma. P16, cyclin D1, bcl-2 and Her2Neu were noncontributory. Ki-67 stained mononuclear tumor cells, but not osteoclast-like giant cells. Follow-up data was available in 5 cases. 1 patient with superficial bladder disease developed a single recurrence and is still alive. 4 patients were dead due to disease within a year (2 had lung metastases; 1 patient had liver and pleura metastases and a renal bed recurrence).

Conclusions: The intimate association of these tumors with urothelial carcinoma along with their IHC profile support an epithelial origin for the mononuclear cells and non-neoplastic reactive histiocytic lineage for the osteoclast-like giant cells. These tumors follow an aggressive clinical course; most patients die of disease within 1 year. Osteoclast-like giant cell tumor of the urothelial tract is a rare carcinoma with divergent differentiation towards the morphology of giant cell tumor of bone.

642 Overexpression of WWOX Protein in Prostate Cancer

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Background: The *WWOX* gene, a candidate tumor suppressor gene, maps to the common fragile site FRA16D on chromosome 16q23.3-24.1. Its mRNA is highly expressed in the testis, prostate and ovary. Loss of 16q has been reported frequently in multiple neoplasias, including prostatic carcinomas. *WWOX* encodes a 46 kDa protein that contains two WW domains and a short-chain oxidoreductase domain, suggesting that it is involved in sex-steroid metabolism. We performed an immunohistochemical study of WWOX protein in malignant and non-neoplastic prostate tissue.

Design: A tissue microarray constructed by 0.6 mm cores from 40 radical prostatectomy specimens was used. It contained four cores from neoplastic and additional four cores from corresponding non-neoplastic regions. Gleason score ranged from 5-9, and pathological stage ranged from T2N0Mx to T3BN1. A polyclonal rabbit anti - WWOX antibody was used for immunohistochemistry. Controls consisted of formalin fixed paraffin embedded cell lines with known positive or negative WWOX protein status. Staining was scored visually taking percent negative, weak, moderate and strong positivity into consideration and these values were compared to those obtained by the Automated Cellular Imaging System (ACIS II) from ChromaVision, Inc.

Results: Normal epithelium was present in 39, atrophy in 12 and tumor in 36 cases. While the secretory layer showed negative or weak staining in most cases, expression in basal cells was often stronger. Most adenocarcinomas (n = 32) expressed WWOX and this staining was generally stronger than benign secretory cells and often stronger

than basal cells. Atrophic glands consistently showed the highest level of expression. Pairwise analysis revealed differences in staining between all tissue types (normal, carcinoma and atrophy) (p value range 0.0023 to 0. 04). Negative staining was detected in only 4 tumors and there was no correlation between staining and tumor stage or Gleason score. There was good agreement between visual scoring and values obtained with the ACIS II.

Conclusions: WWOX was mainly expressed by basal cells in normal glands. Its expression was increased in most prostate carcinomas. The most consistently strong staining was in atrophy. Rare carcinomas lost expression, but overall expression levels did not correlate with other prognostic factors in this study. Overexpression of this proposed tumor suppressor in prostatic carcinoma deserves further investigation.

643 Multi-Institution Automated Image Analysis of PTEN Protein in Prostatic Adenocarcinoma

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Background: The tumor suppressor *PTEN* encodes a protein with dual function phosphatase activity whose action decreases signaling through the PI3 kinase pathway. Prior studies have shown that decreased protein levels are associated with adverse clinical features. We utilize a large number of tissue microarrays (TMAs) from 3 institutions in an attempt to further validate decreased PTEN protein as a prognostic marker in prostate cancer.

Design: TMAs representing more than 1000 radical prostatectomy cases and matched normal samples with 4-fold redundency were immunostained with antibodies specific to Cytokeratin 8 and PTEN proteins. Staing for PTEN was quantified using the ChromaVision ACIS II® workstation after verifying the diagnosis on each image. Thresholds for the ACIS system were adapted on a per-array basis to include all positive pixels with no intensity exclusions. The fraction of epithelium staining positive was calculated by dividing positive PTEN area score by the keratin area score. Percent positive and average intensities were then correlated with tissue type (tumor or normal), Gleason score and pathological stage.

Results: 4410 TMA cores from 374 patients from 3 institutions were visually analyzed thus far. PTEN expression was higher in adjacent normal versus tumor tissue ($p < 0.0000$, normal: mean = 38.42 ± 27.3, range 0.26 - 101.3; tumor: mean = 9.23 ± 15.5, range 0.13 – 94.4). Mean intensity of staining was also higher in normal tissue ($p < 0.0000$, normal: mean = 107.75 ± 32.5, range 60.0 – 209.2; tumor: mean = 80.14 ± 21.3, range 54.0 – 203.0). In the first set of 220 patients analyzed, lower percent positive PTEN staining in tumor tissue correlated inversely with Gleason score ($p = 0.0268$) and inversely with higher pathologic stage ($p = 0.0106$). Average intensity, while lower in tumor samples, did not correlate with Gleason score or pathologic stage.

Conclusions: Analysis of PTEN protein levels in this large data set validates earlier findings that the protein is decreased in a fraction of tumors and that lower overall levels of PTEN correlate with adverse pathologic variables. Further analysis of the remainder of cases as a validation series and of long term followup data will reveal whether decreased PTEN protein has independent prognostic value.

644 p16 Is Upregulated in Proliferative Inflammatory Atrophy of the Prostate

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Background: Focal prostate atrophy is a common lesion in the prostate. Most of these lesions have been shown to be highly proliferative and associated with inflammation and we have referred to them as proliferative inflammatory atrophy (PIA). In the majority of prostate cancers, p16 is paradoxically found to be overexpressed suggesting a loss of its cell cycle regulatory function. In the current study, we examine whether PIA lesions contain p16 upregulation.

Design: Tissue arrays were constructed in two experimental groups. The "test set" arrays were constructed of 50 paraffin embedded radical prostatectomy samples from a series of 300 procedures conducted at the University of Wisconsin. The "validation" arrays were contructed from a series of 73 cases selected from a set of 300 sequential cases performed at Johns Hopkins. Adjacent arrays were immnostained with antibodies against p16, ki-67 and cytokeratin 8 using DAB as a standard chromagen. In the test set, the percent of cells postive for p16 and Ki-67 were recorded using the SNAP imaging microscope. In the validation set, the percent positive for p16 was assessed using the Chromavision ACIS II workstation to calculate percent positive and record average intensity. To determine whether any of the cells co-expressed by p16 and Ki-67, double label immunofluorescence was used.

Results: PIA lesions contained characteristic attenuated, cuboidal epithelial cells with varying degrees of inflammation. In normal appearing epithelium, p16 staining was infrequent and focal, often localized to basal cells. In the test set the mean fraction of cells staining in normal epithelium was 0.63% compared to 16.1% in atrophy ($p = 0.0001$). Similar increased levels were seen in the validation set. The mean Ki67 index (positive staining cells/total number of cells) was also significantly increased ($p = 0.0001$) in PIA lesions (8.21% ± 3.5) versus normal epithelium (1.90% ± 1.1). Co-immunoflourescence did not demonstrate simultaneous staining for p16 and Ki67 within individual cells.

Conclusions: Increased cellular proliferation and altered p16 expression are found in PIA lesions of the prostate. The finding that p16 is elevated in both PIA and prostate cancer supports the hypothesis that some prostate atrophy lesions may be associated either directly as a premalignant lesion, or indirectly, with the development of prostate cancer.

645 Prediction of Clinically Insignificant Prostate Cancer after Single Core Positive Needle Biopsy

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Background: Finding of single core positive biopsy for prostate cancer (pCa) in the current practice of increased PSA testing and extended core sampling may increase the detection of clinically insignificant (CI) pCa on radical prostatectomy (RP). Highly sensitive prediction of pCa based on preoperative clinical and biopsy parameters may be used to rule out patients with pCa and allow the selection of patients who may be followed by surveillance.

Design: Our goal was to ensure that predictive models had high sensitivity and high negative predictive value (NPV). We used a classification tree and logistic regression models to predict pCa, based on combinations of clinical parameters and % core involvement on biopsy. We adjusted the prior probability (for the regression tree) and the cut-off values for predicting cancer (for logistic regression) to ensure high sensitivity. We used a sample of 176 consecutive RPs following biopsies with pCa in a single core on a ten-core biopsy. Initial sample consisted of 123 RP and validation sample consisted of 53 RP. All RP were completely sampled. Tumor volumes were measured using computer image analysis. All biopsies and RP were performed in our institution after 07/00. Clinically insignificant (CI) PCa was defined as organ-confined disease, tumor volume ≤0.5cc and Gleason ≤6.

Results: CI pCa was found in 105 (59.7%) patients. The resulting classification tree tested on the initial sample had high sensitivity and NPV (94.3% and 92.9% respectively), but did not perform well in the validation sample (sensitivity 55.6%, NPV 69.2%). Our final logistic regression model included the following variables (with simplified scoring): age >60 years (score=1), PSA >4 ng/ml (score=2), gland volume >50 cc (score=2), and % core involvement >7.5% (score=2). To use the model as a clinical tool, CI pCa is indicated if the sum of the scores is <3.5. The sensitivity in the initial sample was 96.2% and NPV was 93.8%. It performed reasonably well in the validation sample (sensitivity 72.2%; NPV 77.3%). The final logistic regression model for the entire sample had sensitivity of 90.1% and NPV 87.0%

Conclusions: Previous attempts at predicting pCa on the basis of clinical and biopsy data have had limited success. We constructed predictive models with the deliberate goal of achieving high sensitivity and NPV to rule out patients with minimal disease. The resulting simplified regression model had reasonable sensitivity and specificity on validation and may provide a useful clinical tool for selecting some patients with CI pCa.

646 Regulation of HAI-1 Expression by Androgen and Oncogenic Transformation in the Prostate

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Background: The serine protease inhibitor, Hepatocyte Activator Inhibitor-1 (HAI-1) regulates the proteolytic activation of hepatocyte growth factor (HGF/SF) and its binding to the Met cell surface receptor. Met is expressed in normal prostate epithelial cells, in ~ 50% of invasive prostate cancers and highly in metastases. Therefore, the regulation of HGF/SF/Met activity by HAI-1 may play an important role in Met-stimulated prostate cancer growth, invasion and metastasis.

Design: 5{arrays (TMA) were constructed using three cores of tissue per tissue category and patient. Arrays contained: 1. normal and cancer (69 cases), 2. untreated (20 cases) and androgen-deprived tissues (74 cases) and 3. tissues annotated with PSA recurrence data (640 cases). Antigen was retrieved with citrate buffer, pH 6.0, antibody dilution was 1:50 and Streptavidin-biotin was used for signal amplification. Staining was scored in each category (normal, atrophy, cancer, PIN) by two pathologists on a linear scale from 0-300. LNCaP cells were treated with 10 nM R1881-androgen and HAI-1 surface expression measured by FACS.

Results: HAI-1 is expressed in normal basal and intermediate cells. Consequently, HAI-1 expression is greater in atrophic epithelium (IHC score = 140) than in differentiated secretory epithelium (IHC score = 40). Compared to secretory epithelium, HAI-1 expression is significantly increased in prostate carcinoma (median IHC score = 140). While HAI-1 expression is significantly reduced in androgen-deprived normal epithelium, in prostate cancer it is not affected by androgen deprivation, metastasis formation or androgen-independent growth. Androgen stimulates the release of HAI-1 from the cell surface of LNCaP prostate cancer cells. HAI-1 expression is associated with a relative risk of cancer recurrence of 1.24.

Conclusions: HAI-1 protein expression increases with oncogenic transformation of prostate epithelial cells and is associated with an increased risk of tumor recurrence. Although androgen does not affect HAI-1 expression in cancer cells, it may increase its activity by proteolytic cleavage at the cell surface. In cancers that co-express HAI-1 and HGF/SF/Met, HAI-1 maybe an androgen-responsive regulator of HGF/SF activity and form a link between androgen and tumor invasion and metastasis.

647 Urothelial Neoplasms in Patients 20 Years or Younger - A Clinicopathologic Analysis

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Background: Urothelial neoplasms in young patients are exceedingly rare. There are few studies of bladder lesions in patients ≤20 years of age, and limited data concerning clinical outcomes in these patients.

Design: The Surgical Pathology archives of three large tertiary care institutions were searched for cases of urothelial neoplasms in patients ≤20 years of age. 23 cases were identified and graded using the WHO/ISUP 2004 classification. 21/23 cases were consult cases submitted for expert opinion.

Results: The mean age of the 23 patients was 13.3 years (range: 4-20) and 19 of 23 were male (82.6%). Pathologic grading revealed 2 papillomas, 11 papillary urothelial neoplasms of low malignant potential (PUNLMP), 9 low-grade papillary urothelial cancers, and 1 high-grade papillary urothelial cancer, all lacking invasion. Clinical data was available for 13 patients (follow-up on remaining cases pending IRB approval). Nine of 13 (69%) presented with gross hematuria, two with microscopic hematuria, and one case each of menstrual spotting and abnormal ultrasound during pregnancy. Lesions ranged in size from 0.1 to 6 cm in greatest dimension and were located at the anterior (1), posterior (2), and lateral (2) walls, the trigone (3), the bladder floor (2), and adjacent to the ureteral orifices (3). One of 13 patients smoked and a second had parents who smoked. None of the 13 patients had a history of cancer, a family history of bladder cancer, history of exposure to radiation/chemotherapeutic agents, or parents with an occupational history known to be associated with bladder carcinogenesis. Two of 13 patients had evidence of recurrence with one papilloma recurring 4 times and one PUNLMP recurring after 3 years. All 13 patients are currently alive with no evidence of disease after a mean follow-up of 5.7 years (range: 1 mo. to 10 y).

Conclusions: Urothelial neoplasms in individuals ≤20 years of age more commonly occur in males and are predominantly low grade with a favorable clinical outcome. Prior to the current classification system of bladder cancer, the 11 patients with a diagnosis of PUNLMP would have been classified as having papillary carcinoma. The diagnostic category of PUNLMP allowed 47.8% of patients in this series to avoid being labeled with a cancer diagnosis at a young age.

648 Minute (≤1 mm) Foci of Gleason Score 8-10 Prostate Cancer on Needle Biopsy: Histologic Features and Clinical Significance

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Background: Needle biopsy of high grade prostate cancer typically contains abundant tumor. The histologic features and significance of the rare case with minute high grade cancer on needle biopsy is unknown.

Design: From 1992-2004, 29,388 cases of prostate cancer diagnosed on needle biopsy were identified from the consult service of one of the authors. Of these, 123 patients had a minute (≤1 mm) focus of high grade prostate cancer, defined as Gleason score (GS) 8 (4+4 only), 9, or 10.

Results: Slides from 86 of 123 patients were re-reviewed. Of the 86 cases, 34 (39.5%) were GS8 with cribriform glands (n=13), ill-defined glands (n=9), fused glands (n=4), or combinations of the three (n=8). 32 of 86 (37.2%) cases were GS 9 with single cells and ill-defined glands (n=19), and combinations of single cells, sheets of cells and ill-defined, cribriform, nested, or fused glands (n=13). 20 of 86 (23.3%) cases were GS 10 with single cells (n=17) and single cells with cellular nests (n=3). Clinical data was obtained for 113 patients, with 10 patients lost to follow up. Patients underwent radical prostatectomy [RP] (n=45), radiation therapy [RT] (n=43), hormonal therapy [HT] (n=20), or surveillance (n=5). Statistically significant correlations were noted between: 1: Age and choice of therapy, with median ages of 62 for RP, 71 for RT, and 78 for HT, and 2: PSA and choice of therapy, with median PSA of 6.6 for RP, 10.2 for RT, and 18.5 for HT. Logistic regression analysis demonstrated that both age and PSA were influential in treatment selection. 29/45 (64.4%) of patients undergoing RP were pT2, while 16/45 (35.6%) showed extraprostatic extension (pT3), including one case of seminal vesicle invasion and two cases with lymph node metastases. No significant correlation between age, PSA, or biopsy GS and pathologic stage was noted.

Conclusions: Over the last ten years, we have seen an increased incidence of minute high grade cancers on needle biopsy, possibly as a result of aggressive PSA screening and more extensive sampling. Despite the limited nature of such cancers, pathologists must be attuned to the complex and varied patterns of high grade cancer. Among patients with small foci of high grade cancer who undergo radical prostatectomy the majority have organ-confined disease. Additional follow-up is being obtained to determine whether favorable pathologic stage in this group of unique cases translates to cure or still carries a significant risk of progression.

649 Gene Expression Signatures in Benign and Malignant Epithelial Cells from Formalin-Fixed Paraffin Embedded Prostatectomy

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Background: This study is to develop high through-put gene expression assays using formalin-fixed paraffin-embedded (FFPE) prostate cancer (CaP) tissues. Using OCT embedded frozen prostate tissue specimens, laser capture microdissections (LCM) and GeneChips, we have focused on discovery of (CaP) gene specific expression biomarkers. This is a feasibility study for evaluating CaP associated gene expression signatures in FFPE CaP tissues.

Design: Matched benign and malignant epithelial cells were obtained from a 7-micron section of FFPE whole-mounted prostatectomy, which included the site from which frozen tissue was retrieved for an earlier GeneChip study. LCM derived epithelial cells from benign and malignant glands were analyzed by Affymetrix HG U133a and Human X3P GeneChips and tumor cell specific gene expression signatures were evaluated using the GeneSpring software (Silicon Genetics). The results were compared to gene chip data from corresponding frozen prostate tissue specimens embedded in OCT.

Results: We were able to successfully obtain about 12 thousand fold linear amplification of the mRNA. The preliminary evaluations of the expression patterns in paired benign and cancer cells from FFPE was compared with GeneChip data from corresponding frozen tissue specimens. Analysis of gene expression patterns in LCM derived epithelial cells from FFPE tissues revealed similarity to a subset of genes exhibiting tumor cell specific differential expression in the study of LCM derived benign and malignant cells from frozen OCT-embedded prostate tissues. This subset of 224 genes included HOXC6, SOX4, EIF4E, FOLH1, TMSNB, CDC40, FYN, SUMO2, SHARP, BCL7A, PRDX2, PAP, GATA3 etc. Among these genes, apoptosis regulators, growth/DNA synthesis promoters, antioxidant response modulators, cell-cell adhesion molecules and transcription factors showed similar expression patterns between FPPE and OCT embedded frozen tissues.

Conclusions: Our preliminary studies provide proof of principle that FFPE CaP tissue can be used for cell specific gene expression analysis. This will help us to validate the utility and limitation of FFPE CaP tissues. Utilization of FFPE tissue for GeneChip studies will enhance the pathologic correlations of prostate cancer associated gene expression bio-markers.

650 Prognostic Indicators for Renal Cell Carcinoma; a Multivariant Analysis of Histologic Grade, Ki-67, p53 and Ploidy Status of 130 Patients

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Background: Histologic nuclear grade, performance status, and clinical stage are important prognostic factors in renal cell carcinoma (RCC). Because patients with tumors of similar grade, performance status, and stage may show a wide variation in biologic behavior and clinical outcome, additional prognostic biomarkers are needed to provide further information and possibly offer insight into the mechanisms of the disease. Retrospective studies have previously shown a potential added value for DNA ploidy, proliferation markers and p53 status of tumor as potential predictors of RCC outcome. The goal of this study is to incorporate the added combined information from studying these factors to the histologic grade in predicting outcomes and survival.

Design: 130 patients (87 males and 43 females; mean age 56.92 years), undergoing total nephrectomy for RCC were included in this study. Tumor diameter, TNM stage, and Fuhrman's nuclear grade were defined for each tumor. We correlated the expression of Ki-67 (MIB-1), a marker of cell proliferation, and p53 and DNA ploidy, with grade, stage, and survival in patients with clear cell RCC. Immunohistochemistry was used for p53 and Ki-67, and image analysis for DNA ploidy. Automation was used in scoring p53 and Ki-67 data using CAS-200 and the ACIS imaging system. The prognostic value of the various variables was determined and survival analysis was studied

Results: p53 and Ki-67 are both correlated very well with nuclear grade and tumor size and pathologic stage with significant increase of expression in high grade tumor. In grade I tumor, the over expression of p53 and Ki-67 are 0 and 1%, respectively, and 4.76% and 21.56% in grade IV tumor, respectively. Increased Ki-67 and p53 expression predicted poor cancer-specific survival when a cutoff value for Ki-67 and P53 staining were applied. We also found that DNA diploidy had a significant correlation with nuclear grade, but not with survival rate.

Conclusions: Ki-67, p53 and DNA ploidy are significant and independent predictors of RCC outcome. Combining information obtained from studying those improtant prognostic factors appear to have an added value to nuclear grading. Our results confirm the previously reported value of these factors on an individual basis and highlight the importance of multivariant analysis of these factors in combination with nuclear grade and with each other.

651 Do Increased Biopsy Sampling and Number of Positive Cores Improve the Accuracy of Final Gleason Score (GS) on Radical Prostatectomy (RP)?

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Background: Contemporary series demonstrate improvement of the Gleason score (GS) correlation between biopsy and radical prostatectomy (RP), but the exact prediction on biopsy of the final GS on RP remains variable. It is uncertain whether the prediction of the final GS on RP will be improved with extended biopsy schemes and whether the number of positive cores on biopsy will improve the exact prediction of the final GS.

Design: We correlated the GS in 908 consecutive RPs performed in our institution from 07/00 to 06/04 with the matching biopsy GS and the number of positive biopsy cores. All biopsies were performed using a standard ten-core biopsy protocol.

Results: In 98.1% of the cases the agreement was within ± 1 grade (exact agreement in 63.3%) as shown in the table.

RP GS - Biopsy GS	1 Core %	2 Cores %	3 Cores %	4+ Cores %	Total %
-2	0.0	0.0	0.0	0.3	0.1
-1	5.9	9.5	8.1	7.2	7.5
0	70.2	61.4	58.8	60.3	63.3
1	23.5	28.6	30.9	28.3	27.3
2	0.4	0.5	2.2	3.8	1.8

Biopsy GS was higher from the GS on RP in 7.5% and lower in 27.3% of cases. Neither the level of exact agreement nor overall agreement between GS on biopsy and RP was significantly altered by the number of positive cores (both NS, logistic regression and proportional odds model). Primary Gleason grade remained unchanged on RP in 89.9% of cases, and secondary Gleason grade remained unchanged on RP in 61.8%. Exact agreement of individual scores GS 6, GS 7 and GS≥8 on biopsy and RP was 58.9%, 75.6%, and 47.6%, respectively. The most common discordance in the final GS occurred due to upgrading GS 6 on biopsy to GS 7 on RP (McNemar's χ^2, df=1, p value <0.0001), which correlated with the number of positive cores in patients with GS 6 (1 core: 25.4% vs. 4+ cores: 51%; χ^2 with 3df, p value <0.001). In 92.5% of cases, upgrading of GS 6 on biopsy to GS 7 on RP, was due to changed secondary grade from 3 to 4.

Conclusions: 1). The improved exact and ±1 grade GS agreement on biopsy and RP is not only due to the increased biopsy core sampling, but it is also due to the improved pathologist education, experience, type of practice and the prevalence of cancer seen currently. 2.) The number of positive biopsy cores does not significantly affect the prediction accuracy and the changes of the final GS. 3). Most of the GS discrepancies on biopsy and RP result from GS 6 upgrade to GS 7 due to change of the secondary grade from 3 to 4.

652 No Residual Cancer on Radical Prostatectomy after Positive Ten-Core Biopsy: Incidence, DNA Identity Analysis and Biopsy Findings

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Background: No residual prostate cancer (pCa) or "vanishing cancer" on radical prostatectomy (RP) is a well established finding after a positive transurethral resection or six-core prostate biopsy. It is uncertain whether the more extensive PSA testing and extended biopsy schemes performed currently, that result in detecting smaller pCa volumes, will also increase the incidence of vanishing pCa on subsequent RP.

Design: We identified 11 RPs with no residual pCa in 1095 RPs performed in our institution since 06/00. All positive biopsies were reviewed to confirm pCa and clinical data were retrieved from our institutional database. All RPs were sampled completely and were reviewed by two pathologists before the sign-out. Since 2003, when no residual pCa was found on RP, we have performed PCR testing on formalin-fixed tissue to confirm the identity of the biopsy and the RP. The amplified microsatellite markers were: D1S226, D1S2734, D1S209, D1S468, D19S926, D19S208, and D19S223.

Results: The incidence of no residual pCa on RP was 1%. In 6 cases tested for DNA identity, biopsy and RP samples produced identical allele patterns with no microsatellite instability. Mean patient age was 61.7 years (range 53-73). Mean preoperative PSA was 6.95 ng/ml (range 0.7-17.2) and mean PSA density was 0.12 (range 0.02-0.30). Prostate glands weighed 48 g (mean; range 22-77) and mean gland volume was 55 cc (range 29.5-85.3). Five patients had more than one biopsy prior to RP and all patients had high-grade PIN in the negative preoperative biopsies. Two patients had preoperative hormonal treatment. Positive biopsies contained pCa only in one or two cores: one core in 7 patients and two cores in 4 patients (2 each, unilateral and bilateral). Total pCa volume on biopsy measured 1.8% (2.7 mm; mean) and was ≤1% (≤1.5 mm) in 8 of 11 patients. On biopsy, Gleason score 6 was found in 10 patients and one had Gleason score 8. RPs were sectioned in 7 levels (mean; range 4-13) and 30 slides (mean; range 16-59) were examined per RP.

Conclusions: 1.) The 1% incidence of no residual pCa on RP after ten-core positive biopsy is higher in comparison with the previously reported data. 2.) Vanishing pCa on RP is frequently associated with a small focus of pCa in one core, Gleason score 6 and likely indicates clinically insignificant cancer in most cases. 3.) DNA matching of biopsy and RP formalin-fixed tissue provides a useful test in establishing specimen identity and eliminates the possibility of specimen mishandling.

653 Caveolin-1 Immunohistochemistry Is Useful for Differentiating Chromophobe Renal Cell Carcinoma from Oncocytoma

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Background: Chromophobe renal cell carcinoma (ChRCC) and renal oncocytoma share several histologic features and may mimick each other on routine H+E stains. Although some studies have shown immunohistochemical staining for cytokeratin 7 (CK 7) to be useful in differentiating these two neoplasms, others found significant overlapping pattern, limiting its diagnostic value. We currently investigate the immunohistochemical staining pattern of caveolin-1 (Cav-1), a major structure protein of membrane caveolae, in ChRCC and oncocytoma and compare it to that of CK 7 to evaluate the usefulness of Cav- 1 immunohistochemistry in differentiating these two neoplasms.

Design: Twenty-one cases of ChRCC, including 14 of the classic type and 7 of the eosinophilic type, and 26 cases of oncocytoma were selected. Formalin-fixed paraffin-embedded tissue sections were subjected to immunohistochemical study using monoclonal antibodies against Cav-1 and CK 7 and Dako EnVsion System in a Dako Autostainer. Immunoreactivity was graded as follows: negative, no staining; focally postive, <20% of tumor cells stained; extensively positive, ≥20% tumor cells stained.

Results: All 21 cases (100%) of ChRCC were positive for Cav-1, 20 of which were stained extensively (≥20% tumor cells). The only case stained focally was an eosinophilic variant. In contrast, only 3 of 26 cases (12%) of oncocytoma showed focal positivity (<20% tumor cells) and 23 of 26 (88%) were negative. None of the oncocytoma was extensively positive for Cav-1. A diffuse cytoplasmic staining pattern was observed in almost all positive cases while a membranous pattern in a small number of cases. In the non-neoplastic kidney, positive staining for Cav-1 was detected in the non-glomerular blood vessels and the parietal cells of Bowman's capsules, but not in the tubular epithelium and glomerular capillaries. All 21 cases (100%) of ChRCC were also positive for CK 7, with 18 (86%) stained extensively and 3 (14%) focally. Of the 3 cases stained focally, 2 were the eosinophilic type. Of 26 cases of oncocytoma, 25 (96%) were positive for CK 7, with 7 (27%) stained extensively and 18 (69%) focally.

Conclusions: All ChRCC are immunohistochemically positive for Cav-1 while the vast majority of oncocytoma are negative, strongly suggesting that Cav-1 immunohistochemistry is useful for differentiating these two neoplasms. It is superior to that of CK 7 since there is significant overlapping staining pattern for CK 7 between ChRCC and oncocytoma.

654 Aberrant Expression of Mucin 7 and Cytokeratin 20 in Urothelial Carcinoma

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Background: Mucin 7(MUC7) is a glycoprotein synthesized by glandular epithelium at various site of the human body. Normal urothelium shows no expression of MUC7. Exfoliated urothelial carcinoma cells have been evaluated by nested reverse transcriptase polymerase chain reaction for MUC7. However, no immunohistochemical study has been performed on tissue sections, nor was the grade of urothelial carcinomas ever been correlated with MUC7 expression. CK 20 has been proposed as a new marker for urothelial carcinoma. Non-neoplastic urothelium is negative for CK 20 with the exception of umbrella cells. The number of CK 20 positive cells increases with increasing tumor grade.

Design: Formalin fixed, paraffin embedded tissue from 9 cases of non-neoplastic urothelium (biopsies of patients with tumor elsewhere were used), 25 cases of low grade urothelial carcinoma and 16 cases of high grade urothelial carcinoma were retrieved for the study. After steam heat induced antigen retrieval, paraffin sections were stained with monoclonal antibody for MUC7 (gift of Dr. Clausen, Copenhagen, Denmark), CK20 and p53 and immunoreactivity was visualized by EnVision+HRP on a DAKO autostainer. The pattern of MUC 7, CK 20 and p53 immunoreactivity were semiquantitively evaluated as negative (<1% of cells stained), focally positive (1-10% of cells stained), and positive (> 10% of cells stained).

Results: See table.

Conclusions: The expression of MUC7 increases in higher histological grades. The staining is mainly absent in non-neoplastic urothelium, while the pattern is focal in low grade tumors, changing into a mostly diffuse pattern in high grade urothelial carcinoma. The CK20 expression in non-neoplastic urothelium and urothelial carcinoma is parallel to that of MUC 7 expression. Staining for p53 is more erratic and nonpredictive. Although the expression of MUC7 alone does not correlate with the tumor grade, aberant expression of MUC7 in the bladder mucosa may serve as an additional marker in the diagnosis of urothelial neoplasia.

Diagnosis	Pattern of expression	Immunohistochemical marker		
		MUC7	CK20	P53
Non-neoplastic epithelium (9 cases)	Negative	8 (89%)	7 (78%)	5 (56%)
	Focally positive	1 (11%)	2 (22%)	3 (33%)
	Diffusely positive	-	-	1 (11%)
Low grade urothelial neoplasia (25 cases)	Negative	17(68%)	4 (16%)	16 (64%)
	Focally positive	8 (32%)	7 (28%)	7 (28%)
	Diffusely positive	-	14 (56%)	2 (8%)
High grade urothelial neoplasia (16 cases)	Negative	3 (19%)	1 (6%)	7 (44%)
	Focally positive	8 (50%)	4 (25%)	2 (12%)
	Diffusely positive	5 (31%)	11 (69%)	7 (44%)

655 Morphologic Changes in Pelvic Lymph Node Metastases of Prostatic Adenocarcinoma Following Neoadjuvant Therapy

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Background: Neoadjuvant therapies including chemotherapy (ChTX) and hormonal ablation therapy (HATX) are becoming more common in the management of prostatic adenocarcinoma (PCa). These impart distinctive morphologic changes in the lymph node metastases, which require recognition by the pathologist.

Design: We evaluated the pelvic lymph node (LN) metastases of patients with PCa who received preoperative therapy as part of ongoing clinical trials to establish the spectrum of morphologic changes associated with these therapies. Pelvic LN metastases from 27 patients with PCa were reviewed. The patients received preoperative therapy, which included HATX either alone (6 patients) or in combination with chemotherapy (ketoconazole, doxorubicin, vinblastine and estramustine - 15 patients; imatinib and docetaxel - 6 patients).

Results: The number of lymph nodes examined per case ranged from 1 to 7 with a mean of 2. The size of the metastases ranged from 1.0 to 10.0 mm, with a mean of 4.3 mm. Fibrosis of the lymph nodes with variable cellularity and vessel density was observed in 23 of 27 cases and was moderate to severe in 15. In some cases the fibrosis almost completely obliterated the lymph node, making residual tumor difficult to identify. Metastases consisted predominantly of small glands, cell clusters, or cribriform and fused glands, frequently separated by fibrous bands that imparted an organoid configuration. Varying degrees of cytoplasmic vacuolization and clearing where present in all cases with cytoplasmic clearing being prominent in half of them. Tumor nuclei rarely had prominent nucleoli; nuclear chromatin was pale. Pyknotic nuclei were readily found.

Conclusions: Prostate cancer metastases in pelvic LN following different neoadjuvant therapies exhibit a spectrum of histologic changes that may significantly differ from that of untreated carcinoma. Lack of familiarity with these changes, particularly when complete clinical information is lacking, may lead to diagnostic problems. Knowledge of the histologic alterations observed following neoadjuvant therapy will facilitate the evaluation of these specimens, particularly when extensive fibrosis is present. Immunohistochemical studies may be needed to document the presence of residual tumor. These observations may have particular implications when lymph nodes are submitted for frozen section examination.

656 Reporting of Testicular Germ Cell Tumors: Are the Clinically Relevant Data in the Pathology Report?

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Background: Reporting of the College of American Pathologists (CAP) cancer checklists has been mandated by the American College of Surgeons Commission on Cancer in accredited cancer programs. These checklists consist of "scientifically validated or regularly used data elements" that need to be reported on surgical pathology reports of cancer specimens. We sought to determine the patterns of reporting of these required data elements in testicular germ cell tumors, as mandated by the CAP cancer checklists.

Design: We reviewed 90 pathology reports from patients with testicular germ cell tumors who sought second-opinion for treatment at our referral center. These reports included 45 in-house or inside reports (dated 7/2003 to 3/2004) and 45 corresponding

reports (dated 1/2001 to 2/2004) from referring pathologists. Reporting of the following data elements (required by the CAP checklist) was evaluated: laterality, focality, tumor size, histologic type, spermatic cord margin, venous/lymphatic invasion, and components and percentages in mixed germ cell tumors (MGCT).

Results: Percentages of pathology reports with the reported data elements are listed in table 1. This group of 45 tumors included 14 seminomas and 31 mixed germ cell tumors. Focality and histologic type were mentioned in all reports. The laterality was mentioned in 100% of the referral reports, but in only 93% of inside reports. The tumor size was not reported in 7% of referral reports, and in 26% of inside reports. Status of the spermatic cord margin was not reported in 7% of referral reports and in only 2% of inside reports. Presence or absence of vascular-lymphatic invasion was not mentioned in 27% of reports (both referral and inside). 23% of referral reports lacked mentioned of the % of tumor components of MGCT, compared to only 3% of inside reports.

Conclusions: Overall, the majority of reports on testicular germ cell tumors mentioned clinically relevant data. Of the missing data elements, perhaps the ones with the greatest clinical significance, were the status of vascular-lymphatic invasion (not mentioned in 27% of both referral and inside reports), and % of tumor components of MGCT (not mentioned in 23% of referral reports and 3% of inside reports).

Percentage of pathology reports with reported data elements.

	Laterality	Focality	Size	Histologic type	SC Margin	VLI	% of MGCT Components
Inside Reports	93%	100%	64%	100%	98%	73%	97%
Referral Reports	100%	100%	93%	100%	93%	73%	77%

MGCT: Mixed germ cell tumor. SC: Spermatic cord. VLI: Vascular-lymphatic invasion

657 Gleason Score *vs* Gleason Predominant Grade 4/5 as Predictors of Progression Following Radical Prostatectomy

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Background: There are evidences showing that Gleason grade 4/5 may be a predictor of progression following radical prostatectomy (RP). The aim of this study is to compare Gleason score (<7 or ≥7) and Gleason predominant grade (<4 or 4/5) according to several clinicopathologic findings and biochemical recurrence following RP.

Design: The study population consisted of 200 consecutive patients submitted to radical prostatectomy. The surgical specimens were histologically evaluated by complete embedding and whole mount processing. Gleason score and Gleason predominant grade were compared according to the variables: preoperative PSA, tumor extent, extraprostatic extension (EPE) , seminal vesicle invasion (SVI) and biochemical recurrence. Tumor extent was evaluated by a point count semiquantitative method. The data were analyzed using the Mann-Whitney test for comparison of independent samples and Fisher's exact test for evaluating differences between proportions. Time to PSA recurrence was compared between the groups using the log-rank survivorship analysis.

Results: From the total of 200 patients, 74 (37%) had Gleason score <7 and 126 (63%) Gleason score ≥7; 169 (84.5%) had predominant grade <4 and 31 (15.5%) predominant grade 4/5. Patients with Gleason score ≥7 were more likely to have higher preoperative PSA (p=.0062), more extensive tumors (p<.0001), EPE (p<.0001), and SVI (p<.0001). Patients with Gleason predominant grade 4/5 were more likely to have higher preoperative PSA (p=.0178), more extensive tumors (p<.0001), EPE (p<.0001) and SVI (p<.0001). The mean and median follow-up periods among men without biochemical recurrence were 3.5 and 3.2 years, respectively. During this time, 44 patients (22%) developed a biochemical recurrence. Log-rank analysis revealed no difference in PSA recurrence in patients with Gleason score <7 *vs* ≥7 (p=.1500). However, there was a tendency for shorter time in PSA recurrence in patients with Gleason predominant grade 4/5 compared to those with Gleason grade <4 (p=.0918).

Conclusions: In our study patients with Gleason score ≥7 or Gleason predominant grade 4/5 were more likely to have higher preoperative PSA, more extensive tumors, extraprostatic extension and seminal vesicle invasion. However, only patients with Gleason predominant grade 4/5 had a tendency for a shorter time of biochemical recurrence following radical prostatectomy.

658 Expression of Mucin Antigens in Renal Tumors

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Background: Mucins are large glycoproteins expressed by epithelial cells. The function of mucins in cell adhesion, epithelial integrity and signaling occurs through an association with two other proteins, beta-catenin and E-cadherin. Aberrant expression of mucins has been implicated in epithelial tumors. To enhance our understanding of the role of mucins in renal neoplasia, we studied the immunoprofile of mucins, beta-catenin and E-cadherin in different histologic types of renal tumors.

Design: Seventy three cases of renal cell carcinoma (RCC) (including 42 cases of conventional clear cell type, 24 chromophobe, and 7 papillary), 15 oncocytoma, 7 urothelial carcinoma (UC) and 21 normal kidney tissue from patients who underwent nephrectomy were combined into a tissue microarray (TMA). Immunohistochemistry was performed on TMA slides using antibodies to MUC1, MUC2, MUC5, MUC6, E-cadherin and beta-catenin. Cytoplasmic and membranous immunoreactivity was scored for each antibody.

Results: Cytoplasmic expression of MUC-1 and E-cadherin was observed in epithelial cells of renal tubules in all 21 non-neoplastic kidney tissues. In renal tumors, immunoreactivity for MUC-1 and E-cadherin was observed respectively in 93.3 % (14/15) and 100% (15/15) of oncocytoma, 87.5% (21/24) and 91.7% (22/24) of chromophobe RCC, 59.5% (25/42) and 14.3% (6/42) of clear cell RCC, 71.4% (5/7) and 42.9% (3/7) of papillary RCC, and 71.4% (5/7) and 100% (7/7) of UC. Immunoreactivity for MUC-2, -5, and -6 was not observed in any of the cases while beta-catenin was expressed in all renal tissue including all tumors.

Conclusions: Among the four studied mucins, only MUC-1 is expressed in renal tissue. In renal tumors, the expression of MUC1 is suppressed. This suppression frequently occurs in histologic types of renal tumors with greater malignant potential, specifically conventional clear cell RCC and papillary RCC with the exception of UC probably due to the different histological origin of UC. Loss of MUC1 expression rarely occurs in chromophobe RCC and oncocytoma. Loss of E-cadherin expression is also observed in renal tumors in a similar pattern to MUC1 but at a higher frequency. These results suggest differential roles of MUC1 and E-cadherin in renal neoplasia according to histologic type, and support further investigation of use of these markers in prognostic evaluation.

659 Decreased Expression of PRMT5 Is Associated with Prostate Cancer

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Background: Protein arginine methyltransferase 5 (PRMT5) is an enzyme responsible for methylation of arginine, an irreversible post translational modification. This common modification of protein is implicated in protein trafficking, signal transduction, and transcriptional regulation. To understand the role of PRMT in prostate cancer, we studied the expression of PRMT5 mRNA in prostate cancer by in-situ hybridization and immunohistochemistry.

Design: In situ hybridization and immunohistochemistry (IHC) were performed on paraffin embedded archival tissues from 21 prostectomy specimens. PRMT5 RNA probes were made from the cDNA IMAGE clones using a non radioactive Digoxigenin-RNA labeling kit. IHC was performed using polyclonal PRMT5 antibody (1:500) on a Benchmark immunostainer. The stain signals of neoplastic cells were compared with adjacent non-neoplastic cells in the same section.

Results: Among the 21 studied cases, a decrease of mRNA expression in 11 (52%) cases, no changes in 10 cases. IHC showed a strong nuclear staining in benign prostate glands. In those cases with a decreased expression of PRMT5 mRNA, IHC demonstrate a translocation of PRMT from nucleus to cytoplasm.

Conclusions: Our studies demonstrate decreased expression of PRMT5 mRNA in 52% of prostate cancer cases studied. In intro studies have shown that PRMT5 selectively blocks oncogenic ras-p21 mitogenic signal transduction, and that forced expression of PRMT5 repressed cyclin E1 promoter activity and cellular proliferation. Therefore, PRMT may function as a tumor suppressor gene. Decreased expression of PRMT 5 may contribute to accelerated cell proliferation in prostate cancers, suggests of an important role of PRMT and protein methylation in prostate cancer.

660 Correlation of Prostate Biopsy and Three Time Point Dynamic Contrast Enhanced MRI (3TPMRI) in Men at High Risk for Prostate Cancer (PCa)

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Background: Men with elevated PSA (>4 g/l) or high grade prostatic intraepithelial neoplasia (HGPIN) are considered to be at risk for subsequent detection of a PCa and often need repeated transrectal ultrasound biopsy (TRUS bx). The purpose of this investigation was to correlate the results of biopsies performed in 13 regions of the prostate with corresponding images using 3TPMRI to determine whether this imaging modality would improve specificity for the diagnosis of PCa.

Design: In this prospective study, 26 patients with prior negative TRUSBx with either elevated PSA or prior diagnosis of PIN underwent MRI prior to repeat TRUSBx. 3TPMRI was performed with an endorectal coil (MedRad, Indianola, PA) using a 1.5T system (GEMS, Milwaukee, WI). Axial and coronal T2 weighted images (FSE T2, 3/0mm, 256x192, 14-16 cm FOV, TE 90-102 ms), and axial dynamic T1 contrast enhanced images were performed (3DFSPGR, 256 x 192, 3/0mm, TR/TE 11.1/4.2 ms, flip angle 20 deg, gadodiamide [Omniscan, Amersham, Princeton, NJ], 0.01 mg/kg @ 4 ml/s, 3 time points from 9 phases). Dynamic images were analyzed pixel by pixel with a pharmacokinetic model using 3TP software (3TP LLC, White Plains, NY). Parametric maps were superimposed on the T2 weighted images to identify cancer. Systematic TRUSBx was performed in 13 regions of the prostate, 11 of which were located in the peripheral zone. The biopsies were cut at 6 levels and examined with H and E and with p63 and alpha-methyl Co-A racemase (AMACR) in selected cases. On MRI, each zone was graded for the presence of cancer for the T2 images and then 3TPMRI. Only the peripheral zone was considered for analysis.

Results: An MRI grade of 3 or greater was used as the marker for PCa. In 6 patients, PCa was detected by biopsy and in each case the tumor was also detected by MRI. 3TPMRI was able to correctly localize the tumor focus only to the corresponding right or left side of the prostate. 7 cases considered to be malignant by 3TPMRI did not contain tumor histologically.

Conclusions: In this study 3TPMRI was highly sensitive but not specific for the diagnosis of PCa.

661 p63/AMACR Immunohistochemical Antibody Cocktail Staining of De-Stained Prostate Needle Biopsy Tissue Sections

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Background: We have previously shown that staining prostate needle biopsies with a p63/AMACR antibody cocktail produces results equivalent to those using each antibody individually, a finding that can be utilized when only limited tissue is available for immunohistochemical evaluation of small, diagnostically difficult foci in

prostate needle biopsy tissue. However, one occasionally encounters suspicious foci that are present on only one or two H and E-stained slides that are lost on further sectioning. The aim of this study was to assess the utility of using a p63/AMACR antibody cocktail on de-stained H and E-stained sections.

Design: H and E-stained sections (~ 1 year old) of 61 prostate needle biopsies (randomly selected from a prior retrospective study) were soaked in xylene to remove the coverslips and transferred to charged slides using a comercially available tissue transfer mounting media. Slides were then de-stained in acid alcohol, and subsequently stained with a p63/AMACR immunohistochemical antibody cocktail. The intensities of p63 and AMACR staining were graded semiquantitatively and compared with those obtained with the same antibody cocktail performed using standard sectioning and immunohistochemical methodologies.

Results: There was a substantial level of agreement between the AMACR staining intensity of foci of prostatic carcinoma in de-stained sections and those obtained by standard methodologies (kappa = 0.61; P < 0.0001), with a similar staining intensity in 55 cases (90%), and a decreased intensity in the remainder, including 4 cases (7%) that were completely negative. However, in only 11 cases (19%) was the p63 staining intensity of basal cells in de-stained sections similar to that obtained by standard methods, with a markedly decreased intensity in 50 cases (81%), including 18 (29%) that were completely negative.

Conclusions: Despite prolonged storage and xylene soaking, AMACR immunostaining of de-stained H and E-stained sections is reliable, and is almost equivalent to that performed by standard methodologies. In contrast, p63 immunostaining of such sections appears to be unreliable.

662 Loss of Tumor Suppressor Gene Expression in Normal Prostate Tissue Adjacent to Cancer

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Background: A field defect is postulated to explain the frequent finding that prostate adenocarcinoma is multifocal, and that different areas of tumor within a single patient may exhibit distinct losses or gains in DNA copy number. To gain insight into the molecular nature of the field defect in prostate cancer, we used cDNA expression arrays comprised of 19,700 independent genes to compare morphologically normal prostate tissue from organ donors at the time of accidental death to morphologically normal prostate tissue adjacent to tumors in men with prostate cancer.

