(range 48–64), transitional cell carcinoma histologically confirmed, stage T3/T4, performance status ECOG 0–2. A chemo-radiotherapy regimen consisting of weekly gemcitabine (350 mg/m^2) with 45 Gy of external beam radiotherapy (1.8 Gy/fraction, 5 days/week) was delivered in five weeks on extended fields as appropriate and a boost on the bladder to a median total dose of 65 Gy. Patients were evaluated 4–6 weeks after combined treatment with cystoscopy and CT scans.
Results: 23 patients completed chemo-radiotherapy schedule. 4 patients interrupted the treatment, 3 because of grade 4 toxicity and 1 because of progressive disease. Clinical benefit was found in 19 of the 23 patients (7 complete response, 11 partial response and 1 stable disease). Progressive disease was found in the four remained patients. Adverse effects, especially haematological, were common but manageable. No chemoradiation-associated deaths were observed with this gemcitabine based regimen. Grade 3–4 haematological toxicity (neutropenia and/or trombocitopenia) occurred in 7 and 4 patients respectively. Grade 3 gastrointestinal toxicity (diarrhoea) occurred in 8 patients. Grade 3 cystitis occurred in 6 patients. Median follow-up period was 18 months; at this time 20 patients are still alive and 17 patients remain disease-free.
Conclusions: This schedule of Gemcitabine and radiation therapy is relatively well tolerated and has shown to provide prolonged clinical benefit response and disease stabilization in patients with locally advanced bladder carcinoma. These promising results should be further investigated.

876 POSTER

Combined treatment with Bicalutamide and ZD-1839 may be advisable in human prostate cancer in the early phases

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Background: Combined treatment with Bicalutamide (Casodex) and ZD-1839 (Gefitinib, Iressa) in human Prostate Cancer (PCa) cell lines proved to be effective and hyperadditive (Festuccia et al., Int. J. Cancer, 2005). Relapses after androgen withdrawal in PCa are a significant cause of morbidity and mortality and pose the question of the ideal initial treatment of this very prevalent tumor.
Material and methods: We analyzed by immuno-histochemistry the expression of EGF receptor (EGFR), Erb-B2 (Her2) and PTEN (a tumor-suppressor) in a 50 patient cohort with localized tumors, treated by radical prostatectomy. Among these patients, 21 (group 1) received prostatectomy as initial treatment, whereas the other 29 (group 2) received neo-adjuvant androgen-ablation therapy for 3–6 months based on Casodex (150 mg/die) treatment before surgery. We also obtained primary cultures from 37/50 cases (17 of group 1 and 20 of group 2 patients) to test the Gefitinib antiproliferative/pro-apoptotic effects alone or in combination with Casodex.
Results: We observed a significant increase of EGFR and Her2 in tissues from group 2 patients. This indicates that EGFR/Her2 expression can be regulated in vivo by antiandrogens, as previously observed in cell lines. PTEN expression was lost after Casodex therapy. All PCa primary cultures were sensitive to both Gefitinib and Casodex (IC50 0.2 to 2.0 mM and 0.7 to >4.0 mM, respectively). We observed no differences between the IC50 values calculated in the two groups for Gefitinib indicating that increased EGFR expression was not a pre-requisite for effectiveness. In addition, Gefitinib (0.1 mM) increased the anti-tumour effects of Casodex of 10 fold and, Casodex (0.5 mM) increased the effects of Gefitinib of 2.5-fold. However correlating the IC50 values of single cases with the Her2 and PTEN expression we found that Her2 increase and PTEN decrease can be negative biomarkers of Gefitinib effectiveness.
Conclusions: Our findings favour the clinical development of combination therapies, by early association of Casodex and Gefitinib in newly diagnosed PCa patients by targeting simultaneously EGFR and AR in androgen-dependent/sensitive PCa, since the dual inhibition of AR and EGFR pathways could be useful in naive patients in order to extend the androgen-dependent phase and to delay the onset of EGFR-driven androgen-independence phase of PCa. 'Casodex', 'Gefitinib' and 'Iressa' are trademarks of the AstraZeneca group of companies.

877 POSTER

Apoptosis in urothelial bladder carcinomas and its relation to the expression of caspase 3 and apoptosis regulating proteins bax and bcl-2: prognostic implications

