agents interfering with thymosin β4 (either its up-regulation or actin sequestering function) for the treatment of thymosin β4-overexpressing tumors with high invasive and metastatic potential.

303 Poster

Erythropoietin and steroid membrane initiated actions interact in breast cancer cells leading to enhanced cell survival

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Background: Erythropoietin (EPO) is a hormone primarily involved in erythropoiesis but dotted equally with an array of autocrine/paracrine effects. EPO can regulate major cell functions of normal and cancer cell types including breast cancer, a steroid hormone dependent neoplasm. Steroid effects can be nuclear- and/or membrane-initiated, with the latter depending, among others, to a cross-linking with various growth factor receptors. Previously, in breast cancer specimens we have reported a correlation of erythropoietin, its receptor (EPOR) and membrane androgen sites. In the present work we further explore this interaction and the possible mechanism involved. Material and Methods: We assayed the effect of serum deprivation- and testosterone-BSA-induced apoptosis and cell migration in the presence of erythropoietin and explored the signaling pathways involved. Results: Testosterone-BSA-induced apoptosis and decreased cell migration was reversed by erythropoietin in a dose- and time- related manner. Moreover, the anti-apoptotic effect of EPO was potentiated by the addition of testosterone-BSA indicating an interaction between the two systems. This interaction is not at the membrane-receptor level but is the result of the modulation of specific signaling pathways (switching of p38 and Jnk from pro- to anti-apoptosis and from STAT to Akt and β-catenin signaling), and the enhanced transcription of EPOR by testosterone-BSA. Conclusions: Erythropoietin could be integrated to the ensemble of growth factors that cross-link with membrane steroid receptors, amending tumor cell survival. Their importance to patients' prognosis and selection of the appropriate therapeutic regimen should be considered.

304 Poster

Src activity is increased in liposarcomas and in gastrointestinal stromal tumors—analysis of associations with clinical and molecular tumor characteristics

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Increased activity of the non-receptor protein tyrosine kinase Src can be found in a variety of human cancers in vitro and in vivo. Several studies have shown that elevated Src activity is associated with an increase in tumor malignancy, as well as poor clinical prognosis. The present study was done to determine whether Src activity is also increased in soft tissue sarcomas such as gastrointestinal stromal tumors (GISTs), and liposarcomas, and if Src activity in these tumors correlates with established tumor characteristics, other molecular determinants, or clinical prognosis. Tumors and normal tissues from 29 patients with GIST and from 17 patients with liposarcoma were analyzed for Src activity by immune complex kinase assays. There was a positive correlation for Src autophosphorylation and phosphorylation of MBP, reflecting the ability of Src to activate an external substrate in GISTs (r=0,751; p<0,001) and liposarcomas (r=0,912; p<0,001). Src activity was significantly higher in tumors than in normal tissues within the 16 GIST patients excluding imatinib responders (p=0,017), and in liposarcoma patients (p=0,033). There was a trend for increased Src activity in GISTs to correlate with positive PDGF-R (p=0,066). Elevated specific Src activity was observed as a trend in tumors with high risk of malignant behaviour according to Fletcher (p=0,07), and in those with positive CD117 (p=0,099). Five GIST patients with recurrence and recent surgery were also analyzed for Src activity and, as a statistical trend, Src activity was now lower than in the primary (p=0,08). Furthermore, specific Src activity was significantly lower in GISTs containing spindle cells (p=0,01) than in epitheloid tumors, or tumors containing both cell types. No significant association with clinical prognosis was observed in this series so far, however, this may be due to the duration of follow-up and will be re-analyzed in the future. This study demonstrates that Src activity is significantly increased in GISTs and liposarcomas as compared to normal tissues, and in trend is associated with CD117, PDGF-R, and the score for malignant risk of GIST.

305 Poster

Integrin-Rab21-Rasa1 complex regulates integrin traffic in migrating cells

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During malignancy progression, tumour cells acquire the ability to break the basement membrane and invade underlying tissue, a process called metastasis. The seeding of tumour colonies to different sites in the body requires the activity of integrin cell surface receptors that anchor cells to the surrounding extracellular matrix (ECM). The regulation of cellular migration and adhesion is thereby dependent on the continuous turn-over of integrins that need to be internalized at retracting edges and transported to new adhesion sites of the cell.

We discovered that the small GTPase Rab21 critically regulates the endocytic traffic of integrins (Pellinen et al., J. Cell Biol., 2006). Our aim is now to further elucidate the GAPs (GTPase-activating proteins) and GEFs (GDP/GTP-exchange factors) that are decisive for the control of Rab21-activity. Our recent findings that Rasa1 (GAP) regulates integrin-internalisation and migration of breast cancer cells suggests that Rasa1 is a crucial regulator for Rab21-controlled integrin traffic and has therewith an impact on the motility of transformed breast epithelial cells during metastasis.

