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USCAP & AACR HIGHLIGHTS 2012

[784] Interrogation of *ERG* Gene Rearrangements in Prostate Cancer Identifies Novel Signatures Relative to Disease Progression and with Prognostic Implications

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- **Background:** ERG gene rearrangements have been proposed to be reflective of specific molecular subtype of prostate cancer (PCA) with its prognostic implication. Herein, we investigated gene expression differences between those two classes of tumors to identify potential genetic targets and pathways.
- Design: 6144 informative genes belonging to 46 castration resistant PCA samples were interrogated using the DASL platform. We used bioinformatic approach to identify significant dyregulated genes based on their ERG status (assessed by FISH).
- **Results:** The employed method identified a group of 16 differentially expressed genes between ERG rearranged (19 samples) and ERG non-rearranged tumors (27 samples). We then were able to narrow down those genes to a 10 gene model which was the most accurate to predict prostate tissue sample classification relative to ERG status (77%). Several tumor suppressor genes as well as genes associated with cell growth, apoptosis and cancer metastasis were among the most significant deregulated genes between the two groups. Several of the genes described have never been characterized or have been poorly described in association with prostate cancer. Furthermore, we validated this model using Q-PCR and protein expression using progression TMA. The multi-gene model was also able to confirm significant prognostic value in prostate cancer beyond those of ERG alone when tested on public datasets.
- **Conclusions:** ERG rearrangements tumors represent distinct subclass of PCA that is associated with downregulation of genes related to cell adhesion, and cell motility. This group as well shows downregulation of several tumor suppressor genes. As this represent a distinct subclass of tumors proposed to be associated with more aggressive behavior, analyzing and comparing the genes identified in this study with other genes associated with PCA could lead to better understanding to the molecular mechanism behind the aggressive behavior of ERG rearranged tumors. Furthermore, as the gene panel list identified showed significant association related to patients' prognosis in other tumor types, characterization of those genes could proof significant players in identifying pathways related to cancer progression in general and will help us to identify potential new targets for cancer therapy across several tumor types.

Category: Genitourinary (including renal tumors)

[807] ERG Protein Expression and Genomic Rearrangement Status in Primary and Metastatic Prostate Cancer – A Comparative Study of Two Monoclonal Antibodies

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- **Background:** Overexpression of the ERG protein is highly prevalent in prostate cancer (PCa) and most commonly results from gene fusions involving the *ERG* gene. Recently, an N-terminal epitope targeted mouse and a C-terminal epitope targeted rabbit monoclonal anti-ERG antibody have been introduced for the detection of the ERG protein. Independent studies reported that immunohistochemical (IHC) stains with both monoclonal anti-ERG antibodies (ERG-MAbs) highly correlate with the underlying *ERG* gene rearrangement status. However, a comparative study of both antibodies has not been provided so far. Here, we are the first to compare the mouse ERG-MAb to the rabbit ERG-MAb for their concordance on the same PCa cohort. Furthermore, we assessed if the ERG protein expression is conserved in lymph node and distant PCa metastases, of which a subset underwent decalcification.
- **Design:** We evaluated tissue microarrays of 278 specimens containing 265 localized PCa, 29 lymph node, 30 distant metastases, and 13 normal prostatic tissues. We correlated the *ERG* protein expression with the *ERG* rearrangement status using an ERG break-apart fluorescence in-situ hybridization (FISH) assay and IHC of both ERG antibodies.
- **Results:** ERG protein expression and *ERG* rearrangement status were highly concordant regardless of whether the mouse or rabbit ERG-MAb was used (97.8% versus 98.6%, respectively). Of interest, both ERG antibodies reliably detected the ERG expression in lymph node and distant PCa metastases, of which a subset underwent decalcification. If an ERG protein expression was present in localized PCa, we observed the same pattern in the corresponding lymph node metastases.
- **Conclusions:** This is the first study to comprehensively compare the two available ERG-MAbs. By demonstrating a broad applicability of IHC to study ERG protein expression using either antibody, this study adds an important step towards a facilitated routine clinical application. Further, we demonstrate that the clonal nature of the *ERG* rearrangement is not restricted the genomic level, but proceeds in the proteom. Together, our results simplify future efforts to further eliucidate the biological role of ERG in PCa.

Category: Genitourinary (including renal tumors)

[808] Improved Method of Detecting the *ERG* Gene Rearrangement in Prostate Cancer Using Combined Dual-Color Chromogenic and Silver In-Situ Hybridization

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- **Background:** The recently detected *TMPRSS2-ERG* fusion revealed as a recurrent and prevalent prostate cancer (PCa) specific event, which potentially qualifies it for clinical utilizations. To detect this alteration, fluorescence *in-situ* hybridization (FISH) is the method of choice. However, FISH harbors some disadvantages for widespread adoption in clinical practice. Subsequently, the chromogenic *in-situ* hybridization (CISH), that uses organic chromogens, and the enzymatic metallography silver in-situ hybridization (SISH) emerged as promising bright-field alternatives. Compared to CISH, SISH signals are very distinct and superior with regard to signal clarity and resolution, but rule out a multi-color protocol. However, the precise localization of genomic targets using a dual-color approach is indispensable for gene break-apart and fusion assays. In order to bridge this gap, we aimed to develop a dual-colour combined CISH and SISH (CS-ISH) gene break-apart assay on the example of the *ERG* gene commonly rearranged in PCa.
- **Design:** On the basis of the *ERG* break-apart FISH assay, we established a dual-colour *ERG* break-apart CS-ISH assay and compared these results with those obtained by FISH. We assessed 178 PCa and 10 benign specimens for their *ERG* rearrangement status applying a dual-colour FISH and CS-ISH *ERG* break-apart assay on consecutive sections.
- **Results:** We observed a highly significant concordance (97,7%) between FISH-based and CS-ISH-based results (Pearson's correlation coefficient 0.955, P<0.001).
- **Conclusions:** Our findings demonstrate that the *ERG* rearrangement status can reliably be assesed by CS-ISH. Further, we confirm that the CS-ISH technique combines the accuracy and precision of FISH with the ease of bright field microscopy. We developed a tool which allows a much broader spectrum of applicants to study the biological role and clinical utilization of *ERG* rearrangements in PCa. Moreoever, our study is the first proof-of-principle for bright-field CS-ISH gene fusion or break-apart assays.