Design: Organ donor normal prostate RNA from 12 men, and RNA from an additional 12 men with morphologically normal prostate tissue adjacent to adenocarinoma was hybridized to 19,700 cDNA microarrays. Statistical Analysis of Microarrays (SAM) analysis was used to identify genes with significant under/over-expression in the two patient groups.

Results: We obtained both microarray evidence for specific loss of tumor suppressor genes in the morphologically normal tissue of men with prostate cancer. Among the genes downregulated in this tissue were GAS1 (Growth Arrest Specific 1), a protein known to play a role as a tumor suppressor in Wnt pathway, ALKBH (Alk B Homolog), a protein which plays a critical role in the repair of methylation damage to DNA, and several others.

Conclusions: It is feasible to use gene expression profiling to identify molecular differences between morphologically normal prostate tissue in men with and without prostate cancer. Further work is now indicated to clarify whether the molecular changes observed are the result of aging and senescence of prostate tissue, or whether they confer a predisposition to neoplasia.

663 Evaluation of Immunohistochemical Expression of NB-1, as a Marker for Neoplastic Transformation in the Prostate

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Background: Dendritic cells, their associated immunological mechanisms, and their role in prostate carcinoma has been an area of recent research interest. The process of antigen presentation involves multiple steps. NB1 is part of the proteosomal pathway of antigen processing and presentation.

Design: We evaluated immunohistochemical expression of NB1 in prostatic tissues. A polyclonal antibody to NB1 was used, optimizing results using a Dako CSA 2 kit and antigen retrieval method. A four-slide tissue microarray set was used to stain 800 tissue samples from 146 patients. The TMA set had 352 samples of PRCA, 64 metastatic prostate cancer (28 lymph node (LN) metastases and 36 non-LN metastases), 92 PIN, 88 benign prostatic hypertrophy (BPH), 96 normal adjacent to tumor (NAT) and 64 samples of "true normal" donor prostate. Cytoplasmic staining of glandular epithelium was considered positive. A single observer graded staining intensity on a scale of 0-3 (0: no staining, 1, 2 and 3: cytoplasmic staining seen at 20 X, 10 X, and 4 X respectively).

Results: Assessment of staining demonstrated significantly higher expression in and foci of PIN, prostate carcinoma and metastatic prostate carcinoma. The mean levels of expression were: PRCA: 2.22, PIN: 2.07, metastatic PRCA: 2.07, NAT: 1.45, BPH: 0.99, and donors: 0.96. Statistical analysis demonstrated significant up regulation of NB1 in prostate carcinoma as compared to BPH ($p < 0.0001$), NAT ($p < 0.0001$), and donor prostate ($p < 0.0001$). PIN also showed statistically significant up regulation of NB1 as compared to NAT ($p < 0.001$).

Conclusions: Conclusions: 1. Up-regulated levels of NB1 could be a marker of neoplastic transformation in the prostate. 2. No significant difference in NB1 expression was seen between PIN, PRCA and metastatic PRCA. 3. Further studies will help delineate the precise role of NB1 in PRCA.

NB1 Staining Intensity by Tissue Type

Tissue Type	Mean
Tumor	2.22
PIN	2.07
NAT	1.45
METS	2.07
Donor	0.96
BPH	0.99

664 Needle Core Biopsy of the Kidney for Mass Lesions: A Clinicopathologic Perspective Based on 196 Cases

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Background: Renal needle biopsies (RNB) are being used more frequently in energy-based tumor ablation methods (e.g., cryotherapy) and are often the only procedure to document histologic features of the lesion. Some other indications include locally or systemically advanced renal cell carcinoma (RCC) in patients who may be candidates for adjuvant therapy, widespread metastatic disease and lymphoma. The utility, problems and pitfalls of RNB interpretation from a pathologist's perspective have not been reported.

Design: 196 cases from 1999-2004 were evaluated from 3 large academic medical centers to determine the scope of information that could be determined by pathologic examination.

Results: The mean size of the lesions was 3.9 cm (1-12cm). Cases were categorized in 6 groups based on histologic pattern: Category 1 - **clear cell lesions** [clear cell RCC (CL-RCC), chromophobe RCC (CH-RCC), xanthomatous inflammation]; Category 2 - **oncocytic lesions** [renal oncocytic neoplasm (RON), either favor oncocytoma or cannot exclude CH-RCC, CH-RCC, CL-RCC with predominant granular cytoplasm, unclassified RCC, epithelioid angiomyolipoma (AML) and hepatic and adrenal tissue/lesions]; Category 3 - **tubulopapillary lesions** [papillary RCC (PRCC), mucinous tubular and spindle cell carcinoma (Ca)]; Category 4 - **spindle cell proliferations** (sarcomatoid RCC, reactive processes, predominantly spindled AML); Category 5 -**high-grade infiltrating Ca** (urothelial Ca, metastatic Ca, collecting duct carcinoma, unclassified Ca); and Category 6 - **other** (lymphoma, classic AML, etc.). CL-RCC (37%) was most common, followed by RON (9%) and PRCC (8%). 8% of the cases were non-diagnostic, 14% of the cases had immunohistochemical (IHC) workup as part of routine diagnostic evaluation, and 8% were unclassified Ca. Category 5 was the most problematic, requiring frequent immunostains and clinical correlation.

Conclusions: 1) A wide spectrum of renal neoplasms is encountered and diagnosable by RNB. 2) RNB pathologic evaluation yields diagnostic information sufficient for stratification of patients for clinical management in the vast majority of cases. 3) A pattern-based approach into the above mentioned 6 categories would facilitate approach and provide a diagnostic menu for cases encountered in routine clinical practice. 4) Clinical correlation and contemporary IHC workup is often essential in unclassified cases and would allow for more accurate categorization.

665 Differences in Clinical Outcome between Primary Gleason Grades 3 and 4: An Analysis of 228 Patients with a Pathological Gleason Score 7

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Background: In radical prostatectomy specimens, Gleason score 7 is among the most commonly assigned scores to prostate carcinoma accounting for 30-50% of the cases. Gleason score 7 is different from other more differentiated prostate carcinomas (tumors of Gleason scores 5 and 6), with a significantly worse outcome and higher rate of recurrence.

Design: Five hundred and four patients underwent radical prostatectomy for prostate cancer. Two hundred and twenty-eight of the patients (45%) had a Gleason score of 7. Cases were analyzed for a variety of clinical and pathologic parameters.

Results: Among 228 prostatic adenocarcinomas with Gleason score 7, 91(40%) had a primary Gleason grade of 4 and 137 (60%) had a primary grade of 3. Patients of the former group were more likely to have a higher pathological stage (P=.004), a higher rate of PSA recurrence (P=.008), and a higher incidence of vascular invasion (P=.039). In multiple logistic regression controlling for tumor stage (P = .046), surgical margin status (P = .0003), vascular invasion (P=.033), and preoperative PSA (P=.015), the primary Gleason grade was not an independent predictor of PSA recurrence (P=.141).

Conclusions: Among patients with Gleason score 7, primary Gleason grade 4 carries the likelihood of higher tumor stage, higher rate of PSA recurrence and higher incidence of vascular invasion. It does not however independently predict a worse outcome after controlling for other known prognostic parameters that are associated with disease progression.

666 Small Glandular Proliferations on Needle Biopsies: Most Common Benign Mimickers of Prostatic Adenocarcinoma Sent in for Expert Second Opinion

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Background: The current study aimed to determine the incidence of various benign mimickers of prostatic adenocarcinoma most commonly encountered in a busy consultation practice.

Design: All prostate needle biopsies from the consult service of one of the authors were prospectively evaluated over a 7-month period. Only cases with foci where the contributor questioned malignancy and which upon expert review the entire case was determined to be benign were included in this study. 567 separate suspected atypical foci from 345 patients out of a total of 4046 patients (8.5 %) received in consultation

were identified. The mean age was 61.5 years (range 33-88). Of these, 281 foci (49.5 %) had also immunohistochemical (IHC) studies available for evaluation: High molecular weight cytokeratin (HMWCK) (n=280), AMACR (P504s) (n=45), and p63 (n=34).

Results: The table below shows the incidence of mimickers.

MIMICKERS	N (%)
Partial atrophy	203 (35.8)
Crowded	146 (25.7)
Benign (not otherwise specified)	56 (9.8)
Complete atrophy	55 (9.7)
Radiation atypia	32 (5.6)
Inflammation	26 (4.6)
Adenosis	21 (3.7)
Basal cell hyperplasia	14 (2.5)
Miscellaneous	14 (2.5)

The most common mimicker was partial atrophy. Technically adequate IHC for basal cells was performed in 117 cases of partial atrophy with patchy or patchy/ negative staining seen in 102/117 (87%), with remaining 13% cases completely negative. 15/ 19 (79%) cases of partial atrophy were positive with AMACR. Crowded glands, insufficiently crowded or numerous to warrant a diagnosis of adenosis, was the 2nd most common mimicker. Crowded glands had patchy or patchy/negative IHC for basal cells in 66/81 (81%) cases with remaining 19% cases completely negative. 7/11 (64%) cases of crowded glands were positive for AMACR.

Conclusions: In the past, complete atrophy, adenosis, seminal vesicle, and granulomatous prostatitis were considered the most common mimickers of prostate cancer on prostatic needle biopsies. Our study shows that currently partial atrophy and crowded glands are the most common benign changes causing diagnostic difficulty. Negative or patchy staining for basal cells and positive staining for AMACR may contribute to diagnostic difficulty in these entities.

667 Specialized Stromal Tumors of the Prostate: A Clinicopathologic Study of 44 Cases

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Background: The prognosis of specialized stromal tumors of the prostate is unlcear, some proposing that stromal tumors of uncertain malignant potential (STUMP) are variants of BPH.

Design: 44 cases of STUMPs and stromal sarcomas were studied from the consult files of one of the authors.

Results: Age: Mean: 59 years (29-83). [underline]Presenting signs/symptoms: urinary obstructive symptoms (n=21); abnormal DRE (n=14); hematuria (n=6); elevated PSA (n=6). Histology: **32** pure STUMPs: 14 with stroma showing degenerative appearing giant cells between benign prostate glands; 7 with extensive myxoid stroma w/o glands; 3 resembling glandular-stromal BPH yet with hypercellular stroma; 1 with phyllodes pattern; and 7 with mixed patterns. **8** pure sarcomas: 4 low grade (LG); 4 high grade (HG). **4** STUMPS associated with sarcoma. Histological subtypes of STUMP did not correlate with behavior. Location: Where determinable, 10 STUMPs peripheral zone (3 cases adherent to rectum), 5 transition zone, and 1 both. Follow-up: **9** cases lost to follow-up. **11** STUMPs with radical prostatectomy (RP) soon after diagnosis: 9 organ confined (OC) (mean 3.8 cm.; range; 1.2-7.5 cm.) & 2 no residual tumor (h/o 28 gm. TUR; 275 gm. enucleation). **9** STUMPs no progression following biopsy (7) or enucleation (2) with mean follow-up of 4.2 years (1.3-6 years). **5** STUMPs local tumor growth: 1 case 6 to 7.5 cm in 3 yrs. & 4 cases multiple TURs (n=2, 3, 3, 3) in 1.1, 2, 7, and 8 yrs., respectively. **10** cases with sarcoma (table).

CP= cystoprostatectomy; EPE=extra-prostatic extension

Dx. Mode	Diagnosis (Dx.)	Tx.	Follow-up
N,TUR	STUMP→HG sarcoma over 9 years	CP	18 cm HG sarcoma; dead with mets. to abdomen
ENUC	Combined STUMP/ LG sarcoma		986 gm.; SV invasion
N	Combined STUMP/ LG sarcoma	CP	2.5 cm.; OC
N	Combined STUMP/ HG sarcoma	CP	1.5 & 2 cm. nodules; OC
TUR	LG (low grade) sarcoma	RP	4 cm.; peri-SV invasion; NED at 3 yrs.
TUR	LG sarcoma	RP	4 mm.; OC; NED at 2 yrs.
TUR	LG sarcoma	RP	2.5 cm.; OC; NED at 2 yrs.
TUR X 6	LG sarcoma→HG sarcoma over 12 yrs.	RP	18 cm.; invade bladder and peri-SV; mets. to bone 3, 4, 8 yrs. post-RP
TUR	HG sarcoma	RP	4 cm.: OC; mets. to lung at 9 mos.
TUR	HG sarcoma	RP	7.5 cm.; EPE; vascular invasion

Conclusions: LG sarcoma can locally invade, while HG sarcoma has the potential to metastasize. In contrast to BPH, some STUMPS can recur frequently, occur at a young age, predominantly involve the peripheral zone where they can be stuck to the rectum requiring its removal, and can dedifferentiate to stromal sarcoma. Although STUMPs can be histologically misdiagnosed as BPH, it is important to recognize that these are neoplasms with unique local morbidity and malignant potential.

668 Risk of Prostate Cancer on Re-Biopsy Following a Diagnosis of High-Grade Prostatic Intraepithelial Neoplasia (HGPIN) Is Related to the Number of Cores Sampled

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Background: We aimed to determine whether the extent of needle biopsy sampling both on the initial biopsy that showed HGPIN and on re-biopsy would influence the detection rate of cancer.

Design: 4,237 patients with an initial diagnosis of only HGPIN on needle biopsy were identified; patients who in addition to HGPIN had a focus of atypical glands, suspicious for cancer were excluded. Of these, 937 patients had at least one follow up biopsy and were the subject of this study. The mean age was 67.5 (range from 39 to 87 years). The mean interval from diagnosis of HGPIN to rebiopsy was 4.8 months. In the initial biopsy resulting in a diagnosis of HGPIN, 371 men had ≥8 cores (median 10; range 8-26) and 399 men had 6 core sampling.

Results: Not taking into account the number of cores on rebiopsy, in the 6 core initial sampling group, the risk of cancer on rebiopsy was 22.1% versus 15.1% in the ≥8 core group (p value = 0.013). The table shows the combined influence of numbers of cores in the initial and rebiopsy sampling.

Group	# Cores 1st Biopsy	# Cores Rebiopsy	Risk of Cancer
1	6	6	29/173 (16.8%)
2	6	≥8	26/83 (32.4%)
3	≥8	≥8	44/285 (15.4%)

The differences between groups 1 and 3 as compared to group 2 were statistically significant (p=0.001 and p<0.0001, respectively).

Conclusions: Many cases of HGPIN on biopsy are associated with adjacent unsampled cancer. With relatively poor sampling (6 cores) on the initial biopsy, associated cancers are missed resulting in only HGPIN on biopsy, and with relatively poor sampling on rebiopsy there is also a relatively low risk of finding cancer on rebiopsy. With poor sampling on the initial biopsy and better sampling on rebiopsy, some of these missed cancers are detected on rebiopsy yielding a higher detection of cancer. Sampling more extensively on the initial biopsy detects many associated cancers, such that when only HGPIN is found they often represent isolated HGPIN; rebiopsy even with good sampling does not detect many additional cancers. Our study demonstrates that the risk of cancer following a diagnosis of HGPIN (15.1%) is not that predictive of cancer on rebiopsy if good sampling (≥8 cores) is initially performed. Routine rebiopsy of men with HGPIN may not be necessary in the modern era of more extensive needle biopsy sampling.

669 Chromophobe Renal Cell Carcinoma with Microcystic and Adenomatous Arrangement and Pigmentation

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Background: Chromophobe renal cell carcinoma (CRCC) is a well established entity with a typical morphological and molecular genetic findings. For many years we have been aware of a morphologic variety of CRCC which is not recognized as a variant of CRCC.

Design: We reviewed more than 9000 primary renal cell tumors in our files and selected 20 cases of the CRCC variant which we call chromophobe renal cell carcinoma with adenomatous and microcystic arrangement and pigmentation (CRCCAMP).

Results: Microscopically, the typical feature of the tumors was microcystic arrangement and formation of adenomatous structures. Microcystic areas were composed of smaller eosinophilic and bigger pale cells having cytological appearance typical of conventional CRCC. Cytological features of the adenomatous structures were generally different from the conventional CRCC. They had typical columnar arrangement with nuclei positioned at the base of the glandular structures and small amount of deeply eosinophilic cytoplasm often endowed with brush border facing the lumen of the glands. In addition, all tumors showed brown pigmentation. The pigmentation was located mostly extracellularly. Microscopic calcifications present in all cases formed psammoma bodies or were more extensive, amorphous in shape. Ultrastructurally, the cells showed features characteristic of CRCC: typical cytoplasmic vesicles and mitochondria having tubulovesicular, lamellar or circular cristae. Some tumor cells contained dark, variously sized electron-dense pigment granules typical of ceroid-lipofuscin. Neither melanosomes nor membrane bound neurosecretory granules were seen. Monosomy of chromosomes 1, 2, 6, 10, 13 and 21 was found in 100 %, 36 %, 91 %, 82 %, 82 %, 82 % and 64 % of cases, respectively using FISH. Follow up is known for 19/20 patients in range from 0.2-8 years (mean 2.82 years). All but one patient are well, without signs of further dissemination of the disease.

Conclusions: CRCCAMP have the same ultrastructural and molecular genetic features as the conventional type of CRCC. Because of the excellent prognosis it is important to recognize this type of CRCC with unusual histological architecture, calcification and pigmentation.

670 Oncocytic Papillary Renal Cell Carcinoma. A Clinicopathologic Study of 9 Cases

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Background: Papillary renal cell carcinoma (RCC) exhibits a wide morphological spectrum with papillae covered either by small cells arranged in a single layer or by large eosinophilic cells in a pseudostratified manner. It shows gains of chromosomes 7, 17 and loss of Y and immunostains with racemase (100%) and CK7 (60%). A group of papillary tumors composed of oncocytic cells can be found. Their clinicopathologic and chromosomal data are lacking and it is not demonstrated whether they belong to the papillary RCC group.

Design: Nine tumors composed of papillae covered by oncocytic cells with eosinophilic, granular cytoplasm and round nuclei arranged in a mixture of solid areas and true papillary structures were studied with ultrastructural (2 cases) and immunohistochemical (AE1/AE3, EMA, CAM5.2, anti-mitochondria, Mib1, racemasi, CK7, CK19) (7 cases) methods and with fluorescence in situ hybridization in paraffin sections (9 cases) using centromeric probes for chromosomes 7, 17 and Y (Vysis) diluted 1:100 with tDenHyb1 buffer (Insitus).

Results: Seven patients were males and 2 females, their age range from 40 to 78 yrs (main 64.4), the sizes from 1.7 to 7.5 cm (main 4.2) and the follow up from 1 to 12 yrs (main 6 yrs). AE1/AE3, anti-mitochondria, racemase and vimentin were expressed in 7/7, CK19

in 6/7, EMA in 5/7, CK7 in 3/7, Cam5.2 in 2/7, MIB1 was positive from 0 to 6 cells for high power field. Ultrastructurally, the cytoplasm of the neoplastic cells was filled by mitochondria with lamellar cristae. Three or more signals were frequent in 8/9 neoplasms: chromosome 7 in 7/9 and chromosome 17 in 6/9; 4/7 tumors showed no signal for Y in 4 /7. One tumor metastatized to brain and liver and the patient died 4 yrs after nephrectomy.
Conclusions: 1) oncocytic papillary renal cell neoplasms belong to the papillary RCC histotype; 2) their immunohistochemical profile is similar to the papillary RCC; 3) these tumours share three fluorescent signals for chromosome 7, 17 and loss of Y with papillary RCC; 4) oncocytic papillary RCC can metastatize.

671 Metastasis-Associated Gene 1 (MTA1) Is Associated with Prostate Cancer Specific Death

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Background: The metastasis-associated gene 1 (MTA1) is over expressed in various human neoplasms and is associated with cancer progression. MTA1 is a transcription repressor and also interacts with steroid receptors. In localized prostate cancer (PCA), expression of MTA1 was found to be associated with increased time to PSA recurrence.
Design: To identify the significance of MTA1 expression in PCA and its relation to PCA specific death rather than PSA recurrence, we analyzed MTA1 expression in a cohort of 193 PCA patients who did not receive treatment and were followed by a watchful waiting protocol between 1977 and 2004. MTA1 expression levels were determined by immunohistochemical detection on a tissue microarray (TMA) composed of 435 tissue samples. Protein expression was evaluated using ACISII, a semi-automated image analysis system (Chromavision, CA.). PCA proliferation was determined using Ki67. LNCaP PCA cell line was transfected with MTA1 and flow cytometric analysis was performed at several time point.
Results: Mean MTA1 expression levels ranged between 80.5-143 intensity units (IU). In a univariate analysis, MTA1 expression was significantly associated with PCA specific death (Hazard Ratio 2.8, 95% CI 1.1-7.2; p=0.032). MTA1 was also significantly associated with Gleason score (GS) ≥ 7 (odds ratio 2.4, 95% CI 1.3-4.5, p=0.006). Its ability to predict high GS was further improved when combining GS with the proliferation marker Ki67 (high MTA1 expression and high Ki67 expression, OR 4.24, 95% CI 1.7-10.4; p=0.002). Transient transfection of LNCaP cells with a plasmid coding for MTA1 and subsequent flow cytometric analysis showed an increase in S-phase transition 8 hours after release of a G1 arrest with hydroxyurea compared to controls.
Conclusions: MTA1 expression is significantly associated with higher GS and in a univariate analysis also with PCA specific death. We found experimental evidence that MTA1 expression is associated with PCA proliferation.

672 High-Throughput Analysis of Chromosomal Aberrations in Formalin-Fixed Paraffin Embedded Tissue: A Novel Approach to Define Prostate Cancer Biomarkers from Archival Material

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Background: Chromosomal aberrations such as amplification and loss of heterozygosity (LOH) are critical events in prostate cancer (PCA) progression (e.g., 10q23, 8p, 8q, 22). Combining LOH results might be a useful clinical test to help distinguish indolent from aggressive PCA. Therefore, the goal of this study is to develop methodology to perform high-throughput LOH analysis using formalin-fixed paraffin embedded (FFPE) tissue.
Design: Using known areas of LOH from published reports, we identified target single nucleotide polymorphisms (SNP) that were selected for high rates of LOH in PCA. After digestion with proteinase K, DNA from FFPE tissue was extracted and the region containing the SNP was amplified with an amplicon size of 100-200bp length. Primers for allele-specific primer extension (ASPE) were constructed with a tag sequence at the 5' end and the polymorphic base at the 3' end. Extension was performed with Tsp polymerase, a highly sequence specific enzyme used for genotyping, and incorporating biotin-labeled dCTP in the synthesized and tagged sequences. The tags were hybridized to anti-tags located on the surface of FlexMap beads (Luminex Corp., Austin, TX), then labeled with streptavidin-R-phycoerythrin and run through a Luminex 100 fluorescent analyzer (Luminex Corp., Austin, TX). By selecting multiple neighboring target sequences located on the same chromosomal region, areas of LOH and amplification can be detected by the presence or absence of alleles and relative intensity, respectively.
Results: We have successfully isolated and analyzed DNA from FFPE sample up to 11 years old. Although DNA fragments were smaller than 1kb, amplification of the target sequence areas was possible due to the small amplicon sizes. The detection of alleles present in the sample is successful and highly reproducible. Analysis of the raw data generated with the Luminex 100 analyzer performed well in the identification of chromosomal aberrations located on chromosome 8p in PCA lymph node metastases.
Conclusions: We have developed a method to evaluate LOH and amplifications in a high-throughput manner from archival FFPE tissue samples. This technique provides significantly higher resolution than currently available with FISH and is significantly easier to multiplex than using standard microsatellite markers.

673 Claudin-7 Expression in Renal Epithelial Neoplasms: A Candidate Immunohistochemical Marker for Chromophobe Renal Cell Carcinoma Identified by Gene Expression Profiling

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Background: Claudin-7 (CLDN7) codes for a tight junction protein expressed normally in distal nephron epithelium. In a recent cDNA microarray study of renal epithelial tumors, we identified CLDN7 as a candidate marker to distinguish chromophobe renal cell carcinoma (RCC) from clear cell RCC and renal oncocytoma. While the microscopic distinction of these lesions can be difficult, the differential diagnosis is clinically important because clear cell RCC tends to be more aggressive than chromophobe RCC and may respond differently to systemic immunotherapy, while renal oncocytoma is a benign neoplasm.
Design: Based on differential expression cDNA microarray data for CLDN7 among different renal tumors, this gene product was selected for immunohistochemical studies using a monoclonal anti-CLDN7 antibody (Zymed Corporation 1:400 dilution) and a fixed tissue microarray that contained triplicate specimens each from 6 chromophobe RCC, 6 renal oncocytomas, 19 papillary RCC and 33 clear cell RCC. Steam antigen retrieval was performed prior to immunostaining. Reactions were developed using HRP labeled polymer conjugate secondary antibody (Envision, Dako Corporation). Specificity was verified by negative control reactions without primary antibody as well as appropriate membranous staining reactions in positive colorectal carcinoma control tissues.
Results: Membranous CLDN7 protein expression was detected in 67% of chromophobe RCC, compared to 33% of oncocytomas, 0% of clear cell RCC and 21% of papillary RCC ($p < 0.001$, chromophobe RCC versus clear cell RCC; $p < 0.001$, chromophobe RCC versus all other tumors; Chi-square analysis).
Conclusions: We performed a high-throughput screen for gene products expressed differentially in renal epithelial tumors and identified CLDN7 as a candidate marker to distinguish chromophobe RCC from other tumor subtypes. Overexpression of this distal nephron marker in chromophobe versus clear cell RCC is consistent with histogenetic models of renal carcinomas relating chromophobe RCC to distal nephron intercalated cells and clear cell RCC to proximal nephron epithelium. CLDN7 was detected more frequently in chromophobe RCC than renal oncocytoma, consistent with the original cDNA microarray data. Therefore, analysis of a larger tumor cohort is warranted to determine CLDN7 utility in distinguishing these tumors in the clinical setting.

674 Prostate Cancer Lymph Node Metastases: Gleason Grading Rules

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Background: The number of men treated by surgery for localized prostate cancer (PCA) with concurrent lymph node metastasis (LN+) has decreased to under 1% due to a combination of factors including Prostate Specific Antigen (PSA) screening in the U.S. In other industrialized countries, where PSA screening is not wide spread, the incidence of LN+ is still between 10-15%. Most men with LN+ PCa receive anti-androgen treatment and some can do well despite having locally advanced disease. This study looks at pathology parameters associated with patient outcome.
Design: Of 1148 patients who underwent radical prostatectomy between 1986-2002 at our institution, 119 (10.3%) had LN+ PCA involving at least one pelvic lymph node. All LN+ patients were treated with adjuvant hormone ablation. The majority of these LN+ showed glandular growth architecture within the lymph nodes resembling primary PCA. The LN+ were assigned a Gleason pattern (GP) and evaluated for extra-nodal extension, lympho-vascular invasion (LVI), and size. PSA recurrence after surgery was used as the surrogate outcome (maximum follow-up time: 151 months, mean: 39 months).
Results: GP in the LN+ ranged from pattern 3-5, (GP3=22/119 (19%), GP=4 93/119 (78%), and GP=5 4/119 (3%)). The LN+ GP was significantly associated with the Gleason score of the primary PCA (R^2=0.42, p=0.002) and stage (UICC T-stage, R^2=0.37, p=0.024). The GP was significantly associated with the LN+ tumor area (R^2=0.34, p<0.0001) and time to PSA recurrence (Hazard Ratio 2.5, 95% Confidence Interval 1.1-5.9; p=0.038). T-stage and WHO grading of the primary PCA was also associated with risk of PSA recurrence (HR 3.8, 95% CI 1-14.7, p=0.05 and HR 1.9. 95% CI 1.1-3.25; p=0.026, respectively). After adjusting for T-stage and Gleason score of the primary PCA, GP 4/5 was significantly associated with PSA recurrence (HR 4.2, 95% CI 1.3-13.9; p=0.018). Similarly, LVI was associated with a significant increase in risk of PSA recurrence (HR 1.9, 95% CI 1-3.3; p=0.039). Interestingly, LVI and extra-nodal extension were significantly associated with each other (p=0.004).
Conclusions: The GP of LN+ are histologically similar and often indistinguishable from the primary PCA. GP is associated with tumor burden and tumor stage. In this patient cohort with locally advanced PCA, treated with adjuvant hormone ablation, GP is prognostic for PSA recurrence.

675 The Dual 5-alpha-Reductase Inhibitor Dutasteride Induces Atrophic Changes and Decreases Relative Cancer Volume in the Human Prostate

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Background: The effects of the 5α-reductase inhibitor class of drugs on cancer histopathology at radical prostatectomy have not been previously evaluated in a placebo-controlled multicenter trial. Dutasteride is a new inhibitor of Types 1 and 2 isoenzymes of 5α -reductase.

Design: We blindly analyzed prostatectomy slides from 17 men treated with dutasteride, and 18 men treated with placebo for 5-11 weeks prior to radical prostatectomy. Various histopathologic features including stroma-to-gland area estimates, in benign epithelium, high-grade prostatic intraepithelial neoplasia, and cancer were recorded and treatment effect was scored (0-6+). Digital image analysis was used to measure stroma:epithelium ratio and epithelial height as well as nuclear area in cancer.
Results: In benign epithelium, treatment caused distinctive cytoarchitectural changes of atrophy, and a decrease in epithelial height (p = 0.053). The peripheral zone showed the most marked response to treatment. In cancer tissue, tumor volume was significantly lower in the dutasteride-treated men than in placebo-treated men (mean, 15% versus 24%, respectively, p=0.025), the percent of atrophic epithelium was increased (p = 0.041), and the stroma-to-gland ratio was doubled (p = 0.046). The treatment alteration effect score was doubled (p = 0.055) and this did not correlate with an altered Gleason score.
Conclusions: After short term dutasteride treatment, benign epithelium showed involution and shrinkage in height, while prostate cancer tissue demonstrated a decrease in epithelium relative to stroma. These findings indicate that dutasteride induces significant phenotypic alterations in both the benign and the neoplastic prostate, supportive of a chemopreventive or chemoactive role.

676 Adenoid Cystic/Basal Cell Carcinoma of the Prostate Expresses HER-2/neu

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Background: Adenoid Cystic/Basal Cell Carcinoma (ACBCC) is a rare neoplasm in the prostate. Definitive therapy is warranted, since in 19 cases reported by us, 5 had extraprostatic extension and 4 were metastatic (*Am J Surg Pathol* 2003;27: 1523-9). The expression of HER-2/neu (c-erbB-2) protein expression by prostatic acinar carcinoma was infrequent in several published studies. HER-2/neu reactivity has been described in some salivary gland adenoid cystic carcinomas, but its expression was uncertain in the primary prostatic tumor of this name.
Design: Immunostaining with monoclonal antibody to HER-2/neu (DAKO, Carpinteria, CA) was performed in 14 ACBCC cases (12 from transurethral resection, 2 prostatectomy). As controls, ten acinar adenocarcinomas were also immunostained.
Results: 13/14 ACBCC cases showed strong cytoplasmic reactivity. As with cytokeratin 7, reactivity was predominantly in the adluminal, inner cells of tumor nests (in 50-100% of cells), with little or none in peripheral cells. Three of the cases were mixed ACBCC-acinar adenocarcinoma, and the acinar component of these tumors was consistently negative; rare, weak reactivity was seen in only 1/10 control pure acinar carcinomas. Benign prostatic acini showed weak focal reactivity of the basal layer but secretory cells were negative.
Conclusions: ACBCC of the prostate, a potentially aggressive tumor, expresses HER-2/neu and thus may be responsive to Herceptin (Trastuzumab) therapy, in a manner similar to breast cancer.

677 Are There Different Prognostic Patterns of High Grade PIN? A TMA Study of RACK1 Expression

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Background: RACK1 is an adaptor protein key in determining important cell signaling events. It can determine the specific function of several proteins such a protein kinase C, src or integrins, regulating cell growth and different signaling pathways, leading to carcinogenesis. The aim of this study is to analyse, using a Tissue Microarray design, the RACK1 expression in High Grade PIN -associated or not with prostatic cancer-, as well as in the normal prostate. This is, to our knowledge, the first study to evaluate the molecular expression of RACK1 in prostate.
Design: 55 radical prostatectomies with prostatic cancer and 16 cistoprostatectomies with vesical cancer and concomitant HGPIN without prostatic carcinoma were selected. The clinicopathological data included pTNM state, Gleason pattern, biochemical PSA progression and follow-up (mean 22 months). We designed TMAs to evaluate normal glands and HGPIN in all the cases as well as central and infiltrative areas in the carcinomas. In order to assure the representativity of these areas, each TMA included 3 cores of 1,2 mm by zone. Positivity was semiquantitatively scored, including intensity (from 0 to 3) and percentage of positive staining. We obtained the HScore (range from 0 to 300) for statistical analysis, using SPSS 10.0 software.
Results: RACK1 expression pattern was citoplasmatic in the epithelial cells, with minimal stromal stain around carciomatous glands. Higher expression in HGPIN than in normal tissue was found (p<0,001). However, there were no differences between HGPIN and carcinomatous areas, and neither between central areas of the tumor and infiltrative ones. No different expression was evidenced with pTNM state, PSA or Gleason pattern. Interestingly, RACK1 expression was higher in HGPIN with concomitant cancer than in patients with HGPIN alone (p=0,001). The former group also showed higher RACK1 expression in normal prostate glands (p=0,008). Basal cells, when present, showed high positivity.
Conclusions: We have found significant differences in RACK1 expression in HGPIN and in normal glands related to the presence or not of concomitant cancer. These findings suggest the involvement of RACK1 in the very early steps of malignant transformation in prostate, and support that different prognostic patterns of HGPIN can exist. Further studies have to be done to confirm whether RACK1 expression can be used as a potential marker of progression risk in HGPIN.

678 Cyclin D1 Overexpression in Renal Epithelial Tumors: A Tissue Microarray Study

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Background: Cyclins have an essential role in cell cycle control. Cyclin D is involved in the G1/S transition. The prevalence, biological meaning and prognostic impact of Cyclin D1 (CyD1) expression in renal cell carcinoma (RCC) are controversial. Some studies suggest a favorable role, but other series do not support this conclusion. This is a descriptive study of the immunohistochemical (IHC) overexpression of CyD1 in a RCC/oncocytoma tissue microarray (TMA), and its related clinicopathological variables in different tumor subtypes.
Design: A total of 138 renal epithelial tumors (104 clear cell (CCRCC), 17 papillary chromophilic (PCRCC), and 2 chromophobe carcinomas; 1 collecting duct carcinoma; 14 oncocytomas) were used to construct two different TMA blocks, 200 cores each. Consecutive sections were stained with an antibody for CyD1(DSC-6, DakoCytomation, Glostrup, Denmark). The IHC reaction was visualized with the Envision system (DakoCytomation, Glostrup, Denmark). CyD1 overexpression was defined as a 3+ intensity in more than 20% of the tumor cell nuclei.
Results: We found CyD1 overexpression in 9 (65%) of 14 oncocytomas, 23 (22%) of 104 CCRCCs, and 5 (30%) of 17 PCRCCs. From the 23 CyD1 overexpressing CCRCCs, 19 (82%) were low stage (T1-2), 17 (74%) were low grade (G1-2), and 18 (78%) were alive without disease. In the overall group of CCRCCs, 68% of the tumors were low stage, 57% were low grade, and 66% were alive without tumor. From the 5 CyD1 overexpressing PCRCCs, 4 were low stage, 1 was low grade, and all were alive without disease. In comparison, 65% of all the PCRCCs were low stage and low grade, and 70% were alive without disease.
Conclusions: 1.CyD1 is not uniformly overexpressed in all oncocytomas. 2.The proportion of CCRCCs overexpressing CyD1 is slightly lower than that of PCRCCs. 3.In both tumor types, this feature seems to be associated with more favorable prognostic variables than their respective overall groups. There is a paradoxically increased proportion of high grade cases in overexpressing PCRCC, that is not associated with higher stages or more adverse outcome. 4.CyD1 overexpression is relatively uncommon in RCC, and although it is associated with better outcome, it does not appear to be a useful tool in the prognosis of these tumors.
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679 p53 and PAb 240 Overexpression in Renal Cell Carcinoma: A Tissue Microarray Study

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Background: The prognostic impact of p53 overexpression (OE) in renal cell carcinoma (RCC) is not fully understood. In different series, with diverse immunohistochemical (IHC) and evaluation protocols, variable p53 OE is reported, although association with poor outcome is usually suggested. We investigated the IHC OE of p53 in a RCC tissue microarray (TMA), using strictly standardized methods. We also studied expression of PAb-240, an antibody (Ab) that recognizes a p53 epitope exposed in many mutant forms of this protein. Expression of this Ab in RCC has not been previously reported.
Design: 138 renal epithelial tumors (104 clear cell (CRCC), 17 papillary chromophilic (PRCC), 2 chromophobe, and 1 collecting duct carcinoma; 14 oncocytomas) were used to construct two different TMA blocks (200 cores each). Ab for p53 (DO7, Novocastra, UK), and mutated p53 (PAb 240, DakoCytomation, Denmark) were used. A standardized IHC protocol included mutated cell lines as controls. p53 OE was defined as a 3+ intensity in >20% of the nuclei. PAb 240 was investigated in p53+ and - cases.
Results: p53 OE was observed in 12% (17 of 138 tumors): 30% (5 of 17) of PRCC and 12% (12 of 104) of CRCC. From those, 2 CRCC and 2 PRCC were positive with PAb 240, and the remaining cases in the TMA were negative. From all TMA cases, CRCC were high grade in 43%, high stage in 32% and progressed in 33% of cases. In contrast, 92% (11) of the p53 overexpressing CRCCs were high grade (3 G3 and 8 G4) and 75% (9) were high stage (6 T3 and 3 T4) and died with tumor. Similarly, p53 negative PRCCs were high grade in 20%, high stage in 30% and progressed in 20% of the cases, while 80% (4) of the overexpressing PRCCs were high grade (3 G3, 1 G4) and 40% (2) were high stage (T3,T4), progressing tumors.
Conclusions: 1. In both CRCC and PRCC, p53 overexpression is associated with a marked increase in grade, stage, and tumor progression. 3. Negativity of PAb 240 does not exclude non-productive mutations, but suggests that p53 overexpression is probably not associated with mutation. 3. p53 overexpression is uncommon in RCC, and is more often observed in PRCC than in CRCC. A large number of RCCs should be examined both by IHC and molecular methods to establish the prognostic and pathogenetic role of TP53 in renal cancer. (Funded by Fondo de Investigacion Sanitaria, FIS 99/0736, Spain.)

680 OCT4 Immunoreactivity in Testicular Germ Cell Tumors: Assessment of Diagnostic Utility and Histogenetic Implications

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Background: OCT4 protein (a product of *pou5f1* gene), an octamer-binding transcription factor, is a novel marker expressed in pluripotent undifferentiated embryonic germ and stem cells. Basic research has suggested a role for OCT4 in normal development via regulation of cellular differentiation. In this study, using tissue representing normal testis and entire spectrum of germ cell neoplasia, we assessed the diagnostic utility of OCT4 and its histogenetic implications.

Design: Hematoxylin and eosin stained slides of testicular germ cell tumors (GCT) from 70 patients were reviewed to identify different histologic patterns. A total of 105 representative paraffin blocks were selected and 5 micrometer- thick sections were immunostained with polyclonal goat anti-OCT4 antibody (1:100 dilution).
Results: A total of 159 zones representing various histologic patterns including 35 normal/atrophic testes (NT), 31 intratubular germ cell neoplasia unclassified (IGCNU), 36 seminomas, 30 embryonal carcinomas (1 intratubular and 6 metastatic), 10 yolk sac tumors (YST), 13 teratomas (2 metastatic), 3 choriocarcinomas (CC) and 1 spermatocytic seminoma (SS) were identified. All components of normal testes (spermatogonia, leydig cells and sertoli cells) were entirely negative for OCT4. All IGCNU (31/31), seminomas (36/36), and embryonal carcinomas (30/30) showed diffuse nuclear reactivity for OCT4; whereas all YST (0/10), CC (0/3), teratomas (0/13) and spermatocytic seminoma (0/1) were non-reactive.
Conclusions: 1) In this study, we have established the immunoreactivity profile of OCT4 in normal testis and various components of testicular germ cell neoplasia. 2) OCT4 proves to be a very sensitive marker for pluripotent components of testicular germ cell neoplasia (IGCNU, seminoma and EC). 3) This finding and the overall OCT4 immunoprofile in testicular GCT has significant histogenetic implications. 4) In conjunction with other markers, OCT4 may prove to be a valuable diagnostic aid in the differential diagnosis of germ cell neoplasms.

681 Assessment of the Diagnostic Utility of CD117/c-kit and CD30 in the Differential Diagnosis of Seminomatous and Non-Seminomatous Testicular Germ Cell Tumors

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Background: Precise identification of seminomatous and non-seminomatous components, particularly embryonal carcinoma (EC), in testicular germ cell tumors (GCT) is crucial due to significant therapeutic and prognostic implications. CD117/c-kit, a transmembrane tyrosine kinase receptor, plays an important role in normal spermatogenesis and is a critical member of the signal transduction pathway implicated in the carcinogenesis of seminomas. CD30, a transmembrane cytokine receptor, belonging to the tumor necrosis factor superfamily, is usually preferentially expressed in EC cells. Conflicting data exists regarding CD117/c-kit immunoreactivity in non-seminomatous GCT. Our aim was to clearly establish the diagnostic utility and morphogenetic role of CD117 and CD30 in testicular germ cell neoplasia.
Design: Sections of 105 paraffin-embedded blocks from 70 patients with testicular GCT were reviewed to identify 35 normal/atrophic testis (NT), 31 intratubular germ cell neoplasia unclassified (IGCNU), 36 seminomas, 30 EC (1 intratubular and 6 metastatic), 10 yolk sac tumors (YST) 13 teratomas (2 metastatic), 3 choriocarcinomas (CC) and 1 spermatocytic seminoma (SS). Five micrometer-thick sections were immunostained with polyclonal rabbit anti-CD117 antibody (1:200 dilution) and monoclonal mouse anti-CD30 antibody (1:25 dilution).
Results: Our results are summarized in Table 1-

Table 1- Immunoprofile of CD117 and CD30 in Normal Testis and Testicular GCT

Morphology	No.of Cases	CD117 positive	CD30 positive
NT	35	0	0
IGCNU	31	31	0
Seminoma	35	36 (4 focal)	0
EC	30	0	30 (4 focal)
YST	10	0	0
Teratoma	13	0	0
CC	3	0	0
SS	1	0	0

Conclusions: With these immunoreactivity profiles, CD117 and CD30 appear to be very sensitive and specific markers and may prove to be helpful diagnostic tools in the precise identification and discrimination of various components of testicular germ cell neoplasia. The consistent and exclusive immunoreactivity of CD117 in IGCNU and seminoma implicates its histogenetic role in testicular germ cell neoplasia. In addition, the expression of CD117 in testicular seminomas may signify its therapeutic implications.

682 PAX5 Protein Expression in Bladder Tumors by Tissue Microarray Immunohistochemistry

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Background: PAX5 (B-cell lineage specific activator protein, BSAP) is a B-cell lineage specific transcription factor important in B-cell development and maturation. PAX5 expression has been reported in many B-cell lineage lymphomas, but also in non-hematolymphoid neoplasms, including human transitional cell carcinomas of the bladder. PAX5 mRNA expression in transitional cell carcinomas of the bladder has been studied by reverse transcriptase-polymerase chain reaction (RT-PCR) only. A correlation with de-differentiation has been suggested in these tumors. Detection of PAX5 protein expression in bladder tumors by immunohistochemistry has not, however, been reported in the literature.
Design: A tissue microarray containing 722 tissue cores (each measuring 0.6 mm), including 339 bladder tumors, 325 of which represent transitional cell carcinomas of varying histologic grades, was constructed in the usual fashion. Duplicate cores were included of the 339 tumors as well as 22 cases of normal bladder, placenta and non-bladder tumors as controls. Sections were then stained with a commercially available antibody to PAX5 (BD Transduction Laboratories, Lexington, KY). Staining was interpreted as weakly or strongly positive, negative or uninterpretable. Only nuclear staining was considered positive. Two pathologists (KCJ and GK) jointly scored all cases; scores were confirmed by a third pathologist (JH).
Results: Of the 339 tumor cases, only two showed positive immunostaining for PAX5 (2/339 bladder tumors, 0.59%). Both cases were transitional cell carcinomas (2/325 transitional cell carcinomas, 0.62%) that showed weak immunoreactivity. One case was poorly differentiated; the other, well-differentiated.
Conclusions: We find immunohistochemically detectable PAX5 nuclear protein expression in only a small percentage of transitional cell carcinomas of the bladder (2/325, 0.62%). This does not correlate in our cases to histologic grade or degree of de-differentiation. Immunohistochemical expression of PAX5 is not viable as a diagnostic and/or prognostic tool. Additional studies correlating PAX5 mRNA expression by RT-PCR and protein expression by immunohistochemistry are needed to clarify the role of PAX5 in bladder tumorigenesis and the relationship, if any, to tumor de-differentiation.

683 Cystic Nephroma and Mixed Epithelial and Stromal Tumor of the Kidney: A Spectrum of the Same Entity?

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Background: The recently described mixed epithelial and stromal tumor of the kidney and cystic nephroma are rare benign cystic renal neoplasms that are composed of epithelial and stromal elements. Both lesions have similar clinical and morphologic features. Consensus criteria for distinguishing these entities have not been well established. Our objective in this study was to evaluate cases of cystic nephroma and mixed epithelial and stromal tumor of the kidney to define the morphologic, immunophenotypic, and clinical features of these two entities.
Design: Ten cases diagnosed from 1996 to the present as either cystic nephroma (multilocular cyst) (8) or mixed epithelial and stromal tumor of the kidney (2) were retrieved from the Mayo Clinic files. The gross description, histologic features, and clinical data were reviewed. Immunohistochemical studies were performed on seven cases in which paraffin blocks were available.
Results: The ten patients included eight females and two males with an age range of 44 to 69 years (mean 56). The histologic findings were similar in all cases. Architecturally the lesions were composed of multiple non-communicating cysts lined by a single layer of epithelial cells. All cases had areas with increased stromal cellularity. Eight cases (including one male patient) had ovarian-like stroma present at least focally within the tumor. No stromal or epithelial cell atypia or increased mitotic activity was appreciated, and no blastema or other immature elements were present. The immunoprofile in all seven cases showed epithelial reactivity with keratin and CAM 5.2 and stromal reactivity with ER, PR, SMA, WT-1, and vimentin. The stroma also showed variable intensity and distribution of reactivity with desmin. All cases have acted in a benign fashion with no history of recurrence or metastasis.
Conclusions: We propose that cystic nephroma and mixed epithelial and stromal tumor of the kidney represent a spectrum of the same entity. If the diagnosis of cystic nephroma is limited to cases that are comprised entirely of thin, fibrous walled cysts, then all our cases would be classified as mixed epithelial and stromal tumor. Of note, all ten cases had cystic nephroma like areas. It is probable that "cystic nephroma like" morphology represents the well differentiated end of the spectrum and tumors with abundant cellular stroma and a proliferation of tubules denotes a less differentiated neoplasm at the opposite end of the spectrum. The majority of cases fall in the middle and have combined features.

