508 LYMPHATIC DRAINAGE OF RENAL CELL CANCER (RCC) IN VIVO. AN INTERIM ANALYSIS OF A PROSPECTIVE TRIAL TO IDENTIFY SITE AND DISTRIBUTION OF THE SENTINEL NODE

Bex A.[1], Vermeeren L.[2], Meinhardt W.[1], Prevoo W.[3], Horenblas S.[1], Valdes Olmos R.[2]
[1]The Netherlands Cancer Institute, Dept. of Urology, Amsterdam, The Netherlands, [2]The Netherlands Cancer Institute, Dept. of Nuclear Medicine, Amsterdam, The Netherlands, [3]The Netherlands Cancer Institute, Dept. of Radiology, Amsterdam, The Netherlands

Introduction & Objectives: The lymphatic drainage of RCC is unpredictable and the role of lymph node dissection (LND) in RCC remains controversial despite a randomized study with a median follow up of 12.6 years. In addition, the introduction of targeted agents has revived interest in adjuvant treatment concepts. Accurate LN staging is warranted to determine the risk of recurrence or progression. Here we apply single-photon emission computed tomography (SPECT) in combination with computed tomography (CT) for preoperative identification of sentinel lymph nodes (SNs) and to investigate distribution and site of SN as well as surgical safety of intraoperative sampling.
Materials & Methods: An interim analysis of 21 patients from an ongoing prospective trial to investigate site and distribution of SN in RCC (NL26406.031.08). Patients underwent injection of 99mTc-nanocolloid into the renal tumour for preoperative identification of SN with SPECT/CTand subsequent removal of the tumour and intraoperative sampling using a gamma probe and portable camera. Lymphadenectomy was completed locoregionally.
Results: SPECT/CT detected SN in 15/21 patients (71%), including 4 patients with non-visualisation on planar lymphoscintigraphy. Twenty-seven SN were seen; 17 para-aortic(including interaorto-caval), 4 retrocaval, 2 hilar, 1 celiac trunc,1 internal mammary and 2 mediastinal and pleural. These latter 4 nodes were not harvested according to protocol. All other SN, except for 2 weakly radioactive interaorto-caval nodes, were identified and excised with a mean additional time of 20 minutes. None of the removed SN and locoregional nodes was tumor-bearing.
Conclusions: SN from RCC are mainly localized in the para-aortic region, but aberrant supradiaphragmal nodes receive direct drainage. Intraoperative SN identification and sampling in RCC with preoperative detection on SPECT/CT is surgically safe and feasible. Non visualisation of SN appears in almost a third of the patients. Further studies are required to demonstrate if accurate mapping of lymphatic drainage and extent of lymphatic spread may have diagnostic and therapeutic implications.

509 PROGNOSTIC ROLE OF HISTOLOGIC SUBTYPE IN RENAL CELL CARCINOMA: RESULTS OF THE SATURN PROJECT

Novara G.[1], Antonelli A.[2], Bertini R.[3], Carini M.[4], Cosciani Cunico S.[2], Gontero P.[5], Longo N.[6], Martignoni G.[7], Martorana G.[8], Minervini A.[4], Mirone V.[6], Simionato A.[9], Siracusano S.[10], Volpe A.[11], Zattoni F.[1], Ficarra V.[1]
[1]University of Padua, Dept. of Urology, Padua, Italy, [2]University of Brescia, Dept. of Urology, Brescia, Italy, [3]Vita-Salute University San Raffaele, Dept. of Urology, Milan, Italy, [4]University of Florence, Dept. of Urology, Florence, Italy, [5]University of Turin, Dept. of Urology, Turin, Italy, [6]University Federico II, Dept. of Urology, Naples, Italy, [7]University of Verona, Dept. of Urology, Verona, Italy, [8]University of Bologna, Dept. of Urology, Bologna, Italy, [9]University of Genoa, Dept. of Urology, Genoa, Italy, [10]University of Trieste, Dept. of Urology, Trieste, Italy, [11]University of Eastern Piedmont, Dept. of Urology, Novara, Italy

Introduction & Objectives: To evaluate the prognostic role of histologic in a large multi-institutional series of patients undergoing radical or partial nephrectomy for renal cell carcinoma (RCC).
Materials & Methods: We collected retrospectively the data of 5378 patients who were surgically treated for RCC in 16 academic centers involved in the Surveillance And Treatment Update Renal Neoplasms (SATURN) project.
Results: 4371 (81%) patients had clear cell RCC (ccRCC), 579 (11%) papillary RCC (pRCC), 291 (6%) chromophobe RCC (chRCC), 47 (1%) collecting duct RCC (cdRCC) , and 90 (2%) unclassified RCC (uRCC). At a median follow-up of 42 months (IQR 24–75), 1055 patients (20%) had developed disease recurrence and 786 (15%) were dead of RCC. 5-year cancer-specific survival (CSS) estimates were 78.1% in ccRCC, 85.6% in pRCC, 89.3% in chRCC, 55.6% in uRCC, and 31.9% in cdRCC, respectively (pooled p value <0.0001). All the survival differences among the different subtypes were statistically significant (pairwise p values <0.02). On multivariable Cox regression analyses, histological subtype was an independent predictor of CSS (p <0.0001), once adjusted for the effect of all the other covariates. Specifically, both pRCC (H.R. 0.7; p=0.022), and chRCC (H.R. 0.6; p=0.047) had higher CSS compared with ccRCC. Conversely, both cdRCC (H.R. 2.4; p=0.001) and uRCC (H.R. 1.7; p=0.007) had significantly worse outcome in comparisons with clear cell RCC.
Conclusions: Histological subtypes was an independent predictors of CSS. For the first time, time significant survival differences were demonstrated among all the major subtypes of RCC.