Category: Genitourinary (including renal tumors)

[839] Prognostic Gleason Grade Grouping: Data Based on the Modifed Gleason Scoring System

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- **Background:** There are some problems with reporting Gleason score (GS): 1) GS6 is typically the lowest grade assigned on biopsy (bx). However, GS ranges from 2-10, so men are concerned when told they have GS6 cancer on bx, logically but incorrectly assuming that their tumor is in the mid-range of aggressiveness. 2) Within the literature, GS3+4=7 & GS4+3=7 are often combined as GS7. 3) GS8-10 are also typically lumped together.
- **Design:** We have previously shown that tertiary Gleason patterns in RP influence biochemical free survival rates (BFS), acting as an intermediary between grades. We identifed 8,039 men who underwent RP at our institutution since 2004 without tertiary patterns to study the sole effect of grade derived from the two predominant patterns.
- **Results:** Of 4,510 GS2-6 tumor on bx, 1 had GS2-4 and 7 had GS5. Of 3,548 GS2-6 tumor at RP, 1 had GS2-4, and 44 had GS5. Therefore, 99.8% and 98.7% of biopsy & RP GS2-6 tumors were GS6, respectively, so cases were combined as GS2-6. Prognostic Grade Group (2-6; 3+4; 4+3; 8; 9-10) was among the strongest predictors of biochemical free survival (BFS) in multivariable models. At a median follow-up of two years (range 1-7), 2-year BFS rates for men with 3+3, 3+4, 4+3, 8, and 9-10 at bx were 97.4%, 90.0%, 84.7%, 73.1%, and 46.0% respectively (p<0.001); and 98.8%, 93.6%, 85.6%, 73.7%, and 48.9% (p<0.001), respectively, based on RP pathology.
- **Conclusions:** GS 3+4=7 has a very favorable prognosis with an estimated 2-year BFS of 90.0% and 93.6% for bx and RP, respectively; these results warrant the designation of "moderately differentiated". GS4+3=7 had a significantly worse prognosis than GS3+4=7. The current study shows GS9-10 has almost twice the risk of progression compared to GS8. We propose reporting Gleason grades, along with descriptive terminology, and including Prognostic Grade Groups which accurately reflect prognosis: GS2-6 (*well-differentiated*), Prognostic Grade Group I/V; GS3+4=7 (*moderately differentiated*), Prognostic Grade Group II/V; GS4+3=7 (*moderately-poorly differentiated*), Prognostic Grade Group III/V; GS8 (*poorly differentiated*), Prognostic Grade Group IV/V; and GS9-10 (*undifferentiated*), Prognostic Grade Group V/V. One would still report a case as "Gleason Score 5" or "Gleason score 6", (rather than "Gleason Score 2-6") along with Prognostic Grade Group I. The descriptive terms (i.e. well, moderately, etc.) would only be used along with GS in Pathology reports so that men could better understand their grade. Men will, for example, be reassured with a GS6 that their Prognostic Grade Group is I/V along with a description of the tumor as well-differentiated.

Category: Genitourinary (including renal tumors)

[842] Neoadjuvant Docetaxel Treatment for Locally Advanced Prostate Cancer Affects miRNA Expression: A Pilot Study

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- **Background:** Taxanes are microtubule-stabilizing drugs used investigationally in adjuvant and neoadjuvant settings of prostate cancer (PCA) treatment in attempt to improve systemic control of high risk disease. Understanding mechanism of response to taxanes is essential to develop novel combination therapies. MicroRNAs (miRNAs) are small noncoding RNA molecules that negatively regulate gene expression by binding target messenger RNAs and inhibiting their stability and/or translation. In cancer, miRNAs can act as oncogenes and/or tumor suppressor genes. Our objective was to identify miRNAs that are affected by neoadjuvant docetaxel in high-risk PCA.
- **Design:** Whole cell RNA was extracted from formalin-fixed paraffin-embedded radical prostatectomy specimens from 8 patients with high grade PCA treated with neoadjuvant docetaxel, 8 high grade untreated PCA and their corresponding untreated non-neoplastic tissue. Tumors were matched by Gleason score, patient age and year of surgery. Expression of 88 cancer-associated miRNAs was quantified using a PCR-based miRNA microarray assay.
- **Results:** Thirty-eight (43%) miRNAs (including miR-205, miR-222, and miR-1) were significantly downregulated in untreated PCA compared to untreated non-neoplastic tissue, and one (miR-183) was upregulated ($p<0.05$). In 25 of the downregulated miRNAs, fold regulation was less than 2 (range -2.1 to -8.0). Twenty (23%) miRNAs (including miR-218, miR-124, and let-7b) were significantly upregulated in treated compared to untreated PCA ($p<0.05$). Four of them (miR-125a-5p, miR-222, miR-1 and miR-133b) showed fold regulation greater than 2 (range 2.4 to 5.1). Sixteen of 38 miRNAs downregulated in untreated tumors (including miR-125a-5p, miR-222, miR-1 and miR-133b) were upregulated in treated PCA. Expression levels of 7 of them (miR133b, miR-27b, miR-29b, miR-34a, miR-140-5p, miR-9 and miR-15b) were reverted to values similar to untreated non-neoplastic tissue as a result of docetaxel treatment.
- **Conclusions:** MicroRNAs may play a potential role in PCA response to taxanes. A subset of miRNAs are downregulated in untreated tumors but upregulated in treated PCA to levels comparable to non-neoplastic tissue, suggesting a miRNA modulation towards a non-neoplastic expression profile with neoadjuvant docetaxel treatment. miR-133b has been reported to be involved in the regulation of apoptosis, by functionally targeting members of the BCL-2. Further studies are underway to evaluate the miRNA-mediated effects of taxanes in PCA.

Category: Genitourinary (including renal tumors)

[855] Microsatellite Instability in Prostatic Adenocarcinoma: Association with a Mucinous Phenotype

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- **Background:** The loss of DNA mismatch repair genes and subsequent microsatellite instability (MSI) plays a key role in carcinogenesis of approximately 15% of colonic carcinomas, including patients with Lynch syndrome, as well sporadic cases. A characteristic histologic feature of MSI-associated colonic carcinomas is mucinous change. Several studies have also shown loss of mismatch repair genes in prostatic adenocarcinomas. However no specific histologic phenotype of this tumor has been described in relation to MSI. We examined prostatic adenocarcinomas with mucinous change for their possible association with MSI.
- **Design:** Index cases consisted of 22 prostatic adenocarcinomas with mucinous extravasation involving at least 5% of the tumor. Control cases consisted of 22 prostatic adenocarcinomas (without mucinous change), matched for primary and secondary Gleason patterns, and for pathologic stage. MSI was studied by immunohistochemical staining for MLH1, MSH2, MSH6, and PMS2 by our Translational Research Laboratory.
- **Results:** The Gleason scores of the set of mucinous tumors and of the matching controls were as follows: 16 cases were Gleason score 7; including 4 cases of 3+4, 7 cases of 4+3, and 5 cases of 4+3 with tertiary pattern 5. Two cases were Gleason score 8(3+5) with tertiary pattern 4, and 4 cases were Gleason score 9(4+5). In each set there was 1 case of stage pT2a, 11 cases of pT2c, 7 cases of pT3a, and 3 cases of pT3b. The mean age of the patients with mucinous adenocarcinoma was 64 (range 46-78), and of the control patients the mean age was 62 (range 54-75). MSH6 was absent in 6 (26%) of the mucinous carcinomas, and in 1 (4.5%) of the control cases. MLH1, MSH2 and PMS2 were retained in all cases. This difference is statistically significant ($p=.039$).
- **Conclusions:** Mucinous prostatic adenocarcinomas, characterized by the presence of extravasated mucus, are more likely to be associated with MSI, specifically with the loss of MSH6. This suggests that a subset of patients with loss of MSH6 might be at increased risk for prostatic carcinoma; and that the mucinous phenotype may be a histologic indicator. This association is interesting in light of a recent study which reported that MSH6-associated colonic carcinomas appear to be a distinctive subset of MSI-related tumors. If supported by future studies of larger numbers of cases, these observations may help to identify patients with MSI-related prostate cancer.