684 Sensitive Detection of Small Focal Cancer on Prostate Needle Biopsy by α-Methylacyl-CoA Racemase (AMACR)/P504S Envision Immunohistochemistry

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Background: A major diagnostic challenge for surgical pathologists is establishing a definitive diagnosis of malignancy in prostate needle biopsies with very small foci of adenocarcinoma. α-methylacyl-CoA-racemase (AMACR)/P504S as a positive diagnostic marker for prostatic adenocarcinoma may enhance our ability to diagnose limited prostate cancer. However, there is variability in the reported sensitivity of this technique. Studies report sensitivities ranging from 80 to 98%. Moreover only 66-68% of atrophic and foamy gland carcinomas are reported to be positive for AMACR. It is important to increase the sensitivity and decrease the variability of the AMACR assay. The aim of this study was to evaluate the sensitivity of the EnVision system for AMACR immunohistochemistry to detect small foci of prostate cancer on needle biopsies.
Design: A total of 53 prostate needle biopsies with small foci of prostatic adenocarcinoma (≤1 mm or <5% of a core) including 7 foamy gland and 3 atrophic carcinomas were examined for AMACR expression with P504S monoclonal antibody using DAKO automated immunostainer with an EnVision System immunohistochemistry technique. The Gleason scores were 3 + 3 (6) (N = 50), 4 + 3 (7) (N = 1) and 4 + 4 (8) (N = 2).
Results: AMACR immunoreactivity was found in 53/53 biopsies (100%) of small focal carcinomas including all atrophic and foamy gland cancers with no or minimal staining in benign glands adjacent to malignant glands. The AMACR positivity was detected in >75% malignant glands in 46 (including atrophic and foamy gland carcinomas) out of 53 biopsies, and 51-75% of malignant glands in remaining biopsies. No biopsy showed less than 50% positivity of malignant glands.
Conclusions: Immunohistochemistry of AMACR/P504S with the EnVision technique provides a high sensitivity for the detection of small foci of prostatic adenocarcinoma including the foamy gland and atrophic carcinomas on needle biopsies.

685 Molecular Evidence for Independent Origin of Coexisting Tumors in Multifocal Urothelial Carcinoma of the Bladder

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Background: Human bladder carcinoma is thought to arise from a field change that affects the entire urothelium. Multifocality of urothelial carcinoma is a common

finding at radical cystectomy. Whether these coexisting tumors arise independently or are derived from the same tumor clone is uncertain.
Design: We examined 19 patients who underwent radical cystectomy for urothelial carcinoma. All patients had multiple separate foci of urothelial carcinoma (2 to 4) within the bladder. Genomic DNA samples were prepared from formalin-fixed, paraffin-embedded tissue sections using laser-capture microdissection. Loss of heterozygosity (LOH) assays for 3 microsatellite polymorphic markers on chromosome 9p21 (IFNA, D9S171), regions of putative tumor suppressor gene *p16*, and on chromosome 17p13 (TP53), the *p53* tumor suppressor gene locus, were performed. X-chromosome inactivation analysis was performed on the bladder tumors from 13 female patients.
Results: Seventeen of 19 (89%) cases showed allelic loss in one or more of the bladder tumors in at least one of the three polymorphic markers analyzed. Concordant allelic loss patterns between each coexisting bladder tumor were seen in only 2 of 19 (11%) cases. A concordant pattern of non-random X-chromosome inactivation in the multiple coexisting bladder tumors was seen in only 3 of 13 female patients. A discordant or random pattern of X-chromosome inactivation was seen in the tumors in 8 of 13 female patients. The remaining 2 cases yielded noninformative results on X-chromosome inactivation analysis.
Conclusions: LOH and X-chromosome inactivation assays show that the coexisting tumors in multifocal urothelial carcinoma have a unique clonal origin and arise from independently transformed progenitor urothelial cells, supporting the "field effect" theory for bladder carcinogenesis

686 Visual Estimation of Tumor Extent Is Not an Independent Predictor of PSA Recurrence

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Background: A number of variables such as Gleason grade and tumor stage are well-established prognostic factors in prostate cancer. It is uncertain if the visual estimation of tumor extent (percent of carcinoma) is an independent predictor for prostate cancer recurrence. We analyzed tumor extent as a predictor for biochemical recurrence after prostatectomy using multivariate analysis.
Design: Tumor extent was estimated in the radical prostatectomy specimens from a total of 504 men with clinically localized prostate cancer. Clinical followup data were available for 459 cases. Mean followup was 44.3 month (range, 1.5 month to 144 month). Multivariate analysis was performed to assess tumor extent as a predictor of PSA recurrence.
Results: Of the patients, 157(34.2%) had biochemical recurrence. Mean tumor extent was 35.8% and 24.1% in those with and without recurrence, respectively. Univariate analysis showed a significant association between the visual estimation of tumor extent and PSA recurrence, tumor stage, Gleason grade, surgical margins, extraprostatic extension, seminal vesicle invasion, perineural invasion, and preoperative serum PSA level (all $p<0.001$). However, in a multivariate logistic regression model controlling for pathologic stage, Gleason score, and surgical margin status, the visual estimation of tumor extent is no longer a significant predictor of PSA recurrence ($p=0.32$).
Conclusions: The visual estimation of tumor extent is not an independent predictor of post-prostatectomy biochemical (PSA) recurrence.

687 Molecular Evidence for the Independent Origin of Multifocal Papillary Tumors in Patients with Papillary Renal Cell Carcinoma

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Background: In patients with papillary renal cell carcinoma (RCC), it is common to find two or more distinct tumors or satellite lesions in radical nephrectomy specimens. Whether these multiple papillary lesions result from intrarenal metastasis or whether they arise independently is unknown.
Design: We examined 21 patients who underwent radical nephrectomy for renal cell carcinoma. All patients had multiple separate papillary lesions (2 to 5). Fourteen patients had multiple papillary renal cell carcinomas. Seven had papillary renal cell carcinoma with coexisting papillary adenomas. Genomic DNA samples were prepared from formalin-fixed, paraffin-embedded tissue sections using laser-capture microdissection. Loss of heterozygosity (LOH) assays for 6 microsatellite polymorphic markers for putative tumor suppressor genes on chromosomes 3p14 (D3S1285), 7q31 (D7S522), 9p21 (D9S171), 16q23 (D16S507), 17q21 (D17S1795), and 17p13 (TP53) were performed. In addition, X-chromosome inactivation analysis was performed.
Results: Twenty of 21 (95%) cases showed allelic loss in one or more of the papillary lesions in at least one of the six polymorphic markers analyzed. A concordant allelic loss pattern between each coexisting kidney tumor was seen in only 1 of 21 (5%) cases. A concordant pattern of non-random X chromosome inactivation in the multiple coexisting papillary lesions was seen in 2 of 3 female patients. A discordant pattern of X-chromosome inactivation was seen in the tumors of the other female patient.
Conclusions: Our data suggest that, unlike multifocal clear cell renal cell carcinomas, the multiple tumors in patients with papillary RCC arise independently. Thus, intrarenal metastasis does not appear to play an important role in the spread of papillary renal cell carcinoma.

688 Increased Stat3 and Stat5 Protein Expression Correlates with Aggressive Biologic Phenotype in Prostatic Adenocarcinomas (PACs)

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Background: Signal transducers and activators of transcription (Stats) have been demonstrated to regulate tumor cell proliferation and survival in various cancers including those of breast, head and neck, liver and ovary. However, data on expression of these proteins in prostate cancer is limited with few studies showing increased levels of constitutive Stat3 activation in prostate cancer cell lines and limited numbers of prostate tumors. In this study, we evaluate the expression patterns of Stat3 and Stat5 in human PACs.
Design: Formalin-fixed paraffin-embedded tissue sections from 137 PACs were immunostained by an automated method (Ventana Medical Systems, Tucson, AZ) using monoclonal mouse anti-human Stat3 and polyclonal rabbit anti-human Stat5 antibodies (sc-8019 and sc-836, Santa Cruz Biotechnology, Santa Cruz, CA). Nuclear and cytoplasmic immunoreactivity were each semi-quantitatively scored based on intensity and percentage of positive cells. DNA ploidy was determined on Feulgen stained tissue sections by static image analysis. Results were correlated with morphologic and prognostic variables.
Results: Variable nuclear and cytoplasmic positivity was noted for both Stat3 and Stat5 in the adjacent benign glands in all cases. Increased cytoplasmic Stat3 correlated with high tumor grade [50/60 (83%), $p=0.01$)] and aneuploidy [28/32 (88%), $p=0.02$)]. Tumors that showed both increased cytoplasmic and nuclear Stat3 expression also correlated with high tumor grade ($p=0.03$). Increased cytoplasmic positivity for Stat5 correlated with advanced stage [40/58 (69%), $p=0.002$) while increased nuclear expression correlated with both high grade [31/60 (52%), $p=0.04$)] and advanced stage [33/58 (57%), $p=0.02$)]. Tumors that showed both increased cytoplasmic and nuclear Stat5 correlated with advanced stage [27/58 (47%), $p=0.002$)] and reached near significance with high grade [24/60 (40%), $p=0.06$)]. Neither Stat3 nor Stat5 expression correlated with disease recurrence. On multivariate analysis, advanced stage ($p=0.002$) was an independent predictor of post-surgical biochemical disease recurrence.
Conclusions: These results indicate that increased expression of Stat3 and Stat5 in prostate cancer is associated with aggressive biological behavior and supports the consideration of utilizing these proteins as molecular targets for novel therapies.

689 Immunohistochemical Expression of EZH2 in Prostatic Adenocarcinomas (PACs)

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Background: EZH2 is one of the members of the Polycomb Group (PcG) genes that contribute to cell cycle regulation and oncogenesis. Data on EZH2 protein expression in human cancers is extremely limited. Some previously reported studies have suggested that EZH2 protein expression is associated with aggressive tumor phenotype in breast and prostate cancers. To further elucidate this relationship, we evaluated the expression pattern of the EZH2 protein in human PAC tissue sections.
Design: Formalin-fixed paraffin-embedded tissue sections from 137 PACs were immunostained by an automated method (Ventana Medical Systems, Tucson, AZ) using a polyclonal rabbit anti-human EZH2 antibody (Zymed, San Francisco, CA). Nuclear immunoreactivity was semi-quantitatively scored based on intensity and percentage of positive cells. DNA ploidy was determined on Feulgen stained tissue sections by static image analysis. Results were correlated with morphologic and prognostic variables.
Results: The pattern of EZH2 immunoreactivity was exclusively nuclear and was diffusely present in both the benign and neoplastic elements in all cases. Intense diffuse EZH2 expression was noted in basal and epithelial cells of normal and atrophic glands, seminal vesicles and foci of prostatic intraepithelial neoplasia. 113/137 (83%) PACs showed intense diffuse EZH2 expression. Within the same case, the intensity and distribution of EZH2 expression was similar in both the tumor and benign elements in 112/137 (82%) cases and increased in tumor over benign in 21/137 (15%) cases. In the latter cases, no correlation was found with tumor grade, advanced stage, DNA ploidy or recurrence.
Conclusions: In contrast to previous reports, in the present study of PCA, EZH2 protein expression does not appear to be different in tumor cells versus benign prostatic epithelial cells, did not associate with prognostic variables and did not predict disease outcome.

690 Intratumoral Vascularity in Transurethral Resection Predicts Bladder Carcinoma Metastasis

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Background: Of patients with muscle-invasive bladder cancer, as many as 50% may have occult metastases that usually become clinically apparent within 5 years of initial diagnosis. Most patients with overt metastatic disease die within 2 years, despite chemotherapy. Tumor metastasis has been associated with tumor angiogenesis. In this study we evaluated the significance of intratumor microvessel density (MVD) and expression of VEGF using transurethral resection (TUR) as a predictor for bladder carcinoma metastasis.
Design: Fifty-seven patients who underwent TUR between1997 to 2002 for bladder carcinoma were retrieved from the department of pathology. At the median follow up of 23.5 months (range 2-71 months), 17 developed metastasis, 33 were free of metastasis and 7 had unknown metastatic status. Pathologic stage (pT) of subsequent radical cystectomy / cystoprostatectomy was available for 29 patients (18 pT1/pT2 and 11 pT3/pT4). Tissue blocks were available for 46 cases. One representative tissue block

from each case was used for constructing tissue microarray (TMA) blocks. Each case was sampled in duplicate. Immunohistochemical studies for CD31, CD105 and VEGF were performed according to standard protocol. MVD, detected with anti-CD31 (MVD_{31}) or anti-CD105 (MVD_{105}), was determined from the highest area identified within a single X200 field.

Results: Among the three markers evaluated, only MVD_{31} was significantly associated with bladder cancer metastasis. Median MVD_{31} was 61 (range 20 to 112) and 23 (range 9 to 70) in cases with and without metastasis respectively (p=0.002). Median MVD_{105} was slightly higher in cases with metastasis (18.5 vs 13, p=0.421). VEGF was expressed in 4 of 11 (36.4%) cases with metastasis and 9 of 24 (37.5%) cases without metastasis (p=0.948). Interestingly, MVD_{105} but not MVD_{31} was significantly correlated with VEGF expression. Median MVD_{105} was 32 (range 5 to 59) in VEGF positive tumors in contrast to 13 (range 2 to 45) in VEGF negative tumors (p=0.0276). In addition, tumor metastasis was also significantly correlated with pT of radical cystectomy / cystoprostatectomy. Ten of 11 cases with pT3/pT4 and none of 18 cases with pT1/pT2 disease had metastasis at the last follow-up (p<0.001)

Conclusions: Evaluation of tumor MVD_{31} using TUR specimen is potentially useful in predicting bladder cancer metastasis. MVD_{105} is associated with VEGF expression. However, neither MVD_{105} nor VEGF expression assessed using TUR specimen can predict bladder cancer metastasis

691 Neuropeptide Y (NPY) Receptors as Pathobiological Markers for Renal Cell Carcinomas and Nephroblastomas

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Background: Numerous peptide hormone receptors, as for instance somatostatin receptors, are overexpressed in human cancers, leading to significant clinical applications in tumor imaging and therapy. Among such receptors, those for the neurotransmitter neuropeptide Y (NPY) have recently been found to be highly expressed in selected tumors such as breast and ovarian carcinomas. Knowing that NPY receptor mRNA is present in adult nephrons and in the fetal metanephrogenic mesenchyme, adult and embryonal kidney tumors were evaluated for their NPY receptor expression.

Design: Forty renal cell carcinomas (clear cell, papillary, and chromophobe types) and 18 nephroblastomas were assessed for their NPY receptor expression and/or endogeneous NPY peptide production. NPY receptor expression was investigated on fresh frozen tumor samples by *in vitro* NPY receptor autoradiography using ^{125}I-labelled peptide YY (PYY) in competition with analogs selective for the NPY receptor subtypes Y1, Y2, Y4, and Y5. Receptor density was quantitatively measured using a computer-assisted imaging program. Presence of NPY peptide was assessed immunohistochemically on formalin-fixed tumor samples.

Results: Fifty-six per cent of renal cell carcinomas expressed the NPY receptor subtype Y1 in moderate density, and 80% of nephroblastomas expressed the NPY receptor subtypes Y1 and Y2 in moderate to high density. Y1 receptors were also present in intratumoral blood vessels in high incidence and density. Using immunohistochemistry in selected tumor samples, NPY peptide was observed in nerve fibers in close association with intratumoral blood vessels and tumor cells.

Conclusions: NPY receptors on renal tumor cells and tumor blood vessels may represent a molecular target for endogenous NPY peptide released by intratumoral nerve fibers. With regard to clinical applications, these receptors may also represent *in vivo* targets for a receptor-directed imaging and therapy of renal cell carcinomas and nephroblastomas, for which alternative therapeutic approaches are still required.

692 Could p16, Retinoblastoma and p53 Immunostains Offer New Insights into Prognostic Evaluation of Patients with "Urothelial Atypia of Unknown Malignant Potential"?

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Background: Urothelial atypia of unknown significance" is a diagnostic category, which includes preneoplastic as well as reactive lesions. We examined the expression of suppressor genes in urothelial atypias, comparing them to the main categories of urothelial neoplasia, in an attempt to : 1. gain insight into the role of these genes in urothelial neoplasia; 2. try to stratify the atypias as to their malignant potential.

Design: Twenty cases diagnosed as urothelial atypia were studied: 5 of which were found to have urothelial carcinoma (TCC) 2-12 months later (mean 8 months); 3 were preceded by low grade TCC (4-24 months prior); and 12 were without such histories (1-38 month follow-up, mean 27 months). In addition, the following neoplasms were studied:56 TCC's (31 low grade and 25 high grade) and 18 urothelial carcinomas in citu (CIS's). Standard immunohistochemistry was performed for p16 (INK4A, clone16P04,Cell Marque), cyclin D1(cyD clone DCS-6,DakoCytomation), retinoblastoma (Rb clone Rb1) and p53(clone DO-7).

Results: A "normal" supressor gene profile (p16+,cyD+,Rb+,p53-) was identified in a few cases: 3 low grade TCC's, 2 high grade TCC's,1 CIS and 3 atypias. The most common suppressor gene abnormality in 58% of low grade TCC's, was p53 overexpression (with p16+,cyD+,Rb+). In high grade TCC's and CIS, it was both p16 loss and p53 overexpression (with cyD+,Rb+). The predominant pattern for urothelial atypia was p53 overexpression (with p16+,cyD+,Rb+), similar to low grade TCC, but the pattern of suppressor gene expression did not segregate those atypias, which were associated with TCC from those that were not. The 3 atypias with a "normal " phenotype were all associated with TCC: 2 preceded and 1 followed a diagnosis of TCC.

Conclusions: A majority of low grade TCC was associated with p53 overexpression as the main suppressor gene defect; and p16 loss with or without Rb abnormalities were present in addition to p53 overexpression in high grade tumors. Most atypias had suppressor gene defects, suggesting that they might be preneoplastic, or in fact the CIS stage of a low grade TCC. However the observed suppressor gene profiles did not predict the development of TCC, particularly since all three atypias with "normal" pattern were associated with TCC. These "normal" pattern atypias could have been truly reactive foci, or preneoplastic but driven by a different set of oncogenes.

693 Comparison of Utility of Polyclonal and Monoclonal Antibody to alpha Methyl Acyl Co A Racemase (AMACR) in Work-Up of Prostate Cancer

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Background: AMACR, a new biomarker for prostate cancer (PCa) is increasingly being utilized as an adjunct to morphology and basal cell markers in resolving "atypical" prostate needle biopsies (NBXs). Recent studies using both commercially available monoclonal (P504S) and polyclonal (p-AMACR) antibody have shown variable expression in PCa. The goal of this study is to compare utility of both antibodies in this clinical setting.

Design: 32 NBXs with "atypical " foci suspicious for PCa and 20 NBXs with morphologically unequivocal minute PCa (< 5%) were evaluated. All cases were stained with basal cell marker 'cocktail antibody' (34bE12 +p63), P504S (1:40, Zeta Corp, Sierra Madre, CA) and p-AMACR (1:5000, rabbit polyclonal antibody against recombinant AMACR). Staining was graded as negative, weak, moderate and strong. AMACR staining was considered positive only if staining was significantly stronger than surrounding benign glands.

Results: Of unequivocal minute PCa cases, 70% (14/20) expressed AMACR with both P504S and p-AMACR, 10% (2/20) showed expression with either P504S (1) or p-AMACR (1) alone and 20% (4/20) were negative with both. Basal marker supported the diagnosis in all cases.

In "atypical" group, 17/32 (53%) was classified as PCa, 4/32 (12 %) benign and 11/32 (35%) atypical, suspicious for PCa after review of morphology and basal markers. In "atypical" cases classified as PCa, 4/17 (23%) were negative and 9/17 (53%) were positive with both P504S and p-AMACR antibodies, and 3/17 (18%) and 1/17 (6%) with p-AMACR and P504S alone respectively. Of these PCa cases, 7/17 (41%) underwent radical prostatectomy which confirmed presence of PCa.

Of unresolved "atypical", suspicious for PCa cases, P504S/p-AMACR helped convert diagnosis to PCa in 5/11 (45%) cases. In these cases despite negative basal marker, morphology was less than optimal. Six/11(55%) cases could not be resolved. Of the 5 cases in which AMACR helped in rendering a final diagnosis of PCa, 3 underwent radical prostatectomy which confirmed cancer.

Conclusions: Differences between P504S and p-AMACR appear marginal and clinically insignificant. AMACR was negative in 20% minute PCa with both antibodies. However, as recently reported, when utilized in proper context, AMACR may offer significant advantage in converting "atypical" diagnosis to PCa cases where morphology and traditional basal markers are less than optimal in resolving the diagnosis.

694 Morphologic Subtyping of Papillary Renal Cell Carcinoma: Clinico-Pathologic and Immunohistochemical Analysis

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Background: Separation of papillary renal cell carcinoma (PRCC) into two morphologic subtypes, Type 1 and Type 2, has been proposed (2004 WHO) to have prognostic implications. We evaluated morphological and immunohistochemical features of a series of PRCC to understand the significance, reproducibility and limitations of morphologic sub typing.

Design: 55 PRCC cases diagnosed between 1997-2004 at our institution were evaluated and classified Type 1 when >80% of tumor was covered by small cells with scant pale cytoplasm arranged in a single layer and Type 2 when >80% tumor had voluminous eosinophilic cytoplasm, high Fuhrman nuclear grade (FNG) and/or psuedostratified nuclei. Tumors with features of both types were labelled "Mixed" and those not fulfilling above criteria could not be typed. Pathologic parameters including FNG, TNM stage, cytoplasmic characteristics, multifocality, foamy macrophages, pseudocapsule, psammoma bodies, necrosis, and CK7 expression were assessed and statistically analysed. CK 7 staining was graded as negative, focal (<25%) and diffuse (>25%).

Results: Of 55 PRCC, 30 (55%) were Type 1, 12 (22%) Type 2, 6 (11%) Mixed, and 7 (13%) Untyped. Of Untyped PRCC, 4/7 had extensive clear cell cytoplasm including 1 with mixed features of Type 1 and 2 and the remaining 3/7 had voluminous eosinophilic cytoplasm but no other features of Type 2. All Untyped PRCC were low grade (FNG 1-2) and predominantly (86%) low stage (T1a).

All (100%) Type 2 had high FNG (3-4) vs. 13% of Type 1 (p<0.0001). Of Type 1, 21% presented with high stage (Stage 2 and 3) vs. 36 % of Type 2 (p=0.147). Nuclear stratification (p=0.0001) was significantly associated with Type 2 while foamy macrophages (p=0.009) and psuedocapsule (p=. 04) with Type 1. CK 7 was diffusely positive in 83 % Type 1, 60% Type 2, 60% Mixed and 100% Untyped PRCC. A mean follow-up of 11 months showed progression in 11% cases while 87% were free of disease and 2% died of unrelated causes. High FNG (p=0.05), TNM stage (p=0.01) and decreased CK 7 expression (p=0.05) were associated with outcome, however type of PRCC was not associated with outcome.

Conclusions: PRCC are composed of clinically and morphologically heterogeneous groups than our current understanding. Further molecular investigations and follow-up are needed to fully characterize PRCC.

695 Role of Percutaneous Image-Guided Renal Biopsy (IGRB) in the Management of Renal Masses: Adequacy, Accuracy and Limitations of Pathologic Diagnosis

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Background: With increasing sophistication of the imaging modalities, many small "indeterminate" renal masses (RM), are now detected posing therapeutic dilemmas. As minimally invasive techniques are increasingly utilized in such settings, surgical pathologists are freqently called upon to classify renal mass lesions on biopsies. Histological assessment is also essential before initiating IL-2 therapy in advanced renal cell carcinoma (RCC). We evaluated the adequacy, accuracy and limitations of IGRB in the management of RM.

Design: Eighty-seven IGRB from 86 patients presenting with RM between 1998-2004 at our institution were retrospectively evaluated. The IGRB were blinded to follow-up information and immuno/histochemical stains were performed when necessary. Renal epithelial tumors were classified as per 2003 WHO guidelines.

Results: 80% (70/87) of IGRB were adequate for assessment. The 17 inadequate IGRB showed only benign renal parenchyma (8), insufficient scant tissue (5) or necrotic tissue (4). Of the remaining, 71% (50/70) were primary renal tumors (PRT), 13% (9) urothelial carcinoma, 12% (8) poorly differentiated (PD) malignant neoplasm, 1% (1) squamous cell carcinoma and 3%(2) inflammatory lesions. Of 50 PRT, 60% (30) were conventional RCC with clear and/or granular cell morphology, 14% (7) papillary RCC, 18% (9) with predominant oncocytic cytoplasm (ON), 2% (1) RCC with mixed clear/papillary features, and 6% (3) angiomyolipoma. Of 9 ON, 7 were favored to be oncocytoma, of which 2 underwent radio frequency ablation (RFA) and 1 was resected. Three of 50 (6%) PRT including 1 RCC with mixed clear/papillary and 2/9 ON with mixed oncocytic/clear cytoplasm could not be definitively classified. Resection of 1/2 unresolved ON revealed Chromophobe RCC. Immunohistochemistry resolved all PD neoplasms as lymphomas (3), urothelial (2), RCC (1), melanoma (1) and metastatic breast carcinoma (1). Follow-up was available in 38 cases (44%), which included surgical resection (27), documented metastasis (6) and RFA (5). Resection specimens confirmed IGRB diagnosis in all cases.

Conclusions: IGRB can be utilized in the management of RM with a reasonably good level of adequacy and accuracy. However, caution is advised while evaluating IGRB as sampling, particularly in tumors with mixed oncocytic/clear and papillary morphology, can adversely affect interpretation.

696 Patterns of Metastasis in Renal Cell Carcinoma

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Background: Renal cell carcinoma (RCC) is the most common malignancy involving adult kidneys. The probability of cure is directly related to the stage or degree of tumor dissemination. When distant metastases are present, disease-free survival is poor. Knowledge of the patterns of metastasis in RCC will help design the best strategy for early detection and treatment of metastases.

Design: Patients who were treated at authors' institution for primary and metastatic RCC between 1977 and 2004 were included in this study. The pathological information collected included histological subtypes of RCC, anatomic sites of metastases, and time from detection of the primary RCC to metastasis. Synchronous metastasis was defined as the metastasis diagnosed before or at the same time of the primary RCC while metachronous metastasis was diagnosed after the primary RCC.

Results: 435 patients were included in this study. 268 (61.6%) patients had clear cell RCC, 5 (1.1%) had papillary RCC, 24 (5.5%) had sarcomatoid RCC, and histological subtypes were not available in the remaining cases. Many patients had both synchronous and metachronous metastases. The most common sites for synchronous and metachronous metastases were different (Table). In metachronous metastases, the mean time from diagnosis of the primary RCC to detection of metastasis was 2.52 years (0.06-18.4 years, see Table). Of 40 metastases that developed 5 years after the initial diagnosis of the primary RCC, metastases to lung, bone, soft tissue, liver, adrenal gland, head and neck each accounted for 22.5%, 20% and 20%, 7.5%, 7.5% and 7.5%, respectively. Metastases to different sites also developed at the different times after the initial primary RCC diagnosis (Table)

Conclusions: The patterns for synchronous and metachronous RCC metastases are defferent with the former most commonly involving lymph nodes, adrenal gland and lung, and the latter most commonly involving lung, bone and adrenal glands. Metastases to different sites also develop at the different times after the initial primary RCC diagnosis. Distant metastasis can occur as late as 18 years after the initial diagnosis of primary RCC.

Frequency and time of RCC metastasis to different anatomic sites

	Lymph node	Adrenal gland	Lung	Bone	Soft tissue	Liver	GI tract
Synchronous met	35.9%	19.8%	10.6%	8.5%	7.9%	4.6%	3.3%
Metachronous met	6.1%	5.5%	27.2%	22.0%	8.4%	6.1%	4.9%
Time to metachronous met (year)	2.2	2.3	2.3	2.8	4.1	2.0	1.6

697 Lack of Inducible Nitric Oxide Synthase Expression in Most Prostatic Adenocarcinomas

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Background: Inducible nitric oxide synthase (iNOS) is one of three enzymes that produce nitric oxide from L-arginine. iNOS can be induced in many cell types by signals such as stimulatory cytokines, and has been implicated in the development and progression of a number of tumors, including gastric, pancreatic, colorectal, and lung. Several studies have reported iNOS overexpression in the tumor cells of the majority of prostate cancers. We examined the expression of iNOS protein and mRNA in human clinical prostate tissues as well as prostate cancer cell lines.

Design: Prostate cancer cell lines (DU-145, LAPC-4, LNCaP, and PC-3) were examined for iNOS expression by quantitative RT-PCR and immunoblotting. RNA prepared from 8 matched fresh frozen normal and prostate cancer pairs was examined for iNOS expression by quantitative RT-PCR. Immunohistochemical analysis for iNOS expression with 4 different antibodies was validated by control experiments consisting of transfection of the *NOS2A* coding sequence into the MCF-7 cell line followed by formalin fixation and paraffin embedding. 66 matched normal and prostate cancer pairs from clinically localized tumors were assembled into a tissue microarray (TMA) and assessed by immunostaining. The Gleason scores ranged from 5-9, and the pathologic stages varied from T2N0MX to T3BN1.

Results: The prostate cancer cell lines DU-145, LAPC-4, LNCaP, and PC-3 lacked iNOS expression at the protein or mRNA levels. iNOS expression was undetectable by quantitative RT-PCR in the eight matched normal and prostate cancer pairs. Immunohistochemical analysis of the TMAs revealed only weak, focal iNOS expression in the epithelial cells of 3 prostate cancer cases, with the remainder of the cancers (n = 63) being negative. In a subset of cases, variable numbers of macrophages were positive for iNOS staining in regions of inflammation. Normal prostate epithelium did not stain for iNOS.

Conclusions: Most prostate cancers did not express iNOS, with only weak, focal iNOS staining seen in 3/66 cases. These findings were corroborated by the absence of iNOS mRNA in clinical specimens, the use of transfection experiments to control for antibody specificity, and the use of 4 different antibodies against human iNOS. The staining indentified in macrophages present in benign inflammatory lesions suggests a source of reactive nitrogen species that may facilitate tissue injury and tumorigenesis of the prostate.

698 Plasmacytoid Transitional Cell Carcinoma (P-TCC) of Urinary Bladder: A Clinicopathologic Study in 7 Patients

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Background: TCC having plasmacytoid feature (P-TCC) is rare. We have recently experienced 7 cases of P-TCC and described its clinicopathologic and immunohistochemical findings.

Design: Of the 7 cases, 3 cases were retrieved from the surgical specimens at Asan Medical Center, Seoul, Korea and 4 cases were referred to us from outside hospitals. The pathologic evaluation was made TNM staging system and the tumors were graded based on the WHO classification. Clinical information was obtained by reviewing the clinical records. Immunostainings on all cases were performed with cytokeratin(CK)7, CK20, Ki 67 and p53.

Results: All 7 patient(pt)s were man. Ages ranged from 46 to 81 years (mean; 62). Six pts presented with gross hematuria and the remaining one pt with urgency and microhematuria. Cystoscopic findings revealed a single solid mass with surrounding diffuse papillary lesion in 4 cases and multiple mass-like lesions in 3 cases. Initial diagnosis was made on transurethral resection in 6 cases and on cystoscopic biopsy in 1 case. Radical cystectomy with chemotheraphy was performed in 2/7, radical cystectomy alone in 2/7, chemotheraphy alone in 1/7 and intravesical BCG injection in 1/7. The last case did not receive any further theraphy. Microscopically, the tumors were composed of plasmacytoid cells with eccentrically located nuclei and abundant eosinophilic cytoplasm in variable amounts. The p-component ranged from 30% to 100%. 4/7 were TNM stage I, 2/7 TNM stage II and 1/7 TNM stage III. In 5 cases, high grade (grade 3) invasive TCC or TCIS was variably associated. Immunostaining demonstrated positivity for CK7 and CK20 in both p- and conventional TCC components in all pts. p53 expression was low (5 to 10%) except for one patient (80%). Ki 67 labelling index was from 10 to 40%. 6 pts are alive with no evidence of disease between 2 to 23 months after the diagnosis was made (mean 10.6 months). One patient was lost to follow-up.

Conclusions: When the bladder tumors have extensively plasmacytoid component, a careful search for associated invasive TCC or TCIS should be made for the proper diagnosis of P-TCC. Immunohistochemical studies should be performed for differential diagnoses from plasmacytoma, lymphoma, malignant melanoma and embryonal rhabdomyosarcoma. P-TCC appears to be a morphologic variant of TCC, however, clinical follow-up on these pts was too short to evaluate the biologic behavior of this variant of the tumors.

699 Lymph Node (LN) Involvement by Renal Angiomyolipoma (AML): A Really Rare Phenomenon?

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Background: Renal AML is a benign neoplasm composed of varying admixtures of blood vessels, smooth muscle cells and adipose tissue. There have been a few reports of LN involvement in association with renal AML, and now it is considered to represent a multifocal growth pattern rather than metastasis. The actual incidence and immunophenotypes of LN involvement with renal AML are unknown, because a few patients underwent lymphadenectomy (LND) and there has been no systematic study to look into this phenomenon.

Design: To study the incidence of AML involving LNs and to compare clinical, histological and immunohistochemical (IHC) features of AML cases with and without LN involvement, we retrieved 103 renal AML in the Pathology Files at AMC, YUMC and NCC from January, 1990 to June, 2004. IHC staining was performed against CD10, CD34, CD117, P53, Ki-67, HMB45 and SMA using formalin-fixed and paraffin-embedded tissues in cases with AML having LND.

Results: Of total 103 cases, regional LND was performed in 14 cases (14%). 8 cases (57%) of them showed LN involvement, ranging from 1 to 9 LN involvement, and the

size of involved LNs ranged from 0.2 to 2.3cm. Compared to AML cases without LN involvement, AML cases with LN involvement showed more male predilection, younger age of involvement and larger tumor size (male-to-female ratio, 3:5 vs. 1:5; 36 years (10 to 71) vs. 50.8 years (31 to 63); 12cm (6.5 to 23) vs. 6.1cm (0.2 to 10.7)). Bilateral cases were seen in 2 cases of AML with LN involvement group. Multiple involvements were seen in 3 cases of AML with LN involvement group and in 2 cases of AML without LN involvement group. There were no different histologic features between two groups. The tumor cells were positive for HMB45 and SMA, but negative for CD10, CD34, CD117, P53 and Ki-67 in both groups.
Conclusions: In view of high incidence of LN involvement irrespective of the LN size in AML, it is hypothesized that nodal multifocality might be a common phenomenon during the growth of renal AML. Our results suggested that male sex, multicentricity, large size of primary tumor and young age were more associated with LN involvement than AML without LN involvement in renal AML. Therefore, when AML is large occurring in young aged males, lymph node sampling may be necessary to better define the nodal involvement status. There were no histologic and immunophenotypic differences between two groups.

700 Prostate Re-Biopsies Following the Diagnosis of PIN and ASAP - Numbers and Findings in the Brazilian Population

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Background: The diagnosis of prostatic intraepithelial neoplasia (PIN) and atypical small acinar proliferation (ASAP) are supposed to be followed by a re-biopsy (RB), considering the high probability of diagnosing cancer. The aim of this study is to evaluate the index of RB following the diagnosis of PIN, ASAP or PIN+ASAP, in a reference center of uropathology in Brazil.
Design: Between Jan 2001-Dec 2003, 1420 men were submitted to transrectal prostate biopsy. In 208 (14.7%) the diagnosis was PIN, ASAP or both. We search for a new biopsy until August 31st 2004. The number and findings of RB were evaluated.
Results: PIN was diagnosed in 142 (10%) patients, ASAP in 26 (1.8%) and PIN+ASAP in 40 (2.8%). The mean age of patients diagnosed PIN, ASAP and PIN+ASAP was 64, 66.5 and 65 years old, respectively. Ninety-eight (47%) patients suffered RB, 71, 20, 5 and 2 men were submitted to one, two, three and four RB respectively. The interval for RB ranged from 1 to 1136 days, mean 299.6 days. Following the diagnosis of PIN, ASAP, PIN+ASAP 53 (37.3%), 16 (61.5%), 29(72.5%) patients were submitted to RB. Adenocarcinoma (AC) was diagnosed in 7 (13.2%) 7(43.8%) 12(41.4%) patients, respectively. Mean Gleason score of AC diagnosed in RB was 6 and the mean number of fragments involved by tumor was 3.3.
Conclusion: Only half of patients were submitted to RB after the diagnosis of PIN, ASAP or both. The lesion responsible for the highest number of RB was PIN+ASAP, followed by ASAP and PIN. Cancer was diagnosed in 26.5%, and ASAP and PIN+ASAP, were the most important lesions to predict AC in RB (47.8% and 41.4% respectively).

701 Invasion of Ejaculatory Ducts by Prostate Adenocarcinoma Is Strongly Associated with Non-Organ-Confined Disease

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Background: Ejaculatory duct (ED), formed by the junction of seminal vesicle (SV) and vas deferens, traverses through the central zone of the prostate. Prostate adenocarcinoma (PCa) involving only ED is considered as organ-confined disease, while that involving the SV is considered non-organ-confined. ED and SV cannot be distinguished in prostate needle biopsies based on the histology alone; therefore, the significance of finding ED/SV by Pca in prostate needle biopsy is unknown.
Design: The surgical pathology database at the authors' institution was queried for radical prostatectomies with Pca involving ED from July 1998 to August 2004. For each case, the following pathological parameters were recorded: Gleason score (GS), tumor volume (low: <0.5 cc; medium: 0.5-2 cc; extensive: > 2 cc), presence of extraprostatic extension, involvement of SV and status of pelvic lymph nodes.
Results: Fifty eight radical prostatectomies with PCa invading ED were identified. Gleason score was 6 in 1 case (1.7%), 7 in 29 case (50%), 8 in 4 case (6.9%) and 9-10 in 20 case (34.5%). Four (6.9%) patients received preoperative hormonal ablation. Tumor volume was medium in 11 (19.0%) and extensive in 47 (81%) cases. Metastasis to pelvic lymph nodes was present in 14 cases (24.1%). Extraprostatic extension and SV invasion were present in 57 (98.3%) and 53 (91.4%) cases, respectively. The positive predicative value of ED invasion for extraprostatic extension and SV invasion was 98.3% and 91.4%, respectively.
Conclusions: Invasion of ED by PCa is strongly associated with SV invasion and extraprostatic extension. Therefore, finding invasion of ED/SV by PCa in prostate needle biopsies should be considered highly suggestive of non-organ-confined disease. In addition, the presence of invasion of ED in radical prostatectomy specimens should be classified as extraprostatic extension, rather than organ-confined.

702 Differential Protein Expression in Anatomical Zones of the Prostate

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Background: The prostate has three anatomical zones: the peripheral (PZ), transition (TZ) and central (CZ) zone. The functional roles of these regions are yet unclear. It has been proposed that CZ may be of mesodermal origin, whereas the others are endodermal. Proteome patterns in the zones were characterized to test for differences.
Design: Cells were scraped from macroscopically normal areas of PZ, TZ and CZ in radical prostatectomy specimens. After exclusion of samples with cancer or PIN in Giemsa stained control smears or in corresponding histological sections, 18 cases remained for analysis. Cells were collected in a medium with protease inhibitors, and the protein material was prepared for 2-dimensional gel electrophoresis. Protein spots that differed quantitatively between regions were identified via mass spectrometric fingerprinting of tryptic fragments and selected tandem mass spectrometry sequence analysis.
Results: The scrape technique harvested mainly epithelial cells. Ten proteins with significant zonal differential expression were identified, 8 with underexpression in CZ versus PZ and TZ (arginase II, ATP synthase, cytokeratin 8, lamin A/C, peroxiredoxin 4, protein disulfide isomerase A3, tropomyosin, vimentin) and 2 with overexpression in CZ (peroxiredoxin 2 and creatine kinase B). No significant differences between the PZ and TZ were found.
Conclusions: PZ and TZ, although differing in terms of incidence of cancer and hyperplasia, have epithelium with highly similar major protein expression profiles. However, the protein profile of CZ differs from that of the other regions, suggesting functional differences.

703 Expression of alpha-Methylacyl-CoA Racemase (AMACR)/P504S in Renal Neoplasms

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Background: Alpha-methylacyl-CoA racemase (AMACR), also known as P504S, has recently been utilized as a biomarker for prostate carcinoma. Expression of AMACR is also recognized in normal renal tubules and papillary renal cell carcinoma. The aim of the present study was to evaluate the expression of AMACR/P504S in other subtypes of renal cell carcinoma and renal oncocytoma.
Design: 207 cases of renal neoplasms were selected from the surgical pathology files of MGH, including conventional clear cell type (n=70), papillary (n=56), chromophobe (n=38) and oncocytoma (n=43). Four cores (1 mm in diameter each) of tumor tissue from each case were constructed into tissue microarray blocks. Sections were cut at 4 microns and stained with AMACR/P504S monoclonal antibody on an automated immunostainer. AMACR/P504S staining was evaluated as negative, focally positive (positive in one core), or diffusely positive (positive in two or more cores).
Results: As shown in Table 1, expression of AMACR/P504S was observed in conventional clear cell carcinoma (15.7%), chromophobe carcinoma (21%), and oncocytoma (7%). In addition, 1.8% (1/56) and 92.9 % (52/56) of papillary carcinoma showed focally and diffusely positive for AMACR, respectively.

Table 1. Expression of AMACR/P504S in Renal Cell Neoplasms (Expressed as the Percentage of Cases in Each Category)

AMACR/P504S	Clear Cell	Chromophobe	Oncocytoma	Papillary
Focally positive	1.4 (1/70)	7.9 (3/38)	0 (0/43)	1.8 (1/56)
Diffusely positive	14.3 (10/70)	13.1(5/38)	7(3/43)	92.9 (52/56)
Total	16 (11/70)	21(8/38)	7(3/43)	94(53/56)

Conclusions: Diffuse AMACR staining is present in a significant number of clear cell and chromophobe carcinomas, and even in oncocytoma. Although high expression of AMACR is found in papillary renal cell carcinoma, caution should be exercised in using P504S immunohistochemical staining for the diagnosis of papillary renal cell carcinoma.

704 Cytoplasmic Displacement of p27 Is an Independent Predictor of Biochemical Recurrence in Prostate Cancer after Radical Prostatectomy

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Background: Progression through cell cycle is controlled by cyclin-dependent kinases(CDKs), whose activity is inhibited by the CDK inhibitors (CDKIs) including kinase inhibitor protein (KIP) p27. Decrease or loss of p27 has been associated with disease progression and outcome in human malignancies.
Design: 640 cases of PCa treated with radical prostatectomy were used to build tissue microarrays. Normal prostate tissue, BPH and index tumor were cored in triplicate (0.6 mm). Slides were stained with a rabbit polyclonal antibody to p27 using autostainer and digitized using an automated slide scanner. Correlations between p27 expression and clinical and pathological variables were analyzed by the Spearman test. Survival analysis was performed using Kaplan-Meier analysis, and Cox proportional hazard regression was used to determine the probability of disease recurrence.
Results: Nuclear p27 expression was found in 90.1% of the normal tissues, 54.4% of BPH and 64.4% of PCa. Cytoplasmic expression of p27 was found in 6.4% of the normal prostates, 2.5% of BPH, and 18.80% of PCa. Nuclear p27 expression was correlated with levels of Pre-PSA, while cytoplasmic presence of p27 was associated with more advanced tumor stages. Patients with moderate decrease of nuclear p27 or with cytoplasmic displacement of p27 had worse disease-free survival than those with either high or weak levels of p27. Cytoplasmic presence of p27 was an independent indicator for predicting higher probability of biochemical recurrence in PCa after radical prostatectomy.
Conclusions: We have demonstrated that high levels of p27 are consistently present in normal prostate. Significantly, our data suggest that moderately reduced nuclear p27 might be critical to cell proliferation and disease progression in cancer. Cytoplasmic displacement of p27 might play an important role in regulating nuclear p27, leading to more aggressive disease progression in PCa.

705 Overexpressed and Activated mTOR Pathway in Renal Cell Carcinomas (RCCs)

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Background: CCI-779 is an ester of rapamycin and inhibitor of mTOR protein kinase and is currently in Phase II clinical trials for treatment of patients with solid cancer. The mTOR functions as a checkpoint for cell growth and proliferation, an upstream

Akt and a downstream p70S6K being the two most important mediators. The aim of this study is to evaluate the status of expression and activation of the Akt-mTOR-p70S6K pathway in RCCs, which may further strengthen the rationale of a targeted cancer therapy using a rapamycin analog in RCCs.

Design: Tissue microarray sections containing 128 RCCs (70 clear cell RCC [CRCC], 40 papillary RCC [PRCC], and 18 chromophobe RCC [CHRCC]), 22 metastatic RCC and 24 normal kidneys (NK) were immunostained with monoclonal antibodies to phosphorylated (p)-Akt (Ser473), p-mTOR (Ser2448) and p-p70S6K (Thr389) using an avidin-biotin-peroxidase complex technique. Western blots were performed on three cases of CRCC and the corresponding normal renal tissues using the same antibodies. The scoring system for immunostains was as follows: 1) location – nucleus, cytoplasm, membrane, or a combination of two/three locations; 2) distribution – an estimated percentage; 3) intensity – weak (1+), moderate (2+), or strong (3+). The normal kidneys served as a baseline for comparison.

Results: Expression of p-Akt, p-mTOR and p-p70S6K were seen in 100% (n=24) NK and nearly 100% (n=150) of both primary and metastatic RCCs. The p-p70S6K was located in the nucleus in both NK and RCCs. The p-Akt was observed in the nucleus and cytoplasm of NK and the nucleus and cytoplasm/membrane of RCCs. The p-mTOR was identified in the membrane of NK and the membrane/nucleus of RCCs. The levels of expression of p-p70S6K, p-mTOR and p-Akt were significantly higher in RCCs compared with NK in the overall expression pattern (intensity and distribution, $p<0.05$). Western blots also showed a high level expression of p-p70S6K, p-mTOR and p-Akt in RCCs compared with the corresponding normal kidney tissues ($p<0.05$).

