To develop inhibitors of FGF-2 based on this sequence, computational and MRI approaches were used to identify relevant residues and conformational determinants for the TSP-1/FGF-2 interaction. A pharmacophore model was then designed and used to screen a library of compounds leading to the identification of three FGF-2-binding small molecules, which inhibited angiogenesis in vitro and in the chicken chorioallantoic membrane assay. These lead compounds represent the starting point for the development of new TSP-1-mimetic inhibitors of angiogenesis.
To better characterize the functional role of the FGF-2 binding domain of TSP-1 in modulating tumour behavior, we engineered human tumour cells (1A9 human ovarian carcinoma) to over-express either the whole type III repeats domain or its N-terminal region containing the FGF-2 binding sequence, or its C-terminal region lacking the FGF-2 binding sequence. The coding sequences for the above TSP-1 portions were amplified by PCR and cloned into the p3XFLAG-CMV-13 expression vector for mammalian cells. Then, 1A9 stable transfectants which secreted the sequences of interest were obtained. *In vitro* and *in vivo* studies are ongoing to investigate the consequences of the expression of this TSP-1 domain on the malignant behaviour of the tumour cells and particularly on their angiogenic activity.
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460 Endothelial cell specific chemotaxis regulator (ECSCR) is a novel tumour endothelial marker

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Background: Angiogenesis is the formation of new blood vessels from pre-existing ones. It occurs during embryonic development and in the adult only during wound healing and the menstrual cycle. However, adult angiogenesis can be reactivated in pathological conditions such as cancer. This makes angiogenesis targeting therapies a very appealing research field. The endothelium lines the interior of all vasculature in the body and it is well documented that tumour associated vasculature differs from the normal one. Endothelial cell specific chemotaxis regulator (ECSCR) is a novel transmembrane glycosylated protein upregulated in cancer that is involved in chemotaxis and tube formation. The aims of this work were to elucidate the expression pattern of ECSCR in both developmental and tumour angiogenesis.
Materials and Methods: Paraffin fixed tumour and normal tissues were stained with ECSCR and CD31 or ULEX antibodies and analysed by immunohistochemistry and immunofluorescence. The same techniques were applied to analyse tissue arrays of tumour and normal matched adjacent tissues from the same patient. The expression pattern of zebrafish ECSCR was uncovered by RNA whole mount *in situ* hybridization.
Results: ESCR is differentially expressed in tumour and normal tissues. Furthermore, its location seems to be inside the endothelium line, facing the lumen of the vessels. In the developing zebrafish embryos ECSCR is endothelial specific, being expressed in the major trunk and head vessels.
Conclusions: These data suggest that ECSCR is a good tumour endothelial marker, present at sites of active angiogenesis.

461 Further studies on the antitumour activity of a hybrid synthetic antitumour ester in combination with adriamycin on murine melanoma B-16

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Background: NSC 290205 (A) is a hybrid synthetic antitumour ester which combines a D-lactam derivative of androsterone and nitrogen mustard. In this study cyclophosphamide in the standard CHOP chemotherapeutic regimen was replaced with NSC 290205 (AHOP) and the efficacy of these regimens against murine Melanoma B-16 was compared.
Material and Methods: Melanoma B-16 was used in this study. It was purchased from NCI (USA). Tumour was grown in C57 Bl mice and was transplanted subcutaneously with puncture in the inguinal region. The amount of the transplanted graft was 40−50 mg fragment. The acute toxicity of the compounds was determined and the lethal dose LD10 was used as a therapeutic dose. The antitumour activity was assessed from the inhibition of tumour growth by volume in cm^3 and T/C % oncostatic parameter, according to the protocol of the experimental evaluation of anticancer drugs of the NCI. Treatment was given as an intermitted dose on days 1, 5, 9.
Results: Results show that treatment with steroidal derivative (A) or cyclophosphamide (C) produced almost equal borderline activity. Moreover, both CHOP and AHOP regimens showed significant and comparable antitumour effect. AHOP caused the maximum effect inhibiting tumour growth by 83.0% and producing T/C values of 277.7%. CHOP was less effective producing 53.7% inhibition of tumour growth and T/C 181.9%.
Conclusions: Although the treatment of Melanoma B-16 with cyclophosphamide or NSC 290205 yielded equivalent results, AHOP showed higher antitumour potency than CHOP. It is very likely that the D-lactamic steroid (androstan) alkylator for A, containing the amide group – NH-CO – combined with adriamycin which intercalates between DNA base-pairs, is the explanation for the higher activity of AHOP as compared to CHOP. Preclinical research supports that the aza-steroidal alkylator NSC 290205 demonstrates favorable acute and sub acute toxicity, as well as superior antitumour activity which in combination with adriamycin against Melanoma B-16 justifies further clinical studies.