Category: Genitourinary (including renal tumors)

[872] ERG vs. Alpha-Methylacyl-CoA Racemase Expression in Histologic Variants of Adenocarcinoma of the Prostate

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- **Background:** The utility of the widely used p63/34βE12/alpha-methylacyl-CoA racemase (AMACR) triple stain immunohistochemistry (IHC) can be limited in some variants of prostate adenocarcinoma (PC) including atrophic, foamy gland and pseudohyperplastic PC, where almost a third of these variants do not express AMACR. The recent discovery of TMPRSS2: ERG gene rearrangement in PC and the subsequent development of a highly specific antibody to ERG have generated considerable interest in ERG IHC as an aid in detecting prostate cancers. However, added diagnostic value of ERG expression beyond AMACR expression has not been addressed. The aim of the study was to investigate whether ERG IHC can provide an advantage in diagnosis of these deceptively benign-appearing variants.
- **Design:** 97 radical prostatectomy cases of variant PC, including atrophic, foamy gland, and pseudohyperplastic (PH) adenocarcinomas were selected from our files. All cases had both variant adenocarcinoma and usual acinar adenocarcinoma components. H&E, triple stain with AMACR, and ERG IHC stains were obtained. Both the intensity (0-3) and the percentage of glands stained (1-4)were scored, generating an IHC score ranging from 0-12 for both ERG and AMACR expression. We searched for areas that were either completely negative or stained weakly for AMACR and compared these foci with ERG IHC to assess for difference in comparative staining.
- **Results:** 50 of the 97 cases were positive in ERG IHC. Both ERG and AMACR antibodies marked usual acinar adenocarcinoma with greater intensity as compared to the variants. 15 cases had foci which were completely negative for AMACR including 12 foamy glands, 6 atrophic, 3 pseudohyperplastic, and 4 usual acinar adenocarcinoma foci; these foci were strongly positive with ERG. 16 cases showed significantly weaker staining with AMACR as compared to ERG in foci of variants and usual acinar adenocarcinoma of which there were 10 foci each of usual acinar and foamy glands and 5 foci of atrophic glands. The IHC scores of ERG/AMACR in these 31 cases was 11.6/8.3 for usual acinar, 11.2/6.2 for atrophic, 10.8/6.8 for microcystic, and 9.9/5.2 for foamy gland foci. In 2 cases AMACR was positive in ERG negative foci, which included one focus each of usual acinar and foamy gland PC.
- **Conclusions:** ERG immunostains provide added value beyond AMACR immunostains in a substantial number of cases of prostatic adenocarcinoma with atrophic, foamy gland, and pseudohyperplastic variant histological appearance, that can be deceptively-benign appearing.

Category: Genitourinary (including renal tumors)

[961] Correlation of Immunohistochemical Expression of Protein-Coding Genes RAD23B, SIM2S, Notch3, BID and FBP with Biochemical Recurrence in Patients Following Radical Prostatectomy for Prostatic Adenocarcinoma

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- **Background:** One of the challenges in prostate cancer research is the development of effective predictors of tumor recurrence following radical prostatectomy, to determine whether immediate adjuvant therapy is warranted. This is even more important in patients who have negative margins and may be lost to follow up. To date very few biomarkers have been identified that accurately predict the possibility of tumor recurrence or biochemical failure in this setting.
- **Design:** A panel of five biomarkers (RAD23B, SIM2S, Notch3, BID and FBP) were selected based on cDNA-mediated Annealing, Selection, extension and Ligation (DASL) assay. A TMA was constructed from 94 radical prostatectomy cases with available detailed follow-up data. Five TMA sections were stained with antibodies to RAD23B, SIM2S, Notch3, BID, and FBP. Intensity of the various immunohistochemical stains were scored blindly as follows; 0-negative, 1+ (weak), 2+ (intermediate) and 3+ (strong). The expression of these markers was correlated with biochemical recurrence (BCR) using Fisher's exact test with samples divided into categories 0-2+ vs. 3+ for staining and positive vs. negative for BCR.
- **Results:** Median patient age was 64 yrs (range 47-74yrs). Median Pre-operative PSA was 6.6 (range 1.6-72.6). Gleason scores were as follows: 12/97 cases (3+3=6), 53/97 cases (3+4=7), 23/97 cases (4+3=7), 7/97 cases (4+4=8), 1/97 cases (4+5=9), and 1/97 cases (5+4=9). There were 59 Caucasian and 31 African-American cases, with the remaining 7 of unknown race. There were 61/97 cases without BCR and 34/97 cases with BCR. Increased expression of RAD23B ($p = 0.023$) and SIM2S ($p = 0.021$) demonstrated statistical significance by Fisher's exact test for discriminating between patients with BCR and those without BCR. Expression of BID (two-tailed p-value = 0.357), NOTCH3 ($p = 0.237$), and FBP ($p = 0.237$) did not demonstrate statistical significance between patients with BCR and those without BCR. Interestingly, there was no statistically significant correlation between any of the markers and pathologic stage, Gleason score, patient race, or pre-operative PSA levels.
- **Conclusions:** RAD23B and SIM2S may be useful immunohistochemical biomarkers in the prediction of BCR in patients following radical prostatectomy, irrespective of pathologic stage, Gleason score, patient race, or pre-operative PSA levels. Patients with tumors that demonstrate increased expression of these markers may benefit from close follow-up after radical prostatectomy.

Category: Genitourinary (including renal tumors)

[997] Do Adenocarcinomas of the Prostate with Gleason Score (GS) ≤6 Have the Potential To Metastasize to Lymph Nodes?

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- **Background:** Although rare, GS≤6 in radical prostatectomies (RP) have been reported with pelvic lymph node (LN) metastases. However, there are no studies as to whether pelvic LN metastases occur in tumors GS≤6 using the ISUP modified Gleason scoring system.
- **Design:** We searched the RP databases at 4 large academic centers for entirely submitted RPs GS≤6. 14,444 cases from 1975-2010 were identified. Cases from Institution #1 were split into two groups with December 2004 as the breakpoint, reflecting when the modified ISUP Gleason grading scheme was in press.
- **Results:** Institution #1: 10,935 men were identified with GS≤6 at RP: 7,332 pre-ISUP and 3,603 post-ISUP. In the pre-ISUP group, there were 19 (0.26%) cases with positive LNs, whereas there was only 1 case (0.03%) with a positive LN in the post-ISUP group. 18/19 pre-ISUP cases had non-focal extraprostatic extension (EPE) with 4 invading seminal vesicles. The 1 post-ISUP case had focal EPE. Histological review of 17/19 cases (2 cases unavailable for review) pre-ISUP and the one post-ISUP case showed higher grade than originally reported. Of the 17 pre-ISUP reviewed cases, 3 were upgraded to 4+3=7 with small/large cribriform and poorly formed glands. 10 cases were upgraded to 3+4=7 with either glomeruloid structures, small/large cribriform glands, or ductal features. 3 cases had tertiary pattern 4 with small cribriform glands. 1 case had a prominent colloid component that had been previously left ungraded that currently would be graded as 4+5=9 with large cribriform glands and solid sheets of cells within mucin. The single post-ISUP case had a cribriform gland of tertiary pattern 4. Institutions 2-4: Out of 3,509 men with GS ≤6 at RP, 1 pre-ISUP (slides not available for review) and 1 post-ISUP had a positive LN. The post-ISUP case was called 3+4=7 on biopsy. Although the RP was initially graded as 3+3=6, review showed GS 3+4=7 with small cribriform glands.
- **Conclusions:** Under-grading primarily accounts for lymph node positivity with GS ≤6, which has decreased significantly since the adoption of the ISUP grading system in 2005. Out of over 14,000 totally embedded RPs from multiple institutions, there was not a single case of a GS≤6 tumor with LN metastases. In contrast to prevailing assumptions, Gleason score ≤6 tumors do not appear to metastasize to lymph nodes. Rather, Gleason patterns 4 or 5, as better defined by the current ISUP modified grading system, is required for metastatic disease.