306 Poster

Knockdown of oncogenic microRNA-21 displays cytotoxicity in p53 wild-type colon cancer cells

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Although the number of verified human microRNAs (miRNAs) is still expanding, only few have been functionally described. However, emerging evidences suggest the involvement of altered regulation of miRNA in pathogenesis of cancers and these genes are thought to function as both tumours suppressor and oncogenes. Previous data suggest altered regulation of microRNA-21 (miR-21) expression in CRC. In our study, we examined by Real-Time PCR expression levels of microRNA-21 (miR-21) in 60 colorectal tumors and 40 paired adjacent non-tumor tissues and correlated them to selected clinicopathologic features and survival parameters. We used expression of U6 small nuclear RNA (RNU6B) for data normalization and standard ddCt method for relative quantification of miRNA expression. Levels of miR-21 were significantly higher in tumors comparing to normal mucosa ($p < 0,0001$, Wilcoxon matched-pairs test). High expression levels of miR-21 in tumors (based on high tertile) were associated also with a poor survival (long-rank p=0,043). Up-regulation of miR-21 was previously associated with high potential of invasion, intravasation and metastasis in pre-clinical colorectal cancer models. Till now no data exist focused on miR-21 effects on CRC cells proliferation. To elucidate potential involvement of miR-21 in regulation of colon cancer cells (DLD1, SW837, HCT116 wt-p53, HCT116 null-p53) proliferation we tested effects of synthetic 2´OMe-antisense-miR-21 (anti-miR-21) transfection (2´OMe-EGFP as control) on their growth by use of MTT test. Proliferation was not affected in a null-p53 cell line or cell lines expressing mutated p53 (DLD1, SW837). In a wild-type p53-expressing cell line we observed more than 20% decrease of cells proliferation by MTT test after transfection of anti-miR-21. Now we are testing attenuating effect of anti-miR-21 on CRC cells survival under conditions of p53-directed apoptosis induced by doxorubicin treatment. Simultaneously, we are evaluating also changes in invasive properties of anti-miR-21 transfected cancer cells by matrigel invasion assay. Our results suggest possible role of miR-21 in colorectal cancer pathogenesis. Supported by IGA MZ CR NR/9076 – 4 and project MZ0MOU2005

307 Poster

Differential expression of annexin A1 modulates invasion of melanoma B16 cells

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Identification of proteins involved in melanoma dissemination should complete the knowledge of physiopathology and potentially the prognosis for patients with a primary tumour. We used the B16 mouse melanoma

model: B16F10 and B16Bl6 cells can both induce primary tumours after subcutaneous implantation of cells, but pulmonary metastasis are only found in the mice bearing a B16Bl6 tumour. We performed comparative proteomic studies on these two cell lines, using both cultured cells and subcutaneous tumours. 7 and 12 differentially expressed proteins respectively were identified by mass spectrometry. Especially, annexin A1 (ANXA1) was increased 1,5 to 2 fold in B16Bl6 cells as compared to B16F10 cells, in vivo and in vitro. In an attempt to characterize its role in melanoma B16 spreading, we showed that reducing ANXA1 protein level by siRNA in B16Bl6 cells decreased their in vitro invasion properties on Matrigel® coated chambers. This should be associated with the presence of formyl peptide receptors (FPR), which have been shown to activate invasion in an epithelial cell line SKCO-15 (Babbin et al, 2006). Indeed, we demonstrated by RT-PCR the presence of transcripts encoding for two FPR isoforms (FPR1 and FPRrs) in the two B16 lines without any reliable quantitative difference. These receptors seemed to be functional since B16Bl6 cells incubation with the FPR agonists (fMLP) or antagonists (tBOC) respectively enhanced or decreased Matrigel coated chamber invasion. Furthermore, preliminary data suggested that incubation of B16Bl6 cells with fMLP and tBOC lead to an increase or decrease of ANXA1 steady state level. In conclusion, our results showed that increased ANXA1 expression could be associated at least in vitro with an increased invasion capacity, that might be mediated by the FPR receptors.

308 Poster

Expression of alpha(1,6)fucosyltransferase in the early tumorigenesis of human colorectal cancer

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An elevated level of fucose content in glycoproteins is one of the cancer-related alterations found in patients with malignant diseases. In a recent study we have demonstrated that α(1,6)fucosyltransferase [α(1,6)FT], the enzyme which catalyzes the core fucosylation in these glycoproteins, was enhanced in tumoral colon when compared to healthy adjacent tissues from colorectal carcinoma (CRC) patients. Most CRC arise from neoplastic adenomatous polyps thus we developed the present work in order to determine weather this increase is an early event or it only occurs lately in tumorigenesis.

We investigated the α(1,6)FT expression by means of immuno-histochemistry in 81 adenomas, 13 inflammatory lesions and 9 healthy tissues of free-CRC patients. Tissue sections were stained using anti-human α(1,6)FT MAb and visualized with DAB (3,3´-diaminobenzidine). Negative controls were performed using PBS instead of primary antibody.