Conclusions: Our findings indicate that the correlative over-expression and activation of p-Akt, p-mTOR and p-p70S6K are commonly observed in RCCs, which provide a fundamental support of incorporating rapamycin-like agents in clinical trials for treatment of RCCs. In addition, the Akt-mTOR-p70S6K pathway may play a role in the carcinogenesis of RCCs.

706 Immunohistochemical Detection of MLH1 and MSH2 in Renal Cell Tumors

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Background: Microsatellite instability (MSI) is frequently seen in tumors associated with the hereditary nonpolyposis colorectal cancer syndrome and occurs in 10-15% of sporadic colorectal cancer. MSI in such instances results from defects of DNA mismatch repair genes, and MLH1 and MSH2 are the genes involved in the majority of these cases. The data on the expression of MLH1 and MSH2 in renal cell tumors are limited to relatively small series.

Design: Tissue microarray sections containing 134 renal cell tumors (70 clear cell RCC [CRCC], 29 papillary RCC, 22 chromophobe RCC [CHRCC], and 13 oncocytoma), 20 metastatic CRCC, and 26 normal kidneys were immunostained using monoclonal antibodies to MLH1 and MSH2 with an Envision-HRP kit. The scoring system was as follows: 1) location- nucleus, cytoplasm, or both; 2) distribution- negative, 1+ (<10%), 2+ (11-50%), 3+ (>50%); and 3) Intensity - weak or strong.

Results: For MLH1, weak (1+) nuclear staining was observed in 90-100% of cases in each group except for CRCC. Among 70 cases of CRCC, a significant percentage (43%) was negative. Similarly, for MSH2, nuclear staining was comparable among all study groups except for CRCC. Of 70 cases of CRCC, MSH2 expression was identified as strong, weak and negative in 26%, 57% and 17% cases respectively. Contrastively, 70% (n=14) of metastatic CRCC showed strong nuclear staining (2-3+) for both MSH2 and MLH1. In addition, strong (3+) cytoplasm staining for MSH2 was identified in 95%(n=21) of CHRCC and 77%(n=10) of oncocytoma, but was only occasionally seen in the other groups.

Conclusions: Our preliminary data show that weak to absent MLH1 expression in CRCC and a high level expression of both MSH2 and MLH1 in metastatic CRCC are frequent findings, suggesting a possible role of MSH2 and MLH1 in promoting biologically aggressive progression of CRCC. Additionally, MSH2 appears to be a useful marker in discriminating CHRCC and oncocytoma from CRCC, although further investigation is warranted to confirm this finding. Follow-up data comparing MLH1 and MSH2 expression in primary and metastatic CRCCs may provide answers regarding the predictive value of these markers.

707 Trends in Prostate Biopsies in Recent Years

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Background: The patterns of prostate biopsies have been changed dramatically in the first few years of the 21st century in order to achieve early detection, better Gleason Scoring, and clear documentation of tumor extent. We report our series to demonstrate these changes.

Design: A total of 2255 consecutive prostate biopsies from 25 urologists at multiple community practices from July 2001 to June 2004 were included in this study. The changing patterns every half year with pathology findings are tabulated. The first half of each year was designated as A and the second half year was B. There were 176 cases from 2001B; 267 from 2002A; 414 from 2002B; 372 from 2003A; 445 from 2003B; and 581 from 2004A.

Results: The mean age had been constant as 66 years (ranging from 30 to 100) over the past four years. There was a trend of downward PSA, increasing biopsy cores (from 9.4 to 12.9 per case) with increasing specimen jars (from 4.5 to 10.2 per case). The cancer detection rate was increasing as seen in % positive rate and positive rate to PSA ratio (PR/PSA Ratio), while the overall Gleason Score (GS) was lightly upward. The size of the cancer detected was not decreasing as seen in % positive cores (positive cores/ total cores x 100).

Conclusions: There is a trend of downward PSA, increasing biopsy cores, and increasing tissue submission jars in prostate biopsies over the past four years, while the cancer detection rate is dramatically increased with no evidence of over detection of minimal cancer or low grade cancer.

Prostate Biopsies from July 2001 to June 2004

	Median PSA	Mean # Cores	Mean # Jars	% Positive Rate	PR/PSA Ratio	Mean GS	% Positive Cores
2001 B	6.4	9.4	4.5	26.7	4.17	6.26	24.2
2002 A	7.5	9.8	4.6	37.1	4.95	6.13	29.8
2002 B	6.7	10.7	6.3	27.8	4.15	6.30	24.9
2003 A	6.2	10.2	5.4	30.6	4.94	6.40	28.4
2003 B	6.3	11.3	9.1	40.2	6.38	6.56	30.4
2004 A	5.6	12.9	10.2	35.8	6.39	6.31	27.0

708 Clinical Impact of Changing Practice Patterns in Prostate Biopsies

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Background: With the advent of new technologies and protocols for diagnosis and treatment, clinicians often need to adopt new approaches to patient evaluation. When a new approach or practice pattern is adopted, it is beneficial to measure change in outcomes to verify the efficacy of the new approach. We report two urologists who dramatically improved their biopsy outcomes by changing their practice patterns in prostate cancer detection.

Design: From October 2001 to August 2004, a total of 749 prostate biopsies from the two urologists were included. Before August 2003, Urologist 1 used (limited) sixtant biopsy scheme and submitted the needle cores in two specimen vials for pathology examination. Urologist 2 also submitted the tissue in two vials before January 2004, even though he used 12-core biopsy scheme. Both urologists adopted 12 or more cores (extended) biopsy scheme under an appropriate local anesthesia and submitted the tissue in 12 vials for pathology examination starting in August 2003 (Urologist 1) and January 2004 (Urologist 2). The cancer detection rates were compared before and after the changes of practice patterns.

Results: The comparison of these two groups (limited versus extended biopsies) in terms of serum PSA levels, number of needle cores and jars per case, cancer detection rate, cancer ≤ 1mm, and Gleason Score ≥ 7, are shown in the following table. The cancer detection was significantly different ($P < 0.001$, c2) between these two groups.

Conclusions: Systemic multicore biopsies under a better local anesthesia and tissue submission in site-specific containers for pathology examination can dramatically increase prostate cancer detection rate with a higher number of high grade cancers detected.

Comparison of Biopsy Patterns

	# Cases	Mean Age	Mean PSA	# Cores (Jars)	Cancer Rate	Cancer 1mm≤	Gleason 7≥
Limited	511	66	11.16	9 (2)	23.7 %	28 %	20 %
Extended	238	66	6.14	16 912)	36.6 %	20.7 %	20.4 %

709 Expression of Vitamin D_3 Receptor (VDR) in Primary Kidney Tumors

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Background: The kidney is not only a primary vitamin D target organ, but also is critically involved in vitamin D metabolism. Recent studies have shown that vitamin D has important physiologic effect on proliferation and differentiation in a variety of non-malignant and malignant cells. Our preliminary data showed that VDR was highly expressed in renal distal tubules and renal collecting ducts, whereas, the renal proximal tubule and glomeruli didn't express VDR. The aim of this study was to compare the expression levels of VDR in different types of kidney tumors in order to seek the possible diagnostic utility of VDR.

Design: We constructed paraffin tissue array blocks containing core cylinders from renal cell carcinomas (RCC) of clear cell type (52), papillary (35), chromophobe (20), sarcomatoid (20). The tissue array blocks also contained collecting duct carcinomas (3), oncocytomas (20), normal adult kidneys (12), and normal adult adrenals (6). All donor blocks were sampled with 1.5 mm punchers on Beecher microarray instrument. Immunostaining was performed by using polyclonal rabbit anti-VDR (Santa Cruz Biotechnology) with biotin-free HRP-labeled polymer of envision and detection system (DAKO).

Results: VDR was strongly positive in collecting duct carcinoma (3/3, 100%), papillary RCC (33/35, 94%), chromophobe RCC (17/20, 85%), and oncocytomas (18/20, 90%). The VDR was partially positive in sarcomatoid variant RCC (14/16, 88%). However, 87% clear cell RCC (45/52) were negative for VDR and the remainder (13%) only showed scattered positive cells. VDR was also present in distal convoluted tubules and collecting ducts, but absent in all other structures of normal kidneys and adrenals.

Conclusions: We have shown that VDR is a sensitive marker for primary kidney tumors. The preferential expression of VDR in chromophobe RCC, oncocytomas, and collecting duct carcinomas is in agreement with the concept that these tumors might be histogenetically related to distal convoluted tubules and collecting ducts. The absence of VDR in clear RCC makes VDR valuable in distinguishing clear cell RCC from other types of RCC. Furthermore, studies have shown that the lack of vitamin D_3 is associated with rapid tumor-growth and poor prognosis of RCC patients. Our data may provide very useful information for clinical management of RCC patients, especially VDR negative RCC patients.

710 The "Pushing Pattern" Defines an Underecognized Subtype of T1 Urothelial Carcinoma of the Urinary Bladder

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Background: The question of when an intraepithelial urothelial carcinoma becames invasive into the lamina propria (LP) of the bladder is a matter of controversy, particularly in cases of low-grade carcinoma. Current belief states that tumor cells arranged in slender cords or small nests within the LP is the hallmark for diagnosing invasion, but only high-grade tumors usually do that. Actually, T1G3 is a well recognized category. However, if the pathologist assumes that also G1/G2 tumors may eventually invade the LP, assessment of the invasion can be very difficult if only the "infiltrative pattern" is accepted as LP invasion.

Design: TUR specimens of a series of 200 non-muscle invasive (Ta/T1) homogeneously treated urothelial bladder carcinomas have been retrospectively reviewed by the same pathologist. Ta cases were diagnosed by strictly applying the WHO criteria. T1 tumors were defined when invading in two different forms. First, the "infiltrative pattern", when single cells, cords, or nests invaded the LP, often inducing some stromal reaction. Second, the "pushing pattern", when large tumor nests were seen pushing with broad margins into the LP, often accompanied by stalk occlusion in the surface papilla. Long term follow-up was achieved and 5-year disease free survival was studied.

Results: Males predominated in the series (173M/27F), with a mean age of 63 years (range 13-87). There were 35 Ta and 165 T1 tumors (161 papillary and 4 flat). Among T1 tumors, 39 displayed the infiltrative and 126 the pushing pattern of invasion. All Ta tumors were low-grade. Grade distribution in T1 cases was 71 G1, 80 G2, and 13 G3. When the study was closed, all Ta cases survived and 29 T1 tumors had died of disease (12,7%). Mantel-Haenszel test demonstrated a statistically significant difference in 5-year survival between Ta and T1 tumors ($p<0.001$). However, there was no difference in survival when comparing T1 "infiltrative" versus T1 "pushing" patterns.

Conclusions: The "pushing pattern" is the predominant form of subepithelial connective tissue invasion in T1 bladder carcinomas. The statistical difference in 5-year survival between Ta/T1 tumors this series reveals, together with the absence of a different survival between infiltrative and pushing patterns, support the necessity of its recognition in daily practice and possibly the reconsideration of T1 category in bladder carcinomas.

711 The Combination of the Gleason Index and Millimeters of Cancer in Core Biopsy Increases the Predictable Value of Extraprostatic Extension. A Study of 220 Consecutive Cases

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Background: A transrectal biopsy is the usual procedure for prostate cancer screening and Gleason is the most reproducible, predictable, and widely used grading system. So, the Gleason index (GI) has been directly related to several pathological end points in radical prostatectomies (RP), including the risk of extraprostatic extension (EE). Although the tendency is that the higher the GI the higher the stage, GI <7 in core biopsies does not assure organ confined disease and, conversely, GI >7 is not always associated to EE. Other histological features, i.e., the amount of tumor (TM) in needle biopsy, also correlate with staging but none of them are in themselves absolute predictors of EE. In this work we hypothetisize that the combination of the relative statistical weights of GI and TM in core biopsies may increase the predicted value of EE in RP.

Design: A series of 220 consecutive RP and their previous core biopsies were retrospectively reviewed by three pathologists after previous criteria agreement. GI and TM were evaluated in core biopsies and then correlated with the presence/absence of EE in RP. GI was grouped in 4 categories (<7, 3+4, 4+3, and >7) and TM was defined as the sum of total millimeters of tumor. Statistical analysis included Spearman's correlation, logistic regression, and Pearson's contingency tables.

Results: GI and TM in core biopsies showed medium intensity correlations with EE in RP ($\rho=0.315$ and $\rho=0.374$, respectively). The logistic regression model indicated that all cases with more than 22 millimeters of cancer in biopsy presented EE in RP [coeficient for millimeters= 0.083 (p=0.000), K= -1.835 (p=0.000)]. Additionally, all cases with GI >7 and more than 12 millimeters of cancer displayed EE [coeficient for millimeters= 0.074 (p=0.000), K= -2.043 (p=0.000)]. Finally, Pearson's χ^2 revealed that 80% of cases with GI >7 and 5 or more millimeters of cancer in core biopsy showed EE in RP (p= 0.024, relative risk= 1,79).

Conclusions: GI and TM in core biopsy show a good correlation with EE in RP. However, the combination of both parameters increases the predicted value of this crucial adverse event in the evolution of prostate cancer. So, this combination could be used in daily practice as an additional tool to choose the best mode of treatment.

712 Serum PSA Level Correlates with the Needle Biopsy Extent of Atrophy and Chronic Inflammation, but Not with High Grade Pin and Prostate Cancer

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Background: Serum prostate specific antigen (PSA) is the most common marker used to follow men with and without prostate cancer (PCa) and is used as a guide to initiate prostate biopsies. Recently, the value of serum PSA level in predicting the presence or absence of PCa has been questioned.

Design: One hundred consecutive first time saturation prostate biopsies (PBx) performed by a single urologist between February 2003 and May 2004 and reviewed by two pathologists were included in the study. Biopsy criteria were defined as serum total PSA of 2.5 ng/mL or greater and/or abnormal findings on digital rectal examination. All patients underwent a 24-core biopsy protocol. Patient age, PSA, extent (% of tissue involved) of high grade prostatic intraepithelial neoplasia (PIN), % cancer, % atrophy, % chronic inflammation, and presence of acute inflammation were recorded.

Results: The patients were divided in 3 groups: group A (n=34 with atrophy only), group B (n=29 with PIN and/or atypical glands), group C (n=37 with PCa). Atrophy was detected in all cases, ranging from 1.4 to 62.2% of the tissue (mean 22.5%). Chronic inflammation (CI) was present in 98% of the cases, ranging from 0.2 to 44.6% of the tissue (mean 4.5%). Acute inflammation was present in 61% of the cases. The mean PSA and age of the patients for each group were: 7.4 ng/ml and 60.8 years (A); 5.2 ng/ml and 61.7 years (B); 6.0 ng/ml and 65.4 years (C). The difference in mean age between group A (atrophy) and C (PCa) was statistically significant (p=0.045). No correlation was found between PSA and presence or extent of PIN and/or PCa either in the general population (A+B+C) or in the PCa and PIN group (B+C). The presence of PIN was associated with concurrent prostate cancer (p=0.003). Serum PSA level in the general population correlated with the extent of atrophy (p=0.022) and CI (p=0.009).

Conclusions: In this group of patients, preoperative serum PSA level does not correlate with the presence or absence, and extent of PCa and PIN. Atrophy and chronic inflammation are strong contenders for the PSA released into the serum at an increased level. PSA is a marker useful to follow up men with cancer, although its value as screening and staging tool is questionable.

713 CK7, CK20 and CD15 Can Reliably Differentiate Renal Oncocytoma from Chromophobe Renal Cell Carcinoma

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Background: Distinguishing common epithelial tumors arising in the kidney have significant implications, in terms of therapy and prognosis. Although the majority of renal neoplasms can be distinguished based on histologic examination alone, the morphologic similarities between the eosinophilic variant of chromophobe renal cell carcinoma (ChRCC) and renal oncocytoma (ONC) can sometimes represent a challenge. Hale's colloidal iron, often used in the differential diagnosis (ddx), is not always easy to interpret. Immunostaining for CK7 has recently become a useful tool in the ddx of renal neoplasms. The utility of CK20 and CD15, a proximal nephron marker, deserves further investigation.

Design: Immunohistochemical expression of CK7, CK20 and CD15 was examined in 17 samples of ChRCC and 19 ONC on a tissue microarray (TMA). Per each case three sections of tumor and corresponding normal kidney were available for evaluation. Tumors scored as positive showed at least 5% of the cells staining. Diffuse CK7 staining was defined as >90% of positive cells.

Results: ChRCC revealed diffuse (++) CK7 staining in 11 (65%) and focal (+) reactivity in 6 of the 17 cases (35%). All ChRCC were negative for CK20 and CD15. None of the ONC showed diffuse CK7 staining. Fifteen (79%) ONC demonstrated focal CK7 staining and 4 (21%) were negative. CK20 expression was detected in 10 (53%) ONC. CD15 was positive in 12 (63%) ONC. Using CK7 alone, 65% of ChRCC and 21% of ONC could be correctly classified. However, 35% of ChRCC and 79% of ONC had overlapping pattern of staining, i.e. focal CK7 staining. Based on our results we proposed a ChRCC and ONC immunoprofile as "CK7++ or CK7+/CK20-/CD15-" and "CK7- or CK7+/CK20+ or CK7+/CD15+", respectively. Using the ChRCC immunoprofile we were able to correctly classify 100% of the ChRCC and misclassified 2 (10%) ONC. The ONC immunoprofile correctly classified 90% of ONC and did not misclassify any ChRCC.

Conclusions: CK7/CK20/CD15 immunopanel can reliably differentiate ChRCC from ONC and improves diagnostic accuracy when compared to the use of a single antibody (CK7).

714 Immunostains CD56 and CD10 Aid to Diagnose and Differentiate Small Cell Carcinoma of Urinary Bladder and Prostatic Origin

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Background: Small cell carcinoma (SCC) of GU origin is a rare, but highly malignant tumor and needs to be distinguished for prognostic and differing therapeutic reasons. Frequently, bladder and prostate carcinomas can spread locally, creating diagnostic confusion as to the organ of origin. CD10 and CD56 are antigens expressed chiefly in hematopoietic cells, but are expressed in a variety of non-hematopoietic tissues and tumors. CD10 is a neutral endopeptidase, which inactivates biologically active peptides. Expression of CD10 in renal cell carcinoma and endometrial stromal sarcoma has been reported [Am J Clin Path 2000. 113:374-382]. Expression of CD56 in neuroendocrine tumors has also been recently documented [Am J Clin Path, 2003. 120:64-70]. We employ the CD10/CD56 panel and show that it can distinguish SCC of bladder and prostatic origin.

Design: Search of archived cases for SCC of bladder and prostatic origin yielded 9 cases. Of these, 5 were of bladder and 4 were prostatic in origin. In addition, 1 large cell neuroendocrine tumor of prostatic origin, 1 high grade prostatic adenocarcinoma and 1 high grade urothelial carcinoma were included in our analysis.

Results: All 9 cases of SCC stained positively with either CD56 or CD10 or both. The following patterns were observed: 4 of the 5 SCC arising from the bladder stained positively for CD56 (80%). All 4 SCC of prostatic origin stained positively for CD10 (100%). One of the bladder SCC (which did not stain with CD56) stained for CD10. One of the prostatic SCC stained for both CD10 and CD56 - however, the immunostains were complementary to one another - the tumor fraction that stained for CD56 was negative for CD10, and vice versa. These expression patterns differed from the non-SCC cases studied.

Summary of Immunostain results

	CD56	CD10
SCC of Bladder origin	4/5	1/5
Prostatic SCC	1/4	4/4

Conclusions: Small cell carcinomas of prostate/bladder origin are rare neoplasms. To our knowledge we have compiled the largest case series of small cell carcinoma of lower GU origin.

This study shows that a combination of immunostains CD56 and CD10 can be used to diagnose small cell carcinoma of bladder and prostatic origin. Despite the small number of cases in this series, immunostaining patterns point to the following: **CD10 positivity favors small cell carcinoma of prostatic origin and CD56 positivity favors small cell carcinoma of bladder origin.**

715 t6;11 Renal Cell Tumor. A Clinicopathologic Study of 2 Cases in Adults

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Background: Recently six renal tumors containing a novel t 6;11 (p21.1;q12) has been described. Through this translocation the transcription factor TFEB gene on chromosome 6 is fused to the non protein-encoding *Alpha* gene resulting in an increase of TFEB protein in the tumor cells due to promoter substitution. Five out of 6 previously described t 6;11 tumors occurred in patients between 10 and 18 years old. Four tumors occurred in females and 2 in males. These tumors exhibited epithelioid morphology, immunoreactivity for melanocytic, but not for epithelial markers, features overlapping those observed in renal angiomyolipoma (AML). The follow up (average 16 months) data are limited to 3 patients who were alive and well.

Design: We report the clinicopathologic features of new two cases of t 6;11 tumors of the kidney. We immunohistochemically tested them with the following antibodies: TFEB, TFE3, CK7, CK19, CK8-18, HMB45, Mart1, MUM1, S100, vimentin, actin, CD10 and parvalbumin. We stained for TFEB 15 renal AML including 3 epithelioid and 2 oncocytoma-like variants.

Results: Both patients were female and their age were respectively 52 and 42 yrs at the time of the nephrectomy. The tumors were composed of epithelioid clear cells arranged in a solid pattern; nuclear atypia was occasionally seen but neither mitoses nor necrosis were found. The neoplastic cells expressed TFEB, melanocytic markers (HMB45, Mart1) and were focally positive for CK8-18 and vimentin. TFE3, MUM1, CK7, CK19, actin and S100 were negative whereas CD10 and parvalbumin were expressed in one of the two tumors. One of the two patients showed paratracheal and pleural metastases 3 years after the surgery. None of the AMLs showed immunoreactivity for TFEB.

Conclusions: 1) This is the first description of t 6;11 renal tumors in adults; 2) these tumors can focally express cytokeratins; 3) they can be malignant and 4) TFEB immunostain can be useful to identify them and their metastases.

716 Comparison of Urinary Cytology (UC) and Fluorescence In-Situ Hybridization (FISH) Detection of Urothelial Neoplasia (UN): Analysis of Concordant and Discrepant Cases

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Background: UC sensitivity is limited by UN grade/cellularity, but can be enhanced by ancillary techniques. The Urovysion FISH assay assesses gain of chromosomes (chr) 3, 7 and 17, and loss of 9p as markers of UN in pts undergoing surveillance. The aim of this study is to correlate UC and FISH findings, and to address discrepant cases.

Design: This was a prospective study using Saccomanno-fixed urine from pts undergoing screening (n=23) or surveillance (n=11) for UN (group A-positive/suspicious (n=12); group B-atypical (n=14); group C-negative (n=8)). 16 pts had a bx at $\leq$ 2 months following UC. Cell FISH assessing hyperdiploidy (chr 3, 7, 17) and homozygous loss (9p) was performed on cytospins using the Urovysion assay. Abnormal FISH was defined as: 1) 4 cells with multichromosome gain (MCG; $\geq$2 chr) or 2) 12 cells with homozygous 9p loss. 25 cytologically abnormal cells were scored per case.

Results: Cellular urines with $\geq$10% cytologically abnormal cells included: A (11/12), B (4/14), C (0/8). Positive follow-up histology per group was: A (9/12-all HG-UN), B (6/14-LG-UN (5/6), HG-UN (1/6)), C (1/8-HG-UN). Abnormal FISH per group was: A (9/12; MCG:8/9, -9p:3/9), B (3/14; MCG:3/9, -9p:1/9), C (1/9; MCG:1/9, -9p:0/9). Mean % cells/group with chr 3, 7 and 17 gain were: A (38%, 33%, 30%), B (20%, 14%, 11%), C (14%, 6%, 4%). Isolated chr 3 gain in $\geq$ 10% cells was common: A (5/12), B (6/14) and C (2/9). Isolated gain (7, 9p, 17) or heterozygous loss (3, 7, 9, 17) was rare. Of 5 pts with atypical UC, criteria-normal FISH and isolated chr 3 gain in $\geq$ 10% cells, 1 had a follow-up HG-UN and 1 had h/o HG-UC. One atypical UC with MCG in 3 cells had multiple single chr gain/loss, and follow-up LG-UC. One UC (-) / FISH (-) urine had follow-up HG-UC, and one UC (-)/FISH (+) urine from a pt with chronic UTI had no bx follow-up .

Conclusions: UC and FISH are sensitive for HG-UN, but both are insensitive for LG-UN with few abnormal cells. Cases with highest chr copy number gain are usually HG-UN. Cases with atypical UC having multiple cells with a single chr 3 gain failing to meet abnormal stringent Urovysion criteria may have UN, suggesting the need for additional investigation. Some UN fail to yield abnormal cells or are diploid for all 4 markers, going undetected. Rare UC (-) / FISH (+) cases occur, suggesting either early UN or failure to sample cytologically abnormal cells.

717 Direct In Situ Analysis of Telomere Lengths in Primary Tumors and Corresponding Local and Distant Metastases Obtained Via Rapid Autopsy

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Background: Short telomeres can initiate chromosomal instability thereby fostering malignant transformation however; unchecked telomere loss creates a barrier to tumor expansion. Previous application of direct in situ hybridization assessment of telomere lengths revealed that telomeres indeed shorten early during prostate carcinogenesis (PIN stage) and that telomeres remain short despite the presence of active telomerase in most prostate cancers. In the current study we extend this analysis to tumor samples before and after androgen ablation therapy, including local and distant metastases, along with matched primary tumor tissues obtained via an ongoing rapid autopsy program.

Design: Tissues were obtained at autopsy or during prior surgical intervention from prostate cancer patients enrolled in an ongoing rapid autopsy study at our institution. Tissue micro arrays (TMAs) containing local and distant metastases for 29 autopsy cases and matched primary prostate cancer tissue when available (11 cases) were constructed and stained for telomeres (fluorescent telomere-specific probe), high molecular weight cytokeratins, and DNA. Telomere length, proportional to probe fluorescence intensity, was visually scored on a five point scale, with stromal cells and lymphocytes serving as internal controls. Primary comparisons for telomere length scores included cancer versus normal epithelium, metastases versus corresponding primary tumor, and untreated versus radiation and/or androgen ablation therapy.

Results: A total of 84 metastases and 11 primary tumors from 29 subjects were studied. In agreement with previous results, the majority (16/29) of primary cancers and local metastases displayed abnormally short telomeres. In contrast, in 11/29 metastases telomeres were consistently equivalent (across multiple sites) in length to reference stromal cells, and in 1 case were longer. One case displayed striking heterogeneity in telomere length.

Conclusions: Telomeres are consistently as short in metastases as in primary prostate adenocarcinomas in about 50% of cases. In the other half, telomeres are equal to or longer than the corresponding primary tumors, suggesting that telomere lengthening may have occurred, that stromal telomeres are longer in metastatic sites, or that these metastases arose from subsets of cells in the primary with longer telomeres.

718 Cytokeratin 7 and c-kit Expression in Renal Neoplasms

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Background: Chromophobe carcinoma, accounting for approximately 5% of malignant renal neoplasms, usually presents at a lower stage than conventional renal cell carcinoma (RCC), and appears to be associated with a more favorable clinical prognosis than other subtypes of RCC. The histopathological diagnosis of chromophobe renal cell carcinoma can present a diagnostic challenge, as these tumors can resemble either conventional RCC or oncocytoma.

Design: Tissue microarrrays (TMA) were constructed from 65 renal neoplasms (39 clear cell RCC, 7 papillary RCC, 8 chromophobe RCC, and 11 oncocytomas) obtained from surgical resection specimens of previously untreated patients in the archives of the Department of Pathology at Columbia University Medical Center. Hematoxylin and eosin stained slides were evaluated to confirm the original diagnosis, and the cases typed according to the guidelines of the Heidelberg and UICC/AJCC classifications of renal tumors. The Fuhrman nuclear grade was recorded for each case. Each tumor was represented by three cores in the TMA. TMA sections were stained with antibodies against cytokeratin 7 (CK 7) and CD117 (c-kit).

Results: The rate of immunopositivity of the various tumors is shown in the table below. Clear cell RCC was generally negative for both markers, and positive staining when present was focal; papillary RCC were all diffusely CK7 positive and c-kit negative, Oncocytomas were generally CK7 negative and diffusely c-kit positive, while chromophobe carcinomas were diffusely and strongly positive for both. Using Fisher's exact test, the differences between chromophobe and clear cell RCC were statistically significant for both CK7 ($p<0.001$), and c-kit ($p<0.001$); between chromophobe RCC and oncocytoma for CK7 ($p<0.001$), and between chromophobe RCC and papillary RCC, for c-kit positivity ($p<0.001$).

Conclusions: Immunostaining for CK 7 and c-kit may be a useful adjunct in differential diagnosis between chromophobe RCC and other renal neoplasms.

CK 7 AND C-KIT EXPRESSION

Tumor Type	CK 7	C-KIT
Clear cell RCC	3/39	3/39
Chromophobe RCC	8/8	8/8
Papillary RCC	7/7	0/7
Oncocytoma	1/11	10/11

719 Promoter Hypermethylation of von Hippel-Lindau (VHL) Tumor Suppressor Gene in Sporadic Renal Cell Carcinoma

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Background: Renal cell carcinoma (RCC) is the most common malignancy of the kidney, affecting 32,000 Americans and causing 12,000 deaths annually. It accounts for about 3% of all adult neoplasms. Histologically RCC is quite diverse and the subtypes include clear cell (CC-RCC), comprising about 80% of the cases, and papillary and chromophobe RCC and others, accounting for the remainder. The exact cause of RCC is unknown; however, somatic mutations of the *VHL* gene at chromosome 3p have been implicated in the pathogenesis of approximately 40-60% of sporadic CC-RCC. In addition to somatic

mutations, another mechanism thought to play a role in the development of RCC is the epigenetic phenomenon of gene inactivation, through promoter hypermethylation. In this study we investigated the frequency of *VHL* tumor suppressor gene promoter hypermethylation in RCC.
Design: A total of 38 cases of RCC (30 CC-RCC, 6 papillary, 1 chromophobe and 1 chromophil) and 31 matched non-neoplastic kidney tissues were identified and investigated for methylation of the *VHL* gene. Five (5) micron thick sections were cut from the paraffin blocks and DNA samples extracted using EX-Wax DNA extraction kit. The extracted DNA was modified with sodium bi-sulfite prior to methylation-specific (MSP) PCR using methylation specific primers for the *VHL* gene promoter. DNA modification with sodium bi-sulfite converts unmethylated cytosine to uracil residues, under conditions which do not allow reactivity of methylated cytosine. The converted DNA is then amplified by PCR using primers that are specific for methylated and unmethylated sequences.
Results: All 38 cases of RCC were from men with a mean age of 64.9-years (range, 44-85). Promoter methylation of the *VHL* gene was detected in 7 of 38 RCC (18%), all of these cases being CC-RCC. Two (28%) of the 7 cases showed *VHL* promoter methylation in tissues from adjacent non-neoplastic kidney. *VHL* promoter methylation was not detected in any of papillary or chromophobe RCC.
Conclusions: We conclude that *VHL* tumor suppressor gene is a frequent target for epigenetic silencing in sporadic CC-RCC (18%). This epigenetic alteration may be an early event that precedes the characteristic tumor morphology in a subset of sporadic CC-RCC.

720 Utility of GLUT1 Expression in Distinguishing Renal Cell Carcinoma from Adrenal Tumors

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Background: Primary renal cell carcinoma (RCC) is much more common than primary adrenocortical carcinoma (ACC); however, distinguishing these tumors from each other by microscopic examination can be quite challenging, as the histological and cytological features are often similar. In addition, clear cell RCC may mimic benign lesions such as adrenal adenoma or adrenal cortical hyperplasia; all entities characterized by the presence of cells with abundant clear cytoplasm and small nuclei. Several immunohistochemical (IHC) antibodies, none specific on their own, are used in panels in the diagnosis of RCC or ACC. GLUT1, an erythrocyte-type glucose transporter protein, is highly expressed in juvenile hemangiomas, endothelia in brain, placenta, and by tumor cells in many epithelial malignancies. We investigated, by IHC studies, the differential expression of GLUT1 in RCC and ACC.
Design: A total of 24 cases including 15 RCC, 6 ACC, 1 adrenal adenoma, and 2 cases of adrenal gland hyperplasia, were identified from the archives of the Department of Pathology at the University of Arkansas for Medical Sciences for IHC analysis. After reviewing the histological diagnoses, sections from the paraffin blocks were cut and the slides prepared for immunostaining with anti-GLUT1 (Dako) antibody and the results assessed for GLUT1 immunoreactivity. GLUT1 immunoreactivity was also assessed in non-neoplastic kidney tissue adjacent to areas of malignancy.
Results: The patient age ranged from 31 to 86-years with a mean of 58. The histological diagnosis was confirmed in all cases. GLUT1 immunoreactivity was detected in 12 of 15 RCC (80%), with 8/15 (53.3%) cases demonstrating moderate to strong expression. The staining pattern was restricted to the cytoplasmic membrane of the tumor cells. The endothelia of blood vessels within the renal tumors were negative for GLUT1. Only 1/6 (16.7%) ACC showed moderate staining for GLUT1 while 5/6 (83.3%) cases were completely negative. No GLUT1 expression was detected in non-neoplastic kidney tissue or benign adrenal gland tissue (hyperplasia and adenoma).
Conclusions: We have shown that GLUT1 is differentially expressed between RCC and adrenal neoplastic and non-neoplastic lesions. IHC expression of GLUT1 in RCC may therefore be used, in addition to other markers, to distinguish RCC from otherwise difficult cases of ACC.

721 Variable Expression of α-Methylacyl-CoA Racemase in Benign Prostatic Hyperplasia & Prostate Cancer

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Background: α-Methylacyl-CoA racemase (AMACR) is a cytoplasmic enzyme shown by cDNA expression microarray to be overexpressed in most prostate cancers but virtually undetectable in benign prostate tissue. Immunohistochemistry for AMACR can be used with basal epithelial markers in the diagnosis of prostate cancer. Previous studies have shown positive staining in 83-100% of prostatic adenocarcinomas (defined as moderate or strong diffuse cytoplasmic or continuous subluminal staining). In one study, the mean percentage of stained glands in positive cases was 95.9%, with diffuse (>75% of tumor) staining in 92% of cases. On average 15% of cases of benign prostatic hyperplasia (BPH) show focal weak apical AMACR staining in 2.5-10% of glands. This staining pattern is reportedly easily distinguished from that seen in malignant glands.
Design: Immunohistochemical expression of AMACR was studied using a commercially available antibody (Zeta Corporation) in 57 prostate cancers (51 radical prostatectomies and 6 transurethral resections) and 44 cases of BPH (2 simple prostatectomies and 42 transurethral resections).
Results: AMACR showed strong or moderate staining in 91% of cancers, with diffuse staining (>75% of tumor) in only 54 % of cases. Many tumors showed variable staining with 31% of cases showing weak or absent staining in over 50% of the tumor. The mean percentage of stained glands in positive cases was 71%. 3.5% of cancers were AMACR-negative and 5.0% showed only weak staining. 45% of BPH's showed focal, mainly weak apical staining. However, 11% of cases showed moderate or strong subluminal/cytoplasmic staining and 9% showed weak continuous apical staining. This was present in less than 10% of glands (average 5.4% of glands stained).
Conclusions: This study shows greater AMACR nonreactivity in prostate cancer than previously described, with the mean percentage of stained glands and the number of tumors with diffuse staining being lower than previously reported. Staining variability, with individual tumors showing mixed negative, weak, moderate and strong staining has not been previously described. In addition, BPH cases showed more AMACR reactivity than expected, some with moderate/strong cytoplasmic staining mimicking the malignant AMACR staining pattern. This study emphasizes that AMACR immunostaining is not a stand-alone test, but needs to considered in conjunction with other findings e.g. morphology and basal cell immunohistochemistry to avoid false-positive and false-negative diagnoses when signing out prostate specimens.

722 Renal Cell Tumors Arising in End-Stage Renal Disease (ESRD): A Karyotypic Study with Fluorescent In-Situ Hybridization (FISH)

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Background: Tumors arising in ESRD have variably been reported in the literature as papillary renal cell carcinoma (RCC) or clear cell RCC. However, we have noted five different tumor subtypes arising in ESRD that include clear cell RCC, papillary RCC, chromophobe RCC, acquired cystic disease of kidney-specific RCC (AS-RCC) and clear cell papillary RCC (Lab Invest 2003;83:173A). Trisomies of chromosomes 7 and 17 are considered characteristic of papillary RCC, and losses involving chromosomes 1 and 3 are frequent in chromophobe RCC and clear cell RCC, respectively. We, therefore, analyzed representative tumors from 4 different subtypes in ESRD by FISH, to evaluate if the cytogenetic changes substantiate our morphologic impressions.
Design: A total of 10 cases of renal cell tumors in patients with ESRD were examined. These included 3 cases of clear cell RCC, 2 of AS-RCC, 2 of clear cell papillary RCC, and 3 of papillary RCC. The paraffin-embedded slides were prepared according to the manufacturer's protocol for the Vysis Parrafin Pretreatment Reagent Kit II, and hybridized overnight with Vysis fluorescent centromeric probes for chromosomes 1, 7, 3, and 17. The slides were examined on a fluorescent microscope using the appropriate light filters, and at least 100 cells were counted in each tumor.
Results: One clear cell RCC (1/3) showed monosomies of both chromosomes 7 and 17; both AS-RCC showed monosomies (2/2), one of chromosome 7, and the other of chromosome 17; and one clear cell papillary RCC showed a monosomy of chromosome 7 (1/2). Thus, 4 of 7 tumors other than papillary RCC showed losses of chromosome 7 and/or 17. On the other hand, none of the 3 papillary RCC showed any deletions, and 1 of 3 showed a trisomy of chromosome 7. All the tumors were disomic for chromosomes 1 and 3.
Conclusions: 1. Tumors in ESRD that show variable proportion of papillary architecture but do not resemble the sporadic papillary RCC (i.e., AS-RCC and clear cell papillary RCC), are both morphologically and cytogenetically different from papillary RCC. 2. Except the usual papillary RCC, other renal cell tumors in ESRD show frequent losses of chromosomes 7 and/or 17. This suggests a possible common initiating tumorigenic event for them that requires further study. It is likely that possible secondary genetic events influence their ultimate morphologic phenotype.

723 Small Cell Carcinoma of the Urinary Bladder: A Clinicopathologic Study of 55 Cases

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Background: Small cell carcinoma (SCC) of the urinary bladder is a rare tumor constituting less than 1 % of the primary bladder neoplasms. Large series which include clinicopathologic and outcome data are few.
Design: 55 cases of SCC diagnosed and treated at our institution, with histologic material available, were identified from 1985 to 2002 in our files. Histologic features and clinical outcome were evaluated. In 37 cases where blocs were available, immunohistochemical stains for chromogranin, synaptophysin, desmin and TTF-1 were performed.
Results: 45 (81%) patients were men and 10 (19%) were women. The mean age at dignosis was 69 years (range 42-89). 36 (65%) cases were pure SCC while the remaing 18 (33%) had other minor histologic components (urothelial carcinoma, NOS,10 cases; adenocarcinoma, 2 cases; large cell neuroendocrine carcinoma, 2 cases, both urothelial carcinoma, NOS and large cell neuroendocrine carcinoma, 2 cases; adenocarcinoma and squamous cell carcinoma, 1 case; urothelial carcinoma, NOS and adenocarcinoma, 1 case; adenocarcinoma, urothelial carcinoma, NOS and large cell neuroendocrine carcinoma, 1 case). In-situ carcinoma was identified in 26 cases. 28 patients had cystectomy (24 radical, 4 partial), 9 of which had neoadjuvant chemotherapy. 7 patients were treated with chemotherapy alone and 1 patient received neoadjuvant chemotherapy followed by radiation therapy. Follow-up was available for 36 patients and ranged from 2 months to 183 months (mean= 33.5 months). At last follow-up 27 (75 %) patients were dead of disease, 8 (22.3 %) were alive with no evidence of disease and 1 (2.7 %) was alive with disease.
Immunohistochemical stains were performed on 37 cases. Synaptophysin was positive in 78% (29), chromogranin was positive in 48% (18), TTF-1 was positive 24% (9) of cases. Desmin was positive in only 2 cases.
Conclusions: Small cell carcinoma of the urinary bladder has a poor prognosis. More than half of our cases (67%) were pure small cell carcinoma. Chromogranin and synaptophysin are frequently expressed and remain to be useful markers in this tumor. TTF-1 is expressed in the minority of cases of SCC in this site.

724 Comparative Study of Immunohistochemical Profiles of Fetal and Adult Nephrons Using Renal Tumor Markers

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Background: Renal tumors are heterogenous with distinct histopathology and antigenic profiles that are thought to reflect origin from different regions of the nephron. This antigenic diversity is partly due to the differing embryologic derivation of each region. However, studies on immunoprofile of fetal kidneys are limited. We examined antigenic profiles of fetal and adult kidneys using a panel of antibodies used in the diagnosis of renal neoplasms.

Design: Tissue microarrays(TMA) using two 1mm cores were constructed from 18 fetal(gestational ages 10-15 weeks) and 12 adult kidneys. Immunohistochemistry for CD10, Villin, RCC, CK 7, E-cadherin, CK34βE12, Ulex and P63 were performed on the arrays and scored as positive or negative in the different compartments of the nephron.

Results:

	DT ADULT	DT FETAL	CD ADULT	CD FETAL	URO ADULT	URO FETAL
P63	-	-	-	+	+	+
CKβE12	-	-	-	-	+	-
CK 7	+	-	+	+	+	+
E-cadherin	+	+	+	+	+	+
Ulex	-	-	+	+	+	+

	GLOM ADULT	GLOM FETAL	PT ADULT	PT FETAL
CD 10	+	+	+	+
Villin	+	+	+	+
RCC	-	-	+	+

GLOM: glomeruli, PT: proximal tubules, DT: distal tubules, CT: collecting ducts, URO: urothelium.

P63, CK34βE12, CK 7, E-cadherin and Ulex were negative in GLOM and PT; CD 10, Villin and RCC were negative in DT, CD and URO.

Conclusions: This study showed that markers used in the diagnosis of renal tumors can be divided into two histogenetic groups: proximal nephron (glomeruli, proximal tubules) markers CD 10, Villin, RCC and distal nephron (distal tubules, collecting ducts, urothelium) markers P63, CK34βE12, CK 7, E-cadherin, Ulex. The proximal markers were similarly expressed in the two groups, while the distal markers showed differing expression profiles in fetal and adult nephrons. Based on these results we conclude that CK34βE12 and CK 7 positivity of urothelium and distal tubules respectively are acquired during a later phase of renal development while P63 expression of fetal collecting ducts is lost in the adult kidney.

725 Immunohistochemistry in the Differential Diagnosis of Mucinous Tubular and Spindle Cell Carcinoma and Papillary Renal Cell Carcinoma of the Kidney: Innovations and Pitfalls

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Background: Mucinous tubular and spindle cell carcinoma (MTS Ca), a very rare, recently described distinctive subtype of renal cell carcinoma, may have striking morphologic similarities to the more common papillary renal cell carcinoma (PRCC), particularly basophilic or type 1 tumors. Circumscription, compact tubular architecture, focal papillae, mucin production and foam cells (features seen in PRCC and MTS Ca) as well as a spindle cell morphology have resulted in many cases sent to us in consultation with a diagnosis of possible sarcomatoid PRCC.

Design: A tissue microarray with triplicate samples each from 26 MTS and 20 PRCC was created to simulate experience in renal biopsy specimens. From immunohistochemistry (IHC) data published in the contemporary literature, a panel consisting of CK7, α-methylacyl-CoA racemase (AMACR), epithelial membrane antigen (EMA), high molecular weight cytokeratin (HMCK), renal cell carcinoma antigen (RCC), CD10 and c-kit was designed to test utility in differential diagnosis.

Results:

Immunoreactivity (IR) in MTS Ca and PRCC (results in %)

Tumor	CK7	AMACR	EMA	HMCK	RCC	CD10	c-kit
MTS Ca	85	92*	95	15	4**	12**	5
PRCC	65	90*	88	15	20**	80**	17

* Similar extent of IR in cases ** Overall IR in individual cases greater in PRCC cases

Conclusions: 1) This largest study to date on IHC of MTS Ca dispels the specificity of AMACR for PRCC among renal carcinoma subtypes. 2) The histogenesis of MTS Ca from the distal nephron continues to be debatable, as our study shows expression of proximal convoluted tubule-related markers (AMACR, CD10). 3) The remarkable histologic homology between PRCC and MTS extends to IHC with congruent expression of several IHC markers (AMACR, CK7, EMA, HMCK); however the appreciation of a low-grade spindle cell population and weak to absent IR for CD10 and RCC may be helpful in diagnostically challenging cases.

726 Biochemical Recurrence of Patients with Pathologically Organ-Confined Prostate Cancer (pT2) after Radical Prostatectomy: Analysis of Patients Demographics, Pre-Op PSA and Tumor Parameters in a Cohort of 872 pT2 Patients

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Background: The proportion of patients with pT2 prostate cancer (PCA) has been increasing steadily in recent years comprising in large series the most frequently listed stage category. It is accepted that this group of patients enjoys significant disease free advantage but with variably reported biochemical recurrence rates. The objective of this study was to analyze factors associated with rising PSA after surgery in a large consistently documented and followed cohort.

Design: All patients who underwent radical prostatectomy at our institution whose specimens were completely submitted for microscopic evaluation and showed no evidence of regional nodal metastases, seminal vesicle invasion, extraprostatic extension or tumor extension to surgical margins included. Patients who received hormone or radiation therapy were excluded. Age, race, pre-op PSA, Gleason score and tumor volume of the radical prostatectomy specimens were recorded for all patients. Biochemical recurrence was defined at a PSA level of 0.4 ng/ml.

Results: Eight hundreds and seventy two patients qualified for the study with a mean follow up of 76 (range of 12 to 158) months. Fifty patients (5.7%) experienced biochemical recurrence with the predicting parameters in univariate analysis being older age and higher Gleason score. Patients over 62 years of age and those with a Gleason score of 7 or higher were significantly more likely to recur (p=0.007 and 0.001 respectively). In a multivariate logistic regression, higher Gleason score also correlated significantly with shorter interval to recurrence after surgery. There was no significant correlation between recurrence and race, pre-op PSA or tumor volume.