Category: Genitourinary (including renal tumors)

[1068] Correlation of Urine *TMPRSS2: ERG* and *PCA3* to ERG+ and Total Prostate Cancer Burden

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- **Background:** ERG rearrangement, (most commonly resulting in *TMPRSS2:ERG* (*T2:ERG*) gene fusions), have been identified in approximately 50% of prostate cancers (PCa) and to date is the most specific prostate cancer biomarker. Quantification of *T2:ERG* in post-DRE urine, in combination with PCA3, improves the serum PSA performance for PCa prediction on biopsy. Previously, we have shown significant correlation between urine *T2:ERG* and maximum index tumor nodule dimension at prostatectomy.

  Here we compared urine *T2:ERG* and *PCA3* to both ERG+ and overall tumor burden at prostatectomy to assess the cancer specificity of these urine biomarkers.
- **Design:** Of 301 men presenting for biopsy assessed by transcription mediated amplification (TMA) for *T2:ERG* and *PCA3*, 41 (14%) underwent prostatectomy. All prostatectomies were mapped and all tumor nodules (including suspicious foci) were immmunostained with an ERG antibody shown to be sensitive and specific for ERG rearranged cancer (EPR3864). For each prostatectomy, the total number, greatest linear dimension, Gleason score and ERG IHC status of all tumor nodules was documented. Correlations between clinicopathological data and urine *T2:ERG* and *PCA3* were determined.
- **Results:** The 41 prostatectomies had a median of 3 tumor nodules (1-15) and 2.7 cm of total linear tumor dimension (0.5-7.1 cm). There was no significant difference between the number (p=0.59) or linear tumor dimension (1.2 cm vs. 0.9 cm, p=0.36) of ERG+ and ERG- nodules (p=0.59). Urine *T2:ERG* most correlated with the number of ERG+ foci and total ERG+ linear tumor dimension (both r_s=0.67, p<0.0001). Of patients with 0 cm, >0.1 to 1.0 cm, and >1.0 cm of total ERG+ linear tumor dimension, 1/8 (13%), 4/10 (40%) and 21/23 (91%) had urine T2: *ERG* >30. Urine *PCA3* showed weaker correlation with both tumor nodule number (r_s=0.37, p=0.02) and total linear tumor dimension (r_s=0.27, p=0.08).
- **Conclusions:** We demonstrate a strong correlation between urine *T2:ERG* and total ERG+ tumor burden at prostatectomy. The weaker correlation between urine *PCA3* and total tumor volume suggests that this biomarker may be less cancer specific than *T2:ERG*. Hence, urine *T2*: ERG may be useful for risk stratifying men with elevated serum PSA, prior negative biopsy, or those considering active surveillance.

Category: Genitourinary (including renal tumors)

[852] Tumor Regression after Neoadjuvant Chemotherapy Independently Predicts Survival in Bladder Cancer Patients

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- **Background:** Tumor regression after chemotherapy predicts survival in different cancers. In bladder cancer these studies are still missing.
- **Design:** A cohort of 59 patients with histopatholgically proven urothelial bladder cancer received neoadjuvant chemotherapy (median 4 cycles) before cystectomy and lymphadenectomy. Indications were clinically positive lymph nodes (n=41), advanced primary tumour stage cT4 (n=15) and other reasons (n=3). A tumor regression grade (TRG) was defined similar to the method proposed by Mandard et al. (Cancer 1994;71:2680-6) for esophageal cancer. TRG 1: complete regression without residual cancer and with extensive fibrosis of the tumor bed; TRG 2: presence of residual cancer cells scattered through the predominating fibrosis; TRG 3: residual cancer outgrowing fibrosis or absence of regression. Histopathological characteristics of the untreated tumors (growth patterns, histological subtypes, nuclear size, peri- and intratumoural inflammation, mitotic rate) were correlated with TRG and different parameters of the treated tumors were tested for overall survival (OS) stratification.
- **Results:** Seventeen patients each (28%) had TRG 1 and TRG 2, 25 patients (44%) TRG 3. In the untreated cancers, the only parameter with significant ($p<0.05$) predictive value for therapy response was a high mitotic rate. Higher TRG grades were significantly ($p<0.05$) associated with unfavorable characteristics in surgical specimens (higher ypT and ypN stage, number of positive blocks and diameter of residual tumor). In univariate analysis, TRG, ypT, number of blocks with residual tumor tissue, largest diameter of primary tumor and ypN stratified OS significantly. In multivariate analysis, only TRG predicted OS independently ($p<0.05$).
- **Conclusions:** The suggested tumor regression grade in bladder cancer is an independent predictor of survival. A favorable chemotherapy response is associated with a high mitotic rate in the untreated tumor. This parameter might help to identify patients which benefit from neoadjuvant chemotherapy.

  Category: Genitourinary (including renal tumors)

[907] Differential Expression of SPINK1 in Urothelial Neoplasia: Clinical and Pathological Implications

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- **Background:** High serum level/tissue expression of SPINK1 (Serine protease inhibitor Kazal type I; Tumor associated trypsin inhibitor [TATI]) have been associated with poor outcome in epithelial malignancies. SPINK1 defines an aggressive ETS-negative prostate cancer (PCa) and transcripts can be detected in urine. Recent data suggest that loss of SPINK1 expression in urothelial carcinoma of bladder (UCB) may be associated with advanced stage. SPINK1 is also a potential therapeutic target, given structural similarities with EGF. We assessed the expression of SPINK1 in a wide selection of urothelial neoplasia and benign urothelium.
- **Design:** Tissue microarrays (TMAs) of 390 tissue samples from bladder (n=373), kidney/ureter (n=9), prostatic urethra (n=6) and metastatic sites (n=2) were studied. These included biopsies and resections of high-grade urothelial carcinoma (HGUC) (n=134; 97 invasive) and lymph node metastases (n=2); low-grade urothelial carcinoma (LGUC) (n=77); papillary urothelial neoplasm of low malignant potential (PUNLMP) (n=1); inverted papilloma (IP) (n=4); urothelial carcinoma *in situ* (CIS) (n=83); neuroendocrine carcinoma of bladder (NECB) (n=4); bladder squamous cell carcinoma (n=3); urothelial dysplasia (UD) (n=13); urothelial atypia (UA) (n=18); papillary cystitis (n=1); radiation cystitis (n=1); and normal urothelium (n=49).

  Semi-quantitative SPINK1 IHC evaluation was performed: >20% staining extent and 2-3 intensity (scale 0 to 3) was considered positive.