Poster Session 43
PROSTATE CANCER: NEW MOLECULAR AND GENETIC BIOMARKERS
Sunday, 20 March, 12.15-13.45, Hall I/K

510 QUANTIFICATION OF EPIGENETIC ALTERATIONS IN PROSTATE CANCER

Stadler T.C.[1], Jung A.[2], Schlenker B.[1], Boulesteix A.L.[3], Bernau C.[3], Buchner A.[1], Kirchner T.[2], Stief C.G.[1], Bastian P.J.[1]
[1]Ludwig-Maximilians-University Munich, University Hospital Grosshadern, Dept. of Urology, Munich, Germany, [2]Ludwig-Maximilians-University Munich, Dept. of Pathology, Munich, Germany, [3]Ludwig-Maximilians-University Munich, Dept. of Medical Informatics, Biometry and Epidemiology, Munich, Germany

Introduction & Objectives: DNA methylation is an essential epigenetic modification in carcinogenesis. In this study, methylation patterns in promotor-/exon-1-areas of selected genes were analyzed and quantified with the pyrosequencing technique to evaluate their applicability as potential diagnostic markers in prostate cancer.
Materials & Methods: We analyzed 71 prostate cancer samples (Gleason grade 6-9) from prostatectomy specimens and compared them to normal prostatic tissue from the same patients. Additionally, cell lines LNCaP, PC-3 and DU-145 were used as system controls. After DNA isolation from paraffin sections applying QiaAMP FFPE micro kits and bisulfite conversion using EZ Amp Gold kits we performed pyrosequencing to evaluate CpG-island hypermethylation in 10 selected gene loci (APC, DAPK, Endoglin, GADD45a, GSTP1, p14, RASSF1A, RUNX3, 14-3-3 Sigma and TNFRSF10C).
Results: Successful DNA isolation, bisulfite conversion and pyrosequencing could be performed in all samples. On the basis of quantitative distinctions in promotor-/exon-1-CpG-island methylation the following genes allowed significant determination of benigne and malignant tissue: GSTP1 (p<0.01), APC (p<0.01), RASSF1a (p<0.01), TNFRSF10C (p<0.01). Gene loci APC, RASSF1a and Endoglin showed slight associations between methylation patterns and disease recurrence.
Conclusions: Pyrosequencing is the ideal procedure for the quantitative evaluation of epigenetic alterations in normal and malignant prostatic tissue. Our data suggest that gene promotor methylation patterns in GSTP1, APC, RASSF1a and TNFRSF10C may facilitate the determination of transformed and normal prostatic tissues. Methylation status in specific promotor-/exon-1-areas (APC, RASSF1a, Endoglin) might be even of prognostic value.

511 EPIGENETIC SILENCING OF RASSF10 IN PROSTATE CANCER AND ITS FUNCTIONAL CONSEQUENCES

Dansranjav T.[1], Wagenlehner F.[1], Steger K.[1], Sturm K.[2], Weidner W.[1], Dammann R.H.[3], Schagdarsurengin U.[1]
[1]University Clinic Giessen and Marburg, Dept. of Urology and Pediatric Urology, Giessen, Germany, [2]University Clinic Giessen and Marburg, Dept. of Pathology, Giessen, Germany, [3]Justus-Liebig University, Institute of Genetics, Giessen, Germany

Introduction & Objectives: Several members of Ras association domain family (RASSF) are frequently silenced in human cancer and supposed to be tumor suppressors. The objective of this work was to characterize functional significance of epigenetic inactivation of RASSF10, a newly identified member of RASSF, in prostate carcinoma (PCa).
Materials & Methods: To evaluate the role of DNA-methylation in silencing of RASSF10, three PCa cell lines (DU145, 22RV1, LNCaP), 83 PCa primary tumor samples (51 pT1-2, 32 pT3-4) and 52 corresponding non-tumorous samples were subjected to RASSF10 bisulfite pyrosequencing. Furthermore, we analyzed the effect of 5-aza-2-deoxycytidine (5-aza-dC) and trichostatin A (TSA) on RASSF10 mRNA expression in PCa cell lines. To investigate the functional role of RASSF10 in PCa malignancy, we introduced pCMV-Tag1 plasmid containing full-length RASSF10 cDNA into LNCaP cells lacking endogenous RASSF10 expression. The effect of RASSF10 overexpression was examined by colony formation test and apoptosis assay (TUNEL).
Results: Among cell lines, LNCaP exhibited the highest RASSF10 methylation grade (median=77%) followed by 22RV1 (20.7%) and DU145 cells (6.14%). A weak expression of RASSF10 was detected in DU145 cells and a complete silencing in 22RV1 and LNCaP. A significant increase of RASSF10 expression was induced by TSA in all three PCa cell lines and additional gain of expression with the combination of 5-aza-dC/ TSA. Comparing to LNCaP cells baring empty vector, the RASSF10 transfected cells formed significantly fewer colonies (101.6±13 vs. 56±15.5, p=0.0018) and showed more than two fold increase of apoptosis rate. Among matched malignant and non-malignant prostate tissue, significantly more tumor samples (11) showed increased RASSF10 methylation then non-tumoral tissue (3) (21.1% vs. 5.8%, p=0.041). Hypermethylation of RASSF10 correlated with higher Gleason score (≥7 vs. <7; 30.4% vs. 10%, p=0.039) and with advanced tumor stages (pT3-4 vs. pT1-2; 28.1 % vs. 7.8%, p=0.027). The methylation rate of RASSF10 was significantly higher in elderly patients (age of onset 68-78 years, n=48) compared to younger patients (age of onset 52-67 years, n=35) (22 9% vs. 5.7 %, p=0.037).