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Background: Apoptosis is the most significant component of programmed cell death that complements cell proliferation in maintaining normal tissue homeostasis. Bax protein accelerates apoptosis by antagonizing the apoptosis repressor bcl-2. Caspase 3 is the final step of the apoptosis-inducing protease pathway. We determined the association of apoptosis with the apoptosis related proteins bax, caspase 3 and bcl-2, as well as their interaction with prognosis in urothelial carcinoma (UC) of the urinary bladder.
Material and methods: Using immunochemistry we investigated the expression of bax, caspase-3 and bcl-2 in 88 primary UC bladder specimens. Apoptosis was detected by staining with a MoAb recognizing exposed single-stranded regions in the DNA of apoptotic cells (anti-ssDNA) and the apoptotic index (AI) was expressed as the percentage of the immunoreactive neoplastic nuclei. Kaplan-Meier survival curves were compared in order to define their possible prognostic role in disease-free survival (DFS).
Results: Positive staining for bax, caspase 3 and bcl-2 was noted in 50%, 90.8% and 55% of cases, respectively. Well differentiated UCs showed overexpression of bax and caspase 3 ($p < 0.05$), as well as a trend for strong expression of bcl-2 ($p > 0.05$). We noted a positive relation between bax and caspase 3 ($p < 0.05$), but no statistical association could be detected between the above proteins and bcl-2. AI increased with increasing grade and stage ($p < 0.05$), but was unrelated to the expression of the apoptosis related proteins. Log-rank test showed that high grade, T stage and increased AI had an adverse impact on DFS ($p < 0.05$), while patients with bax overexpression exhibited significantly longer DFS times ($p < 0.001$).
Conclusions: Apoptosis increases along with progression of the neoplastic lesions of the bladder epithelium. Although bax and bcl-2 are strongly expressed in urothelial bladder carcinomas, they don't seem to be the major regulators of apoptotic activity. The lack of relation of caspase 3 expression with degree of apoptosis may be due to the inability of immunohistochemistry to discriminate between the active and inactive forms of caspase. The adverse prognostic role of apoptotic rate is possibly the result of the loss of normal mechanisms controlling cell death, facilitating the survival of cells with increased ability to resist in unfavorable growth conditions. With regard to disease-free survival, Bax protein emerges as a promising favorable indicator.

878 POSTER

The expression of pentaspan membrane glycoprotein Prominin-1/CD133 is not limited to prostatic stem cells and is down-regulated in prostate cancer

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Background: Prominin-1 (CD133) is known as a cell surface marker of neural and hematopoietic stem/progenitor cells. One report has shown that Prominin-1 carrying the AC133 epitope can also be used to identify the prostatic basal stem cells (Richardson et al., J. Cell Sci. 2004, 117:3539). Furthermore, the expression of Prominin-1 is up-regulated in malignant hematopoietic diseases as well as in certain types of solid tumors such as those derived from the brain and kidney, which prompted us to evaluate whether Prominin-1 can be used as a prognostic and/or predictive clinical marker of prostate cancer.
Methods: The expression of Prominin-1 in normal adult human prostate as well as in 25 prostate cancer samples was monitored by immunohisto-chemistry.
Results: The analysis of human prostate revealed hE2, but not AC133, immunoreactivity on the apical side of prostatic epithelial cells whereas the AC133 immunoreactivity is restricted to a small population of cells

distributed throughout acini. Interestingly, hE2 immunoreactivity is also associated with small particles found in the acinar lumen, which is in agreement with the presence of small membrane particles containing Prominin-1 in seminal plasma. The analysis of several prostate cancer samples revealed a down-regulation of the hE2 immunoreactivity in the tumor region, independent of their Gleason score (5–10). In those tissues however we found that the hE2 immunoreactivity is up-regulated in luminal cells in the vicinity of the tumor, especially in the areas of inflammation or intensive proliferation of basal cells.
Conclusions: These data showed that the overall expression of Prominin-1 in prostate is not limited to the basal stem cells as assumed from a previous study with mAb AC133, but only the Prominin-1 molecule carrying the AC133 epitope appears to label these stem cells. With regard to the prostate diseases, our pilot screen shows that Prominin-1, as detected by hE2 immunoreactivity, is down- and up-regulated in the tumor and inflammatory regions, respectively. Further studies are needed to determine the potential application of Prominin-1–containing particles as a novel biomarker for human prostate cancer.

879 POSTER

Value of the innovated agarose cell block technique in improving the diagnostic sensitivity of urine cytology in cancer bladder cases

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Background: Proper handling and processing of urine sample have great impact on improving diagnostic sensitivity. Agarose cell block (ACB) technique is an innovated technique by Mansy (2004) based on the use of melted agarose gel as an embedding media, for the processing of the sediment of urine sample in block manner.
Objective: The aim of this work is to investigate the validity of ACB technique in processing urine samples simultaneously for light and electron microscopic (EM) examination with the prospect to enhance the quality of diagnosis.
Material and methods: The material of this study consisted of 45 voided urine samples collected from 30 patients (Pt) with bladder carcinoma, 14 Pt with non specific cystitis and one Pt who underwent transurethral resection of the primary tumour (TUR-T) followed by adjuvant immunotherapy with BCG. The sediment of the collected urine from each case was processed for the performance of Papanicolaou (Pap) stained smear and the preparation of ACB. The solidified agarose block was divided longitudinally into two halves. One half was processed for paraffin, hematoxylin and eosin (H&E) stained sections and the other half for EM examination.
Results: Significant increase in the number of sedimented urothelial cells in the ACB paraffin prepared sections versus the corresponding Pap stained smear was noticed. Moreover, the diagnostic sensitivity of urine cytology was improved with the application of ACB technique. Out of the 30 malignant cases confirmed by histopathology of bladder biopsies (two grade I Ta, one grade I T1, two grade II Ta, five grade II T1, eleven grade II T2, two grade III T2, three grade III T3a, four grade III T3b), 70% were diagnosed by Pap stained urine smear versus 90% by ACB paraffin H&E stained sections and 100% by ACB EM processed samples. Furthermore, simultaneous processing of the same sample for light and EM examination allowed proper study, facilitated the discrimination between normal, dysplastic and malignant urothelial cells, the identification of type of malignancy and the accurate diagnosis of controversial cases specially those revealing severe urothelial dysplastic changes and patient under adjuvant immunotherapy after TUR-T.
Conclusion: Thus, ACB technique could be considered a useful technique which helps in increasing the sensitivity of urine cytology and opens a new prospect for cytomorphological study.