  FISH was performed in 116 cases using SPINK1 locus/control and 5'/3' split probes.
- **Results:** SPINK1 overexpression was present in 50% of HGUC with loss of expression in 55% of invasive tumors. SPINK1 overexpression was present in 53% of LGUC and in a PUNLMP, 82% of CIS, 31% of UD, 22% of UA, 50% of NECB, and 33% of SCC. The inverted papillomas were negative. SPINK1 expression was seen in umbrella cells of normal urothelium (76%) and papillary and radiation cystitis. SPINK1 overexpression was also noted in urothelial samples from kidney/ureter and prostatic urethra. SPINK1 amplification or gross rearrangements were not seen by FISH.
- **Conclusions:** SPINK1 is expressed in a wide range of urothelial neoplasia and in umbrella cells of benign urothelium. This should be considered if urine-based tests for detection of SPINK1 in both urothelial and prostatic carcinomas are implemented. SPINK1 appears to be a very specific marker of CIS. Loss of SPINK1 expression, especially in invasive tumors, is noteworthy.

  Category: Genitourinary (including renal tumors)

[927] p16 Expression Is Not Associated with Human Papillomavirus (HPV) in Urinary Bladder Squamous Cell Carcinoma

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- **Background:** Squamous cell carcinoma of the urinary bladder is an unusual histologic type of bladder neoplasm with unknown etiology. There is a well established association between human papillomavirus (HPV) infection and the development of uterine cervical and head/neck squamous cell carcinomas. However, the role of HPV in the pathogenesis of squamous cell carcinoma of the urinary bladder is uncertain. The purposes of this study are to investigate the potential role of HPV in the development of squamous cell carcinoma of the urinary bladder and to determine if p16 expression is a surrogate marker for HPV in this malignancy.
- **Design:** Twenty-three cases of squamous cell carcinoma of the urinary bladder and 15 cases of urothelial carcinoma with squamous differentiation are included in this study. HPV infection is analyzed by both in situ hybridization at the DNA level and immunohistochemistry at the protein level. p16 protein expression is analyzed by immunohistochemistry.
- **Results:** HPV DNA and protein are not detected in 23 cases of squamous cell carcinoma (0%, 0/23) and 15 cases of urothelial carcinoma with squamous differentiation (0%, 0/15). p16 expression is detected in 8 cases (35%, 8/23) of squamous cell carcinoma and 6 cases (40%, 6/15) of urothelial carcinoma with squamous differentiation. There is no correlation between p16 expression and the presence of HPV infection in bladder squamous cell carcinoma and urothelial carcinoma with squamous differentiation.
- **Conclusions:** HPV does not play a role in the development of squamous cell carcinoma of the urinary bladder and urothelial carcinoma with squamous differentiation. p16 expression should not be used as a surrogate marker for evidence of HPV infection in either squamous cell carcinoma of the urinary bladder or urothelial carcinoma with squamous differentiation as neither HPV DNA nor protein is detectable in these neoplasms.

Category: Genitourinary (including renal tumors)

[985] Nephrogenic Adenoma: An Immunohistochemical Study

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- **Background:** Nephrogenic adenoma (NA) is a rare benign lesion commonly occurring in the urinary bladder. NA may be derived from detached renal tubular cells implanting along the urothelial tract in previously injured areas. NA can be confused with lesions with papillary architectures including urothelial papilloma, papillary urothelium neoplasm with low malignant potential, and low-grade papillary urothelial carcinomas. NA involving deep lamina propria and/or superficial muscle can mimic metastatic prostatic adenocarcinoma, urothelial carcinoma with bland histology including nest variant of urothelial carcinoma and microcystic urothelial carcinoma, and clear cell adenocarcinoma.
- **Design:** Twenty one cases of NAs were retrieved from the archives of hospital of the University of Pennsylvania. Immunohistochemistry for PAX8, p63, CK903 and PSA were performed on routine tissue sections according to the standard immunohistochemistry protocols. Intensity of the nuclear staining for PAX8 and p63 and the cytoplasmic staining for CK903 and PSA were evaluated and graded as 0, 1+, 2+, and 3+. Extent of staining was assigned as focal (<25%), nonfocal (25% to 75%), or diffuse (>75%).
- **Results:** PAX8 showed diffuse moderate to strong (2+/3+) nuclear staining in all of NAs (n=21) and negative in the normal urothelium (n=15). Nuclear staining for p63 was not seen in any case of NAs examined (n=19) and was diffuse and strong (3+) in the normal urothelium (n=14). High molecular weight keratin CK903 showed weak (1+) diffuse staining in all of the nephrogenic adenomas examined (n=19) and diffuse and strong positivity in the normal urothelium (n=16). PSA staining was negative in both of the NAs (n=21) and normal urothelium (n=16).
- **Conclusions:** We evaluated a panel of immunohistochemical markers in 21 cases nephrogenic adenomas. A panel composed of PAX8, p63 and PSA appears to be sensitive and specific in differentiating NA from its mimics of urothelial and prostatic origins.

Category: Genitourinary (including renal tumors)

[1033] Histopathological Tumor Features Associated with Her2 Amplified Urothelial Bladder Cancers

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- **Background**: Approximately 10% of invasive urothelial bladder cancers (UBC) are Her2 amplified. Anti-Her2 targeted therapies might become an option for these UBC, however, little is known about their morphological features.
- **Design:** A cohort of 150 patients with UBC was evaluated by fluorescence in situ hybridization (FISH) for Her2 amplification. The histopathological features of the 13 amplified primary tumors (8.6%) were compared with 13 matched non-amplified primary tumors.
- **Results:** Urothelial carcinomas with Her2 amplification presented with a broader variety of histomorphological subtypes than the control group, showing particularly a high frequency of micropapillary tumor components (10/13 patients). In contrast, the Her2 non-amplified tumors mainly presented the classical solid type of urothelial carcinoma and apart from two cases with sarcomatoid growth patterns, did not show a particular trend towards growth of any morphological subtype. In addition, Her2 amplified carcinomas demonstrated a high degree of intratumoral chronic inflammation while peritumoral inflammation was low.
- **Conclusions:** UBC with Her2-amplification are morphologically heterogeneous, frequently demonstrate micropapillary growth and show strong intratumoral and week peritumoral chronic inflammation. These features may help pathologists to identify UBC harboring Her2 amplification.

Category: Genitourinary (including renal tumors)

[1071] CD44 Full-Thickness Immunoreactivity Is More Sensitive Than CK5/6 for the Diagnosis of Flat Urothelial Lesions with Atypia

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- **Background:** Flat urothelial lesions with atypia can pose a diagnostic dilemma when attempting to make the distinction between reactive urothelial atypia (RUA) and carcinoma in situ (CIS). A recent study suggested that CK5/6 may be a useful biomarker to help in this differential diagnosis. The aim of this study is to determine the diagnostic utility of CK5/6 in comparison to CD44 immunostain in the evaluation of flat urothelial lesions with atypia, since both immunostains demonstrate similar immunoreactive patterns.
- **Design:** Thirty-seven transurethral resection of bladder (TURB) biopsies were evaluated. Twenty-eight (76%) cases comprised RUA with benign clinical follow-up and 9 (24%) CIS cases with classic histomorphologic features. All cases were evaluated by immunohistochemistry for CK5/6 (DAKO, RTU) and CD44 (DAKO, 1:25) using the LSAB method. Intensity and staining patterns were determined for each marker. Sensitivity and specificity for the diagnosis of RUA was determined.
- **Results:** Full-thickness staining for CK5/6 was observed in 20 RUA cases. Negative or weak basal staining for CK5/6 was observed in 8 RUA cases and in all 9 CIS cases. Full-thickness staining for CD44 was observed in all 28 RUA cases and in 1 case of CIS. Negative or weak basal staining for CD44 was observed in 8 CIS cases. Sensitivity and specificity for the diagnosis of RUA were 71% and 100% for CK5/6 immunostain, and 100% and 89% for CD44, respectively.
- **Conclusions:** Full-thickness staining for CD44 is more sensitive than full-thickness staining for CK5/6 for the diagnosis of RUA. CK5/6 does not add diagnostic value in this setting, and therefore should not be used as a substitute for CD44 in the traditional triple stain panel (CD44, CK20 and p53) employed in the differential diagnoses of flat urothelial lesions with atypia.