Conclusions: Most patients with pathologically organ-confined disease have excellent disease free survival at 5 and 10 years interval. A closer monitoring for older pT2 tumor patients and those with a Gleason score of 7 or higher may be indicated.

727 Shortened Telomeres in Clear Cell and Papillary Renal Cell Carcinomas

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Background: Telomere shortening, a possible crucial event leading to genetic instability and development of cancer, has been reported in multiple types of human cancers. In this study, we evaluated telomere length in two most common types of renal neoplasms: clear cell and papillary renal cell carcinomas by using fluorescence in situ hybridization (FISH).

Design: Telomere length was analyzed in clear cell (n=20) and papillary (n=16) renal cell carcinomas with a FISH probe specific for the mammalian telomere repeat sequence . The telomere signals were counted in an average of 20 cells per field. Telomere length was measured using the method previously reported as: very short, short, normal, long and very long.

Results: Comparing to matched normal appearing kidney tubules, telomere shortening was observed in all 36 cases of clear cell and papillary renal cell carcinomas. The majority of clear cell carcinomas (80%) had very short telomeres as compared to 18.75% of papillary carcinomas. The results are summarized in the Table.

Conclusions: We demonstrated the telomere shortening in renal cell carcinomas with clear cell type more severe than papillary type. Telomere length abnormalities appear to play a significant role in development of both clear cell and papillary renal cell carcinomas. This may lead to a better understating of renal carcinogenesis. The shortened telomeres may also serve a marker of renal tumor development.

Telomere Analysis in Renal Cell Carcinoma

Tumor Type	No.	Short	Very Short
Clear Cell RCC	20	4/20 (20.0%)	16/20 (80.0%)
Papillary RCC	16	3/16 (81.3%)	13/16 (18.7%)

728 Urothelial Carcinoma with Rhabdoid Features: Report of Five Cases

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Background: Extrarenal rhabdoid tumors have been described in a variety of primary sites with only rare case reports of urothelial carcinomas with rhabdoid features in the literature. In this report we describe the clinicopathologic characteristics including clinical follow-up on five cases of urothelial carcinoma with prominent rhabdoid features.

Design: Four cases were retrieved from the consultation files of one of the authors. One of the cases was retrieved from the surgical pathology files at our institute.

Results: The patients were all men with ages ranging from 53 to 86 years (mean = 67.4 years). Patients initially presented with hematuria or obstructive symptoms. The sites included bladder (n=4) and renal pelvis (n=1). In addition to the rhabdoid component, multiple coexistent histological components were seen including in situ urothelial carcinoma (CIS) and high grade papillary urothelial carcinoma (n=1), poorly differentiated carcinoma with small cell features (n=1), sarcomatous areas (n=1) and a myxoid component (n=2). All cases in this series had focal or diffuse positive staining with one or more cytokeratin markers (EMA, Cam 5.2, AE1/AE3). Three of the five patients were treated initially with surgery (radical cystoprostatectomy, n=2; radical nephrectomy, n=1).Two of five patients died within one month, while a third patient died within four months. Only two patients were alive at 3 and 9 months after diagnosis.

Conclusions: The histologic and immunohistochemical findings in this study serve to broaden the morphologic spectrum of urothelial carcinomas with prominent rhabdoid features and add further evidence as to their poor prognosis.

729 Pleomorphic Adenocarcinoma of the Prostate with Bizarre Giant Cells: Report of 6 Cases

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Background: Pleomorphic tumors with giant cells have been described in a variety of primary sites. However, only a couple of isolated cases have been described amongst prostatic carcinomas and none on diagnostic biopsy material.

Design: 5 cases were retrieved from the consultation files of one of the authors. One of the cases was retrieved from the surgical pathology files at our institute.

Results: Patient ranged in age from 59-76 years (mean = 65.8 years). The diagnosis was made on prostate biopsy (n=3), urethral biopsy (n=1), TURP (n=1) or radical prostatectomy (n=1). In all cases, giant, bizarre, anaplastic cells were present. In 4 of the cases, the marked pleomorphism occupied 5% of the specimen, with 20% and 70% bizarre giant cells in the other 2 cases. In one case, the bizarre cells had atypical mitotic figures, with other cases showing no mitoses in the markedly pleomorphic cells. In addition to the pleomorphic giant cell component, multiple coexistent histological components were seen including Gleason score 9 conventional prostate cancer (n=6), small cell carcinoma (n=1), squamous differentiation (n=1), and prominent ductal differentiation with intraductal spread (n=1). Immunohistochemically, 4 were for negative for PSA in the giant cells, 1 had 5% and the other 50% positivity. Staining for PSA in the conventional prostate carcinoma component was 1%, 5%, 20%, 50%, 100%, and 100%. The bizarre giant cells were strongly positive for cytokeratins AE1/AE3 and/or Cam 5.2 (n=3). Two cases had a history of conventional prostate cancer 4 years prior to the giant cell component, 1 treated with Lupron and the other with radiation. Follow-up after diagnosis of the giant cell component: Case 1: dead in 1 year; Case 2: progressive metastases in 2 years; Case 3: alive at 1 year; Case 4: large perineal recurrence after radiation at 3 years; Case 5: radical prostatectomy with extra-prostatic extension and seminal vesicle invasion; and Case 6: recently diagnosed.

Conclusions: Conventional prostate cancer, even when very high grade, typically consists of cells with relatively uniform nuclei. Our study expands the histology described in prostate cancer to include in very rare cases the presence of prominent pleomorphism and bizarre giant cells. This giant cell component heralds a particularly aggressive clinical outcome.

730 Insulin-Like Growth Factor Binding Protein 3 Is a Marker of Clear Cell Renal Cell Carcinoma

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Background: With the advance of cDNA microarray technology, there has been progress in finding specific markers for renal cell epithelial neoplasms. We recently demonstrated that insulin-like growth factor binding protein 3 (IGF-BP3) mRNA transcription levels are 5.6 times higher in clear cell renal cell carcinoma (RCC) compared to other renal epithelial tumors. Using a polyclonal antibody directed against IGF-BP3, we performed immunohistochemistry on 127 renal epithelial neoplasms, including 58 clear cell RCC, to evaluate IGF-BP3 protein expression and to see if IGF-BP3 staining intensity correlates with Fuhrman nuclear grade.

Design: Tissue microarrays were constructed with formalin-fixed, paraffin-embedded tissues from 127 renal epithelial tumors. Hematoxylin and eosin stained sections were reviewed to confirm diagnosis and Fuhrman nuclear grade. Staining intensity was scored on a 0 to 3 scale.

Results: Seventy-four percent (43/58) of clear cell RCC showed positive IGF-BP3 staining compared to 9% (6/63) of other renal neoplasms, p<0.0001. In addition, other neoplasms did not have high intensity IGF-BP3 staining (63/63). High grade clear cell RCC (15/17, Fuhrman grade 3 and 4) more often demonstrated high intensity IGF-BP3 staining than low grade clear cell RCC (8/41, Fuhrman grade 1 and 2), p=0.0004.

IGF-BP3 staining intensity

IGF-BP3 intensity	clear cell RCC	Fuhrman 3&4	Fuhrman 1&2	chromophobe RCC	papillary RCC	oncocytoma
3	14	11	3	0	0	0
2	9	4	5	0	0	0
1	20	2	18	1	3	2
0	15	0	15	17	30	16

Conclusions: We confirmed our molecular analysis of IGF-BP3 as a marker of clear cell RCC (74%, 43/58 positive) when compared to other renal epithelial tumors (9%, 6/63 positive). Also, we demonstrated that IGF-BP3 staining intensity was stronger in high grade clear cell RCC (88%) than in low grade clear cell RCC (19%).

731 Study of KIT Exon 11 Mutations in KIT-Positive Sarcomatoid Renal Cell Carcinoma Specimens

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Background: Sarcomatoid renal cell carcinoma (SRCC) is not a distinct histologic entity and represents high-grade transformation in different subtypes of Renal Cell Carcinoma (RCC). Clinically, this pattern of dedifferentiation is associated with poor prognosis because of its local aggressiveness and high metastatic rate. Recently, immunohistochemical expression of KIT in SRCC has been documented. Previous studies have shown that Imatinib, a selective tyrosine kinase inhibitor of KIT, is highly effective in KIT-positive gastrointestinal stromal tumours (GIST), especially those that have gain-of-function mutations in the KIT exon 11, that encodes a juxtamembrane domain of this protein.The aim of our study was to assess exon 11 KIT mutational status in 12 SRCC cases that showed KIT overexpression, in order to explore the possibility of the use of Imatinib as a target therapy for this neoplasm.

Design: 12 cases of SRCC that showed immunohistochemical expression of KIT (rabbit polyclonal A4502, DAKO, dil. 1:100) in the sarcomatoid areas, were selected for this study. The epithelial part of SRCC were classified, according to WHO classification 2002, into Clear cell RCC (6 cases), Papillary RCC (2 cases), Chromophobe RCC (2 cases) and Unclassified RCC (2 cases).The expression of KIT in the sarcomatoid areas was diffuse (positivity in >50% of tumoral cells) in 5 cases (42%), moderate (positivity in 10-50% of tumoral cells) in 2 cases (16 %), and focal (positivity in 2-10% of tumoral cells) in 5 cases (42 %). The pattern of KIT immunostaining was cytoplasmic in 8 cases (67%), nuclear in 3 cases (25 %) and both in 1 case. For the evaluation of KIT mutational status, genomic DNA was extracted from selected sarcomatoid areas of formalin-fixed, paraffin-embedded cases. DNA was amplified by polymerase chain reaction using the primers corresponding to KIT exon 11 and the product was directly sequenced and analysed for KIT exon 11 mutations.

Results: No mutations have been found in exon 11 KIT in the sarcomatoid areas of all 12 SRCC analysed that previously showed KIT immunohistochemical expression.

Conclusions: KIT oncoprotein expression in SRCC is not correlated with activating mutations in KIT exon 11. In analogy to GIST, our results could imply that SRCC patients would not benefit from treatment with Imatinib. However, to assess this fact, further studies need to be done in order to analyse oncogenic kit mutations in other parts of KIT gene.

732 Optical Illusion in the Histological Diagnosis of Prostate Carcinoma: Assignment of Who Scores Is Governed by Growth Pattern and Not by Nuclear Morphology

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Background: In grading of prostate carcinoma, Gleason (growth pattern) and WHO scores (nuclear features) compete in terms of predictive power for progression of disease. In Germany, a third score has combined growth pattern and nuclear features (Helpap score), but has never proven its superiority over Gleason or WHO scores. In a retrospective analysis of 183 radical prostatectomy cases, we found Gleason, WHO, and Helpap scores to yield virtually identical sensitivities and specificities in respect to PSA rebound as a marker of tumor recurrence (60-65%). To find out why the combination of scores assessing two distinct tumor characteristics failed to improve predictive power, we hypothesized that the growth pattern (seen at low power) imposes an irresistable diagnostic bias in the subsequent estimation of nuclear features (at high power).

Design: A student ignorant to the working hypothesis (MP) digitized 3 random HPF from the H&E sections of 183 prostate cancers and selected 10 nuclei per HPF. For each selected nucleus, we used Photoshop-based image analysis to quantify maximal nuclear diameters, surface area, degree of heterochromasia and roundness of the nuclear contour. All nuclei were then individually copied into a PowerPoint presentation, and 3 pathologists assigned nucelar grades, this time precluding a potential bias of the growth pattern.

Results: During analysis of microscope slides, assignment of nuclear features by all 3 pathologists was independent of true nuclear morphology (e.g. r=0.019 for nuclear diam., P = 0.77) but governed to almost 100% by the tumor growth pattern (r=0.85, P<0.0001). Yet, when the same nuclei were regarded in PowerPoint without the context of growth pattern, the nuclear scores reflected true nuclear morphology (e.g. r=0.584 for nuclear diam., P<0.0001).

Conclusions: We propose that much (if not all) of the good predictive value of the WHO score truly reflects architectural features rather than true nuclear features. We propose that the equation - WHO score = Gleason score - formulated in the recent TNM classification does not reflect tumor biology but is due of a diagnostic bias. The predictive power of combined histological scores (such as Helpap and others) will have to be critically reevaluated.

733 Cystic Adult Renal Lesions - Radiologic and Pathologic Correlations

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Background: Cystic renal lesions are relatively common with >50% of men and women >50 years of age developing renal cysts. Most of these cysts are benign and require neither treatment nor surveillance. In between the simple cyst and the obvious malignancy lies a complex renal cyst, which can be either benign or malignant. The current classification of cystic renal lesions, by Bosniak, is based on their radiologic appearance. Class I & II encompass simple and minimally complex cysts and class III & IV, suspicious complex cysts and malignant renal cysts respectively. While class I is always benign, class II is almost always benign, class III can be malignant 50% of the time and class IV is almost always malignant. However, distinction between class II & III, based on radiology, is not always straightforward.

Design: Our aim was to determine the correlation between the Bosniak classification of cystic renal lesions and the pathologic findings. Our study included 92 patients presenting with cysic renal lesions during the period 2002-2004. There were 62 males and 30 females. Age ranged from 36-84 years for males and 29-84 years for females. Four patients with Bosniak II lesions opted for surgery instead of observation, 7 patients with Bosniak III and 8 patients with Bosniak IV lesions underwent surgery. All lesions were examined microscopically.

Results: Four patients with Bosniak II, all men, had 2 benign and 2 malignant lesions: 2 cystic nephroma and 2 cystic papillary renal cell carcinoma (RCC) . Among 7 cases of Bosniak III, all men, there were 6 malignant lesions (clear cell RCC x3, papillary RCC

x2, chromophobe RCC x1) and one benign lesion - cystic nephroma. Among 8 Bosniak IV lesions (4 females + 4 males) there were 3 benign lesions, mixed epithelial and stromal tumor of the kidney (MESTK), all females, one cystic clear cell RCC (female) and 4 papillary RCC (all males).

Conclusions: In our series, 6/7 Bosniak III lesions were malignant, 4/8 of Bosniak IV lesions were malignant and 2/2 Bosniak II lesions were malignant. Among 12 malignant lesions, 11 were diagnosed in men (male:female ration 11:1). MESTK, a recently recognized benign tumor, mimics malignancy in females. Although Bosniak classification is very helpful in distinguishing clearly benign cystic lesions from malignant ones, there is still a category of benign lesions of the kidney whose nature is difficult to predict based on this classification.

734 Solitary Fibrous Tumors of the Kidney: A Clinicopathologic Study of Seven Cases

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Background: Solitary fibrous tumors (SFT) are uncommon spindle cell neoplasms that predominantly develop in the pleura. Extrapleural SFT have been reported in a variety of locations with renal involvement being rare. The purpose of this study is to investigate renal SFT and further define these tumors by their histopathology and immunophenotype.

Design: The study cohort consisted of 6 renal SFT and one perirenal SFT. Clinical and histopathologic features were reviewed. Immunohistochemical stains for actin, bcl-2, calretinin, CAM 5.2, CD31, CD34, CD99, c-kit, cytokeratin AE1/AE3, desmin, EMA, HMB-45, S-100 protein, smooth muscle actin, vimentin and wide-spectrum cytokeratins were performed on all cases.

Results: All SFT presented as grossly circumscribed masses with a mean tumor size of 5.7 cm (range 2.2 to 10.1 cm). Mean patient age at diagnosis was 52.6 years (range 29 to 79 years). Five tumors were incidental and two presented with flank pain. All tumors consisted of spindle cell proliferations arranged haphazardly and in short fascicles with alternating hypercellular and hypocellular regions. Variable foci of densely collagenized tissue were present, intermixed with thin and thick-walled blood vessels that focally formed hemangiopericytoma-like vascular patterns. Four renal SFT demonstrated entrapment of non-neoplastic renal tubules and 3 renal SFT showed focal involvement of perirenal adipose tissue, despite appearing grossly circumscribed. All SFT had a low mitotic rate and lacked nuclear atypia and foci of tumor necrosis. All tumors exhibited immunoreactivity for vimentin and 6 SFT (86%) were positive for CD34 and CD99. Only 2 SFT were bcl-2 positive. Three SFT demonstrated p53 immunoreactivity. All SFT were negative for actin, calretinin, CAM 5.2, CD31, c-kit, cytokeratins, desmin, EMA, HMB-45, S-100 protein, and smooth muscle actin.

Conclusions: Renal SFT are rare, often incidental, spindle cell neoplasms that are morphologically and immunophenotypically similar to pleural-based SFT. Despite being grossly circumscribed, renal SFT are infiltrative neoplasms that require complete surgical excision with the assessment of resection margins becoming increasingly important as more segmental nephrectomies are performed. Although no overt malignant features were identified in these renal SFT, these tumors should be assessed for histologic features of malignancy and differentiated from other spindle cell neoplasms of the kidney.

735 Prostate and Pancreatic Cancers Cause Increased Neurite Outgrowth *In Vitro*

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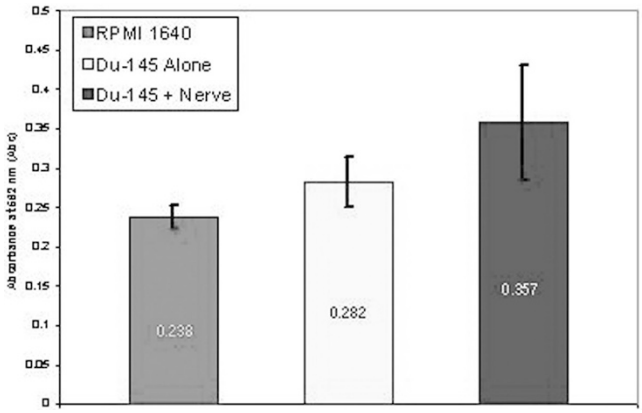
Background: Previous *in vitro* studies and *in vivo* morphologic evidence suggest that prostate and pancreatic cancer and nerves engage in mutually beneficent growth interactions. This phenomenon appears similar to angiogenesis.

Design: PC-12 rat pheochromocytoma cells induced to neural growth pathways were cultured on removable membranes with the supernatant from 6 culture conditions: Du-145 human prostate cancer cells co-cultured with mouse dorsal root ganglia (DRG), MiaPaCa human pancreatic cancer and DRG, Du-145 alone, MiaPaCa alone, DRG alone, and serum-free RPMI growth medium. After 48 hours of culture, membranes were removed and washed. PC12 cell bodies were removed by physical shearing, leaving only neurites within the membranes, which were stained and placed in neurite lysis buffer (Chemicon) for 5 minutes. Lysates were collected and absorbance measured at 562 nm.

Results: Du-145 + DRG had the most neurite growth, which was significantly higher ($p<0.05$) than either Du-145 alone or plain RPMI.



MiaPaCa + DRG had significantly higher neurite growth than MiaPaCa alone.

Conclusions: This quantitative method shows that neurite growth is increased in presence of supernatant of cancer/nerve co-culture. This evidence supports the hypothesis that prostate and pancreatic cancers cause neurogenesis. This effect is being studied in other cancers as well.

736 Relationship between Prostate Cancer and Tuberculosis in the Inhabitants of the Semipalatinsk Region in Kazakhstan

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Background: The incidence of tuberculosis is higher in some areas of Kazakhstan as compared to the US. The relationship between prostate cancer (PCa) and tuberculosis (TB) has not been examined.

Design: A series of 676 prostates that were obtained from sequential autopsies of inhabitants of Semipalatinsk region in Kazakhstan were examined for the presence of neoplastic and non-neoplastic disease.

Results: The cause of death was connected with PCa in one case and related to TB in 121 patients (17.9%). However, only 4 patients had histological proof of TB in the prostate, 3 of which died of TB and one of ulcerative colitis. Among people without cancer the incidence of TB was 20.2% as compared to people 12.1% in people with cancer. Conversely, the incidence of cancer among people with TB was 19.0%. Among people without TB, the incidence of Cancer was 30.1% (p=0.014).

Conclusions: It is apparent that patients who die of TB have a lower incidence of PCa than the general population of this region of the world. The paradoxical inverse relationship between cancer and TB could be due to the fact that patients with TB die earlier.

737 Ethnic Peculiarities of Latent Cancer of Prostate of the Inhabitants around Former Semipalatinsk Nuclear Test Site (Kazakhstan)

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Background: The study of prevalence, pathogenesis and morphogenesis of prostate cancer (PCa) is relatively restricted in countries of Asia. Ethnic variations in cancer incidence in the region are unknown.

Design: Morphological analysis of prostates was performed on sequential gross autopsy material of histotopographic sections among 676 inhabitants of Semipalatinsk region in eastern Kazakhstan. The population in this area consists mainly of Asians (32.1%, mean age at death: 50 yrs) and Caucasians (67.9%, mean age at death: 60.6 yrs).

Results: Cancer of prostate was discovered in 190 cases. PCa occurred 3 times more often among the persons of Caucasian origin, than among the Asians (35.3% vs. 12.9%, $p<0.000$). Of interest also is that in Europeans PCa incidence increased with age, while among the Asians it increased only up to the 5th decade, and later decreased (p=0.000). HGPIN was also more frequent in the Caucasian population than Asians (37.7 vs. 16.1, p=0.000). However Asians had more HGPIN in the 4th and 5th decades and Caucasians in the older population. Low Gleason scores (Gl5) were more frequent in the younger Asians (0% vs 28.6 % in the 30s; 9.5% vs. 42.9% in the 40s) (p=0.025). Also Caucasians tend to have more foci of cancer than Asians.

Conclusions: Ethnic peculiarities were discovered among the inhabitants of Semipalatinsk region, Kazakhstan with latent cancer of prostate. Asian patients have a lower incidence of PCa that happens earlier in life and is less aggressive biologically. This is further evidence of ethnic predisposition to the cancer of this organ.

738 Expression of Smad4 in Prostatic Adenocarcinoma (PAC) Whole Mount Sections Correlates with Tumor Gleason Grade

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Background: Smad family members have been identified as intracellular signaling components of the transforming growth factor-beta 1(TGF). Smad2 and 3 complex with Smad4, that translocate to the nucleus to regulate transcription of target genes. The role of Smad4 has been studied in pancreatic, colorectal, endometrial and lung cancer and to a lesser extent in prostate cancer, but Smad4 status has not been studied as a potential prognostic factor in PAC.

Design: Formalin-fixed paraffin-embedded whole-mount radical prostatectomy specimens from 207 patients with PAC were evaluated for Smad4 expression by immunohistochemistry using a monoclonal antibody (sc-7966 Santa Cruz Biotechnology, Santa Cruz, CA). Hematoxylin and Eosin stained slides were reviewed and tumors graded based on the Gleason grading system. Tumors were scored for Smad4 expression semiquantitatively based on the staining intensity and distribution. The pattern of staining (nuclear vs cytoplasmic) was also analyzed. These results were compared with Gleason grade and clinical and pathologic stage.
Results: Smad4 cytoplasmic expression significantly correlated with tumor Gleason score. High cytoplasmic expression of Smad4 was seen in 89% (168/188) of tumors. 96% (90/94) of high grade tumors with Gleason score of 7 or more showed high cytoplasmic expression (p=0.005). High Gleason score tumors also frequently showed acquisition of nuclear expression of Smad4. 74% (76/103) high grade PAC showed high nuclear expression of Smad4 (p=0.03). Finally when the results of staining intensity, distribution and pattern were compared with stage, Smad4 expression did not show a statistically significant relationship with stage (p=NS).
Conclusions: This newly described association of Smad4 overexpression, nuclear and cytoplasmic with high Gleason score tumors suggests a possible role for Smad4 in prostate cancer progression. Given this correlation of Smad4 expression with Gleason grade, known predictor of disease outcome, further studies of the prognostic significance of Smad4 in PAC appear warranted.

739 Low-Grade Mucosa-Associated Lymphoid Tissue Lymphoma Involving the Kidney: A Clinicopathologic Study of 3 Cases

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Background: Low-grade B-cell lymphoma of mucosa-associated lymphoid tissue (MALT) involving the kidney is extremely rare. A review of the literature only identifies 12 isolated cases. Clinical and biological behavior and pathologic features of the tumor are not well defined.
Design: 3 cases of MALT lymphoma arising in the kidney were retrieved from the consultation and pathology files at our institution covering a period of 5 years (1999-present). Clinicopathologic features and immunophenotypes of these 3 cases were evaluated.
Results: The patient's age ranged from 53 to 83 years (mean 69) and 2 patients were male. The most common presentation was a unilateral renal mass discovered incidentally on imaging studies for nonspecific symptoms or for evaluation of chronic diseases. All three patients denied a history of hematuria or other urologic symptoms. Two patients underwent nephrectomy while the other one performed retroperitoneal exploration with biopsy of perirenal mass. Grossly (2 cases), the masses were unencapsulated but well-demarked, ranging from 3.0 to 7.5cm in diameter. Cut surface revealed yellow-tan and brown tumors without necrosis or hemorrhage. Microscopically, the involved kidneys showed architecture effacement by nodular or pseudonodular infiltrate of relatively uniform small to medium-sized lymphocytes. At high power, the neoplastic cells resembled centrocyte-like or monocytoid cells with slightly irregular to round nuclear contours, clumped chromatin, inconspicuous nucleoli and abundant eosinophilic cytoplasm. Mitotic figures were rare. These cells infiltrated renal tubules and glomeruli to form lymphoepithelial lesions (1 case). Immunohistochemical studies showed that the infiltrated cells were positive for CD20, CD79a with immunoglobulin κ light chain restriction in all 3 cases and negative for CD5, CD10. One patient is receiving chemotherapy without complications. The second patient underwent a partial nephrectomy and was asymptomatic at the subsequent follow-up. The third patient developed a pulmonary embolism following nephrectomy and further follow-up is not available.
Conclusions: MALT lymphoma rarely affects the kidney. Since low-grade MALT lymphoma often have an indolent clinical course with a tendency to be localized at the time of diagnosis and to be curable with local therapy, it is important pathologically to distinguish MALT lymphoma from other types of primary renal lymphomas that pursue a more aggressive clinical course.

740 Prostate Cancer: Should Stage T2 (2002 TNM Staging System) Be Subclassified?

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Background: In the 2002 TNM staging system tumors are subclassified as T2a (less than one half of one lobe involvement), T2b (more than one half of one lobe involvement), and T2c (bilateral involvement). Recent studies question the existence of a true pT2b tumor as well as a difference related to biochemical failure after radical prostatectomy (RP) between pT2a and pT2c tumors.
Design: The study population consisted of 142 stage pT2 consecutive patients submitted to RP. The surgical specimens were histologically evaluated by complete embedding and whole mount processing. Tumor extent was evaluated by a point count semiquantitative method. Organ confined (pT2) tumors were subclassified as pT2a (unilateral involvement and tumor extension corresponding to less than half of the area), pT2b (unilateral involvement and tumor extension corresponding to more than half of the area), and pT2c (bilateral involvement). pT2a and pT2c patients were compared according to the following variables: age, preoperative PSA, Gleason score (GS), Gleason predominant grade (GPG), positive surgical margins, and biochemical recurrence. The data were analyzed using the Mann-Whitney test for comparison of independent samples and Fisher's exact test for evaluating differences between proportions. Time to PSA recurrence was compared between the groups using a log-rank survivorship analysis.
Results: From the total of 142 patients, 25 (17.60%) had pT2a tumors and 117 (82.39%) pT2c tumors. No patient had pT2b stage. Patients with pT2c tumors were more likely to have a higher GS (p=.0141) and positive surgical margins (p=.0097), compared to those with pT2a tumors. There was no significant difference in age (p=.8240), preoperative PSA (p=.1533) and GPG (p=1). The mean and median follow-up periods among men without biochemical recurrence were 3.5 and 3.3 years, respectively. During this time, 29 patients (20.42%) developed a biochemical recurrence. Log-rank analysis revealed no difference in PSA recurrence between men with pT2a and pT2c tumors (p=.4378).
Conclusions: The results of our data showed that no unilateral tumor extended to more than half of the area. Patients with pT2c tumors had higher Gleason score and positive surgical margins. However, PSA recurrence for pT2a tumors was similar to pT2c tumors. The data of our study favor a consideration to modifying the TNM staging system to eliminate subclassification of T2 tumors.

741 Gene Expression Profiling of Renal Angiomyolipoma with Oligonucleotide Arrays

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Background: Renal angiomyolipoma is a mesenchymal neoplasm histogenetically related to perivascular epithelioid cells and is typically composed of variable amounts of mature adipose tissue, smooth muscle and atypical thick-walled blood vessels. Immunohistochemically and ultrastructurally the neoplastic cells show features of melanocytic and smooth muscle differentiation. Angiomyolipoma is associated with the autosomal dominant genetic disorder, tuberous sclerosis, caused by mutations in the TSC1 or TSC2 genes on chromosomes 9q34 and 16p13. The gene expression programs that determine the unique biology of angiomyolipoma are not well known; we, therefore, compared gene expression profiles in angiomyolipoma vs renal epithelial tumors using Affymetrix oligonucleotide microarrays.
Design: The case cohort included 6 angiomyolipoma, 13 clear cell renal cell carcinoma (RCC), 5 papillary RCC, 4 chromophobe RCC and 3 renal oncocytoma. Total RNA samples from frozen, grossly dissected tumors were hybridized to Affymetrix HG Focus microarrays, which contained probes for 8746 genes. Microarray results were analyzed by unsupervised hierarchical average linkage clustering and supervised Significance Analysis of Microarrays (SAM) algorithms to identify distinct expression profiles in angiomyolipoma. Genes identified by SAM were analyzed with the GOstat program to identify biological processes highly represented in angiomyolipoma expression profiles.
Results: Angiomyolipoma was clearly discriminated from renal epithelial tumors based on distinct gene expression profiles; this tumor subtype overexpressed 409 and underexpressed 108 genes significantly at a median false discovery rate of 0.76%. Several genes related to muscle development ($p=3.6 \times 10^{-4}$), lipid biosynthesis ($p=4.5 \times 10^{-3}$) and pigmentation ($p>0.05$) were overexpressed in angiomyolipoma. These tumors also overexpressed vascular endothelial growth factors B and D (VEGFB, VEGFD) and underexpressed VEGFA and VEGFC.
Conclusions: Angiomyolipoma can be distinguished from other renal tumors by unique gene expression profiles. Myoid, adipose and melanocytic marker overexpression is consistent with the histogenesis and immunohistochemical profiles of angiomyolipoma. Overexpression of VEGFB and VEGFD compared to VEGFA and VEGFC could be related to the distinctive angiogenesis in these tumors. Atypical angiogenesis has been described in other tuberous sclerosis-related neoplasms. Future studies are indicated to determine if this finding is related to VEGFB or VEGFD overexpression.

742 Ultrastructural and Immunohistochemical (IHC) Appraisal of Tubulocystic Carcinoma (TCCa) of the Kidney: Histogenetic and Diagnostic Implications

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Background: Fewer than 12 cases of a distinctive tubulocystic renal neoplasm have been reported in the literature, variously named as "RCC, collecting duct type," "Bellini duct carcinoma" and "low-grade collecting duct carcinoma." Our ongoing experience with 33 such cases, which we have termed tubulocystic carcinoma (TCCa), indicates that less than 10% have a malignant outcome after nephrectomy. The ultrastructural features of this neoplasm have not been analyzed to date.
Design: Ultrastructural studies were performed from material retrieved from paraffin-embedded formalin-fixed blocks from 3 cases, and IHC studies were performed on 14 cases using an extensive panel to assess histogenetic and diagnostic considerations.
Results: The relatively homogenous cytoarchitectural appearance of TCCa (tubulocystic spaces lined by cells containing moderate to abundant eosinophilic cytoplasm and round nuclei with irregular chromatin and prominent nucleoli) was recapitulated in the striking ultrastructural similarity in the 3 cases. Tumors showed abundant microvilli with brush border organization similar to cells of proximal convoluted tubule (PCT) but with shorter microvillous height and cytoplasmic interdigitation [similar to intercalated cells of the collecting duct (IC-CD)]. Abundant mitochondria (correlating with oncocytic cytoplasm) was noted. Microvesicles normally seen in IC-CD were not evident. IHC studies showed expression of PCT-related markers [CD10 (85%), AMACR (77%)] and markers related to distal nephron [parvalbumin (100%), 34ßE12, HMWCK (15%), CK19 (100%)].
Conclusions: 1) The ultrastructural features of TCCa are distinctive, and their characteristic microvillous/brush border appearance in the context of a tubulocystic architecture suggests the diagnosis. 2) The histogenesis of this tumor continues to be enigmatic, as ultrastructural and IHC findings show features of both PCT and intercalated cell differentiation. 3) These findings support the deletion of "collecting duct" or "Bellini duct" from the nomenclature of this tumor.

743 Prospective Cytogenetic and Histopathologic Correlation of Renal Epithelial Neoplasms: A Series of 67 Cases in Vermont

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Background: Chromosomal abnormalities have been well documented in renal cell carcinoma (RCC). It is known that specific cytogenetic changes correlate with the histologic subtypes of RCC, serving as a useful diagnostic tool for the surgical pathologist. Our study evaluates the cytogenetic changes in a series of renal epithelial tumors, identifying and reviewing cases with discrepant histopathology and cytogenetics results.

Design: Conventional metaphase cytogenetics was performed on fresh tissue from 74 renal tumors over a period of three years. Cases with a histological diagnosis of RCC or oncocytoma were selected for further study. Surgical pathology and cytogenetic reports were reviewed for 67 cases. Cases with cytogenetics results not supporting the histopathological diagnosis were identified, the histology reviewed, and divided into three groups: Group I, papillary RCC misclassified as conventional RCC; Group II, chromophobe RCC misclassified as conventional RCC; and Group III, cases with concordant morphologic diagnoses but unusual cytogenetic features.

Results: Histologic diagnoses included 48 conventional RCCs, 7 papillary RCCs, 3 chromophobe RCCs, 1 "hybrid" RCC, 1 cystic RCC, 1 "oncocytic" RCC, and 6 oncocytomas. Chromosomal changes were observed in 43 out of 67 cases (64%), the remainder having normal karyotypes. Conventional RCCs showed loss of chromosome 3 (27%), translocations of chromosome 3 (27%), and trisomy 5 (23%). Papillary RCCs showed trisomy 17 (50%) and loss of Y (67%). Chromophobe RCCs showed loss of 2, 6, and 17 (67% each). Oncocytomas showed loss of 1 and Y (33%). Thirteen cases had a histologic diagnosis that was discordant with the cytogenetic findings, including 3 in Group I, 2 in Group II and 8 in Group III. Five (12%) of these cases were originally misclassified upon histologic review.

Conclusions: Cytogenetics is a useful tool in subtyping RCC. Our series shows that subtyping RCC on H&E alone can be challenging, with a 12% misclassification rate. Cytogenetic-histologic correlation revealed areas of difficulty, such as chromophobe RCC and papillary RCC with a solid architecture and/or clear cell cytomorphology. This correlation also serves to educate surgical pathologists on the histologic subtleties of these neoplasms.

744 Nuclear Expression of Phospho-Akt in Prostatic Adenocarcinoma, Prostatic Intraepithelial Neoplasia and Benign Nodular Hyperplasia

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Background: Cytoplasmic phospho-Akt (p-Akt) expression has been reported in prostate adenocarcinoma (PAC), with increased expression in advanced disease. Actively studied in the cardiovascular literature, nuclear expression of p-Akt has not been reported in prostate tissue. We aim to document the nuclear expression pattern of p-Akt in PAC, high-grade prostate intraepithelial neoplasia (HGPIN) and benign nodular hyperplasia (BNH), and explore possible correlations between nuclear p-Akt expression and tumor progression in PAC.

Design: Formalin-fixed paraffin-embedded tissue from 30 archival prostatectomy specimens were re-cut, H&E-stained and immunohistochemistry performed for p-Akt (polyclonal, Cell Signaling Technologies, Beverly, MA, 1.5 mcg/ml) using an Envision+ detection system (Dako) with blocking buffer as negative control. Positive cases were scored (1-4): 4 = strong in >50% of cells, 3 = weak to moderate in >50% of cells, 2 = strong in <50% of cells, 1 = weak to moderate in <50% of cells. Well differentiated PAC (Gleason score 5 or 6), moderately differentiated PAC (Gleason score 7), poorly differentiated PAC (Gleason score 8 or 9), HGPIN and BNH were scored separately.

Results: Nuclear p-Akt expression was observed within PAC and HGPIN in 12 of 30 cases (40%, 2.8 average score). Nuclear p-Akt expression was also seen in the BNH apical epithelium in 11 of these 12 cases (92%, 2.6), and was absent in the remaining 18 cases. Nuclear p-Akt expression was observed in a greater number of moderately (67%, 2.7) and poorly (60%, 3.0) differentiated tumors compared to well-differentiated tumors (8%, 3.0). More Stage 3 tumors expressed nuclear p-Akt (53%, 2.7) compared to Stage 2 (29%, 3.0). Similar cytoplasmic p-Akt expression was observed in BNH apical epithelium (100%, 3.5) and malignant epithelium (100%, 3.6), and the level of cytoplasmic expression did not vary with tumor grade or stage.

Conclusions: This study identifies a subset of patients with nuclear p-Akt expression in their PAC, HGPIN and BNH. Within the invasive tumor, nuclear p-Akt expression was found more often in advanced disease, indicating that it may have prognostic utility in prostate cancer. These findings suggest that nuclear translocation of p-Akt could be involved in the cancer progression pathway in a subset of patients with PAC.

745 The Morphological Spectrum of Penile Condylomatous Tumors. A Report of 44 Cases and a Re Evaluation of the Buschke Lowenstein Tumor-Giant Condyloma Concept

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Background: Classification of penile condylomatous tumors is problematic and terminology so inconsistent as to make impossible to compare series. A major problem has been the variable description and interpretation given to giant condyloma (GC) and or the Bushcke Lowenstein tumor (BLT).

Design: 150 tumors diagnosed as condylomas, GC, BLT, warty carcinomas (WCa) and verrucous and papillary carcinomas were reviewed. 44 cases were selected. They all showed condylomatous papillae with koilocytosis and a central fibrovascular core. Factors evaluated were: age, tumor site and size, atypias and the interphase of tumor with stroma.

Results: We found 4 categories:1- **usual condylomas**: the koilocytotic atypia was restricted to surface, 2- **atypical condylomas**: the atypical cells involved two thirds of papillae, 3- **non invasive warty (condylomatous) carcinoma**: atypical cells comprised the full thickness of the papillae,4- **Invasive warty (condylomatous) carcinoma**: in addition to full thickness atypia there were jagged invasive tumor borders.

Types	# of cases	Age	Preferential location	Size
Usual condyloma	20	35	F	0.4
Atypical condyloma	3	38	Gl, COS, F	5
Non-invasive warty ca	3	49	Gl, COS, F	2.5
Invasive warty ca	18	62	Gl, COS, F	5

F: foreskin; Gl: glans: COS: coronal sulcus.

Conclusions: There is a morphological spectrum of penile condylomatous lesions. We are proposing a simple morphological classification system in four distinctive groups. Because of the poor definition of the Buschke Lowenstein tumor - giant condyloma concept, which have been used for condylomatous or verrucous tumors, we recommend the abandonment of the terminology for penile condylomatous lesions.

746 Increased Expression of Fatty Acid Synthase in Prostate Adenocarcinoma Following Total Androgen Ablation

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Background: Fatty acid synthase (FAS) is essential for prostate cancer (PCa) survival. In addition, tumors overexpressing FAS are biologically aggressive. Although androgen regulated, FAS is overexpressed in androgen-independent PCa metastatic to bone. We assessed whether androgen ablation resulted in FAS upregulation in residual tumor cells following neoadjuvant chemical castration.

Design: FAS expression was evaluated by immunohistochemistry in prostate biopsies (PBx) and corresponding radical prostatectomies (RP) from 85 patients with PCa treated with total androgen ablation (TAA) before surgery for a mean of 3.73 months (range 1 to 24 months). Intensity of staining was scored as 0, 1+, 2+, and 3+ and the percentage of cells staining with different intensity was recorded. Only 2+ and 3+ staining was considered positive and the sum was recorded. FAS overexpression was defined by an increase (+Δ) of at least 10% of cells in RP versus PBx. A +Δ of less that 10% of staining was considered as no change (NC).

Results: The mean age of the patients was 65.9 years (range 50-78 years). The biopsy Gleason score (GS) was 6 (55%), 7 (34%), and 8 (11%). Benign prostatic glands were negative for FAS. FAS protein was detected in 96% of high-grade prostatic intraepithelial neoplasia lesions (PIN), 75% of untreated PCa (PBx), and 97% of treated PCa (RP). FAS expression increased in 53% of RP relative to PBx. In 9 PCa (11%) FAS levels in RP remained comparable to PBx. In 31 cases (36%) FAS expression in RP was reduced relative to PBx. Eighty-nine percent of GS 8 PCa showed FAS overexpression after TAA.

Conclusions: FAS expression persists or is increased in the majority of PCa, particularly in high grade, aggressive tumors, after androgen ablation. Since FAS inhibitors result in selective apoptosis of prostate cancer cells, FAS may constitute an additional therapeutic target alongside TAA.

747 Smad4 Protein Is Expressed in Prostatic Adenocarcinomas (PACs) and Correlates with Grade, Stage and DNA Ploidy

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Background: The tumor suppressor gene Smad4 (DPC4) has been localized to chromosome 18q21.1 and is a member of the Smad family which mediates the TGFbeta signaling pathway suppressing epithelial cell growth. However, variable expression of this protein has been reported, with a loss in some cancers and increased expression in others. Given both the variability and lack of concensus reported regarding Smad4 expression in prostate cancer, we assessed Smad4 immunoreactivity in PACs.

Design: Formalin-fixed paraffin-embedded tissue sections from 133 PACs were immunostained by an automated method (Ventana Medical Systems, Tucson, AZ) using a monoclonal mouse anti-human Smad4 antibody (sc-7966 Santa Cruz Biotechnology, Santa Cruz, CA). Nuclear and cytoplasmic immunoreactivity were each semi-quantitatively scored based on intensity and percentage of positive cells. DNA ploidy was determined on Feulgen stained tissue sections by static image analysis. Results were correlated with morphologic and prognostic variables.

Results: Variable nuclear and cytoplasmic Smad4 positivity was noted in the adjacent benign glands in all cases. 64/133 (48%) PACs featured increased nuclear and 68/133 (51%) featured increased cytoplasmic protein expression. Nuclear Smad4 overexpression correlated with tumor grade (p=0.02), stage (p=0.04) and DNA ploidy (p=0.04). Cytoplasmic overexpression correlated with tumor grade (p=0.04) and DNA ploidy (p=0.04) while showing a trend for correlation with tumor stage (p=0.08). Neither nuclear nor cytoplasmic Smad4 overexpression correlated with post-surgical biochemical disease recurrence.

Conclusions: Smad4 protein expression persists in PACs compared to benign glands with both nuclear and cytoplasmic overexpression correlating with tumor grade, stage and DNA ploidy. Given the significant reported variablity of Smad4 in several different cancers, further studies in prostate and other tumors appear warranted to elucidate its role in tumorigenesis.

748 Decreased α-Methylacyl CoA Racemase Expression in Localized Prostate Cancer Is Associated with an Increased Rate of Biochemical Recurrence and Cancer Specific Death

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Background: α-Methylacyl CoA racemase (AMACR) was identified by differential display and expression array analysis as a gene over expressed in prostate cancer relative to benign prostatic tissue. AMACR expression is highest in localized prostate cancer and decreases in metastatic prostate cancer. Herein we explored the use of AMACR as a biomarker for aggressive prostate cancer.

Design: AMACR protein expression was determined by immunohistochemistry using a semi automated image analysis system (ACIS II System, Chromavision, San Juan Capistrano, CA) using a scale between 0 and 255 chromogen intensity units on tumor samples from over 440 men with localized prostate cancer at time of diagnosis. The two clinical cohorts consisted of 204 men treated by radical prostatectomy and 190 men who were followed expectantly without localized treatment ("watchful waiting"). The endpoint for the surgical cohort was an elevation in serum PSA following surgery over 0.2 ng. /ml (biochemical failure) with up to eight years follow up and the watchful waiting cohort was followed for up to 20 years for all cause and cancer specific death. A cutpoint for AMACR protein expression was determined using a regression tree method taking all parameters including AMACR into account for both cohorts separately. Cross validation of the optimal cutpoints was performed.

Results: Lower AMACR expression was associated with worse outcome independent of clinical parameters in both patient populations. The cutpoint for AMACR developed using PSA biochemical failure as a surrogate endpoint did not predict cancer specific death. However, the AMACR cut point developed using cancer specific death as the endpoint predicted PSA failures independent of Gleason score, serum PSA, and surgical margin status.

Conclusions: This is the first study to show that the quantitative evaluation of AMACR is significantly associated with prostate cancer progression. This study also suggests that not all surrogate endpoints may be optimal to define biomarkers of aggressive prostate cancer.

749 Prostatic Adenocarcinoma with Mucinous Components a Clinicopathological and Immunohistochemical Study

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Background: Mucinous adenocarcinoma of the prostate is a rare variant of prostatic adenocarcinoma characterized by lakes of extracellular mucin in at least 25% of a resected tumor. Limited data is available on the significance of mucinous components (MC) in prostatic adenocarcinoma. Clinicopathological profiles of 9 cases of prostatic adenocarcinoma with MC including 3 cases of mucinous prostatic adenocarcinoma were analyzed.

Design: We searched surgical pathology files [5/03- 8/04] for patients with prostatic adenocarcinoma with MC. Clinical characteristics examined included age, PSA and clinical presentation. Gleason score [GS], pathological stage and the surgical margin [SM] status were evaluated in completely embedded radical prostatectomy specimens. Immunohistochemical features were assessed using paraffin sections. Staining was recorded as focal [1-10%], moderate [11-50%] or extensive [51-100%].