After the immunohistochemical assay, no positive expression was found in the healthy and inflammatory tissues. In the case of adenomas, 13 of the 81 polyps analysed (16%) were positive for α(1,6)FT expression whereas the percentage of positive expression in tumor tissues (that we previous described) was the 61.3%. After the statistical analysis, we found significant differences for tumour vs. adenomas, inflammatory lesions and control healthy tissues ($p<0.01$). We also analysed the possible association between the α(1,6)FT expression in the neoplastic polyps and the histological type or the grade of dysplasia in this polyps. A significant increase of expression was observed in the cases without dysplasia with respect to the dysplasic ones ($p=0.05$), whereas a correlation between the expression and the histology was not found.

In conclusion, the absence of α(1,6)FT immunohistochemical expression in the inflammatory and healthy tissues of patients without CRC clearly suggests that the alteration of the enzyme expression is specific of the malignant transformation and is not related to the inflammatory process normally associated to the cancer. On the other hand, the low rate of positive expression obtained in pre-cancerous lesions indicates that the α(1,6)FT expression increase is a late event in the tumorigenesis strongly associated with a total cell transformation of the colorectal tumor.

309 Poster

Endo180 expression by tumour cells with an invasive phenotype correlates with prostate cancer progression

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The purpose of this study was to investigate whether stromal or epithelial expression of Endo180 (CD280; MRC2; urokinase-type plasminogen activator receptor-associated protein, uPARAP) can contribute to metastatic prostate cancer. The prognostic and functional roles of Endo180 were characterised using tissue microarray (TMA) and immunofluorescent costaining with pan-cytokeratin (pCk) to allow quantitative analysis of stromal (pCk-) and epithelial (pCk+) cellular expression. Expression of Endo180 and its function-associated partners in collagen remodeling and tumour cell migration and chemotaxis: membrane type-1 matrix metalloproteinase (MT1-MMP) and urokinase-type plasminogen activator (uPA)-uPA receptor (uPAR) respectively; were quantified in human prostate tissue clinically graded as benign prostate hyperplasia (BPH) (n=29) or with good (n=26), intermediate (n=96) or poor (n=18) tissue differentiation. Significant differences or correlations between categorical variables, including serum prostate specific antigen (PSA), were determined using two-sided statistical tests and 95% confidence intervals. Increases in Endo180+/pCk- and Endo180+/pCk+ cells confirmed both stromal and epithelial upregulation of Endo180 respectively. The increase in epithelial expression of Endo180 displayed linear correlation with advanced clinical grade and greater prognostic capability than serum PSA. The differential patterns of stromal and epithelial Endo180 coexpression with MT1-MMP and uPAR-uPA with increased clinical grade revealed the potential for a distinct sequence of their molecular and functional interplay during the different stages of prostate cancer progression. Taken together these data support the use of Endo180 as a potential biomarker for the prognosis of prostate cancer tissue biopsies and as a target to prevent tumour cell migration, chemotaxis, invasion and tissue remodelling during prostate cancer metastasis.

310 Poster

Candidate genes for the recurrence of glioblastoma multiforme identified by microarray

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Background: Glioblastoma multiforme (GBM) is the most aggressive and most lethal primary malignant brain tumor, correlated with survival rates of less than one year from the time of diagnosis. Current surgical procedure attempts to remove the bulk of the tumor mass, whereas GBM frequently recurs within 1-3cm from the primary tumor resection site. Molecular mechanisms involved in the recurrence of the tumor are still poorly understood. The aim of the study was to define the molecular signature of GBM surrounding white matter (WM) in order to better understand the molecular mechanisms involved with tumor relapse.

Material & Methods: Human GBM tumor bulk and surrounding tissue (1-3cm from the border of the tumor) were obtained from five patients who underwent total tumour resection, while normal white matter was harvested from patients who underwent surgical procedure for nonmalignant pathologies. Samples were processed for hybridization on the Affymetrix Human U133A arrays and data were examined with the GeneSpring analysis software. A subset of interesting genes was further validated by RT-PCR.

Results: Gene expression analysis of the samples was done in 2 independent steps. First, molecular profiling comparison of GBM surrounding WM and normal WM resulted in 59 genes differentially expressed between both tissues. Among these, numerous genes expressed by mature neural cells were down-regulated in GBM surrounding WM. Moreover, KLRC1, a specific natural killer receptor naturally involved in the activation of antitumoral cells was drastically repressed in GBM surrounding WM, suggesting that the antitumoral immune surveillance is compromised in this tissue.

Second, we focused our study on genes specifically regulated in GBM periphery respectively to GBM core.

The highest up-regulated gene in GBM surrounding tissue codes for DTX4, a regulator of NOTCH signalling pathway described for its key role in maintaining neural progenitors in an uncommitted state.

Conclusion: This study revealed unique molecular characteristics of GBM surrounding tissue, showing the dysregulation of genes involved in immune surveillance along with genes associated to stemness maintenance. All together, these data may help to understand the molecular mechanisms associated with GBM recurrence.

311 Poster

MYC inhibition of p27-induced erythroid differentiation is mediated by the repression of erythroid master genes and uncoupled from its cell cycle promoting activity

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Inhibition of differentiation has been proposed as an important mechanism for the tumorigenesis mediated by MYC, but the mechanisms involved are