Category: Genitourinary (including renal tumors)

[1079] Clear Cell Renal Cell Carcinomas That Respond to Tyrosine Kinase Inhibitor Sunitinib Have Distinct microRNA Expression Patterns from Non-Responders

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- **Background:** The tyrosine-kinase inhibitor sunitinib has emerged as one of the standards of care for metastatic clear cell renal cell carcinoma (mCCRCC). However, lack of therapeutic benefit and significant toxicity are seen in a significant proportion of patients receiving the treatment. Therefore, identification of biomarkers that predict clinical benefit is critical. Recent studies have shown that microRNAs (miRNAs) play critical roles in the development and metastasis of human cancers. We aimed to study whether miRNA expression patterns may predict response to sunitinib in patients with mCCRCC.
- **Design:** Twenty-three patients with mCCRCC were treated with sunitinib and categorized as "responders" (duration of disease control on sunitinib > 12 months) and "non-responders" (lack of any disease control on sunitinib). Whole cell RNA was extracted from formalin-fixed paraffin-embedded (FFPE) pre-treatment nephrectomy specimens. Using high-throughput real-time PCR-based miRNA microarrays, the expression profiles of 768 miRNAs were compared in these two groups.
- **Results:** The expression levels of 12 (1.6%) miRNAs were significantly different between the responders and non-responders ($p<0.05$). The fold change was greater than 2 for 7 miRNAs. MiRNAs miR-187, miR-191*, miR-302c, miR-632, miR-19a* and miR-1257 were significantly overexpressed in sunitinib responders compared with non-responders and the fold change was 2.1, 2.3, 1.9, 1.9, 1.5 and 1.8, respectively (p values all <0.05). MiRNAs miR-9, miR-138, miR-9*, miR-376a*, miR-144* and miR-223* were significantly down-regulated in sunitinib responders, and the fold change was 0.20, 0.23, 0.21, 0.53, 0.49 and 0.44, respectively (p values all <0.036).
- **Conclusions:** By whole genome miRNA screening, 12 miRNAs were found to have differential expression patterns between CCRCC that responded to sunitinib treatment and non-responders. Several of these miRNAs were reported in the literature to affect the maturation of immune regulatory bone marrow derived dendritic cells (miR-223) and hypoxia inducible pathways (miR-138). These findings suggest that miRNAs could be informative biomarkers for predicting response to tyrosine kinase inhibitors in patients with mCCRCC.

  Category: Genitourinary (including renal tumors)

RIÑÓN

[827] Expression of Parafibromin in Renal Tumors and Its Potential Correlation with Tumor Prognosis

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- **Background:** Parafibromin, encoded by HRPT2 gene, is a recently identified tumor suppressor. Complete and partial loss of parafibromin expression has been found in the parathyroid carcinoma, the hyperparathyroidism-jaw tumor (HPT-JT), lung carcinoma and breast carcinoma. So far, little has been known about its role in renal tumors. Parafibromin expression has only been examined in a small series of chromphobe renal cell carcinomas and oncocytomas of kidney. In this study, we report the expression of parafibromin in a large series of renal tumors, including clear cell renal cell carcinoma (RCC), papillary RCC, chromophobe RCC and oncocytoma, using tissue microarrays (TMA).
- **Design:** 19 cases of renal oncocytoma, 23 cases of chromophobe RCC, 37 cases of papillary RCC, and 61 cases of clear cell RCC, from 1991 to 2009, were retrieved from the pathology archives of the Hospital of the University of Pennsylvania and used for the construction of multiple renal tumor TMAs. The constructed renal tumor TMAs were sectioned, incubated with primary and secondary antibodies and visualized with the avidin-biotin-peroxidase complex method. The staining was classified into 3 patterns according to Gill et al. Diffuse staining in over 95% nuclei with strong intensity was considered "diffusely strong positive." No nuclear staining in all (>99%) of tumor tissue was defined as "Negative staining". All other staining patterns were "Weak staining".
- **Results:** All of the 19 cases of oncocytoma showed strong nuclear staining (100%). 12 of the 23 chromophobe RCC showed positive nuclear staining (52%); 11 were negative. Among the 37 cases of papillary RCC, 15 are type 1 papillary RCC, 1 of them was positive for parafibromin stain; 22 are type 2 papillary RCC, 6 of them are positive for parafibromin stain; the overall positive cases are 7 (19%). Out of the 61 clear cell RCC cases, only 3 of them were positive for parafibromin stain (5%).
- **Conclusions:** Parafibromin expression varies significantly among the different types of renal tumors. Parafibromin may be a helpful marker in the differential diagnosis of renal tumors. Similar to parathyroid carcinomas, parafibromin appears to have tumor suppressor functions in renal carcinomas. Preserved expression of parafibromin is associated with benign tumors in the spectrum- oncocytoma; whereas loss of its expression is associated with malignant tumors bearing more adverse prognosis. These findings may indicate a potential role of parafibromin as a prognostic marker in renal tumors.

  Category: Genitourinary (including renal tumors)

[843] Concordance of *TMPRSS2-ERG* Fusion Status by Quantitative PCR with ERG Protein Expression by Immunohistochemistry Using Anti-ERG Monoclonal Antibody EPR3864

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- **Background:** *TMPRSS2-ERG*, the most common gene fusion in prostate cancer (PCA), is associated with expression of a truncated protein product of the oncogene ERG. We recently demonstrated that ERG detection by immunohistochemistry (IHC) in PCA was highly predictive of *ERG* rearrangement as assessed by FISH. The objective of the current study was to compare ERG IHC with *TMPRSS2-ERG* fusion mRNA expression by quantitative PCR in a large cohort of patients treated with radical prostatectomy (RP) for clinically localized PCA.
- **Design:** 187 RP specimens from patients with clinical stage T1/T2 PCA treated with RP between1987-2004 were included in the study. RNA was extracted from manually dissected formalin-fixed paraffin-embedded sections obtained from selected RP blocks and expression of *TMPRSS2-ERG*a and *TMPRSS2-ERG*b was quantified using RT-PCR. A prespecified cutpoint was used to classify samples as fusion positive or negative. The same RP blocks were used to build 10 Tissue Microarrays (TMA) composed of three representative 1.5 mm tissue cores from each tumor using the antibody EPR3864. Any nuclear staining was considered indicative of ERG expression. Endothelial cells were used as a positive control because they are strongly positive for ERG. To compare the accuracy of IHC ERG protein detection in determining the *TMPRSS2-ERG* fusion status with assessments by RT-PCR (standard reference), sensitivity, specificity, positive (PPV) and negative (NPV) predictive values, with corresponding 95% confidence intervals (CI) were calculated.
- **Results:** *TMPRSS2-ERG*a and/or *TMPRSS2-ERG*b fusions were present in 110 (59%) analyzed tumors by RT-PCR. ERG IHC was detected in 112 (60%) tumors, 106 (95%) of which were positive for fusion by RT-PCR. We identified 6/112 (5%) tumors demonstrating ERG protein expression without any of the *TMPRSS2-ERG* fusions as assessed by RT-PCR. Conversely, 4/110 (4%) cases with *TMPRSS2-ERG* fusions by RT-PCR had no detectable ERG protein expression in any of the informative cores. ERG IHC was highly concordant with the *TMPRSS2-ERG* RT-PCR, with a sensitivity of 96% (95% CI: 91%-99%), specificity 92% (84%-99%), PPV 95% (89%-98%), and NPV 95% (87%-99%).
- **Conclusions:** ERG detection by IHC showed a high concordance with *TMPRSS2-ERG* mRNA overexpression measured by RT-PCR in a large cohort of RP patients. The consistency of these results supports the utility of both methods for assessing *TMPRSS2-ERG* fusion status. IHC may be a useful adjunctive tool since it is easier to perform and less costly.