Results: Excluding cases with only needle biopsy, MC were seen in 9 cases [0.64%] of 1402 cases of prostatic adenocarcinoma. These included 3 cases of mucinous prostatic adenocarcinoma [0.21%). The patients' mean age was 53 years [range, 50-56 years] in those with mucinous adenocarcinoma and 65 [range, 55-80 years] in those with <25% MC. All patients presented with PSA elevation. The 3 patients with mucinous adenocarcinoma had a mean PSA of 15.6 ng/ml [range, 5.8-29 ng/ml] and the other 6 cases had a mean PSA of 9.5 ng/ml [range, 5.2-16.6 ng/ml]. Histologically, MC were composed of cribriform islands, tubules and cords of tumor cells floating in mucin. GS 4+ 3, pT2c and negative SM were found in all 3 mucinous adenocarcinomas with 30%, 40% and 70% mucinous areas. MC were seen in 5% to 20% in the other 6 cases. GS 4+ 3 or 3+ 4, pT2c and negative SM were found in 5 patients whereas 1 patient had GS 4+ 3 with a third component of pattern 5, pT3a and positive SM. Immunohistochemical staining was similar in MC and in in non-mucinous areas with PSA (extensive strong staining in all]. CK 7 staining was seen in MC of 6 cases [66%] [focal in 3, moderate in 2 and extensive in 1] and in the non- mucinous components of the same cases [focal in 4 and moderate in 2]. CK 20 was focally positive in 6 cases [66%] in MC and five cases in non- mucinous areas (55%). CEA was positive focally in 1 case in MC [11%] and in four cases in non-mucinous areas [44%].

Conclusions: Prostatic adenocarcinoma with any MC appears to be high-grade carcinoma. Knowledge of the immunoprofile is useful when differentiating this tumor from mucinous carcinoma of non-prostatic origin

750 JAGGED1 Expression Is Associated with Prostate Cancer Metastasis and Recurrence

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Background: The androgen receptor (AR) plays a critical role in prostate cancer (PCA) biology. While withdrawal of androgens leads to a rapid decline in PCA growth, tumor cells emerge which are refractory to hormonal depletion. Recent studies have identified JAGGED1, a NOTCH receptor ligand, as a highly androgen-responsive gene product in the PCA cell line LNCaP and raise the possibility that JAGGED1 may play a role in tumor progression and as a marker of clinical outcome.

Design: To study the expression of JAGGED1 in the progression and outcome of PCA, immunostaining was performed on TMAs containing a wide range of prostate tissues from 167 men with hormone naive and refractory PCA. Staining intensity was quantified using a semi-automated Imaging System (Chromavision, San Juan Capistrano, CA).

Results: JAGGED1 staining intensity was increased significantly in clinically localized PCA (score = 94.2; SE = 1.8) versus benign prostate tissue (score = 79.6; SE = 2.8) (p value <0.001), and again in metastatic PCA (127.5; SE = 4.6) as compared with localized prostate cancer (p value <0.001). Univariate analysis demonstrates a strong association between PSA recurrence (defined as a PSA increase >0.2 ng/ml) and high levels of JAGGED1 staining (relative risk 3.05 with 1.8 staining intensity cutoff). Moreover, the best multivariate model predictive of PCA specific recurrence after radical prostatectomy for clinically localized PCA included extraprostatic extension (p value = 0.0005, RR = 3.94), preoperative PSA (p value = 0.042 , RR = 1.97) and JAGGED1 protein expression (p value = 0.016, RR = 3.51). All images for this study are available online: http://rubinlab.tch.harvard.edu/supplemental_data/JAGGED1/index.jsp

Conclusions: These findings support a model in which dysregulation of JAGGED1 protein levels plays a role in PCA progression and metastasis, and suggest that JAGGED1 may be a useful marker in distinguishing indolent from aggressive PCA.

751 Prostate Cancer and Prostatic Intraepithelial Neoplasia Detection Rate by Saturation Needle Biopsy

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Background: Although the optimal extent of prostate biopsy (PBx) is still controversial, there is a trend to increase the number of cores taken to increase the detection rate of prostate cancer (PCa). The aim of this study was to investigate the detection rate of PCa and high-grade prostatic intraprostatic neoplasia (PIN) by first time saturation PBx**.**

Design: Between February 2003 and May 2004, 100 consecutive saturation PBx performed by a single urologist and reviewed concomitantly by two genitourinary pathologists were included in the study. Indications for saturation PBx were abnormal digital rectal examination and/or a serum PSA over 2.5 ng/ml. All patients underwent a 24-core biopsy protocol. Patient age, PSA, percentage (%) of PIN, % cancer, %atypical glands, % adenosis, Gleason score (GS), PIN type, number of cores involved by PIN and cancer, bilaterality, extra-capsular extension (ECE) and perineural invasion (PI) were recorded.

Results: Mean age and PSA of the patients were 63.8 years (range 41-80) and 6.2 ng/ml (range 1.49-67), respectively. PCa was detected in 37 (37%) patients. GS was 6 in 25 (68%), and 7 in 12 (32%) cases. ECE and PI were identified in 2 and 8 cases, respectively. The mean number of cores with cancer was 5.3 (range 1 to 19) and the mean percentage of tissue/case involved by cancer was 8.7% (range 0.2 to 36%). In 10 cases (27%), PCa was detected in only one core, in 5 of which the tumor involved ≤10% of the length of the core. In 18 cases (48.6%) PCa was present bilaterally and in 26 cases (70%) was associated with PIN. Of 100 patients 51 (51%) were positive for PIN: flat (8%), micropapillary (22%), and tufting (92%). The mean number of cores with PIN was 2.7 (range 1 to 8) and the mean percentage of tissue/case involved by PIN was 1.1% (range 0.1 to 14.3%). In 17/51 cases (33.3%) PIN was present bilaterally. PIN was the only finding is 22 cases (22%). Atypical glands were found in 22 cases: 15 concomitant with PCa, 3 with PIN and 4 (4%) as the only finding. Adenosis was detected in 2 patients (2%).

Conclusions: The detection rate of PCa by saturation PBx was 37%. PIN was present as only finding in 22% of the patients. Approximately one-forth of the PCa (tumor involving a single core) may have remained undetected by conventional sextant biopsy. Saturation biopsy increases the detection rate of PIN and PCa.

752 Nox Gene Expression in Renal Cell and Prostate Carcinoma

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Background: Antioxidant therapy for cancer has become increasingly widespread in recent years, and there are multiple clinical trials of antioxidants for both renal and prostate cancer. The Nox family of genes encodes peptides that enzymatically produce reactive oxygen species (ROS). We have previously demonstrated that Nox 5 regulates growth in the prostate cancer cell line DU-145, and that Nox 1 overexpression enhances tumorigenesis in this cell line. In order to determine whether these pro-oxidant enzymes are activated in genitourinary malignancy, we have studied gene expression of Nox isoforms in clinical samples of prostate and renal cancer, with this study representing the first known detailed report of Nox in renal cell carcinoma.

Design: Tissues were harvested and frozen at the time of surgery following either radical prostatectomy or radical nephrectomy. Gene expression was assayed using Affymetrix chips or quantitative RT-PCR. In the case of the Affymetrix chip, gene expression was compared to a universal RNA standard, and in the case of RT-PCR, cyber green technology was used to quantitate message levels, normalized to actin expression. We analyzed 60 prostate cancers and 24 renal cell cancers.

Results: Nox 1 is significantly overexpressed in the majority of both prostate and renal cancers. Expression of one of the key Nox regulatory proteins, Nox A1, correlates highly with Nox 5 expression in higher-grade prostate cancer. This relationship is not apparent in lower-grade prostate cancers. In clear cell (conventional) renal cell carcinoma, Nox 1 and Nox 4 are overexpressed in most cases, while Nox 3 is underexpressed in 85% of the cases.

Conclusions: Nox genes encode enzymes responsible for the generation of reactive oxygen species in both renal and prostate cancers. Overexpression of Nox 1 is a common cancer-associated finding in both tumor types. It appears that Nox 5 enzyme and Nox A1 regulatory proteins are co-regulated, but only in high-grade prostate cancer. Nox 3 is down regulated in the vast majority of clear cell renal cell carcinoma. Thus, the pro-oxidant Nox enzymes may play an important role in the pathophysiology of both of these common urologic malignancies. Within the Nox gene family, there appears to be both organ-specific and grade-specific isoform expression.

753 Prostate Cancer (Ca) c-Myc (8q24) Amplification Detected by Fluorescence In Situ Hybridization (FISH) and MIB-I Proliferation in Needle Biopsy (NBx) Predict Risk of Clinical Progression after Radical Prostatectomy (RP)

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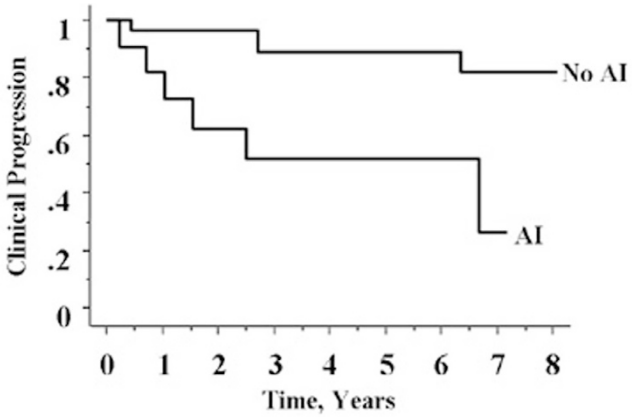
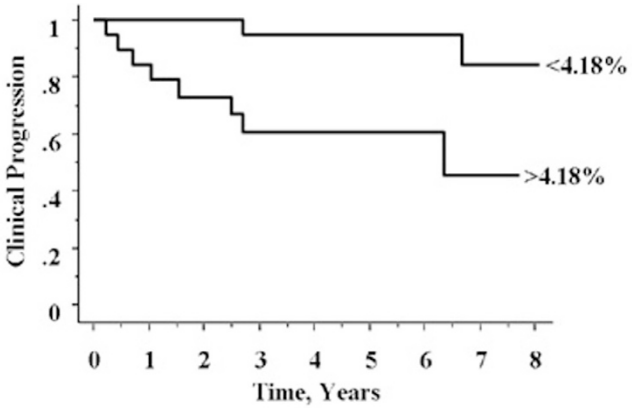
Background: Additional increase (AI) of 8q24 is prevalent in high grade and stage ca and is associated with biochemical failure in prostate ca. The c-myc oncogene is contained within the 8q24 region and amplification is associated with ca proliferation. Studies have shown the nuclear proliferation immunomarker, MIB-I, to be linked to prostate ca progression.

Design: We analyzed 41 nbx containing Gleason score 8 (n=24; 58%) or 9 (n=17; 42%) ca and their matching RP specimens for chromosome 8 alterations (C8A) including 8q24 AI using FISH. The RP result was the gold standard for determining the reliability of C8A detection on nbx (Kappa statistic). These same ca on nbx were quantified using a CAS200 analyzer for the nuclear immunostain MIB-I and expressed as %nuclear area. The results of the C8A and MIB-I levels were correlated with each other (Spearman rank) and with clinical progression (CP) (Kaplan and Meier method; KMM), defined as biochemical failure, local recurrence, or systemic progression (n=10).

Results: C-myc AI was seen in 12 (29%) nbx and 11 (27%) RP with 100% sensitivity and 97% specificity (Kappa=0.94). Mean ca MIB-I=6.17% (median=4.18%; range 0.02-40.3%). Ca with c-myc AI tended to have higher MIB-I levels (p=0.07). Median patient (pt) follow-up was 6.0 yrs (range=1-8). Figures 1 and 2 show results of KMM tracking nbx c-myc AI (p=0.009) and MIB-I levels (p=0.002) with CP, respectively (KM curves illustrative only).





Conclusions: C-myc (8q24) AI can be identified on nbx with high sensitivity and specificity, is associated with high MIB-I levels, and confers an increased risk of CP after RP. This method may help identify pt at risk of CP before treatment is initiated, potentially altering management.

754 Epidermal Growth Factor Receptor (ErbB1) Expression in Prostate Cancer Progression: Correlation with Androgen Independence

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Background: Once Prostate Cancer (PCa) progresses to androgen independent state, therapeutic options are limited and prognosis is invariably poor. The molecular mechanisms for the development of androgen resistance likely involve peptide growth factors and their receptors. ErbB receptor tyrosine kinases (RTK) have been implicated in the progression of a wide variety of human cancers. In this study, we investigated the potential role of epidermal growth factor receptor (ErbB1) in PCa progression by comparing its expression in primary hormone-naïve clinically localized vs. advanced metastatic androgen-independent PCa.

Design: Tissue micro arrays containing 433 cores from 112 radical prostatectomies performed for clinically localized hormone-naïve PCa and 323 cores from 30 patients died of advanced hormone refractory PCa at our institution were evaluated for the protein expression of ErbB1 receptor (31G7, Zymed). Staining was predominantly localized to cell membrane and was evaluated on a scale of 1(negative), 2(weak), 3(moderate) and 4 (strong) staining intensity (SI). Mean EGFR = 2 and EGFR >2 expression groups were analyzed for association between SI and different clinical parameters and for androgen state between hormone-naïve and hormone-refractory PCa.

Results: EGFR expression was strongly associated with hormone-refractory status (odds ratio=6.1, CI=(2.6, 14.5), p<0.0001). It was not associated with tumor differentiation (p=0.44), positive margins (p=0.53), seminal vesicle invasion (p=0.69), extraprostatic extension (p=0.10) or PSA biochemical failure (p=0.5) in hormone naïve group. In hormone-refractory group there was slight evidence that higher EGFR staining (>2) was associated with worse survival as measured time from chemotherapy to death, however it is not statistically significant (p=0.44).

Conclusions: Our preliminary findings suggest that EGFR expression increases during the natural history of prostate cancer progression, in particular with androgen-independent state. This correlation with progression to androgen-independence suggests that EGFR-targeted drugs could be of therapeutic relevance in the management of advanced PCa.

755 Heterogeneous Androgen Receptor (AR) Protein Expression in Metastatic Androgen-Independent Prostate Cancer: Implications for Complex AR Mechanisms in the Progression to Androgen Independent Prostate Cancer

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Background: Since intracellular AR, a ligand-dependent transcription activator mediates androgen action; abnormalities in AR are believed to play an important role in the prostate cancer (PCa) progression. How PCa becomes androgen independent however is largely unknown and information of AR expression in hormone refractory (HR) PCa is limited. We investigated the AR protein expression in HR metastatic PCa with the goal to understand its importance in the development of androgen independent disease.

Design: A tissue micro array (TMA) was constructed to represent all metastasis sites and prostate from 30 rapid autopsies of men who died of HR PCa at the University of Michigan. TMA was immunostained for monoclonal antibody AR-clone AR 441 (Neo Markers, Fremont, CA). Immunohistochemistry was quantitatively evaluated (percentage expression of 0-100%) using Chromavision ACIS II version. Comparisons were made between patients as well as within the same patients.

Results: Consistent with its role as a transcription factor, AR was usually localized in the nuclei. AR expression demonstrated significant heterogeneity across disease sites, with 31% (83 of 265) tumor samples expressing > 50% AR and 41.5% (100/265) expressing <10% AR. Overall AR expression was down regulated with median AR expression of 20.04% (range, 0-100%, standard error 34.28). AR expression also ranged markedly between different disease sites in similar patients (2-50 fold). AR expression was not predictive of survival time as measured from initiation of chemotherapy to death (p=0.99).

Conclusions: Androgen independent metastatic PCa frequently demonstrate high amount of AR staining despite the fact that they are no longer responsive to androgen deprivation therapy. Heterogeneity in AR expression with frequent AR positive and AR negative tumor populations between and within same patients, suggest alternate molecular mechanisms play an important role in the progression of androgen independence.

756 Correlation of Prostate Biopsy and Prostatectomy Gleason Score: Relationship of Number of Positive Biopsies, Highest Volume and Highest Score

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Background: The Gleason score (GS) of prostate biopsies (bx) is a pre-operative predictor of radical prostatectomy (RP) GS, clinical stage, and prognosis. However, bx GS does not perfectly correlate with RP GS. Overgrading and undergrading still occurs. A particular problem area is choosing the most predictive bx GS when bx of differing GS are present.

Design: Bx GS was correlated with RP GS for 306 RP cases in which pre-operative bx material was available from 1998 - 2004. Total number of bx sites, number of bx sites involved with carcinoma, GS and % of each bx site involved with carcinoma, and GS of RP were recorded. Correlation of the bx and RP GS were done for cases with 1 positive bx site and >1 positive bx site with the same GS. In cases with >1 positive bx site and varied GS, correlation between highest GS bx and RP, and highest volume bx and RP were evaluated.

Results: 175 cases had 1 positive bx site. The correlation between bx GS and RP GS was 128/175 (73%). Of the 47 non-correlated cases, 36 were undergraded (77%) and 11 cases were overgraded (23%). The difference was 1 GS unit in 42/47 of the cases. 131 cases had >1 positive bx site. 94 of these had all sites with the same total GS and 37 had differing GS. The correlation between bx with same GS and RP GS was 64/94 (68%, P=0.4, NS from 1 + site above). Of the 30 non-correlated cases, 26 were undergraded (87%) and 4 cases were overgraded (13%), and the difference was 1 GS unit in 27/30 cases. 37 cases had differing GS between bx sites, but in 28 cases, the bx with the highest GS was the bx with the highest volume of cancer**.** The correlation for these 28 cases was 75% (P=0.82, NS compared to 1 positive site; P=0.46, NS compared to >1 positive site same GS). In the remaining 9 cases with differing bx GS, the bx with the highest GS correlated with RP GS in 4 cases, the bx with highest tumor volume correlated with RP GS in 3 cases, and in 2 cases neither correlated.
Conclusions: Multiple positive bx of same GS are no more predictive of RP GS than single positive bx. When multiple positive bx with different GS are present, in most cases the bx with the highest GS is the bx with the highest volume. For the few cases in which the GS of the bx of highest volume of cancer differed from the GS of the bx with highest GS, there was no statistical difference in correlation with subsequent RP GS grade. The current practice of urologists to use the bx of the highest GS to predict outcome appears reasonable in these relatively rare events.

757 Renal Cell Neoplasm with Dual Differentiation of Proximal and Distal Nephron: An Immunohistochemical Studies

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Background: Renal epithelial neoplasms are currently classified into clear cell, papillary, chromophobe, collecting duct, unclassified and oncocytoma based on their morphologic and cytogenetic features. It is generally considered that each group is derived from or has terminal differentiation toward proximal or distal portion of the nephrons. However, morphologic overlap of different histologic types and combinations of two or more types are not uncommon. In this study, we characterized the phenotypic features of 123 renal neoplasms using RCC marker, a marker for proximal tubules and Ksp-cadherin, a new and specific marker for distal nephron.
Design: Sequential or double immunostain for RCC marker and Ksp-cadherin was performed on paraffin-embedded tissue sections of 123 renal cell neoplasms including clear cell RCC (n=59), papillary RCC (n=30), chromophobe RCC (n=14), oncocytoma (n=20) using the standard procedure. Non-neoplastic kidney tissues (n=45) were also included in the studied.
Results: Within the normal kidney, RCC marker stains the proximal tubule with a apical surface pattern and Ksp-cadherin stains the cells of the distal portions of nephron with a basolateral pattern. Double immunostain of RCC marker and Ksp-cadherin showed staining of the entire renal tubular system. The expression of RCC marker was seen in 89.9%, 96.8%, 14.3%, and 0% for clear cell, papillary, chromophobe RCCs, and oncocytomas, respectively. The expression for Ksp-cadherin was seen 18.6%, 16.1%, 100%, and 95% for clear cell, papillary, chromophobe RCCs, and oncocytomas. Interestingly, 11 of 59 (18.6%) clear cell RCCs and 5 of 31 (16.1%) papillary RCCs showed staining for both RCC marker and Ksp-cadherin. Furthermore, the clear cell RCCs with dual staining showed a distinctive cytomorphology characterized by tubulocystic or tubulopapillary architecture, higher nuclear grade, and more eosinophilic cytoplasm. All papillary RCCs with dual staining were tumors with type 2 morphology.
Conclusions: Ksp-cadherin is a highly selective marker for the distal portions of the nephron. It stains over 95% of the chromophobe RCC and oncocytoma. A subset of clear cell RCC and papillary RCC expresses both RCC marker and Ksp-cadherin, suggesting dual differentiation and appear to have distinct cytomorphology.

758 Incidental Prostatic Carcinoma in 237 Cystoprostatectomy Specimens for Bladder Cancer Evaluated by Whole Mount Sections

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Background: Removal of the prostate with the bladder cancer during a radical cystectomy for bladder cancer is standard practice to prevent a local recurrence of transitional cell carcinoma. In addition, it may provide additional benefit of removing any "incidental" prostatic adenocarcinoma. Previously published studies have shown that the incidence of prostate carcinoma ranges from 38 to 46% by selective sections or whole mount sections of prostate in smaller number of cases. In this study, we have examined the histopathologic features of incidental prostatic carcinoma in 237 radical cystoprostatecomy specimens for bladder cancer and compare with that of radical prostatectomy for prostate cancer.
Design: The entire prostate glands of 237 men undergoing cystoprostatectomy for bladder cancer from 1988 to 2003 at one large university hospital were examined by whole-mount sections at 5-µm intervals to identify unsuspected prostatic adenocarcinoma. The common histopathologic features such as Gleason score, status of extracapsular extension (ECE), seminal vesicle involvement (SVI), and positive surgical margin (SM) were recorded and compared with that of radical prostatectomy for prostate cancer.
Results: Of 237 patients who had cystoprostatectomy for bladder caner, prostatic carcinoma was detected in 123 patients with an incidence of 52%. Sixty-nine percent of prostatic carcinoma was of Gleason score of 6 or higher and 17% was Gleason score of 7 or higher. ECE, SVI and positive SM of prostate cancer were seen in 9.8%, 4.9%, and 4.1% of the cases. In contrast, in radical prostatectomy (n=712) for prostate cancer, 51.2% cancers were of higher than Gleason score of 7, had more frequent ECE (30%), SVI (10.4%) and positive SM (10.7%).
Conclusions: Prostatic carcinoma is a very common finding in radical cystoprostatectomy specimen. Although the cancers had lower grade, less extracapsular extension, less seminal vesicle invasion, and less positive surgical margin than that of radical prostatectomy for prostate cancer. Many of them may be of clinically significant. We recommend that the prostate of radical cystoprostatectomy for bladder cancer should be examined similarly as for radical prostatectomy for prostate cancer.

759 Prostatic Involvement by Urothelial Carcinoma in Patients with Bladder Cancer: Patterns and Significance

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Background: Prostatic involvement by urothelial carcinoma (UCa) in patients with bladder cancer is frequently diagnosed. It is considered to be T4a in the TMN classification regardless of the depth and pattern of involvement in the prostate. However, the patterns of involvement and their relationship with bladder cancer and prognostic significance were not thoroughly investigated.
Design: Whole mount prostate sections from 214 cases of cystoprostectomy specimens were reviewed for incidence and patterns of UCa involvement of prostate including (1) urethral/ductal CIS; (2) lamina propria invasion; (3) prostatic stromal invasion; (4) extracapsular/seminal vesicle invasion or direct penetration from bladder cancer. The patterns of prostatic involvement by UCa were correlated with bladder tumor stage, presence of multifocal vesical CIS, location of bladder cancer, and lymph node metastasis.
Results: UCa was detected in 69 prostates of 214 (32.2%) radical cystoprostatectomy specimens. Thirty of 69 cases (43.5%) were CIS only and 39 (56.5%) were invasive UCa. Ten of the invasive UCa were direct penetration from bladder cancer and the remaining 29 cases arose from prostatic urethra/ducts, of which 11, 13 and 5 of them were lamina propria, prostatic stromal, and extracapsular or seminal vesical invasion, respectively. The frequency of lymph node metastasis was significantly higher for cases with prostatic UCa involvement (43.5%) than that of no prostatic UCa (15%) ($p<0.001$). Moreover, the frequencies of lymph node metastasis were associated with the patterns of prostatic UCa involvement, which were 23.3%, 27%, 69.2%, 80% for CIS, lamina propria, deep stromal, and extracapsular/seminal vesicle invasion or direct penetrating. The prostatic involvement by UCa was associated with multifocal vesicle CIS ($p<0.05$) and presence of bladder tumor at trigone ($p<0.01$).
Conclusions: Four patterns of prostatic involvement by UCa can be recognized and each appears to have different prognostic implications. The occurrence of prostatic UCa is associated with mutifocal vesicle CIS and bladder tumor at trigone area. Our results also suggested that prostate involvement by CIS or laminal propria invasion should not be considered T4 disease in bladder cancer staging.

760 Androgen Receptor CAG Tract Heterogeneity in Benign and Malignant Prostatic Glands

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Background: Shorter CAG repeat tracts(CRT) of the androgen receptor(AR) gene have been correlated to increased AR transactivation and increased risk of prostate cancer(Pca). Previous studies were performed on easily accessible but *non-androgen responsive tissues* such as leucocytes and fibroblasts. We analyzed and sequenced CRT in the ***prostatic tissues themselves*** enabling us to look at Pca and the different zones of benign prostate.
Design: Lesions of PCa and benign peripheral(PZ), transition(TZ) and central(CZ) zones were mapped on four whole mounted radical prostatectomy specimens. Tissue microarrays(TMA) were constructed from these regions using duplicate/triplicate 1 mm cores. An average of 1670 cells per benign zone and 3200 cells per PCa lesion were collected from TMA using laser capture microdissection. DNA was isolated and amplified by nested PCR. PCR products were separated on a polyacrylamide gel and directly sequenced. Immunohistochemistry for AR and PSA was performed on TMA.
Results: 1. The *number* of different sized CAG tracts examined was heterogeneous in all groups with:

NUMBER OF DISTINCT CAG TRACTS VS REGION OF PROSTATE

REGION	Mean # of CAG tracts/ Patient	Range
TZ	4.25	2-6
PZ	5.00	3-7
CZ	5.00	2-7
PCa	4.75	3-7

2. Complete *deletions* of the CAG tract were identified in 3 patients: 1 from PZ and Pca, 1 from TZ only, 1 from CZ only.
3. The CAG tract *length* showed wide variation:

CAG TRACT LENGTH VS REGION OF PROSTATE

REGION	Mean	Range*
TZ	17.1	11-23
PZ	19.2	16-23
CZ	17.4	14-23
PCa	17.3	11-22

*Range excludes deletions where CAG tract length=0

Benign and malignant prostatic tissues were all immunoreactive for AR and PSA.
Conclusions: 1. Previous studies on leucocytes showed a *unique* CAG repeat length per patient. Our data showing subpopulations with *different sized* CAG tracts within each zone of benign prostate and PCa suggests somatic instability and mosaicism of the AR.

2. Other novel findings, including entire CAG tract deletions in histologically "benign" prostate, suggests that genetic heterogeneity of the AR precedes histologically recognizable, phenotypic changes.
Further study is indicated in determining whether clones with shortened or missing CAG tracts have a growth advantage in prostatic neoplasia.

761 Immunoreactivity of Anti-P501S, a Novel Prostate Specific Marker, and Anti-PSA in Human Metastatic Prostate Cancer

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Background: P501S, also called prostein, is a prostate specific protein identified by cDNA library subtraction and high throughput microarray screening. Expressed in the majority of both normal prostate and prostate adenocarcinoma, P501S is predicted to be a type IIIa plasma membrane associated protein. A previously published report involving approximately 4700 normal and malignant tissues of various types demonstrated that anti-P501S immunoreactivity was confined to normal and malignant prostatic tissue. Prostate-specific antigen (PSA), a member of the kallikrein family of proteases, is a secreted protein produced by the prostatic epithelium and the lining of the periurethral glands. Used in both serum testing and immunohistochemistry, PSA is found in normal and malignant prostatic tissue. Published reports have also identified PSA in other cancers, such as adrenal neoplasms and renal cell carcinoma. We investigated the immunoreactivity of anti-P501S compared to anti-PSA in human metastatic prostate cancer samples to determine the relative utility of these markers in aiding in metastatic prostate cancer recognition. The identification of metastatic prostate cancer is vital to the treatment and survival of prostate cancer patients.
Design: Formalin fixed paraffin embedded (FFPE) human metastatic prostate cancer tissue samples were obtained from various organs, including lymph node and bladder. The sectioned slides were pretreated with heat-induced epitope retrieval, and immunohistochemistry was performed using mouse monoclonal antibodies to P501S and PSA and a universal negative control reagent. Bound antigen was detected using the DakoCytomation Envision + Detection System.
Results: The P501S and PSA antibodies demonstrated immunoreactivity in similar areas of the metastatic prostate cancer tissues. Anti-P501S produced a granular cytoplasmic staining pattern, while anti-PSA uniformly stained the cytoplasm of cells. The P501S immunostaining was confined to cancer cells in the specimens tested, while on a subset of samples PSA immunostaining was observed in serum and other non-malignant regions of the specimen, where PSA leakage may have occurred.
Conclusions: Both anti-P501S and anti-PSA stained metastatic prostate cancer tissue samples, with anti-P501S demonstrating a slightly more discrete staining pattern. Due to previous reports describing PSA expression in other non-prostatic cancers, a panel of antibodies including both anti-P501S and anti-PSA could aid in the identification of metastatic prostate cancer.

762 Aurora A: A Biomarker of Aneuploidy in Bladder Cancer

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Background: Aurora A is a mitotic kinase encoding gene that controls chromosomal segregation. When overexpressed, it causes centrosomal amplification resulting in misegregation of chromosomes and aneuploidy.
Design: Urothelial cells from voided urines of 23 patients with bladder cancer were tested for Aurora A copy number using a gene-specific FISH probe. The gene expression levels were subsequently tested on 31 paired RNA samples corresponding to bladder tumors and their adjacent urothelium and correlated with gene copy number, DNA ploidy, and tumor characteristics. These correlative observations were confirmed by the functional studies in which in vitro grown SV-40 transformed human urothelial cells were transfected with an Aurora A containing viral construct. The transfected cells were analyzed for post-transfection centrosome and chromosome copy numbers as well as DNA ploidy based on measurements of total nuclear DNA content.
Results: Studies on voided urines revealed that tumors with low levels of Aurora A amplification (3-4 copies) showed minimal deviation in their chromosome copy number and diploid or near-diploid total nuclear DNA content. Tumors with higher levels of Aurora A amplification (>4 copies) had a pronounced increase of chromosome copy number and total nuclear DNA content (aneuploidy). The low level of gene amplification (3-4 copies) was ubiquitously present in the voided urine samples of all patients with bladder cancer. The high level of Aurora A amplification was strongly associated with such parameters of clinical aggressiveness as high histologic grade and muscle invasion. Functional studies on Aurora A transfected urothelial cells revealed polyploidization of centrosomes, increased copy numbers of selected chromosomes (3, 7, and 17) and total DNA content gradually increasing over time post-transfection.
Conclusions: Aurora A when overexpressed in urothelial cells causes misegregation of chromosomes resulting in aneuploidy. The degree of aneuploidy is proportional to its degree of amplification/expression. Increased copy number of Aurora A is ubiquitous in bladder cancer and can be detected in exfoliated urothelial cells from urine sediments.

763 Value of CDX2, Villin, and α-Methylacyl Coenzyme A Racemase Immunostains in the Distinction between Primary Adenocarcinoma of the Bladder and Secondary Colorectal

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Background: Primary adenocarcinoma of the urinary bladder is an uncommon neoplasm, and sometimes causes diagnostic dilemma because it can be indistinguishable morphologically from adenocarcinoma of colorectal origin secondarily involving the bladder by direct extension or metastasis. The aim of this study was to evaluate whether CDX2, villin and α-methylacyl coenzyme A racemase (AMACR), which are immunomarkers preferentially expressed in colorectal adenocarcinoma, can be used in aiding the differential diagnosis.
Design: Formalin-fixed paraffin-embedded tissue sections from 17 enteric type primary adenocarcinomas of the urinary bladder, 17 colorectal adenocarcinomas secondarily involving the bladder, 23 primary colorectal adenocarcinomas, and 14 conventional urothelial carcinomas were used in this study. The expression of CDX2, villin and AMACR was evaluated immunohistochemically employing commercially available antibodies. A tumor was recorded positive if more than 5% of the tumor cells exhibited nuclear staining for CDX2, apical and/or cytoplasmic staining for villin, or cytoplasmic staining for AMACR.
Results: CDX2 and villin appeared to be less frequently expressed in primary bladder adenocarcinomas than in colorectal adenocarcinomas ($P<0.0001$ and $P=0.0019$, respectively). The frequency of AMACR expression was similar between these two types of tumor (Table). Coexpression of CDX2/villin, CDX2/AMACR and CDX2/villin/AMACR was observed in 7 (41%), 5 (29%) and 4 (24%) primary bladder adenocarcinomas, and 39 (98%), 27 (68%) and 26 (65%) colorectal adenocarcinomas, respectively ($P<0.0001$, $P=0.0103$ and $P=0.008$, respectively).

	Primary bladder adenoCa (n=17)	Secondary colorectal adenoCa (n=17)	Primary colorectal adenoCa (n=23)	Urothelial carcinoma (n=14)
CDX2	8 (47%)	17 (100%)	23 (100%)	0
Villin	11 (65%)	16 (94%)	23 (100%)	0
AMACR	11 (65%)	13 (77%)	14 (61%)	2 (14%)

Conclusions: Primary adenocarcinoma of the urinary bladder differs from urothelial carcinoma by expressing immunomarkers commonly detected in colorectal adenocarcinoma. However, our data suggest that these markers may still have diagnostic value in distinguishing primary adenocarcinoma of the bladder from secondary colorectal adenocarcinoma, particularly when used in combination.

764 Biomarker Identification for Testicular Tumors using Cation -Exchange ProteinChips

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Background: Different types of testicular tumors require different treatment regiments with varying prognosis. Accurate and early detection of these tumors, therefore profoundly affects management outcomes. The recent developed surface enhanced laser desorption/ionization time-of-flight mass spectrometry (SELDI-TOF MS) allows rapid and reproducible detection of novel tumor biomarkers. Application of this powerful technology in tumor research will shed new light on tumorigenesis, help in early and differential diagnosis, as well as patient monitoring leading to improved patient care.
Design: 23 frozen testicular samples from our tumor bank were used in the study, including 2 diffuse large B cell lymphomas, 2 non-seminomatous germ cell tumors (1 embryonal carcinoma and 1 yolk sac tumor), 7 seminomas, 8 mixed germ cell tumors, and 3 normal testicular tissue samples. Tissue homogenates were prepared and aliquots were analyzed on SELDI-TOF MS CM10 chips. Protein spectra were generated under different laser power settings. Data analysis was performed using Proteinchip software, Biomarker Wizard and Biomarker Patterns software (by Ciphergen Biosystems, Inc.).
Results: Up to 450 clusters of peaks were yielded ranging from 1000 Da to 71000 Da. Peaks with statistically significant expression changes between sample groups were identified as potential biomarkers. Classification trees were created using multivariate analysis to classify samples into lymphoma, germ cell tumor or normal tumor-free tissue with negligible error rates. Peaks (proteins/peptides) at m/Z values of 9808 Da, 7325 Da ad 1075 Da separated lymphomas from the others with a prediction rate of 100%.
Conclusions: Our preliminary study has identified several potential biomarkers in testicular tumors. Further characterization of these protein markers would include protein purification, sequencing and functional study. These studies could eventually result in better understanding of the tumorigenesis, early tumor detection, and more effective treatment.

765 Allelotyping of Clinical and Pre-Clinical Prostatic Carcinomas

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Background: Allelic imbalance such as loss of heterozygosity (LOH) is an important process for prostatic carcinogenesis. In the past, higher frequencies of LOH at 13q, 6q, 8p have been reported in the clinical prostate cancer. Although, in fully developed cancers, it is difficult to identify which alterations are specifically important in which stages of the cancer development. To address this issue, especially to know which genetic changes are making crucial roles in the development of cancer from pre-clinical to clinical stage, it is necessary to compare the LOH frequencies of clinical and pre-clinical cancers including precursor lesion.
Design: Twenty-two cases of PIN, 27 of latent cancer (LC), 18 of incidental cancer (IC) from cyst-prostatectomy for bladder cancer, and 70 of clinical cancer (CC) were collected and the LOH frequencies at 13q(14, 21, 33), 6q (16-22), and 8p (22-23.2) were compared in these 4 categories. Also, the association of incidence with Gleason score (GS) and other clinicopathological findings were investigated.
Results: Frequencies of LOH were similar in 3 categories of PCa. No LOH was found in PIN (Table 1). In CC, LOH at 13q and 6q were more frequent in stage D disease than in B or C. LOH at 8p was more frequent in stage B than in stage C and D (Table 2). LOH at 8p was more frequent in lymphatic/venous invasion negative and capsular penetration negative cases than in those positive cases of CC. Such tendencies were not given at 13q or 6q.

Conclusions: LOH at 13q and 6q seem to make important roles in both early development of prostatic carcinogenesis, local extension of cancer and metastases. Meanwhile, LOH at 8p seems to be more important at the pre-clinical stage through the local extension of cancer rather than in metastatic process. PIN might be irrelevant to the clinical cancer on the basis of LOH phenomenon.

Table 1 LOH frequencies in PIN, latent, incidental and clinical cancers

Cases	13q14-33	6q16-22	8p22-23
PIN	0/22 (0%)	0/22 (0%)	0/22 (0%)
Latent PCa	15/27 (55.6%)	16/30 (53.3%)	7/21 (33.3%)
Incidental PCa	5/14 (35.7%)	7/14 (50.0%)	3/14 (21.4%)
Clinical PCa	30/65 (46.2%)	35/75 (46.7%)	8/57 (14.0%)

Table 2 LOH frequencies by stages in clinical PCa cases

Stage	13q	6q	8p
B	9/20 (45%)a	9/21 (43%)d	7/18 (39%)g
C	13/35 (37%)b	15/38 (40%)e	1/28 (4%)h
D	8/10 (80%)c	11/16 (69%)f	0/11 (0%)i

a vs. c: p<0.05; b vs. c: p<0.01; d vs. e: p<0.01; e vs. f: p<0.05; g vs. h: p<0.005

766 Expression of MAP Kinase and Akt in Clear Renal Cell Carcinoma (RCC), Including Sarcomatoid RCC

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Background: MAP kinase (MAPK) and Akt are protein kinases (PK) that play an important role in the cell cycle. Clinical significance of these PK is due to the therapeutic use of small molecules targeting specific cell cycle protein pathways. As there is limited data on expression of these PK in renal cell carcinoma (RCC), we sought to determine the immunohistochemical expression of MAPK, phosphorylated MAPK (pMAPK), Akt, and phosphorylated Akt (pAkt), in different subsets of clear renal cell carcinoma (CRCC).

Design: A tissue microarray composed of duplicate 1.0 mm cores from 60 cases was used (total: 160 cores). These included 20 CRCC with sarcomatoid dedifferentiation (SRCC) including separate cores from epithelial and sarcomatoid components, 20 Fuhrman nuclear grade (FNG) 2 CRCC, and 20 FNG 3-4 CRCC. Immunohistochemical analysis of MAPK, pMAPK, Akt and pAkt was performed. Expression was rated by two observers on % of positive tumor cells (0= <5%, 1= 5-25%, 2= 26-75%, 3= >75%) and staining intensity (0= none, 1= weak, 2= moderate, 3= strong). Sum of % staining and intensity was used for determining positive and negative; sum ≥ 2 was considered positive.

Results: Results of immunohistochemical expression and pair-wise statistical comparisons are listed in tables 1 and 2, respectively. The expression of pAkt in the epithelial component of SRCC is significantly different from the expression in CRCC and sarcomatoid component of SRCC.

Conclusions: Majority of CRCC express MAPK, and only a subset express pMAPK, Akt and pAkt. These data suggest that specific targeting of the MAPK and Akt pathways may play a role in the treatment of a subset of patients with CRCC.

Results of Immunohistochemical Expression

	Akt (% positive)	pAkt (% positive)	MAPK (% positive)	pMAPK (% positive)
FNG 2 CRCC	35	50	95	40
FNG 3-4 CRCC	40	40	90	55
Epithelial SRCC	35	10	75	30
Sarcomatoid SRCC	30	35	95	55

P-Values of Pair-wise Comparison of IHC Expression

	Akt	pAkt	MAPK	pMAPK
FNG 2 vs FNG 3-4	1	0.75	1	0.53
FNG 2 vs Epithelial SRCC	1	0.014	0.18	0.2
FNG 3-4 vs Epithelial SRCC	1	0.065	0.41	0.74
FNG 2 vs Sarcomatoid SRCC	1	0.52	1	1
FNG 3-4 vs Sarcomatoid SRCC	0.74	1	1	0.53
***Epithelial vs Sarcomatoid**	0.13	0.005	0.005	0.44

*McNemar's test. All others, Fisher's exact test.

767 Transforming Growth Factor-β Receptor Expression in Clear Cell Renal Cell Carcinoma

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Background: Transforming growth factor-β (TGF-β) regulates diverse biological functions. Alterations in the TGF-β signaling pathway are thought to impact cellular processes implicated in neoplastic transformation. Of the 3 TGF-β receptors (TBR) (types I, II, and III), loss of TBR2 has been reported in renal cell carcinoma (RCC). We have also previously reported (Oncogene 22:8053-62, 2003) the down-regulation of TBR2 and TBR3 mRNA in clear cell RCC (CRCC) and now evaluate the protein expression (using immunohistochemistry) of TBR 1, 2, and 3 in all TNM stage CRCC and metastatic CRCC.

Design: Immunohistochemical stains for TBR types 1, 2, and 3 were performed on formalin-fixed paraffin embedded tissue from 10 normal kidneys, 40 primary CRCC (10 TNM stage I, 10 TNM stage II, 10 TNM stage III and 10 TNM stage IV), and 10 metastatic CRCC (matched metastasis from the TNM stage IV CRCC).

Results: Results of immunohistochemical expression are listed in Table 1. TBR3 was detected in all 10 normal kidneys, but not in any of the CRCC. TBR1 on the other hand was detected in all tumors and normal kidneys. TBR2 was detected in all normal kidneys and almost all (19/20) clinically localized (TNM stage I and II) CRCC. TBR2 was not detected in the majority of metastatic CRCC and in some (3/10) of the locally advanced (TNM stage III) CRCC.

Conclusions: There is loss of expression of the TGF-β receptor type 3 in clear cell RCC. This loss may represent an early event in the carcinogenesis of clear cell RCC, as this receptor is expressed in the normal kidney. The type 2 receptor can be detected in the clinically localized clear cell RCC, but is lost in clear cell RCC that are locally advanced or have metastasized. This may imply a role for TRB2 in the progression of clear cell RCC. Finally, The type 1 receptor does not seem to be affected in CRCC.

Table 1: Results of Immunohistochemical Expression of TGF-β Receptors

	TBR1 (no. positive)	TBR2 (no. positive)	TBR3 (no. positive)
Normal Kidney (n=10)	10/10	10/10	10/10
Stage I CRCC (n=10)	10/10	10/10	0/10
Stage II CRCC (n=10)	10/10	9/10	0/10
Stage III CRCC (n=10)	10/10	7/10	0/10
Stage IV CRCC (n=10)	10/10	2/10	0/10
Metastatic CRCC (n=10)	10/10	2/10	0/10

TBR= Transforming growth factor β receptor

768 PCA3 ^{DD3} Gene Expression Is a Marker of Prostate Cancer

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Background: PCA3 ^{DD3} gene has been identified by differential display analysis comparing mRNA expression of normal prostate and prostate cancer tissue and has been found by quantitative RT-PCR to be very specific for prostate cancer. This study was aimed at localizing PCA3 ^{DD3} gene expression on prostate cancer sections.

Design: In situ hybridization has been performed using ^{35}S mRNA probes on paraffin-embedded tissue sections. PCA3 ^{DD3} gene expression has been evaluated semi-quantitatively in cancer cells, stromal cells, basal cells, benign glands and in PIN separately.

Results: One representative section of 48 radical prostatectomy specimens from patients with prostate cancer has been selected. Of which, 28 sections had cancer and 20 had no tumor present. Of the 28 cancer sections, cancer cells were positive in 26 (93%), of which 19/26 (73%) had more than 50% cancer cells expressing the marker. Of the 20 sections without tumor cell, benign glands were positive in only 2 cases (10%). Furthermore, PIN was present on 28 sections and strongly expressed the marker on 27 (96%). Stromal cells and basal cells were negative in all cases.

Conclusions: In patients with prostate cancer, PCA3 ^{DD3} gene is expressed by cancer cells in almost all cases. Benign glands are positive in a minority of cases, suggesting that those cells are molecularly abnormal. Strong expression has been observed in PIN cases. PCA3 ^{DD3} gene may potentially be very helpful to confirm or exclude the presence of cancer cells on biopsies with atypical small acinar proliferation (ASAP) or following radiation therapy.

769 Pathological Characterization of Residual Prostate Cancer in the Neoadjuvant Surgical Model: A Proposal for Standardization of Morphologic Features of Treated Prostate Cancer

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Background: Novel therapeutic strategies are being tested in the neoadjuvant setting in patients (pts) with prostate cancer (PCa). Establishing reproducible morphologic parameters to characterize the residual PCa will be essential to evaluate the relative efficacy of different therapeutic trials.

Design: We previously analyzed the pathologic features of PCa following chemotherapy (ketoconazole, doxorubicin, vinblastine and estramustine) and hormonal ablation therapy (HATX) in the radical prostatectomy specimens (RPS) from 57 pts. We established by hierarchical cluster analysis of the architectural and stromal features that the residual PCa could be divided into three groups characterized by 1) predominance of cell clusters and cords (Group A: 5/57), 2) predominance of small and fused glands (Group B: 11/57) and 3) presence of PCa with cribriform areas and/or intraductal spread (Group C: 41/57). In the current study we expanded our evaluation to include pts who received preoperative HATX only (20) and pts who received imatinib, docetaxel and HATX (20).

Results: Residual PCa following different genotoxic therapies shows a spectrum of changes that can be divided into three groups: A (9/97, 9%), B (18/97, 19%) and C (70/97, 72%). There is a significant difference in the distribution of the pathologic stage among groups. 21/27 (78%) of the tumors in Groups A and B were organ confined or exhibited extraprostatic extension. In contrast, 56/70 (80%) of the RPS within Group C had seminal vesicle invasion or lymph node metastasis. (p= 0.04)

Conclusions: In this expanded series of the neoadjuvant surgical model, which includes three different therapeutic regimens (TR) including HATX alone or in combination with two different chemo TR, treated PCa could be divided in three distinct groups. This supports the concept that the groups observed are independent of the therapeutic regimen and reflect the spectrum of PCa response to different genotoxic challenges. Future studies will need to establish the reproducibility of the criteria applied and their biological relevance by linking them to genotypes and therapy response.

770 Tyrosine Kinases in Seminomas

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Background: Tyrosine kinases have been identified as major pathogenetic mechanisms in the development of malignant diseases. A novel approach to cancer treatment is the development of drugs that target Tyrosine Kinases; the best known of its kind is STI571 (Gleevec). c-kit has been demonstrated to be expressed in seminomas. We have investigated the presence of other tyrosine kinases in these tumors.