880 POSTER

Clonal origin of multifocal renal cell carcinoma as determined by microsatellite analysis

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Purpose: 3% of all carcinomas are renal cell carcinomas. The reported incidence of satellite tumor lesions in renal cell carcinoma (7% to 25%) suggests that there is a risk of local recurrence after nephron sparing surgery. It remains largely unknown whether small satellite tumors show malignant features and whether they are metastases from the primary tumor. Therefore, we determined the clonality of multifocal tumors by molecular genetic analysis.
Materials and methods: A total of 20 multifocal clear cell renal cell carcinomas were investigated by microsatellite analysis using 6 markers for chromosome 3p, namely D3S1560, D3S1289, D3S1766, D3S1300, D3S1566 and D3S1663. Polymerase chain reaction was performed according to standard protocols, followed by gel electrophoresis and automated analysis using an automated DNA sequencer (Li-Cor, Lincoln, Nebraska).
Results: All primary clear cell tumors were characterized by loss of heterozygosity on 3p. Multifocal tumors showed identical microsatellite alterations with at least 2 marker in all cases. 14 out of 20 matched completely in all 6 marker.
Conclusions: Identical loss of heterozygosity detected in different tumors in the same kidney strongly suggest that multifocal clear cell renal cell carcinomas have a common clonal origin in most cases. These findings indicate that satellite tumors are the result of intrarenal metastasis from the primary tumor. The clinical implications of these results need further investigation.

881 POSTER

The value of PTEN expression in smears of prostate cancer; correlation with prognostic factors and disease outcome

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The PTEN (phosphatase and tensin homolog deleted on chromosome 10) tumour suppressor gene is located on chromosome 10q23, a genomic region frequently lost in human cancers. Complete inactivation of the PTEN tumour suppressor gene is extremely common in advanced cancer, including prostate cancer (CaP). The aim of this study was to examine the expression of PTEN protein in prostate carcinoma cell samples and its association with clinicopathological parameters.
Materials and methods: eighty imprint smears were obtained at surgery and studied immunocytochemically using anti-PTEN antibody. Cases were considered positive when granular cytoplasmic staining was seen in all tumour cells, mixed when areas of both positive and negative tumour cell clones were seen, and negative when adjacent benign prostate tissue but not tumour tissue showed positive staining. The PTEN expression pattern was correlated with histopathological findings in the same samples. The results were correlated with postoperative Gleason score, preoperative Serum Prostate Antigen (PSA) and pathological stage.
Results: Thirteen smears (16.2%) of prostate cancer were positive, 50 (62.5%) were mixed, and seventeen (21.3%) were negative. Positive correlation between PTEN expression with Gleason score 7 or higher was observed ($p < 0.0001$). There was also significantly higher PTEN expression in smears from patients with PSA value $\geqslant 10$ ($p = 0.0003$) and poorly differentiated prostate carcinomas with Gleason score >7($p < 0.001$). Relationship was also observed between PTEN expression and disease outcome.
Conclusions: PTEN protein is correlated with pathological parameters of poor prognosis and could be a good marker for biological behaviour of prostate carcinomas.

882 POSTER

Expression Protein Kinase C in prostate hyperplasia and carcinomas in relationship with clinicopathological parameters

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Objective: Protein kinase C (PKC) comprises a family of serine/threonine kinases that plays a key role in the signal transduction pathways. It consists of at least 12 isoforms with different tissue expressions, substrate specificity, and subcellular localization that are related to specialized cell functions, including cell proliferation, differentiation, and apoptosis. Recent evidences prove that PKC isozymes play an important role in the transition from an androgen-dependent to an androgen-independent status The aim of this study was to investigate the PKC expression (PKC alpha and PKC delta) in smears of patients with benign hyperplasia or carcinomas in order to evaluate the malignant potential role of these diseases.
Methods: Sixty imprint smears (30 invasive carcinomas) and (30 hyperplastic prostates) were obtained at surgery and studied immunocytochemically using anti-PKC alpha and delta antibodies. The PKC expression was correlated with histopathological findings in the same samples.