Category: Genitourinary (including renal tumors)

[914] Prognostic Relevance of mTORC1 Pathway Components in Papillary Renal Cell Carcinoma

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- **Background:** The mammalian target of rapamycin complex 1 (mTORC1) pathway is dysregulated in many human cancers and agents targeting the mTORC1 are being clinically used. mTORC1 pathway interacts with effectors of cell cycle progression and ultimately regulates protein translation and cell proliferation. We undertook this study to ascertain if Papillary Renal Cell Carcinoma (PRCC) demonstrates significant expression of mTORC1 pathway components and if the expression of activated mTORC1 has any prognostic significance.
- **Design:** Standard immunohistochemical analysis was performed for p-S6, p-mTOR, p-4EBP1, HIF-1a, p-AKT, PI3K, PTEN on sections of tissue microarrays constructed from 76 primary PRCC treated at our hospital with nephrectomy (1998-2008). Duplicate 1.0 mm cores of representative tumor were obtained from each case to construct the tissue microarrays. Cytoplasmic expression was assessed for each marker as the percentage of positive cells (0-3) and intensity of staining (0-3). A final Histo-score was calculated as the product of intensity and percentage and correlated with clinic-pathologic parameters using T-test, Fisher's exact test, Pearson's correlation, or log-rank (Mantel-Cox) test.
- **Results:** In our cohort, M:F ratio was 3.00 and mean age at diagnosis was 58.67 years. Mean tumor size was 5.08 cm. Fuhrman's nuclear grade was G1 in 7, G2 in 41, G3 in 25, and G4 in 3 cases. 9 (11.84%) patients had high pathologic stage (pT3-4), 10 (13.16%) developed subsequent metastases/ recurrence, and 5 (6.58%) died of the disease. Compared to normal proximal renal tubules, we found increased expression of p-S6 in 14 (18.4%) tumors, p-mTOR in 70 (92.1%), p-4EBP1 in 43 (56.6%), HIF-1a in 34 (44.7%), PI3K in 40 (52.6%), and p-AKT in 41 (53.9%). PTEN expression was reduced in 40 (52.6%) tumors. Increased expression of p-S6 was significantly associated with higher tumor grade (P=0.03). Higher grade tumors also showed reduced expression of HIF-1α (P=0.01) and increased expression of PTEN (P=0.00). There was a trend for tumors with metastases/ recurrence to show higher expression p-S6 (P=0.08). Increased expression of p-S6 also correlated with poor patient survival (P=0.01).
- **Conclusions:** In this current work in clinically annotated PRCC where the patients had received no previous treatment, we found that increased expression of p-S6 correlates with higher grade, metastases, and poor patient outcome. Our results support consideration of therapeutically targeting mTORC1 pathway in PRCC.

Category: Genitourinary (including renal tumors)

[944] Macrophage Related Markers Expression in MITF/TFE Family Renal Translocation Carcinoma, Melanotic Xp11 Translocation Renal Cancer and Pure Epithelioid PEComa (so Called Epithelioid Angiomyolipoma) of the Kidney

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- **Background:** Cathepsin K is a lysosomal protease recently described in MITF/TFE family renal translocation carcinomas (tRCC) and angiomyolipoma of the kidney both classic and epithelioid (pure epithelioid PEComa (PEP)). Both tRCC and angiomyolipoma are neoplasms expressing MITF/TFE family transcription factors. Moreover t(6;11) TFEB+ tRCC and PEP constantly immunostain for the melanogenesis markers HMB45 and MART1 such as melanotic Xp11 tRCC. These three tumors can show overlapping morphological and immunohistochemical features and their differential diagnosis can be challenging. In this study we investigated the expression of macrophage markers (CD68-PGM1, CD68-KP1, cathepsin K, CD163) in a series of tRCC, PEP and melanotic Xp11 tRCC, in order to assess their utility to distinguish them.
- **Design:** We studied the immunohistochemical expression of CD68-PGM1, CD68-KP1, cathepsin K and CD163 in 21 tRCC (including 9 PRCC TFE3+ tRCC and 12 t(6;11) TFEB+ tRCC), 5 melanotic Xp11 tRCC and 13 PEP.
- **Results:** All PEP, all t(6;11) TFEB+ tRCC, and all but two, PRCC TFE3+ tRCC express strongly and diffusely cathepsin K whereas the 5 melanotic Xp11 tRCC were weakly positive. CD163 resulted positive in 3 out of 7 PEP and in none of 4 t(6;11) TFEB+ tRCC, 4 PRCC TFE3+ tRCC and 1 melanotic Xp11 tRCC. CD68-KP1 was expressed in all 22 tested tumors (13 PEP, 4 t(6;11) TFEB+ RCC, 1 melanotic Xp11 tRCC, 4 PRCC TFE3+ tRCC) whereas CD68-PGM1 immunostained all 13 PEP, but none of the other neoplasms.
- **Conclusions:** 1) Cathepsin K is consistently expressed in PRCC TFE3+ tRCC, t(6;11) TFEB+ tRCC, melanotic Xp11 tRCC and PEP; 2) CD68-PGM1 is a useful tool for the differential diagnosis between PEP and all the other tRCC, including the HMB45 positive t(6;11) TFEB+ tRCC; 3) the CD68-PGM1 negativity in melanotic Xp11 tRCC seems to relate this tumor more closely to tRCC rather than PEP.