Design: Immunoexpression of tyrosine kinases was evaluated in fifty-five seminomas. In all of the cases paraffin blocks were available. c-Kit, PDGFR-alpha, PDGFR-beta and c-abl were investigated in all cases. Staining was assessed as follows: 6-25%, 1+; 26-50%, 2+; 51-75%, 3+; and 76-100%, 4+. Clinical information and follow-up was available in 50 patients.

Results: The age of the patients ranged from 17 to 69 (mean 34). Thirty-two patients had stage I disease, 15 stage II and 3 stage III. Follow-up was available in 50 patients and varied from 3 to 120 months. Recurrences were seen in 12 patients. There was no relationship between c-kit, PDGFR-alpha, PDGFR-beta or c-abl and age, stage or recurrences.

	c-kit	PDGFR-α	PDGFR-β	c-abl
4+	11	44	45	41
3+	6	9	5	5
2+	7	1	2	5
1+	17	1	3	4
Neg.	13	0	0	0
	41/54 (76%)	55/55 (100%)	55/55 (100%)	55/55 (100%)

Conclusions: In our study, 55/55 (100%) of seminomas were positive for PDGFR-alpha/beta and c-abl and 41/54 (76%) were positive for c-kit. Overall, 100% of the tumors in this study were positive for at least three of these markers. The staining was strongly and diffusely positive in approximately 80% of cases. Targeting tyrosine kinases could be a therapeutic strategy in some cases of seminomas. Studies should investigate the prospect of treating seminomas with tyrosine kinase inhibitors, especially the subset of patients who fail with conventional therapy or develop metastatic disease.

771 CD10 Expression in Urothelial Tumors: Comparison with K903 and P63

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Background: CD10 antigen is a cell membrane-bound 100-kDa-cell surface zinc metalloendopeptidase involved in inactivation of a variety of biologically active peptides and found in a range of tissues. We investigated 34 archival cases of urothelial tumors for CD10 expression and compared it with established urothelial markers K903 and P63.

Design: Formalin-fixed, paraffin embedded tissue from 13 cases of low-grade non-invasive, 15 cases of high-grade invasive, and 6 cases of sarcomatoid urothelial carcinoma stained with CD10 (CellMarque), monoclonal K903 (DakoCytomation) and anti-P63 4A4 (Santa Cruz Biotechnology). Positivity was quantitatively scored as follows: 0: less than 5%, 1+: 5-25%, 2+: 25-50%, 3+: >50% staining of tumor cells. CD10 staining was also performed on normal urothelium (5 cases).

Results: CD10 expression was detected in: 8 cases (61%) of low-grade tumors (n=13), mostly low intensity, (5 cases 0, 5 cases +1, 2 cases +2, 1 case +3); 12 cases (80%) of high-grade tumors (n=15), mostly with higher intensity, (3 cases 0, 3 cases 1+, 5 cases 2+, 4 cases 3+); 2 cases of sarcomatoid carcinoma (n=6) stained with low intensity (4 cases 0, 2 cases 1+). The staining patterns overall were cytoplasmic and occasionally Golgi.

K903 was positive in: 100% of low-grade tumors (11 cases 2+, 2 cases 3+); 100% of high-grade tumors (2 cases 1+, 7 cases 2+, 6 cases 3+); 67% of sarcomatoid carcinoma (2 cases 0, 4 cases 1+).

P63 expression was seen in: 100% of low-grade tumors (13 cases 3+); 73% of high-grade tumors (4 cases 0, 4 cases 1+, 2 cases 2+, 5 cases 3+); 67% of sarcomatoid carcinoma (2 cases 0, 4 cases 1+).

CD10 staining on 5 cases of normal urothelium showed negative staining in 4 cases and 3+ staining in 1 case.

Conclusions: CD10 is expressed in high-grade urothelial carcinomas, excluding the sarcomatoid variant, and seems to be equal in expression to P63 and somewhat less sensitive than K903. P63 and K903 seem to be more sensitive markers for low-grade and sarcomatoid urothelial carcinoma.

CD10 should be considered in a panel of immunohistochemical stains for high-grade urothelial carcinoma.

With limited evaluation of normal urothelium, it appears that CD10 metalloendopeptidase expression is acquired in high-grade invasive urothelial carcinoma and may play a role as a mediator of invasion.

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772 Expression of KIT in Hereditary Renal Cell Carcinoma

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Background: The c-kit proto-oncogene encodes a transmembrane receptor tyrosine kinase (KIT, CD117). After binding to the stem cell factor, KIT plays an important role in cell survival, proliferation, and differentiation. Recent reports have postulated that KIT is overexpressed in specific types of Renal Cell Carcinoma (RCC). However, no studies have been performed in hereditary syndromes with predisposition to renal tumors, especially in Birt-Hogg-Dubé (BHD), where patients present with chromophobe RCC, oncocytomas, and hybrid tumors.

Design: 74 tumors from patients with hereditary and sporadic types of RCC were assessed for immunohistochemical expression of KIT. The hereditary conditions included: BHD, von Hippel-Lindau (VHL), Hereditary Papillary RCC (HPRCC), and Hereditary Leiomyomatosis and RCC (HLRCC). Distribution pattern and intensity were recorded.

Results: 7 Chromophobe RCC (hereditary and sporadic), showed strong membranous overexpression of KIT. 12 Hybrid tumors displayed a complex expression pattern in which the more eosinophilic cells were strongly positive (+) whereas the pale cells were negative or weakly (+). 7 sporadic oncocytomas were strongly (+) for KIT. One sporadic type 2 PRCC was focally (+). There were 3 (33%) clear cell RCC (CCRCC) focally (+) for KIT. Morphologic re-evaluation showed chromophobe areas in all these clear cell tumors. The 3 CCRCC in the setting of BHD were negative. All other tumors studied were negative: 6 VHL – CCRCC, 4 HPRCC, 12 HLRCC, 4 sporadic Type 1 PRCC, 2 sporadic Type 2 PRCC, 9 sporadic CCRCC, 6 sporadic CCRCC with sarcomatoid features, 2 collecting duct carcinoma, 3 multicystic RCC, and 1 metanephric adenoma.

Conclusions: KIT is overexpressed in chromophobe RCC and oncocytomas in both sporadic and familiar settings. Hybrid tumors, which display a characteristic chromophobe/oncocytoma mixed morphology, and are almost exclusive of the BHD syndrome, were (+) for KIT. They display an intermediate immunostain pattern, which add more evidence to the postulated common pathogenesis of chromophobe RCC and oncocytomas. CCRCCs, even in BHD patients, were always (-). It seems that a focal KIT expression in CCRCC is related to areas of chromophobe morphology and therefore may assist in recognizing chromophobe RCC. The overexpression of KIT in a subset of benign and malignant renal tumors present in a background of a genetic condition (BHD) could imply that KIT is not directly related to malignant phenotype in these tumors.

773 Radical Prostatectomy (RP) after Single Core Positive and Minimal Prostate Cancer (PCa) on Ten-Core Biopsy

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Background: Single core positive needle biopsy or minimal PCa (≤5% or ≤1mm) on biopsy are common findings in the current diagnostic practice. RP findings after single core positive biopsy or minimal PCa on biopsy have not been examined in a larger study with extended biopsy sampling.

Design: We reviewed 180 consecutive RP performed in our institution after 07/00 in patients who had PCa in a single core on ten-core biopsy. All RPs were completely sampled and the clinical data were retrieved from our institutional database.

Results: A subset of 87 (48.3%) patients had minimal PCa on biopsy and 93 (51.7%) had non-minimal PCa on biopsy (>5% or >1mm). Patients with minimal PCa on biopsy were younger than patients with non-minimal PCa (59.1 vs. 61.1; means; p=0.073)(all Wilcoxon) and had lower PSA (6 vs.7 ng/ml; means; p=0.092) and PSA density (0.16 vs. 0.19; means; p=0.023). On RP, GS ≤6, GS 7 and GS≥8 were found in 119 (67.6%), 50 (28.4%), and 7 (4%) patients, respectively. GS was identical on biopsy and RP in 119 (67.6%) patients and was increased on RP in 43 (24.4%) and decreased in 14 (8%) patients. Patients with GS ≥7 on RP compared with patients with GS ≤6 had more PCa on biopsy (15% vs. 5% core involved; medians; p<0.001), had higher PSA (8 vs. 5.8 ng/ml; means; p<0.001), and PSA density (0.24 vs. 0.14; means; p<0.001) and were older (62.9 vs. 58.9; means; p<0.001). Extraprostatic PCa (pT3) was found in 11 (6.1%) patients, while 169 (93.9%) had organ-confined disease (pT2). Patients with pT3 disease had more PCa on biopsy than patients with pT2 disease (30% vs. 5% core involved; medians; p<0.001), had higher PSA (9.5 vs. 6.3 ng/ml; means; p=0.006) and PSA density (0.26 vs. 0.17; means; p<0.001) and were older (65.4 vs. 60; means; p=0.002). Positive margins were found in 30 (16.7%) RPs. Patients with positive margins compared with patients with negative margins had more PCa on biopsy (21.5% vs. 13.5% core involved; medians; p=0.006), higher PSA density (0.22 vs. 0.16; means; p=0.015) and were older (62.4 vs. 59.7; means; p=0.068). Two patients (1.1%) showed seminal vesicle invasion and 1 (0.5%) had positive regional lymph nodes. No residual PCa was found on RP in 4 (2.2%) patients.

Conclusions: 1.) Majority of patients with single positive core or minimal PCa on ten-core biopsy have organ-confined disease and GS 6. 2.) Adverse findings on RP (GS≥7, stage pT3, positive margins) following single core positive biopsy are associated with non-minimal PCa on biopsy, PSA density >0.20 and age over 60 years.

774 Single Core Positive and Minimal Prostate Cancer (PCa) on Needle Biopsy: Correlation with Tumor Volume and Clinically Insignificant Cancer

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Background: A significant proportion of PCa diagnosed currently on needle biopsy demonstrates single core involvement or minimal PCa (≤5% or ≤1mm) in one core. Finding of single positive core on extended biopsy schemes in the era of increased prostate specific antigen (PSA) testing may increase the detection of clinically insignificant PCa on radical prostatectomy (RP).

Design: We evaluated tumor volumes in 180 consecutive RPs in patients who had single core positive biopsies. All biopsies and RP were performed in our institution after 07/00. The biopsies were performed using a standard ten-core sampling. All RPs were completely sampled. Tumor volumes were measured using computer assisted image analysis (Image Pro-Plus). Clinically insignificant (CI) PCa was defined as organ-confined disease, tumor volume ≤0.5cc and Gleason ≤6.

Results: Minimal PCa on biopsy was found in 87 (48.3%) patients and 93 (51.7%) patients had non-minimal PCa on biopsy (>5% core involvement or >1mm). In 119 (66.1%) patients tumor volume was ≤0.5 cc and 61 (33.9.%) patients had tumor volumes >0.5 cc [30 (16.7%) had tumor volumes >0.5 to ≤1 cc)]. Patients with minimal PCa on biopsy had smaller tumor volumes (0.4 cc mean/0.14 cc median) in comparison with patients who had non-minimal PCa on biopsy (0.9 cc mean/0.46 cc median; p<0.0001)(all Wilcoxon). CI PCa was found in 105 (58.3%) patients overall, which included 63 (72.4%) patients with minimal PCa on biopsy and 42 (45.2%) patients with non-minimal PCa on biopsy (p<0.013). In the studied cohort, the patients with CI PCa in comparison with the patients with non-CI PCa had lower mean PSA (5.75 vs. 7.55 ng/ml; p=0.0024), lower mean PSA density (0.14 vs. 0.23; p<0.0001), smaller mean gland volumes (47.9 cc vs. 40.51; p=0.009) and were younger (58.7 vs. 62.2 years; p=0.002). We found no significant differences regarding the location (apex 66%, base 52.8%, mid-zone 58.6%) and the side (left 60%, right 56.5%) of the positive biopsy followed by CI PCa.

Conclusions: 1.) CI PCa is found in 58.3% of patients after single core positive biopsy and in 72.5% of patients after minimal PCa on biopsy. 2.) Two thirds of the RPs performed after a single positive core in ten-core biopsy reveal tumor volumes of ≤0.5 cc. 3.) The extent of PCa in single core positive biopsies may be used, in addition to patients' age, PSA and PSA density, to better select patients who will benefit from conservative surveillance treatment.

775 CD90/Thy1 Is Overexpressed by Stromal Cells Associated with Primary Prostate Carcinoma

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Background: There is increasing evidence that stromal cells can affect the biology of human epithelium, both in development and in carcinomas (Cunha et. al. Int J Cancer, 2003). Although some of these studies have characterized the phenotype of cancer-associated stromal cells, most of these studies that used human tissue involved cell culture and animal model-based work. We have begun to characterize the phenotype of cancer-associated stroma in primary human prostate carcinoma to identify genes important in regulating cancer growth and progression.

Design: Thirty primary prostate carcinomas from radical prostatectomy specimens of a range of Gleason grade were immunostained for > 180 CD antigens using an indirect immunoperoxidase method applied to frozen sections of cancer specimens. Distribution and intensity of staining (on a three-point scale) were assessed for each CD antigen. Based on the finding that CD90/Thy-1 was immunohistochemically overexpressed in cancer-associated stroma, an independent set of 10 primary prostate carcinomas were analyzed for mRNA overexpression using laser microdissected tumor cell samples from frozen sections and CD90/Thy-1 RNA levels by quantitative RT-PCR.

Results: In all immunostained cancers an approximately 10-cell wide layer of CD90-positive stromal cells was found adjacent to the cancer. Intensity and extent of CD90 stromal cell expression was invariant to Gleason grade. Quantitative RT-PCR confirmed overexpression of CD90 in 8 of the 10 samples compared with benign gland-associated stromal cells, which had been handled identically.

Conclusions: We have found a stromal cell gene (CD90) that distinguishes prostate cancer-associated stroma from normal prostate gland-associated stroma. The function of CD90 (Thy1), which is known to be expressed in primitive hematopoietic progenitor cells, thymocytes, and fibroblasts, is unknown in the prostate stromal cell. CD90 can be used to isolate cancer-associated stromal cells to begin to understand mechanisms by which the stroma affects growth of cancer. In addition, CD90 might serve as a molecular target for therapy to regulate the growth of primary prostate cancer and as a diagnostic tool for atypical foci of prostate glands that are challenging to diagnose definitively.

776 Gleason Patterns of Primary Prostate Carcinoma Have Unique Transcriptomes

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Background: Gleason grade of primary prostate carcinoma is a parameter important for both prognostic and therapy decisions. Although the molecular changes of prostate carcinoma have been characterized using cDNA arrays by several groups, the molecular basis of Gleason patterns has not been specifically characterized. Knowing the molecular phenotype of Gleason patterns might provide clues to mechanisms of prostate cancer biology and potential molecular targets for therapy that would be specific for each Gleason grade.

Design: 30 snap frozen, OCT-embedded blocks of primary prostate carcinomas (10 of each Gleason pattern 3, 4, and 5) were used for laser capture isolation of 2,000 – 5,000 tumor cells of each pattern and of normal prostate luminal cells. The tumor cell and normal luminal cell RNA underwent two rounds of linear amplification, Cy3/Cy5 dye labeling, and hybridization to 40,000-gene cDNA microarrays. Array results were statistically analyzed with SAM, using as criteria for over- or underexpression at least twofold differences between cancer and normal, and a false discovery rate of <1.0%. Array results were validated using antigen-specific immunoperoxidase staining on an independent set of 40 prostate carcinomas.

Results: The array data showed overexpression by >90% of cancer cells of both AMACR/P504S and hepsin, proteins previously reported to be overexpressed in all prostate cancers, and > 100 genes that differentiate pattern 3 from pattern 4⁄5 cancer cells (since RNA profiles of pattern 4 and 5 tumors were indistinguishable, their data were combined). Immunohistochemical characterization of 40 tumors of different Gleason scores confirmed overexpression of two of these genes – CD10 by pattern 4⁄5 cancers and CD26 by pattern 3 cancers.

Conclusions: 119 genes (most upregulated) distinguish Gleason patterns 4 and 5 prostate cancer from Gleason pattern 3 cancer. Some of these differentially expressed genes could serve as immunohistochemical markers that are more precise prognostic biomarkers than Gleason pattern. Of these differentially expressed gene products, a subset are membrane proteins, i.e., CD10 (CALLA, enkephalinase) and CD26 (dipeptidylpeptidase IV). Either these proteins, their substrates, or products in downstream metabolic pathways might serve as specific molecular targets for therapy.

777 Pathology Findings in 6825 Ten Core Prostate Needle Biopsies: A Single Centre Community Experience

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Background: Extended prostate needle biopsy has become a standard biopsy in many community-based practices. In the era of mostly PSA-driven prostate biopsies, it is uncertain whether the increased core sampling will increase the biopsy rates of prostate cancer (pCa), high-grade prostatic intraepithelial neoplasia (PIN) and diagnostic atypia (ATYP).

Design: We evaluated the pathology findings in 6825 biopsies performed in our centralized community-based practice during a four-year period (07/00-06/04). The biopsies were routinely performed using a standard ten-core biopsy that included additional bilateral cores from the base and the mid-zone. The biopsies were performed in a setting without organized PSA screening and were read by 8 pathologists in one hospital. We reviewed the biopsy reports and we classified the diagnostic findings into four main categories: 1.) pCa, 2.) High-grade prostatic intraepithelial neoplasia (PIN), 3.) Atypical (ATYP), 4.) Benign.

Results: The distribution of diagnostic findings on an annual basis from 07/00 to 06/04 is shown in the table:

	Year 1 (%)	Year 2 (%)	Year 3 (%)	Year 4 (%)	Total (%)
pCa	592 (39.0)	810 (43.9)	732 (42.1)	729 (42.3)	2863 (42.0)
PIN	310 (20.4)	231 (12.5)	226 (13.0)	203 (11.8)	970 (14.2)
ATYP	75 (4.9)	65 (3.5)	97 (5.6)	92 (5.4)	329 (4.8)
Benign	543 (35.7)	740 (40.1)	682 (39.3)	698 (40.5)	2663 (39.0)
Total	1520 (100)	1846 (100)	1737 (100)	1722 (100)	6825 (100)

Atypical (ATYP) category in the table includes all cases with atypical findings (suspicious, uncertain significance) (3.9%) and all cases with atypical PIN (0.9%).

Conclusions: 1.) pCa detection rate of 42% with ten-core biopsy sampling is similar to previously reported pCa rates based either on six-core or variable core sampling in academic and community practices in North America. 2.) Stable annual rates of the biopsy findings in a community practice reflect the prevalence of the disease in the community and also provide quality assurance in a large volume prostate biopsy practice. 3.) Possible reasons to account for the higher PIN rate in the first year include the increased biopsy sampling introduced in that year and the less stringent quality assurance during the same year, which resulted in reporting some low-grade and atypical PIN cases as high grade PIN.

778 Predicting Pathologic Stage for Prostate Cancer: Different Gleason Grade in the Same Lobe as a Novel New Prognostic Marker

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Background: The ability to predict pathologic stage prior to definitive local treatment is an important step in rational treatment planning. Nomograms developed by Partin and by Kattan have been very useful for such planning, and have relied primarily on biopsy Gleason score, PSA, clinical stage, and potentially, biopsy indicators of tumor burden. We have examined a novel new marker of high risk of extraprostatic extension (EPE), the presence of different Gleason grades in the same prostatic lobe (DGGSL).

Design: We evaluated the prognostic utility of this marker in a large retrospective series of 1836 patients operated on by a single surgeon. Logistic regression models were used to predict organ-confined or EPE status for individual patients, and the prognostic utility of resulting models was evaluated by the concordance index (a multivariable measure comparable to the area under the curve for single prognostic markers).

Results: In a model that also included biopsy Gleason grade, clinical stage, PSA, perineural invasion, and maximum percentage of biopsy occupied by tumor, the presence of DGGSL was the strongest independent predictor of EPE, with an odds ratio of 20.7 (95% CI: 4.9, 86.9), p<0.0001. Because the treatment decision is especially difficult for patients with biopsy Gleason=7, we also evaluated DGGSL in 359 patients with this tumor grade. In these patients, the only conventional prognostic markers that were significant predictors of EPE were PSA (>10 vs. ≤ 10) and perineural invasion. When DGGSL was added to this model it was overwhelmingly the strongest predictor, with odds ratio of 16.1 (95% CI: 3.8, 68.0), p=0.0002. DGGSL significantly enhanced the overall predictive ability for these patients with concordance index=0.74, compared to 0.67 for the model that only included PSA and perineural invasion.

Conclusions: These results indicate that the presence of DGGSL is a very strong risk factor for EPE. Furthermore, in patients with biopsy Gleason grade=7, DGGSL significantly enhances the preoperative ability to predict pathologic stage.

779 Expression of alpha Methyl- Acyl-CoA Racemase (AMACR) in Prostatic Carcinomas (PC), High Grade Prostatic Intraepithelial Neoplasia (HGPIN), and Benign Glands (BG) in Japanese People

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Background: AMACR (P504S) has been identified as a relatively sensitive and specific marker of PC. However, most of reports are from western countries and there is no report about utility of AMACR in prostate biopsy in Asian peoples. The objective of this study is to examine the diagnostic utility of AMACR in the evaluation of prostate needle biopsies in Japanese people.

Design: All patients were Japanese. We reviewed formalin-fixed, paraffin-embedded tissue (134 cases, 738 cores): 60 PC cases (PC: 162 cores of 366 cores), 14 HGPIN cases (HGPIN: 19 cores of 75 cores), 45 cases without neoplastic lesions (BG: 297 cores) were selected. Sections were serially sectioned and stained with HE and the antibodies to P504S and p63. The extent of immunostaining of PC and HGPIN was recorded as percentage of positive cells. The extent of immunostaining was recorded as 0 (no staining), 1+ (partial staining, not circumferential), 2+ (circumferential staining) and counted the positive glands. BGs were categorized as Group A (BG in PC cases), Group B (BG in HGPIN cases), and Group C (BG without neoplastic lesions). Group gland category comparisons were performed ANOVA, paired and unpaired t-test.

Results: PC of 162 cores showed more than 1+ reactivity in 141 cores (87.0%) and 2+ reactivity in 141 cores (75.3%). Average AMACR expression of 1+ and 2+ positivity in PC per cores was 77.1% and 62.8%. HGPIN of 162 cores showed more than 1+ reactivity in 13 cores (68.2%) and 2+ reactivity in 10 cores (52.6%). 878 BGs (48 cases, 188 cores) showed more than 1+ reactivity and 385 BGs (35 cases, 94 cores) showed

2+ reactivity for AMACR in Group A. 661 BGs (13 cases, 43 cores) showed 1+ reactivity and 253 BGs (9 cases, 24 cores) showed 2+ reactivity for AMACR in Group B. 590 BGs (32 cases, 106 cores) showed 1+ reactivity and 67 BGs (9 cases, 19 cores) showed 2+ reactivity for AMACR in Group C. There is significant difference among these groups ($p < 0.0001$). BGs with patchy basal cell distribution showed significantly high ratio of reactivity for AMACR compared to those of continuous basal cell distribution in every three groups (p<0.0001).

Conclusions: Sensitivity and specificity of AMACR for PC in Japanese patients are lower than previously reported. The current study demonstrated that some of BGs showed strong positivity for AMACR same as PC, which glands tended to show patchy basal cell distribution. Interpretation of AMACR staining should be executed with caution in Japanese people.

780 Carbonic Anhydrase IX as a Highly Sensitive and Specific Marker of Clear Cell Renal Cell Carcinoma: A Comparative Immunohistochemical Study Using a Panel of Commonly Utilized Antibodies in the Differential Diagnosis of Renal Cell Tumors

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Background: Various immunohistochemical (IHC) markers, alone or in combination, have been used in the differential diagnosis of renal cell tumors (RCT). While most show both sensitivity and specificity in differentiating low-grade tumors, their usefulness diminishes in most-needed situations, ie., in the differential diagnosis of high-grade/poorly-differentiated tumors whether primary or at metastatic sites. We investigated the utility of a panel of IHC markers, including carbonic anhydrase IX (CA9)- a recently described sensitive marker for clear cell renal cell carcinoma (ClrC), in the differential diagnosis of RCT.

Design: A tissue microarray consisting of 15 ClrC, 18 chromophobe (ChrC) and 16 papillary (PapC) renal cell carcinomas, 13 renal oncocytomas (RO), and 3 renal cell carcinoma, unclassified/collecting duct carcinoma (Unc/CDC) was constructed, and used in the study. Two of the ClrCs had areas of sarcomatoid differentiation. IHC staining was performed using the antibodies against CK7, CD10, vimentin, c-kit, AMACR, and CA9. Results were graded as 0, 1+ (≤ 10% positive cells), 2+ (>10 to 50% cells), or 3+ ([>50% cells).

Results: All 15 ClrCs stained positive for CA9. Fourteen of these, including the sarcomatoid areas in 2 of 2 tumors, showed 2+ or 3+ staining. Among others, only 5/16 PapC stained 1+; all ChrC, RO, Unc/CDC showing no staining.

CK7 was positive in 13/16 PapC, 14/18 ChrC, and 3/15 ClrC.

CD10 showed focal to diffuse membranous positivity in 12/15 ClrC. 13/16 PapC were also positive, but 8 of these showed a luminal positivity alone. 7/18 ChrC and 1/13 RO were positive but with predominantly cytoplasmic granular staining.

Vimentin stained 13/15 ClrC, 9/15 papC, and 1/13 RO.

c-kit stained all 18 ChrC and 10/13 RO, while all the other tumors were negative.

AMACR was strongly positive (2+ or 3+) in all 16 PapC, but also stained 7/15 ClrC and 4/18 ChrC, albeit generally at lower intensity.

Conclusions: 1. Among the panel of antibodies tested, CA9 is the most sensitive marker for ClrC. The strong staining in even the sarcomatoid areas of ClrC makes it potentially highly useful in the differential diagnosis of RCT.

2. c-kit is specific for ChrC and RO, but does not help in their differentiation.

3. CD10, CK7 and AMACR, although useful, particularly when used in combination and with consideration of staining patterns, do not appear to be highly specific.

781 Microvessel Density and Regulators of Angiogenesis in Prostate Tissue

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Background: Neovascularization, which is under the control of inhibitors and stimulators of angiogenesis, is a critical factor for the progression and enlargement of solid tumors. Correlation between increased angiogenesis and invasion as well as metastasis of many tumors has emerged that, increased vascularity of tumors may be an important indicator of prognosis.

Design: The predictive value of clinicopathological features and factors of angiogenesis were evaluated. Microvessel density (MVD); VEGF, bFGF, stimulator of tumor angiogenesis; p53 and TSP-1 were assessed in radical prostatectomy specimens. Histological slides of prostatic cancer (PC) patients were reviewed according to the Gleason grading system. MVD in PC, benign prostatic hyperplasia (BPH), and in prostatic intraepithelial neoplasia (PIN) were evaluated Microvessels were identified by CD34 expression of endothelial cells. VEGF, bFGF, TSP-1 and p53 immunoreactivity were graded and intensity of staining were rated semiquantitatively.

Results: VEGF, TSP-1, p53 expressions and MVD of PC were significantly higher than PIN and BPH areas. VEGF scores and MVD of BPH were significantly lower than PIN areas. TSP-1 scores of PIN and BPH were not different. All tissues expressed bFGF in diffuse manner without significant difference, p53 expression of PIN was significantly higher than BPH.

Conclusions: These data demonstrate that there is increasing levels of MVD and exspression of VEGF, TSP-1 and p53 in prostatic tumorigenesis. The level of MVD and the other regulators of angiogenesis in PC are predictors of malignant transformation.

782 Cystic Nephroma (CN) and Mixed Epithelial and Stromal Tumor (MEST) of the Kidney: Are They One and the Same Entity?

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Background: CN and MEST are rare benign renal neoplasms that have overlapping clinical and morphologic features, including predominance in middle aged women, variably cystic architecture, eosinophilic cells and hobnail cells lining the cysts and ovarian-type stroma. The aim of this study was to analyze and compare the histologic features and immunohistochemical profile of these tumors.

Design: We studied 33 cases from 4 large academic institutions. Representative tissue sections were immunostained for estrogen receptors (ER), progesterone receptors (PR), CD10, calretinin, and inhibin.

Results: Nineteen tumors were diagnosed as CNs, 17 in females and 2 in males, their age ranged from 24-63 (mean 44) years. Fourteen tumors were diagnosed as MESTs, all in females, their age ranged from 26-56 (mean 42) years. Histologically, all tumors were well-circumscribed except for 1 MEST. The stromal/epithelial ratio was approximately 2.3 in MESTs vs 0.3 in CNs; prominent ovarian-type stroma composed 45% of the stroma in MESTs and 12 % of the stroma of CNs. Stromal hyalinization was prominent in both. Five MESTs showed stromal luteinization. In the epithelial component, the relative amount of large cysts, medium to small cysts and phyllodes-type glands was: 67%/26%/7% in CNs vs 26%/40%/34% in MESTs.The epithelial component ranged from flat to cuboidal to hobnail cells in both, flat cells being predominant in CNs. No significant atypia of either component was seen, although the epithelial cells showed reactive changes. In the stromal component, ER and PR staining was 62% and 85% in MESTs vs 20% and 36% in CNs, CD10 positivity was seen in 77% vs 46% of cases, calretinin was seen in 69% vs 40% of cases, and inhibin in 42% vs 31% of cases, although the staining was focal. Uninvolved kidney was negative for ER, PR and calretinin, and positive for CD10 (glomeruli and proximal convoluted tubules) and inhibin (distal convoluted tubules).

Conclusions: This study confirms the remarkable similarity between CN and MEST in sex predilection and morphologic attributes of both the epithelial and stromal components and immunohistochemical profile albeit with variation in individual categories. The presence of ovarian-type stroma and mullerian related immunohistochemical markers raises the possibility that these tumors may originate from mullerian remnants misplaced during embryogenesis.

783 High-Resolution Whole-Organ Mapping with SNPs and Its Significance to Early Events of Carcinogenesis

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Background: Identification of chromosomal regions containing genes involved in the development of clinically and microscopically occult preneoplastic lesions may provide extremely valuable clues to the incipient events of human carcinogenesis.

Design: For high-resolution mapping with SNPs, the sequence-based maps spanning approximately 27 and 5 Mb corresponding to microsatellite-defined regions in 13q14 and 17p13 respectively were assembled. The integrated gene and SNP maps of 13q14 and 17p13 were used to select 661 SNPs mapping within the 27-Mb segment around the chromosome 13q14 region and 960 SNPs mapping within the 5-Mb segment around the chromosome 17p13 region. SNPs were genotyped using an automatic pyrosequencing instrument. 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 permitted us to narrow the deleted segments from 4.6 to below 0.1 Mb scale and disclosed that loses represented clusters of discontinuous segments ranging in size from 0.1 to 4.6 Mb separated by nondeleted regions. In both tested loci the deleted segments involved not only the model tumor suppressor gene such as RB1 or p53 but also extended to its flanking regions, implying that other genes mapping to these regions may play a role in the development of preneoplasia.

Conclusions: The combination of whole-organ histologic maps showing the distribution of preneoplastic *in situ* lesions with high-resolution genotyping using SNPs identified sites of early clonal expansion of human preneoplasia. The high resolution of this map and its precise integration with genome sequences bladder was a prerequisite for positional targeting of genes involved in early phases of neoplasia.

784 Seminoma with Microcystic and Tubular Patterns: A Study of 28 Cases

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Background: Seminoma is the most common testicular germ cell tumor, and its diagnosis is usually straightforward. Unusual patterns, however, may cause confusion. The microcystic pattern of seminoma has received scant attention in the literature and is prone to misinterpretation as yolk sac tumor (YST)

Design: We identified 28 seminomas with cystic spaces of variable nature. From 1-27 slides of the tumors were reviewed for features of the cystic spaces, associated tubular pattern, extent of lymphocytic infiltrate, presence of a granulomatous reaction, stromal hyalinization, and cytological features. 13 tumors were immunostained for cytokeratins (AE1/AE3), α-fetoprotein (AFP) and OCT3/4. 12 microcystic testicular YSTs were compared with the cystic seminomas.

Results: The 28 seminomas occurred in men 21-55 years old. The cystic spaces ranged from small and closely packed to more dilated and dispersed. Edema fluid was identified in the spaces of 16 cases, although many spaces in these same cases lacked edema. 12

tumors were accompanied by tubular patterns of solid and hollow nature. 2 tumors with prominent stroma had a striking "jigsaw" arrangement of anastomosing islands with numerous small spaces. 13 tumors either lacked lymphocytes (n=8) or had few of them (n=5). A granulomatous reaction occurred in 3 tumors and stromal hyalinization in 4. The cytological features of typical seminoma were retained in all tumors, with the characteristic polygonal cells. In 13 tumors, stains for cytokeratin and AFP were uniformly negative, whereas OCT3/4 stains were strongly positive. By comparison, the microcystic spaces of the YSTs were usually more irregular and anastomosing and typically lined by flattened tumor cells. The nuclear features were more variable than those of the seminomas.

Conclusions: Microcysts in seminoma may occasionally be a striking feature of this tumor and are often seen with tubular patterns. Ancillary seminoma features, including lymphocytes and granulomas, are often absent or inconspicuous in these cases. Distinction from YST depends on identification of the characteristic cytological features of seminoma cells, a less variable pattern than in YST and absence of other YST patterns. Immunostains are also diagnostic.

785 Diagnostic Utility of Cytokeratin 5/6 in Differentiation of Benign from Malignant Glands on Prostate Needle Biopsies

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Background: Differentiation of small foci of prostate carcinoma from benign lesions on prostate needle biopsy is one of major diagnostic challenges in daily surgical pathology practice. Since prostate carcinoma lacks basal cells, loss of immunostaining for basal cell specific markers such as high molecular weight cytokeratin 34βE12 (CK903) and p63 has been used to distinguish benign from malignant glands. Cytokeratin 5/6 (CK5/6) is a recently recognized marker for basal cells in prostate. However, the data regarding the diagnostic utility of CK5/6 on prostate needle biopsy is limited. In this study, we evaluated the use of CK5/6 as an effective alternative to 34βE12 and p63 on prostate needle biopsies.

Design: One hundred twenty-five needle core biopsies were obtained from the surgical pathology files of UMMHC. Two sequential sections from each biopsy were immunostained with either CK5/6 antibody or 34βE12 and p63 cocktail antibodies on an automated immunostainer. Immunohistochemical stains were graded semi-quantitatively as negative (no glands positive), 1+ (less than 50% of glands positive), and 2+ (greater than 50% of glands positive). 34βE12 cytoplasmic staining and p63 nuclear staining were evaluated separately on the same slide stained with 34βE12 and p63 cocktail antibodies

Results: Twenty-six out of 125 needle biopsies showed prostatic adenocarcinoma with Gleason scores ranging from 6 to 8. Ninety-nine out of 125 needle core biopsies were negative for carcinoma. As shown in Table 1, all benign biopsies demonstrated 2+ immunostaining for CK5/6, p63, and 34βE12, except one biopsy that showed 1+ staining for 34βE12. All carcinoma glands showed negative staining for CK5/6, 34βE12, and p63 (Table 2).

Table 1: Immunohistochemical Staining Results for CK5/6, 34βE12, and p63 in Benign Biopsies (n=99)

	Negative	1+	2+
CK5/6	0/99	0/99	99/99
34βE12	0/99	1/99	98/99
p63	0/99	0/99	99/99

Table 2: Immunohistochemical Staining Results for CK5/6, 34βE12, and p63 in Biopsies (n=26) with Carcinoma

	Negative	1+	2+
CK5/6	26/26	0/26	0/26
34βE12	26/26	0/26	0/26
p63	26/26	0/26	0/26

Conclusions: Our data demonstrated that CK 5/6 is a good marker for basal cells in the prostate, and it can be used as a reliable and effective substitute for 34βE12 or p63 to discriminate benign from malignant prostate glands on difficult needle biopsies.

786 A Sensitivity of P504S / α-Methylacyl-CoA Racemase (AMACR) Immunohistochemistry for the Detection of Prostate Carcinoma on Storage Needle Biopsies

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Background: A monoclonal antibody to P504S / α-methylacyl-CoA-racemase (AMACR) has been proven to be a positive marker for the detection of small focal prostate carcinoma on needle biopsies. However, a recent report has described a variation in the sensitivity of AMACR in the detection of small focal cancer on prostate needle biopsies between in-house (100%) and consult cases (80%). This variation raises a question concerning the effect of storage time of unstained sections of prostate needle biopsies on the sensitivity of AMACR immunostaining. The purpose of this study was to determine if there was variability in the detection of prostate cancer on needle biopsies between stored, unstained glass slides and freshly-cut sections of formalin-fixed, paraffin-embedded tissue.

Design: A total of 63 prostate biopsies with prostate carcinoma were transferred onto glass slides, baked, and stored for 1.6 to 9.2 months. The Gleason scores were 3+3(6) (N=40) including 10 small focal carcinoma (≤1 mm), 4+3(7) (N=16), and 4+4(8) or high (N=7). The slides were then stained with a P504S monoclonal antibody to AMACR. Staining intensity of fresh and stored sections was compared to sections cut from the same blocks just prior to immunostaining, without an intervening storage period.

Results: All 63 biopsies of prostate adenocarcinomas expressed AMACR, regardless of the length of the storage interval. There was no loss of sensitivity of AMACR for prostate adenocarcinoma in baked and stored sections. The sensitivity was preserved and was independent of Gleason scores. The sensitivity of AMACR for small foci of adenocarcinoma was also not affected by the length of storage. Overall, stored slides had a slightly increased intensity of immunostaining with no observable increase in non-specific background staining over freshly-cut sections.

Conclusions: The time interval between the mounting and staining of sections did not affect the sensitivity of the AMACR immunohistochemical stain in the detection of prostate carcinoma including small foci of cancer on needle biopsies. Other technical factors may influence the variations in the sensitivity of AMACR immunohistochemistry.

787 VHL Gene Hypermethylation Is Detected in a Subset of Papillary Renal Cell Carcinoma with Extensive Clear Cell Changes

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Background: Different subtypes of renal cell carcinoma have characteristic genetic changes with chromosomal 3p alteration seen in clear cell renal cell carcinoma (CCRCC) and trisomy 7 and 17 in papillary renal cell carcinoma (PRCC). PRCC with extensive clear cell changes (CCC), although rare, poses significant diagnostic challenge. Only two studies in the literature suggested these lesions had genetic changes characteristic of CCRCC. We studied the genetic and epigenetic changes of 6 such cases.

Design: PRCC with extensive CCC were retrieved from the surgical pathology archive from the authors' institute. The areas with papillae lined predominantly with clear cells were dissected for isolation of DNA for molecular studies. Each of the 3 exons of the VHL gene was PCR-amplified and sequenced using an automated sequencer. Sequences derived from the amplified samples were compared to the wild-type VHL sequence to identify and characterize the presence of mutations. Methylation status was determined using VHL methylation-specific PCR primers after DNA bisulfite modification. Whole tissue sections were used to perform fluorescent in site hybridization (FISH) using the Urovysion kit (Abott Vysis) containing probes for the pericentromeric regions of chromosomes (chr.) 3, 7, 17 and 9p21. At least 25 nuclei were examined to calculate the copy numbers of chromosomes and loss of 9p21.

Results: Six PRCC with CCC were identified. The clear cell component ranged from 10 to 100%. Four cases stained for CD10 and cytokeratin 7 were strongly positive for both markers. By FISH, trisomy 7 was detected in 4 of 4 informative cases (chr. 7 copy number=3.2, 3.3, 2.9 and 2.6, respectively), and trisomy 17 was present in 1 of 3 informative cases (chr. 17 copy number=2.7). 9p21 locus was deleted in 3 of 3 cases. VHL gene sequence was obtained in 4 informative cases and no mutation was detected in any case. Methylation status of the VHL gene promoter region was available in all 6 cases and 2 had hypermethylation.

Conclusions: PRCC with extensive CCC has genetic changes characteristic of PRCC, namely, trisomy 7 and in some cases trisomy 17. No mutation in VHL gene is found. However, a third demonstrate hypermethylation involving VHL gene promoter region. Studies are currently underway to determine whether VHL protein expression is abolished by its promoter hypermethylation.

788 KIT and RCC Are Useful in Distinguishing Chromophobe Renal Cell Carcinoma from the Granular Variant of Conventional Renal Cell Carcinoma

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Background: The distinction between chromophobe renal cell carcinoma (CRCC), the granular variant of conventional renal cell carcinoma (GRCC), and renal oncocytoma is a common diagnostic dilemma. We systematically investigated the usefulness of antibodies to KIT (CD117), CD10, RCC (renal cell carcinoma antigen), and RON, as well as colloidal iron stains, in distinguishing these three most likely to be confused renal epithelial tumors.

Design: Eleven CRCC, 6 GRCC, and 12 renal oncocytomas were retrieved from the University of Virginia, Department of Pathology electronic database. All GRCC had at least one microscopic focus of classic clear renal cell carcinoma morphology.

Immunohistochemical staining using rabbit polyclonal antibody to KIT (DAKO, 1:100), mouse monoclonal antibody to CD10 (Novocastra, 1:20), RCC (Novocastra, 1:50), and RON (Transduction Laboratory, 1:750) was performed according to the manufacturer's instructions. Positive and negative controls were performed simultaneously. Colloid iron staining was performed using the modified Muller-Mowry method.

Results: KIT was positive in all CRCC (11/11) and renal oncocytomas (12/12) with identical staining patterns, but was absent in all GRCC (0/6). RCC was observed in over 80% of the GRCC (5/6), but was negative in all CRCC (0/11) and renal oncocytomas (0/12). CD10 was expressed in 100% of the GRCC (6/6), 72% of CRCC (8/11), and 58% of renal oncocytomas (7/12). RON was positive in all CRCC (11/11) and renal oncocytomas (12/12), but was only 50% positive in the GRCC (3/6). Colloid iron was diffusely and strongly positive in over 80% of the CRCC (9/11), focally and weakly positive in 41% of the renal oncocytomas(5/12), but was negative in all GRCC (0/6).

Conclusions: 1. KIT is a very sensitive marker for both CRCC and renal oncocytoma;
2. Immunohistochemistry using antibodies to KIT and RCC was sufficient to discriminate between CRCC/oncocytoma and GRCC;
3. Neither RON, KIT, nor any combination of other antibodies in our study can distinguish CRCC from renal oncocytoma.
4. Colloid iron staining aided in the distinction of the majority of the CRCC (over 80% strongly positive) from renal oncocytoma (41% focal weak positive, remainder negative).

789 Renal Papillary Adenoma - A Possible Precursor Lesion of Papillary Renal Cell Carcinoma

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Background: The precursor lesions of renal cell carcinoma are not well understood. In the recent WHO classification of kidney tumors, papillary adenomas (PA) have been defined as epithelial lesions with a tubulopapillary architecture measuring less than 5 mm and of low nuclear grade. Most published studies of this tumor are based on autopsy material. Limited reported cases of PA arising in a background of bilateral multicentric papillary renal cell carcinomas (PRCC) raise the possibility that PA may be related to carcinoma. The purpose of this study is to determine the incidence, histomorphological, and immunohistochemical features of PA, including microscopic papillary foci (hyperplasia), and therefore elucidate their possible relationship to renal cell carcinoma (RCC).

Design: 542 nephrectomy specimens consecutively resected in the last 8 years were reviewed. Immunohistochemistry using antibodies specific for alpha-methylacyl-CoA racemase (AMACR, PRCC marker) and GST-alpha (clear cell RCC marker) was performed.

Results: Thirty-eight (7%) nephrectomies histologically showed evidence of adenoma(s) or papillary hyperplasia. Of the 38 cases, 18 (47%) arose in the setting of PRCC (18/71 (25%) of PRCC). 5 (13%) occurred in polycystic kidney disease, 5 in clear cell RCC, 2 in chromophobe RCC, 2 in end stage kidney disease, 1 in oncocytoma, and 2 in renal stromal tumors. Histomorphologically, the PAs were characterized by varying proportions of papillae and tubules formed by cuboidal cells with scant basophilic cytoplasm similar to those in type 2 PRCC. No evidence of clear cell, chromophobe, spindle or oncocytic morphology was identified. Adenomas associated with PRCC tended to be multiple in number (61% of cases had > 2 adenomas, mean = 5). In contrast, all PA arising in other conditions had $\leq$ 2 adenomas. In four cases, more than 10 adenomas present. The majority of adenomas (33/38, 87%) stained strongly for AMACR in a fashion similar to PRCC. The 5 AMACR negative cases all arose in the setting of adult polycystic kidney disease. GST-alpha was positive in only 16 % of adenomas.

Conclusions: The high coincidence, multifocality, histological and immunohistochemical similarities between PA and PRCC suggest that the two are a continuum of one biological process. Whether the coexistence of PA with other renal lesions is incidental or consequential remains to be determined.

790 Differences in Telomere Length between Chromophobe Renal Cell Carcinoma and Oncocytoma

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Background: Telomeres consist of a tandemly repeated DNA sequence located at chromosomal ends. Telomere shortening has been found in multiple malignant tumors and is implicated in cellular senescence and carcinogenesis. To determine the differences, if any, of telomere length in chromophobe renal cell carcinoma and oncocytoma, and to see whether telomere shortening may play a role in renal carcinogenesis, we performed fluorescent in situ hybridization (FISH) for telomere length in surgical specimens of 22 oncocytomas and 15 chromophobe renal cell carcinomas.

Design: Paraffin-embedded, formalin-fixed surgical specimens of 22 oncocytomas and 15 chromophobe renal cell carcinomas were examined for the presence of telomeric DNA using a Cy3-labeled telomere-specific peptide nucleic acid probe and counterstaining with DAPI for visualization by fluorescent microscopy. Adjacent non-neoplastic renal tubules were used as an internal control. The intensity of telomere staining, which has previously been shown to be linearly-related to telomere length, was assessed.

Results: We found marked telomere shortening in 100% (15/15) of chromophobe renal cell carcinomas. In contrast, only 31% (7/22) of oncocytomas showed telomere shortening whereas the majority (69%) were characterized by normal or elongated telomeres.