  Category: Genitourinary (including renal tumors)

[957] Expression of Novel Renal Tubular Associated Markers in Nephrogenic Adenoma (NA) of the Urothelial Tract: Potential Utility in Distinction from Its Malignant Mimics

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- **Background:** NA of the bladder, urethra and renal pelvis is believed to represent renal tubular satellites/outposts in response to injury or metaplastic response to urothelial injury. Lesions have a range of histologic patterns, form a mass lesion, may have an infiltrative growth and morphologically mimic malignant lesions such as clear cell carcinoma of the bladder (CCC), urothelial carcinoma with glandular differentiation or nested pattern (UCa) and prostatic adenocarcinoma (PCa). This study aims at analyzing the utility of novel renal tubule-associated markers in differentiating NA from its malignant mimics.
- **Design:** hKIM-1, Pax2, Pax8, racemase and S100A1 are renal tubule associated markers. The entire renal tubular system including the Bowman's capsule expresses Pax2, Pax8 and S100A1. hKIM-1 is expressed in injured proximal renal tubules and racemase is expressed primarily in proximal convoluted tubules. We analyzed the utility of a panel of immunohistochemical (IHC) markers [renal-associated markers - hKIM-1, Pax2, Pax8, racemase, S100A1; urothelial-associated marker S100p and MIB-1 (Ki67)] in the distinction of NA (n=39) from its histologic mimics: CCC (n=4), UCa (n=6) and PCa (n=14). The staining results were recorded in a semiquantitative fashion (0: negative, 1+: 1-25, 2+: 26-50, 3+: 51-100% and intensity as weak, moderate and strong). A MIB-1 (Ki-67) proliferation index (% positive in 100 cells counted) was ascertained for each case.
- **Results:** IHC results are summarized in the table expressed as percentage positive.

  13% of NA negative or weak for Pax2, Pax8 and racemase were strongly positive for hKIM-1. The range of MIB-1 proliferation index for NA, CCC, PCa, UCa was 1-4%, 20-50%, 5-30% and 10-40%, respectively.

Categories	hKIM-1	Pax-2	Pax-8	Racemase	S100A1	S-100p	Mean Ki-67
NA	67	87	74	59	95	0	1
CCC	0	25	0	50	50 (weak)	100 (usually 1+)	25
PCa	0	0	0	100	0	0	7
UCa	0	0	0	33 (weak)	0	0	25

- **Conclusions:** The consistent IHC expression of renal tubular markers supports morphologic observations and cytogenetic data that NA of the urothelial tract is of renal tubular origin. Depending on the histologic pattern of NA, a judicious IHC panel of hKIM-1, Pax2, S100A1 (supporting NA), S100p (supporting UCa), PSA and PSMA (supporting PCa) and high MIB-1 proliferation (supporting CCC and UCa), has diagnostic utility in the distinction of NA from its malignant mimics.

  Category: Genitourinary (including renal tumors)

[1053] Unclassified Renal Cell Carcinoma and Invasive High Grade Urothelial Carcinoma: Is the Distinction Possible by Immunohistochemistry and Clinically Important?

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- **Background:** The differential diagnosis for a poorly differentiated carcinoma in the kidney includes renal cell carcinoma (RCC) and urothelial carcinoma (UC). Such distinction is presumed to be clinically important as the chemotherapeutic and surgical regimens for them are different. This study aimed to investigate: 1) whether a panel of immunostain markers could differentiate between poorly differentiated RCC and UC, and 2) whether the distinction between the two is clinically important?
- **Design:** A tissue microarray was constructed to include 11 unclassifiable RCC (including 3 with features of collecting duct RCC), 2 renal UC and 16 invasive high grade pelvic UC and was stained with a panel of markers (PAX-8, K903, p63, CK7, CK20). Clinical information was obtained by chart review and patient contact.
- **Results:** The mean age of RCC and UC patients was 60.3 (range 45-81) years and 70.1 (range 54-95) years. The male/female ratio was 9/2 for RCC and 13/5 for UC patients. PAX-8, K903, p63, CK7 and CK20 were positive in 10, 0, 0, 2 and 2 of 11 RCC, and 3, 9 18, 17 and 7 UC, respectively. Therefore, a PAX-8 positive/K903 negative/p63 negative phenotype identified 10/11 (90.9%) RCC, and a PAX-8 negative/K903 or p63 positive phenotype identified 15/18 (83.3%) UC. The follow-up for RCC patients was an average of 11 months, and in UC patients was an average of 10.4 months. 9/11 (81.9%) RCC patients and 13/18. (72.2%) UC patients died of disease, which was not found to be statistically significant (p=0.55).
- **Conclusions:** Immunostains using a panel of markers (PAX-8, K903 and p63) could reliably distinguish poorly differentiated RCC and invasive high grade UC. However, all these patients had extremely poor prognosis; therefore, the distinction between the two tumors seem to be of limited clinical prognostic significance.

  Category: Genitourinary (including renal tumors)

TESTÍCULO

[962] CDX2 Is Superior to Alpha-Fetoprotein in Yolk Sac Tumors (YST) Both in Adult and Pediatric Patients: Study with Emphasis on Morphologic Patterns

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- **Background:** YST can be diagnostically challenging due to its diverse morphologic patterns. Alpha-fetoprotein (AFP), a traditional YST marker, has also some limitations. CDX2, an antibody often used in intestinal adenocarcinomas, has recently been reported to be positive in adult testicular and ovarian YST, but it has not been studied in pediatric YST. We studied CDX2 expression both in adult testicular and pediatric YST.
- **Design:** We compared the expression of CDX2 and AFP in 28 GCT: 22 adult testicular YST and 6 pure pediatric testicular (1) and extratesticular (5) YST. In adults, YST component ranged from 10% to 100%. The YST patterns were as follows: microcystic (75%), glandular (39%), myxomatous (36%), papillary (32%), macrocystic (25%), endodermal sinus (21%), solid (21%),and polyvesicular vittelline (11%). We used as negative controls, the non-YST components of mixed GCT and 6 additional pure GCT (4 seminoma, 2 embryonal), all confirmed by positive OCT 3/4. Extent of staining was scored as 0 (<5%), 1+ (5%-10%), 2+ (11%-50%) and 3+ (>50%). Staining intensity was graded from 0-3+.
- **Results:** CDX2 was positive in 100% (6/6) of pediatric YST: No cases 0 or 1+, 3 cases each with 2+ and 3+. Mean intensity was 3. AFP stained 83% (5/6) pediatric YST: 1 with 0, 1 with 1+, 3 with 2+ and 1 with 3+. Mean intensity was 2.6. In adults, 91% (20/22), expressed CDX2: 2 (9%) with 0+, 4 (18%) with 1+, 8 (36%) with 2+ and 8 (36%) with 3+ staining. The mean intensity of CDX2 staining was 2.5. In adult YST, AFP stained 95% (21/22) cases: 1 (5%) with 0+, 6 (27%) with 1+, 11 (50%) with 2+ and 4 (18%) with 3+ staining. The mean intensity of AFP staining in the adult YST component was 2.5. Regarding the expression of CDX2 vs AFP in different YST patterns, CDX2 was superior in myxomatous (70% vs 0%), papillary (89% vs 55%) and macrocystic (71% vs 14%) patterns. Both antibodies were comparable in microcystic (90% vs 95%), endodermal sinus (66% vs 66%), polyvesicular (66% vs 33%) and solid (66% vs 66%) patterns. Seminomas and embryonal carcinomas were uniformly negative for both antibodies. CDX2 was expressed only in intestinal component of teratomas.
- **Conclusions:** We found that CDX2 is an excellent marker for pediatric YST, which was not reported previously. We also confirmed that CDX2 is a useful marker identifying YST component in adult GCT. CDX2 is superior to AFP in detecting the commonly overlooked YST patterns such as myxomatous papillary and macrocystic. In contrast to AFP, CDX2 has distinct nuclear expression without background staining.

  Category: Genitourinary (including renal tumors)