Conclusions: Telomere shortening with induction of chromosomal instability appears to play a role in the development of chromophobe renal cell carcinoma but not in the majority of oncocytomas. This finding is consistent with previous studies of telomere dysfunction in human cancers and in particular supports our recent finding of significant multiple chromosomal losses in chromophobe renal cell carcinoma (Furge et al, Cancer Res 2004). The absence of telomere shortening and the elongation of telomeres in oncocytoma may be responsible for its benign behavior. Telomere length assessed by FISH may be a useful marker in distinguishing chromophobe renal cell carcinoma from oncocytoma.

791 p53 Expression in Small Cell Carcinoma of the Urinary Bladder

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Background: Small cell carcinoma of the urinary bladder is a rare and highly aggressive tumor. p53 expression has been shown to be associated with poor prognosis in a variety of tumors. This study was undertaken to investigate p53 expression in a large series of small cell carcinomas of the urinary bladder and to correlate the findings with clinicopathologic parameters and clinical outcome.

Design: This study included 50 cases of small cell carcinoma of the urinary bladder. Pathologic findings were reviewed and correlated with clinical findings and follow-up information. Immunostaining for p53 was performed on paraffin-embedded tissue sections using the avidin-biotin-peroxidase method. Results were recorded as positive expression (≥10% of cells with nuclear staining) or negative expression (<10% of cells with nuclear staining). SAS was used for the statistical analysis.

Results: The series included 40 males and 10 females. All 50 patients except one had advanced disease (T2 or above) at presentation. Pathologic stages were as follows: T1 in 1, T2 in 25, T3 in 21 and T4 in 3 patients. During a median follow-up of 12 months (range: 1 month to 122 months), 38 patients died of cancer. Two-year and 5-year cancer-specific survival rates were 45% and 16%, respectively. p53 positive expression was present in 27 out of 50 (54%) cases (7 with 10-25% staining, 4 with 25-50% staining, 11 with 50-75% staining and 5 with 75-100% staining); conversely, p53 staining was negative in 23 out of 50 (46%) of cases (19 with no staining and 4 with <10% staining). No correlation was demonstrated between the level of p53 expression and survival (P=0.16). For p53 expression rate greater than or equal to 10%, 5-year cancer-specific survival was 16.6%; for p53 expression rate less than 10%, 5-year cancer-specific survival was 14.7%. There was no correlation between p53 expression and other clinicopathologic characteristics, including age (P=0.20), gender (P=0.84), history of smoking (P=0.25), pathologic T stage (P=0.38), clinical stage (P=0.60), lymph node metastasis (P=0.17), and distant metastasis (P=0.88).

Conclusions: Our data indicate that p53 is frequently expressed in small cell carcinoma of the urinary bladder. However, no correlation is found between p53 expression and clinicopathologic parameters, including long-term survival.

792 The Non-Steroid Anti-Inflammatory Drug Sulindac Sulfide Induces Apoptosis and Growth Arrest in Prostate Cancer Cells

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Background: An increasing number of studies have demonstrated that many non-steroid anti-inflammatory drugs (NSAIDs) such as Sulindac Sulfide, at clinically tolerable concentrations, are effective in prevention and treatment of several types of cancer. Sulindac sulfide has been shown to induce prostate cancer (CaP) apoptosis *in vitro* and *in vivo*. However, the underlying molecular mechanisms have not yet been identified.

Design: In order to select the target genes, we used microarray transcriptional profiling. The treatment of CaP cell DU145 and PC3 with 50μM of Sulindac Sulfide for 24hrs resulted in a 2-9 fold induction of GADD45alpha and GADD45gamma in the transcriptional profile and they were confirmed by real time PCR and western blot analysis and further elvaluated by inhibitors and siRNA assays.

Results: We found a significant changes in IL-24, c-jun N-terminal protein kinase (JNK), and cdc2. Sulindac treatment induced tumor suppressor gene IL24 and activated JNK. Reduction of GADD45alpha and gamma transcriptions using siRNA abrogated apoptosis induction, blocked JNK activity and restored cdc2 kinase activity.

Conclusions: These data demonstrate that IL24 and JNK are implicated in Sulindac mediated program cell death. GADD45 family genes are critical mediators for Sulindac induced apoptosis and growth arrest and play an important role in CaP cell proliferation and apoptosis.

793 Gene Expression Profiles Using Formalin Fixed, Paraffin-Embedded Benign and Prostatic Carcinoma Tissues

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Background: Profiling prostatic carcinoma gene expression requires large amounts of fresh or frozen tissue. The use of formalin fixed, paraffin embedded (FFPE) tissue yields fragmented and small quantities of RNA and is of limited value in gene expression studies using previous conventional methods. We performed gene expression analysis using archived FFPE prostatic carcinomas with Illumina's novel fiberoptic bead arrays, and its proprietary cDNA-mediated, Annealing, Selection, and Ligation (DASL™) assay.

Design: Eighty-one cases of paired benign and prostate carcinoma FFPE blocks (N=173) were retrieved from The VA San Diego Healthcare system. 500 to 2000 ng of highly fragmented total RNA (average fragment size is 130 nt) was extracted from five 5 um paraffin slices using Roche's High Pure RNA Paraffin kit. Areas of tumor and benign tissues were scored as a percentage of the total tissue. The RNA was converted to cDNA without additional RNA amplification. Quality control was performed using quantitative PCR with housekeeping gene RPL13A. Tissues with a Ct value higher than 29 cycles by qPCR were determined unsuitable for array analysis. 231 tumor and 290 stroma specific genes (3 probes per gene) were selected from a comprehensive literature review and the oligo probes were hybridized onto the bead arrays. Gene pair ratios and absolute signals were used to correlate tumor and stromal content.

Results: The first 48 samples were used as a training set and subsequent samples were used for validation. We were able to depict the most frequent tumor and stromal specific genes and gene pairs, such as Tumor: AMACR/ANXA2, GSTP1/IL-1R, HOXC6, HPN, LIM; Stroma: SPDEF, BCL2ß, and GSTP1. The gene expression reproducibility between the replicates has R^2 values > 0.93. The reproducibility improves to 0.99 with decreasing Ct values.

Conclusions: Using Illumina's DASL system we were able to profile gene expression in prostate carcinomas and benign prostate tissues from archived FFPE samples. The system affords custom designed oligo probes and has potential for disease specific gene profiling.

794 Carbonic Anyhdrase II in Oncocytoma and Chromophobe Renal Cell Carcinoma

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Background: Carbonic anhydrase II (CAII), one of the isoenzymes catalyzing bicarbonate dehydration in renal tubules, plays a role in renal acidification. CAII was first suggested as a possible marker of chromophobe renal cell carcinoma and oncocytoma in our previous finding of a significant increase of carbonic anhydrase II mRNA levels by gene expression microarray analysis. To investigate the value of this marker in pathologic diagnosis, we analyzed CA II expression in 105 kidney tumors on tissue microarrays and conventional sections by immunohistochemistry.

Design: Tissue microarrays containing 87 renal cell neoplasms, including 17 oncocytomas, 14 chromophobe renal cell carcinomas, 32 clear cell renal cell carcinomas, and 24 papillary renal cell carcinomas were constructed. In addition, conventional sections from 11 cases of oncocytoma and 7 cases of chromophobe renal cell carcinoma were analyzed for comparison. Immunohistochemistry was carried out with a monoclonal antibody specific for carbonic anhydrase II. Staining intensity was scored on a 0 to 3 scale.

Results: Ninety two percent of oncocytomas (26/28) and 100% of chromophobe renal cell carcinomas (21/21) showed moderate to strong, diffuse cytoplasmic CAII immunoreactivity with intensities ≥2, while only 13% (7/56) of other renal cell carcinoma subtypes stained with intensities ≥2.

Conclusions: Our immunohistochemical study confirms the finding of increased CAII mRNA levels by gene expression microarrays. We conclude that CAII immunostaining cannot be used to distinguish oncocytoma from chromophobe renal cell carcinoma but may be helpful in differentiating chromophobe renal cell carcinoma and oncocytoma from other renal neoplasms.

795 Cancerous and Precancerous Processes in Prostates of the Inhabitants of Semipalatinsk Region (East Kazakhstan)

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Background: Cancer of the prostate (PCa) is not a leading cause of oncological mortality in East Kazakhstan. The determination of prostate disease frequency in this population might help elucidate the underlying causes of disease.

Design: Morphological analysis of prostates was performed on sequential gross autopsy material of histotopographic sections among 676 inhabitants of Semipalatinsk region in eastern Kazakhstan. The population in this area consists mainly of Asians and Caucasians.

Results: 67.9% of the series corresponded to Caucasian patients and 32.1% to Asians. The mean age at death was 51.8 years with the youngest at 17 and the eldest at 87. The Asian population was younger at death than the Caucasian ($p<0.05$). The most common cause of death was cardiovascular disease (34.8%), followed by tuberculosis (17.9%) and pulmonary disease (11.4%). HGPIN was found in 30.8 % of the patients (190 cases). Latent cancer of prostate was discovered at every third inhabitant of Semipalatinsk region (28.1%). Only 2 cases with PCa were found in the third decade. The number of PCa cases increased with age up to the seventh decade when they decreased (20s:1.1%; 30s:5.8; 40s:17.9%; 50s:21.6%, 60s:30%; 70s:19.5%; 80s:4.2%). Most cancers had low Gleason (G) score (14.8% had G5; 66.7% had G6; 16.9% had G7 and 1.6% had G8). Extra capsular extension (ECE) was found in only 69.5 %, but only 8.4% had ECE level 3.

Conclusions: Prostate cancer and HGPIN prevalence is lower in the population of the Semipalatinsk region in eastern Kazakhstan than historical autopsy series in the US. The incidence increases in age only to certain decades and later decreases, which is also different from historical controls. The cancers were mostly indolent and not related to the cause of death.

796 Clinical Significance of Tertiary Gleason Pattern 5 in Gleason Score 7 Radical Prostatectomies

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Background: The Gleason grading system in reporting prostate cancer accounts for the primary and secondary grades seen most prevalently, however there is no consensus on how to account for a tertiary (third most prevalent) grade. Two prior studies have shown that there is prognostic significance of tertiary Gleason patterns of higher grade in terms of worse pathologic stage and higher progression rates. These studies, however, were based on only retrospective data utilizing original diagnoses and were limited due to possible grading inconsistencies or non-routine reporting practices of a tertiary Gleason pattern.

Design: We analyzed the clinical significance of a tertiary grade 5 pattern within 214 radical prostatectomy (RP) specimens with Gleason score 7 (3+4 or 4+3) in terms of pathologic stage (PS) and biochemical recurrence free survival (bRFS) (PSA>0.2 ng/mL). Two pathologists re-reviewed all cases to confirm initial diagnoses and to ensure standardization of grading criteria and the reporting of a tertiary grade 5 pattern. Both pathologists were blinded to clinical outcome. No patients included in the study received neoadjuvant therapy. Six patients who received adjuvant therapy were excluded from the survival analysis.

Results: Patients with Gleason score 7 and tertiary grade 5 cancer (GS7+5) (n=37) have significantly higher PS than patients with Gleason score 7 without tertiary grade 5 cancer (GS7) (n=177) ($p<0.001$). This includes 6% of GS7+5 patients with lymph node positive (LNP) stage compared to only 2% of GS7 patients with LNP stage. The overall percentage of patients with GS7+5 also increases as the PS worsens. Patients with GS7+5 (n=33) have significantly decreased bRFS compared to patients with GS7 (n=175) ($p=0.0005$). GS7+5 patients have a mean bRFS of 54 months compared to GS7 patients with a mean bRFS of 121 months.

Conclusions: We advocate routine reporting of tertiary grade 5 patterns in RP specimens as patients with GS7+5 cancer have significantly more advanced PS and decreased bRFS compared to patients with GS7 cancer. The overall percentage of patients with GS7+5 cancer increases as the PS worsens. Finally, patients with a tertiary grade 5 pattern could be considered for adjuvant therapy protocols.

797 Clinical Significance of Benign Glands at the Surgical Margins in Robotic Radical Prostatectomy Specimens

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Background: The use of the Da Vinci Surgical Robotic System (DSRS) for retropubic radical prostatectomy is increasing. The robotic retropubic radical prostatectomy (RRRP) is performed in an antegrade fashion compared to conventional open retropubic radical prostatectomy (ORRP), which is done in a retrograde fashion. Electrocautery is used more liberally at RRRP. Such differences in surgical techniques may result in alterations in the radical prostatectomy specimens (RPS) distinct to those found with ORRP. Benign glands at the margins of RPS may contribute to a post-operative rise in PSA levels. We compared the frequency and clinical significance of the presence of benign prostate glands at the surgical margins in the RPS obtained by RRRP or ORRP performed by the same surgeon.

Design: Thirty-six consecutive RPS from patients with biopsy proven prostate cancer were reviewed. Of these, 23/36 (64%) were obtained from RRRP and the rest 13/36 (36%) from ORRP. All slides from the RPS were carefully reviewed for the study by a dedicated pathologist and each case was analyzed for Gleason score, perineural involvement, margin status including the presence of benign glands and the extent at the surgical margin, extraprostatic extension (EPE) and tumor size.

Results: Mean age for entire cohort was 61 yrs. Gleason scores were evenly distributed between the two groups except for greater number of cases with Gleason >7 in the RRRP group. Comparative results between the two surgical groups are presented below

Surgery Group	Median follow up (months)	EPE+	Cancer at inked margin	Extent of positive margin (mm)	Benign glands at margin	Median pre-operative PSA	Post-operative PSA nadir <0.1
RRRP (N=23)	4.43	7 (30%)	6 (26%)	6.00 (1-10)	12 (52%)	8.6	12/13 (92%)
ORRP (N=13)	11.5	5 (38%)	1 (8%)	1.5 (N/A)	2 (15%)	6.85	8/8 (100%)

Conclusions: The incidence and extent of benign prostate glands in patients undergoing RRRP is significantly increased (52%) compared to ORRP (15%). Early clinical follow up results suggest that patients undergoing robotic radical prostatectomy for prostate cancer achieve a PSA nadir of <0.1 irrespective of the presence of benign prostate tissue at the surgical margins. While longer follow up is needed to clearly establish the significance of the benign prostate glands at the surgical margins, the more liberal use of electrocautery at RRRP may provide for additional several millimeters of tissue destruction beyond the surgical margins.

798 A Morphologic and Immunohistochemical Study of Renal Tumors in End-Stage Renal Disease (ESRD): Emphasis on Tumors of Unusual Morphology

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Background: Renal cell tumors arising in the background of ESRD show a wide spectrum of morphologic features. While some of these tumors resemble those seen in sporadic settings, others appear unique to ESRD (Lab Invest 2003;83:173A).

Design: A detailed morphologic evaluation was performed on 58 cases of renal tumors in ESRD. From the recent contemporary published data, we constructed an appropriate panel of antibodies (AMACR, CK7, parvalbumin and vinculin) and performed immunohistochemical (IHC) analysis on representative tumors from each morphologic group (total 31 tumors), to evaluate the utility of IHC staining in discriminating between the different tumor subtypes.

Results: Based on the morphologic features, 5 different tumor types, as described previously, were identified. Three subtypes were similar to those seen in sporadic settings (11 papillary RCC; 8 clear cell RCC; 5 chromophobe RCC), while there were 2 unique tumor types [20 acquired cystic disease of kidney-specific RCC (AS-RCC); 13 papillary clear cell RCC]. AS-RCC were only seen in the kidneys with acquired cystic disease, and 2 of these also showed sarcomatoid differentiation.

In addition, 2 types of possible precursor lesions were present in abundance; a) papillary adenomas (PA), and b) dilated oncocytic tubules, sometimes clustered together, with AS-RCC like cytologic features.

IHC profile of different tumor types was as follows- papillary RCC: AMACR+ (diffuse 5/6), CK7+ (diffuse 6/6), parvalbumin- (focal 1/6), vinculin+ (6/6); AS-RCC: AMACR+ (diffuse 11/11), CK7- (focal 3/11), parvalbumin+/- (6/11), vinculin+ (11/11); clear cell RCC: AMACR-/+ (focal 4/8), CK7- (focal 2/8), parvalbumin- (weak, focal 5/8), vinculin- (weak, focal 4/8); papillary clear cell RCC: AMACR- (0/4), CK7+ (diffuse 4/4), parvalbumin- (focal 1/4), vinculin-/+ (weak, focal 4/4); and chromophobe RCC: AMACR- (0/2), CK7+ (2/2), parvalbumin+ (2/2), vinculin+ (2/2). Additionally, PA stained like papillary RCC, and oncocytic dilated tubules like AS-RCC.

Conclusions: 1. Most renal tumors in ESRD are tumors unique to this setting (AS-RCC and clear cell papillary RCC), with fewer tumors resembling those in sporadic setting. Distinct precursor lesions exist for AS-RCC and papillary RCC. 2. IHC profile is distinctive between morphologic subtypes of tumors in ESRD, and it may be useful in appropriate categorization of these tumors.

799 Pathological Features of Incidental Prostatic Adenocarcinoma in Radical Cystoprostatectomy Specimens Removed for Bladder Cancer

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Background: Pathobiological relationship between incidental prostate cancer (PCa) and clinically detected PCa is unknown. To evaluate incidental PCa and topographical relationship between PCa and high-grade intraepithelial neoplasia (HGPIN) or postatrophic hyperplasia (PAH), we examined these relationships in cystoprostatectomy specimens.

Design: We studied 96 men who underwent radical cystoprostatectomy for bladder cancer by one surgeon (SPL) at the Baylor College of Medicine from 1992 to 2003. All prostate specimens were separated from bladder were processed by whole mount step-section technique. Detailed pathological evaluation of all cancers was performed by single pathologist.

Results: Of the 96 patients, 54 (56%) had an incidental PCa with a median of 0.04 cc in volume, ranging 0.01 to 6.14 cc. Extracapsular extension (ECE) was identified in 5 (9%), seminal vesicle invasion in 1 (2%) and none had positive lymph node. A Gleason pattern 4/5 component was recognized in 11 (20%) patients. Of these 11 patients, 6 (54%) had >0.2 cc in volume and 5 had <0.2 cc with a minimum of 0.01 cc. Also, the frequency of Gleason pattern 1 or 2 component was relatively high, n=15 (28%). Overall, 16 (30%) patients fit with our criteria of clinically significant cancer (tumor volume>0.5 cc, ECE, or a Gleason pattern 4/5 component). Main foci of these significant cancers was distributed in apex (6, 38%), mid (9, 56%) and base (1, 6%), similar to 367 (34%), 642 (60%) and 59 (6%) of 1068 clinically detected cancers which we reported. PCa in this study was more likely to be located in peripheral zone (70%). Overall, 66(69%) had HGPIN and 28(29%) had PAH. The patients with HGPIN were more likely to have PCa (70%) than those without HGPIN (27%). Of cancers with HGPIN, 29(63%) were associated with HGPIN (within 2mm of a focus of HGPIN). In contrast, of 19 cancers with PAH, only 3 (16%) were associated with PAH (within 2mm of a focus of PAH).

Conclusions: Because the craniocaudal and zonal distribution of prostate cancer in cystoprostatectomy specimens was similar to those of clinically detected cancer and the frequency of Gleason grade 1-2 cancers was relatively high, these data support the hypothesis that clinical PCa dedifferentiates from well differentiated PCa. HGPIN appeared to have topographical association with PCa, in contrast to PAH in cystoprostatectomy specimens.

800 Reactive Stroma in Needle Biopsies Predicts Biochemical Recurrence-Free Survival in Patients after Radical Prostatectomy

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Background: We previously reported that the quantity of reactive stroma and the diameter of perineural invasion (PNI) in prostatectomies are significant predictors of recurrence in patients treated with radical prostatectomy. However, the prognostic significance of reactive stroma and PNI in needle biopsies remains unclear.

Design: 224 cases of prostatic needle biopsies were diagnosed as prostatic carcinoma at the Baylor College of Medicine from 1988 to 1998. The H.E. slides were reviewed by single pathologist (NY). The largest area of tumor was decided as index cancer (IC), and Gleason Score (GS) was also evaluated in IC. Reactive stromal grade (RSG) was evaluated based on following: Grade 0, IC area with no or up to 5% reactive stroma; Grade 1, 6 to 15%; Grade 2, 16 to 50%, and Grade 3, 51 to 100%. The diameter of PNI was measured with an ocular micrometer. 221 cases were followed up from 1 to 131 months.

Results: Number of H.E. slides in each case varied from 1 to 30 slides, with 7.58 +/- 7.73 (mean +/- standard deviation). One case (0.04%) had RSG 0; 149 cases (66.5%), RSG 1; 2, 59 cases (26.3%), RSG 2, and 15 cases (6.7%), RSG 3. PNI were found in 38 cases (17.0%), ranging from 0.05 to 0.26mm in diameter. RSG in biopsies was correlated with clinical stage (p<0.0001), lymph node metastasis (p=0.0041), extracapsular invasion (ECE, p<0.0001), seminal vesicular invasion (SVI, p<0.0001), surgical margin (p=0.0351) and GS (p=0.0021) in radical prostatectomy. Diameter of PNI in biopsies also correlate with clinical stage (p=0.013) and ECE (p<0.0001). Interestingly, there was a close correlation between RSG and diameter of PNI in biopsies (p=0.0044). By Kaplan-Meier survival analysis, patients with RSG 1 and 2 had better survival than those with 0 and 3 (p=0.0034), but the diameter of PNI was not associated with recurrence-free survival. By Cox proportional hazard analysis, RSG was an independent predictor of recurrence (p=0.0174).

Conclusions: Prostatic carcinoma with either no to little reactive stroma (RSG 0), or with abundant stroma (RSG 3) showed reduced recurrence-free survival with significance, which was consistent with our previous data from radical prostatectomy specimens.Therefore, quantitation of reactive stroma in biopsy is useful to predict biochemical recurrence in prostate carcinoma patient treated with radical prostatectomy.

801 Proliferation and Apoptosis in Prostate Cancer and the Perineural Space: A Phenotypic Change Related to Survival

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Background: Proliferation and apoptosis are involved in prostate carcinogenesis. Ki67 expression, as a marker of proliferation, has been shown to be an excellent prognostic indicator. The value of Ki67 and apoptosis in perineural invasion (PNI) has not been determined.

Design: Prostates from 640 of 5400 patients who had undergone radical prostatectomies were used to build tissue microarrays. The index tumor (largest and/or highest Gleason) was cored in triplicate (0.6 mm). A separate array of 230 PNI of the same patients was also built. Slides were stained with a rabbit polyclonal antibody to Ki67 and stained with TUNNEL. Slides were digitized using an automated slide scanner. Proliferation and apoptotic indices were obtained by visual counting of cells in the array of PCa primary tumor and the array of PNI. Kaplan-Meier analysis and log rank test were used to determine the probability of disease recurrence defined as a serum prostate-specific antigen (PSA) level >0.4 ng/ml after radical prostatectomy.

Results: Ki67 and TUNNEL staining was nuclear. The mean Ki67 in PCa was 1.07 while in PNI 3.44 (p<0.05). The mean apoptotic ratio was decreased in PNI as compared to PCa (0.66 vs 1.71, p<0.05). High proliferative index in PCa was predictive of a higher probability of recurrence [p=0.000, HR 1.117(1.05-1.189)] on multivariate analysis. Ki-67 in PNI was not. Inversely, a lower apoptotic ratio in PNI was predictive of a higher probability of recurrence [p=0.0344, HR 0.213(0.051-0.892)]. Apoptotic index in PCa was not predictive.

Conclusions: A high proliferation index in the primary tumor and a low apoptotic index in PNI are associated with reduced survival. These data suggest that molecular changes might be responsible for this phenotypic change and associated with the microenvironment in the perineural space.

802 Expression of the Tumor Suppressor 14-3-3 Sigma Protein in Renal Epithelial Neoplasms

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Background: Tumor suppressor 14-3-3 sigma is a p53-regulated G2/M inhibitor, which regulates numerous cellular signaling pathways involved in cell cycle control, DNA repair and apoptosis. It was originally identified as an epithelial marker down-regulated in breast cancer cells. Recently, studies have indicated that the 14-3-3 sigma gene is inactivated mainly through promoter hypermethylation in breast and hepatocellular carcinomas. However, expression of the 14-3-3 sigma protein in renal epithelial neoplasms has not yet been studied.

Design: To further understand the pathogenesis of renal epithelial neoplasia, we have immunohistochemically evaluated the expression of the 14-3-3 sigma protein in 93 renal epithelial neoplasms. Four tissue microarrays consisted of 23 renal clear cell carcinomas (CCRCC), 20 renal papillary carcinomas (PRCC), 17 renal chromophobe cell carcinomas (ChRCC), 19 renal oncocytomas and 14 renal transitional cell carcinomas (TCC). Twenty cases of normal kidney cortex were included as controls.

Results: The 14-3-3 sigma protein was moderate to strongly expressed in cell membrane and cytoplasm of proximal and distal tubules in all 20 normal kidneys. Collecting duct epithelium was negative for 14-3-3 sigma. Strong to moderate expression of 14-3-3 sigma was found diffusely in 16/19 (84%) oncocytomas. Expression of 14-3-3 sigma was dramatically decreased in renal cell carcinomas, including 2/23 (8.7%) of CCRCC, 1/17 (11.1%) of ChRCC and 5/20 (25%) of PRCC. There was a significant difference of 14-3-3 sigma expression between oncocytoma and CCRCC, or ChRCC or PRCC (P<0.001). Among those renal carcinomas expressing 14-3-3 sigma, the immunostaining intensity was mild to moderate compared to normal renal tubules and oncocytoma. Decreased expression of 14-3-3 sigma was also seen in 2/14 (14.3%) of TCC.

Conclusions: Loss of expression of the tumor suppressor 14-3-3 sigma was found in 75-92.3% of renal carcinomas. Our study indicates that loss of 14-3-3 sigma may be involved in the development of renal epithelial carcinomas. The molecular mechanism of 14-3-3 sigma down-regulation in renal carcinomas warrants further analysis. Strong expression of 14-3-3 sigma protein was seen in about 84% of oncocytomas. Expression of 14-3-3 sigma protein may be useful in facilitating the distinction among renal oncocytic tumors.

803 Expression of Novel Markers in Small Cell Carcinoma of the Prostate: Possible Therapeutic Targets

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Background: Small cell carcinoma of the prostate (SCCP), a form of neuroendocrine (NE) differentiation, is a rare and aggressive form of prostatic carcinoma (PC) similar in morphology to small cell carcinoma (SCC) in other organs. SCCP is usually diagnosed at an advanced stage and conventional PC treatment (hormone/anti-androgen, radiation, surgery) is non-curative. Treatment with chemotherapeutic agents used in SCC of lung has shown limited response but no improvement in survival. Novel therapeutic agents, including small molecules and humanized antibodies, targeting specific proteins involved in cancer are effective in other tumors and their efficacy against PC is being studied. This study was designed to evaluate the expression of three novel therapeutic markers: C-Kit, BCL-2 and EGFR, in SCCP.

Design: Eighteen cases of SCCP were collected from a multi-institutional review of primary and metastatic PC cases, including autopsies, radical prostatectomies, trans-urethral resections, and biopsies. The diagnosis of SCCP was confirmed morphologically and with NE markers. Ten cases of high-grade undifferentiated prostate carcinoma (UPC) were selected for comparison. Sections were stained with antibodies for C-Kit, BCL-2 and EGFR. Due to tissue volume constraints, not all cases were stained for all three markers . Staining of > 10% in tumor cells was defined as positive.

Results: A higher percentage of SCCP cases had positive staining for C-Kit, BCL-2 and EGFR, compared to positive staining found in UPC cases and staining was more intense in SCCP cases than in UPC cases. Results are summarized in table 1.

IHC Results

	SCCP (%)	UPC (%)
C-Kit	17/18 (94)	3/10 (30)
BCL-2	18/18 (100)	2/10 (20)
EGFR*	8/11 (83)	4/10 (40)

*not all cases stained with all 3 antibodies

Conclusions: 1. SCCP overexpresses C-Kit, BCL-2 and EGFR in a majority of cases. This provides an immunohistochemical basis for further study to evaluate possible therapeutic implications.
2. The overexpression of C-Kit, BCL-2 and EGFR is more common in SCCP than in UPC, and therapeutic agents targeting these proteins maybe more effective in SCCP than in UPC.
3. Further study of the roles of C-Kit, BCL-2 and EGFR, in the pathogenesis of SCCP is warranted.

804 Expression of Alpha-Methylacyl-CoA Racemase (AMACR) and C-kit in Renal Angiomyolipoma

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Background: A-methylacyl-CoA racemase (AMACR) is a recently identified molecular marker for prostate cancer. Its utility has also been investigated in renal epithelial neoplasms. Expression of this marker has not been previously studied in renal angiomyolipomas (AML) which may show overlapping histological features with renal epithelial neoplasms. C-kit expression has also been recently studied both in renal epithelial neoplasms and renal AML and conflicting results have been reported. The aim of this study is to investigate the expression of AMACR and C-kit in renal AML.

Design: Immunohistochemical stains for AMACR and C-kit were performed on paraffin-embedded tissue sections from 21 renal AML. Intensity (0 to 3+) and percentage of immunoreactivity was scored. Results were categorized as negative (<5% positive cells), focally positive (5-50% positive cells) and positive (>50% positive cells).

Results: 61% (13/21) cases of renal AML demonstrated positivity ranging from focal to diffuse. Immunoreactivity for c-kit was identified in 29% (6/21) cases and the majority of the cases (5) showed focal staining. AMACR and C-kit both exhibited cytoplasmic staining profile and the intensity ranged from 1+ to 2+. The results are summarized in the table.

	Positive (>50%)	Focal Positive (5-50%)	Negative (<5%)
AMACR	19% (4/21)	42% (9/21)	38% (8/21)
C-KIT	5% (1/21)	24% (5/21)	71% (15/21)

Conclusions: 1- Current study shows that renal AML may show immunoreactivity for AMACR and should be included in the differential diagnosis of AMACR positive renal tumors.
2- In contrast to some recent published reports the majority of the renal AML in our study were negative for C-kit.

805 Evaluation of p16^{INK} Immunohistochemical Expression as a Marker for Neoplastic Transformation in the Urinary Bladder

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Background: Mutations of cell cycle regulatory genes, e.g. INK4a, are found in bladder urothelial carcinoma (UC). INK4a encodes p16INK4a and p19ARF that are involved in p53 degradation. These changes have been described at a molecular level and are seen associated with the evolution of urothelial carcinoma. The purpose of this immunohistochemical study was to investigate p16^{INK} protein expression in different urothelial diseases and assess possible utility in distinguishing reactive versus neoplastic changes.

Design: Immunohistochemical expression of p16^{INK} was investigated using paraffin sections from five different groups of patients. These were organ donors with chronic cystitis (OD) [7 patients], bladder biopsies showing chronic cystitis (CC) [5], urothelial carcinoma-in-situ (CIS) [3], high-grade urothelial carcinoma (HG-UC) [7] and normal adjacent to tumor (NAT) [9]. Cytoplasmic and nuclear staining of urothelial cells was seen. A single observer graded staining intensity on a scale of 0-3 (0: no staining, 1, 2 and 3: cytoplasmic/ nuclear staining seen at 20X, 10X, and 4X, respectively). A composite score was calculated (S % positive x Intensity).

Results: CIS had the highest mean expression of 2.13. HG-UC mean expression was 1.58. OD and CC were 1.28 and 1.25, respectively. NAT was 0.09. p16^{INK} immunohistochemical staining decorated CIS cells. The surrounding non-neoplastic urothelial cells showed minimal p16^{INK} expression. A similar pattern of staining was on assessing p16^{INK} expression in foci of urothelial carcinoma and adjacent areas of NAT.

Conclusions: 1. p16^{INK} seems to be a useful marker for distinguishing between CIS and reactive atypia, with significantly higher expression in foci of CIS. 2. p16^{INK} also clearly separates areas of NAT from neoplastic foci, thus helping demarcate extent of lesion. 3. The current sample set is small. Additional cases need to be evaluated to substantiate the current findings.

806 Detection of PTEN Deletion in Prostate Cancer but not Prostatic Intra-Epithelial Neoplasia (PIN) by Fluorescence In-Situ Hybridization (FISH) on Tissue Microarrays

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Background: Phosphatase and tensin homolog (PTEN), is a tumor suppressor protein that inhibits the phosphatidylinositol-3-kinase (PI3K)/Akt/protein kinase B (PKB) pathway and is one of the most frequently deregulated genes in a wide range of cancers. Although somatic PTEN alterations have been reported in prostate cancer, including loss of heterozygosity or homozygous deletions, point mutations, and promoter hypermethylation, the relationship between genomic alterations of PTEN and prostatic neoplasia remains unclear. The purpose of this study was to determine whether PTEN deletion, as assessed by FISH, is associated with more aggressive or advanced forms of prostate cancer.

Design: Archival formalin-fixed, paraffin embedded tissues from 25 radical prostatectomy specimens were used for tissue microarray assembly. Arrayed tissues were examined by H and E staining and scored according to Gleason grading criteria. Standard dual-color FISH was performed using commerically-available DNA probes for band 10q23 (PTEN locus) and band region 10p11.1-q11.1 (centromere of chromosome 10) (LSI PTEN/CEP 10, Vysis, Inc.). At least 100 nonoverlapped intact interphase nuclei were scored in areas of cancer, PIN, benign glandular epithelium and stroma. The signal counts for areas of neoplasia were compared against those obtained from benign and stromal cell nuclei using standard statistical methods.

Results: 3/3 (100%) cases of benign hyperplasia and 18/18 cases (100%) of PIN were found to be PTEN deletion-negative while 17/25 (68%) of cancer samples were PTEN deletion-positive ($P < 0.01$). In addition, homozygous PTEN losses appeared to be uncommon in the PTEN deletion-positive group (11.8%). PTEN deletion tended to prevail in high-stage T3 cancers (77%) and 3 of 4 (75%) of recurrent cases.

Conclusions: These observations support the hypothesis that a relative imbalance of PTEN is likely to be an important factor in the progression of prostate cancer. Future studies should include larger cohorts with long-term follow-up data.

807 Correlating Pathological Evaluation of Neurovascular Bundle to the Intraoperative Findings

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Background: Radical prostatectomy is an effective treatment option offered to those patients with prostate carcinoma. During the procedure, neurovascular bundles can be preserved, partially damaged, or totally resected. The extent of neurobundle preservation is directly correlated to the patient's subsquent erectile fuction. However, the assessments of neurovascular bundle by the surgeons and pathologists may be varied, and this correlation is not well documented.

Design: Fifty one radical prostatectomy wholemount specimens, removed by one urological surgeon, were retrieved from the pathology files in The Methodist Hospital and Baylor College of Medicine. The specimens were reviewed by the blinded pathologists to score the neurovascular bundle volume at the apex, mid and base on the right and left sides, respectively. The pathological scoring system is defined as none to minimal, moderate, and total resection of the neurovascular bundle. The surgeon's intraoperative assessment was scored as preserved, partially damaged, or totally resected, which was recorded on each patient's procedure document. The correlation was performed between these two findings at apex, mid and base of the prostate, respectively.

Results: Fifty one wholemount specimens with bilateral assessment led to 102 observations for each location, including apex, mid and base. There were 25 observations of neurovascular tissue (25%) at the apex, 24 observations (25%) at mid , and 59 observations (58%) at the base, in which the pathology findings indicated more neurovascular tissue than those claimed by the surgeon's intraoperative assessment.

Conclusions: To our knowledge, this is the first pathological correlation of a surgeon's intraoperative estimation of neurovascular bundle preservation following radical prostatectomy. Our data indicated that there is a positive correlation between pathological and intropeative evaluation of the neurovascular volume at the apex and mid, although the pathological evaluation tends to find more nerovascular tissue on the specimen than that claimed by the surgeon; for the base of the prostate, there tends to be even more neurovascular tissue than at the apex and mid glands. The significance of the neurovascular tissue at different areas such as apex, mid, and base on the radical prostatectomy specimen will be determined by the careful followup for the patient's erectile function after surgery, and this is under investigation.

808 Upregulated Expression of Androgen Receptor (AR) Specific Transcription Corepressors in Prostate Cancer

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Background: Androgens play a role in the growth and progression of prostate cancer. The binding of androgen to androgen receptor (AR) triggers the translocation of AR from cytoplasm to the nucleus and binding to its specific androgen response elements to the promoters of androgen-responsive genes. The activity of AR is regulated by transcriptional coactivators and corepressors, which facilitate the interaction between AR and transcription factors including basal transcription machinery and chromatin. Recently, several AR specific corepressors, including HB01, SHP, Smad3 and PIASy have been identified. Their expression in prostate cancer has not yet been explored. To address, we studied the expression of these 4 corepressors in prostate carcinoma and HGPIN by in situ hybridization (ISH).

Design: 77 cases were studied consisting of 20 cases with whole tissue sections and 57 cases in a prostate tissue microarray. Probes were prepared by subcloning a 500-1000bp cDNA fragment from the original plasmids into pBbluescript vector which contains T3 and T7 promoter. Dioxin-labeled probes were synthesized using T7 or T3 DNA polymerase. ISH was performed according to a standard protocol.

Results: Increased mRNA expression was observed in carcinoma for corepressors as follows: HB01 (25%), SHP (69%), Smad-3 (35%) and PIASy (40% of cases). Only SHP mRNA was increased in HGPIN, whereas the levels of expression for HB01, Smad3 and PIASy mRNA in HGPIN were unchanged compared with adjacent benign prostatic epithelium.

Conclusions: The upregulated expression of these 4 transcription factors in carcinoma from radical prostatectomy specimens suggests that androgen receptor activation occurs in hormone naïve primary prostate cancer. Interestingly, the increased mRNA expression for SHP in HGPIN suggests it may play a role in prostate cancer initiation and early progression. Further studies of the expression of these genes at different stages of prostate cancer are planned to determine their relative in initiation and progression of prostate cancer.

809 Potential Roles of Cytologic T-Lymphocytes and Nature Killer Cells in Prostate Basal Cell Layer Disruptions and Tumor Invasion

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Background: The physical disruption of the basement membrane and basal cell layer is a pre-requisite for prostate tumor invasion. The disruption of the basement membrane is believed to result from elevated proteolytic enzymes, while the mechanism of basal cell layer disruptions remains elusive. As our previous studies with antibodies to basal cell specific antigens and leukocyte common antigen (LCA) revealed, focal basal cell layer disruptions are consistently surrounded by or adjacent to LCA positive cells (Man et al, Cancer Detect Prev, In press). This current study attempts to assess whether these cells belong to a cytotoxic cell population.

Design: Consecutive tissue sections from human prostate tumors (n=30) with co-existing normal, hyperplastic, *in situ*, and invasive components were double immunostained with two different chronogens for the high molecular weight cytokeratin 34bE12 to identify basal layer disruptions, and each of the following markers, CD4, CD8, CD56, microphage, perforin, and mast cell tryptase, to elucidate the correlation of these molecules with focal basal layer disruptions.

Results: Multiple focal basal cell layer disruptions were seen in each of the cases. These disruptions were consistently surrounded by or adjacent to CD8, CD56, perforin, and mast cell tryptase positive cells. Tumor cells overlying focally disrupted basal cell layers often displayed distinct morphological alterations in cellular density and polarity, as well as the nuclear size and shape, compared to the adjacent cells within the same duct, but away from the disruption. The CD4 and microphage positive cells also appeared to be associated with basal cell layer disruptions, but the number of these cells varied substantially and the association was less consistent.

Conclusions: The consistent detection of CD8, CD56, perforin, and mast cell tryptase positive cells near focal basal cell layer disruptions suggest that cytotoxic T lymphocytes, nature killer cells and mast tryptase positive cells are likely to promote basal cell layer disruptions and tumor invasion. The development of specific agents to target these cells may have significant clinical value in treatment and prevention of tumor invasion.

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810 Interferon Signal Transduction Pathway Is Disturbed in a Subset of High Grade Invasive Urothelial Carcinomas

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Background: Interferons are known to have a variety of biological activities including antitumor, antiviral and immunomodulatory effects. Recent cell culture and gene expression profiling studies have suggested that interferon signaling pathways may play a key role in the development and progression of high grade urothelial carcinoma. This study evaluated the expression of several key genes in the interferon signaling pathways in low grade non-invasive (LGTCC) and high grade invasive urothelial carcinoma (HGTCC).

Design: Construction of tissue microarray: 33 invasive HGTCC, 6 LGTCC and 3 benign urothelium. Immunohistochemical staining and evaluation: Immunostains were performed with antibodies against NFkB (P50), Stat3, Phospho-Stat3, and ISGF-3g (p48). Benign urothelium served as control. Stains significantly higher or lower than that of benign urothelium were considered as positive or negative for that marker. A case was scored as negative only if all 3 tissue cores from the same case were negative. A case was scored as positive if any of 3 tissue cores was positive. For NFkB and Stat3, the cytoplasmic staining was evaluated. For phospho-Stat3, the nuclear staining was evaluated. For P48, both cytoplasmic and nuclear staining was evaluated.

Results: Normal urothelium was negative for NF kB (P50) and Stat3 expression, but showed consistent positive nuclear stain for phospho-Stat3 and both cytoplasmic and nuclear staining for P48. The expression of P50, Stat-3, Phospho-Stat3 and P48 was summarized in the Table. Expression of phospho-Stat3 and P48 was heterogeneous in TCC, with many tumor cells having lost expression and only a small fraction of tumor cells retaining expression.

Conclusions: About 1/3 of HGTCC are positive for P50 and Stat3, in contrast to 0% of LGTCC, suggesting these two genes play an important role in the development of high grade, more aggressive disease in a subset of transitional cell carcinoma. There is an increased loss of phospho-Stat3 and P48 in HGTCC (although not statistically significant due to small sample size), suggesting a possible role for these two genes in the development of HGTCC.

	P50	Stat3	Phospho-Stat3	P48
HGTCC	10/33 (30.3%)	13/33 (39.4%)	7/33 (21.2%)	12/33 (36.4%)
LGTCC	0/6 (0%)	0/6 (0%)	3/6 (50%)	4/6 (67%)
p value	0.12	0.06	0.15	0.20

Expression of P50, Stat3, phospho-Stat 3 and P48 in TCC

811 Lack of Association between Epidermal Growth Factor Receptor Overexpression and Disease Recurrence in Clear Cell Renal Cell Carcinoma

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Background: The prognostic significance of epidermal growth factor receptor (EGFR) overexpression in clear cell renal cell carcinoma (CCRCC) is controversial. Some studies have shown it is an unfavorable prognostic factor, while other studies found no association between EGFR overexpression and prognosis. Recent reports that anti-EGFR therapy was ineffective for CCRCC further raised the question about the biological function of EGFR in CCRCC. We studied EGFR overexpression in a cohort of CCRCC with long-term follow-up (> 10 years).

Design: 44 CCRCC were used to construct a tissue microarray (TMA). All patients were treated with radical/partial nephrectomy and had been followed for >=10 years. Eleven had cancer recurrence within 1 year, 22 within 1-10 years, and 11 were recurrence-free more than 10 years aftersurgery. The TMA was stained with anti-EGFR antibody. EGFR overexpression was scored using two methods. 1. Simple scoring- strong circumferential membranous stain (3), weak circumferential membranous stain (2), weak partial membranous stain (1) and negative staining (0). Only staining intensity 2 and 3 were considered positive for EGFR overexpression. 2. Composite scoring-the staining intensity (0-3) was multiplied by the percentage of positive cells with EGFR overexpression score ranging from 0 to 300.

Results: Using the simple scoring method, EGFR overexpression was found in 7/11 (63.6%), 18/22 (81.8%) and 5/11 (45.5%) of patients with recurrence within 1, 1-10 and >10 years after surgery (p>0.05). There was no correlation between EGFR overexpression and tumor recurrence status or tumor stage (p=0.37 and 0.55). However, EGFR overexpression correlated with Furhman nuclear grade (r=0.372, p=0.036). Using the composite scoring method, mean EGFR overexpression score was 120$\pm$78, 122$\pm$77 and 82$\pm$85 for patients with recurrence within 1, 1-10 and >10 years after surgery respectively (p>0.2). There was no correlation between EGFR overexpression level and disease recurrence status, tumor stage or Furhman nuclear grade.

Conclusions: EGFR overexpression correlates with Furhman nuclear grades in CCRCC. However, it is not significantly different between patients with recurrence within 1, 1-10 and >10 years after surgery. There is no correlation between EGFR overexpression and prognosis.

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812 Prognostic Significance of Peritumor Lymphatic Vessel Density in Early Stage Squamous Cell Carcinoma of the Cervix

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Background: The role of angiogenesis in the development and progression of cervical carcinoma is established. However, in cervical cancers the earliest feature of disseminated disease is regional lymph node involvement. Despite its role in tumor dissemination, little is known about the role of tumor lymphangiogenesis in metastases and whether lymphatic spread occurs via pre-existing lymphatic channels or vessels newly formed by lymphangiogenesis. The recently developed monoclonal antibody D2-40 was reported to be a selective marker for lymphatic endothelium useful in identifying lymphatic invasion in various malignant neoplasms.

Design: We examined the intra- and peritumor lymphatic vessel density (LVD) in a series of 112 FIGO stage I and II cervical squamous cell carcinomas using D2-40 immunohistochemistry on formalin-fixed paraffin-embedded tissue sections. The lymphatic vessel density within the tumors (intratumor LVD) and within 2 mm of the edge of the tumors (peritumor LVD) was determined in 10 high power fields (X400) with the highest number of D2-40 positive lymphatic vessels. The intra- and peritumor LVD was correlated with clinicopathologic tumor features, D2-40 immunoreactivity in tumor cells and patient outcome.

Results: Intra- and peritumor LVD was significantly higher compared to benign squamous cervical mucosa (p<0.0001). Peritumor LVD (9.56 ± 0.47, mean ± SEM) was significantly higher compared to intratumor LVD (7.84 ± 0.49) (p<0.01). High peritumor, but not intratumor, LVD was significantly associated with low D2-40 immunoreactivity of tumor cells (p=0.005), presence of lymphatic invasion (p<0.0001), nodal metastasis (p=0.026) and FIGO stage (p=0.032). Intra- and peritumor LVD showed no correlation with patient age, tumor size or grade. High peritumor, but not intratumor, LVD was highly significantly associated with poor recurrence-free (p<0.0001) and overall survival (p<0.0001) in both uni- and multivariate analysis.

Conclusions: The monoclonal antibody D2-40 specifically labels lymphatic vessels and is a useful marker for determination of lymphatic vessel density. Cervical squamous cell carcinomas showing high peritumor LVD are associated with more advanced, aggressive disease and poor outcome. Determination of peritumor LVD may serve as an independent prognostic and/or predictive factor in cervical cancers.