462 Serum N-glycome biomarker for monitoring progression of DEN-induced hepatocellular carcinoma in rat

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Backgrounds and Aims: The diagnosis and certainly the follow-up of liver diseases such as cirrhosis and HCC remains a heavily debated problem. So, a more sensitive and specific non-invasive serological marker is needed for the early diagnosis of HCC and for monitoring treatment. Because most serum N-linked glycoproteins are synthesized by the liver, any changes in serum total N-glycans could reflect an alteration of liver physiology. Thus, changes in the quantity and type of N-glycans in serum could be exploited for the non-invasive diagnosis of liver diseases. In the present study, we studied glycomics during development of HCC in rat model.
Methods: Rat HCC was induced by diethylnitrosamine (DEN), a hepatocarcinogen, which results in the sequence of fibrosis and cirrhosis encountered in human hepatocarcinogenesis. N-glycans were profiled using the DSA-FACE technique developed in our laboratory. Glycan structures and glycan genes associated with progression of HCC in liver were analysis by westen lectin blot and qPCR.
Results: In comparison with control rats, two glycans (R5a and R5b) in serum total N-glycans of DEN rats increased gradually but significantly during progression of liver cirrhosis and cancer, whereas a biantennary glycan (P5) decreased. The log of the ratio of R5a to P1(NGA2F) and R5b to P1, [log(R5a/P1) and log(R5b/P1)], were significantly ($p < 0.0001$) elevated in HCC rats, but not in cirrhosis, fibrosis and control animals. We thus propose a GlycoTest model using the above serum glycan markers for monitoring the progression of cirrhosis and HCC in the DEN-treated rat model. These serum glycan markers were validated in a rat model involving prevention of tumour development by using an antitumour drug (S-trans-trans-farnesylthiosalicyclic acid; FTS). DEN-treated rats were subsequently treated with FTS leads to prevent progression to HCC. We found that GlycoTest markers (P5, R5a and R5b) in the FTS treated DEN rats reverted towards non-DEN levels, while HCC-specific markers, log(R5a/P1) and log(R5b/P1), normalized completely. Moreover, we found an increase in core-α-1,6-fucosylated glycoproteins in serum and liver of HCC rats by western lectin blot, demonstrating altered fucosylation during progression of HCC.
Conclusions: By analyzing N-glycomics during progression of HCC, we identified serum N-glycan biomarkers (GlycoTest model) that can be used to monitor progression of HCC and to follow up treatment of liver tumours in the DEN rat.

463 Autocrine regulation of receptor for advanced glycation endproducts (RAGE) by S100A4 promotes migration and invasion in A375 melanoma cells

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Background: The calcium-binding protein S100A4 is associated with metastasis of different cancer entities, including melanoma. The multiligand receptor for advanced glycation endproducts (RAGE) has been suggested to interact with extracellular S100A4 protein. We hypothesized that the interaction between RAGE and S100A4 plays an important role in activation of growth, adhesion, motility and migration in a human melanoma cell line with high metastatic potential.
Materials and Methods: In order to investigate the cellular role of the RAGE-S100A4 interaction *in vitro*, we produced recombinant S100A4 and soluble RAGE (sRAGE). Furthermore, we established A375 melanoma cells stably transfected with S100A4 using vector pIRES2-AcGFP1 (A375-S100A4). The overexpression of S100A4 has been verified by western blot and flow cytometry. Assays for determination of migratory, invasive and adhesive behaviour of A375-S100A4 cells were performed. Furthermore, specific

interaction of S100A4 with RAGE was characterized by surface plasmon resonance spectroscopy using immobilized sRAGE.
Results: The overexpression of S100A4 did not influence growth properties and adhesive behaviour of the A375-S100A4 cells; however, it affects their motility and migratory activity in comparison to mock-transfected cells. A375-S100A4 cells show an increased secretion of S100A4 into the extracellular space and, in consequence, an enhanced RAGE protein expression. Molecular interaction studies revealed high affinity (lower micromolar range) of S100A4 towards immobilized sRAGE, suggesting a biochemical rationale for the observed effects.
Conclusion: This investigation shows that overexpression of S100A4 influences the metastatic behavior of A375 melanoma cells. The enhanced secretion of S100A4 leads to an autocrine upregulation of RAGE expression and synthesis in A375-S100A4 cells. The findings support the supposed functional role of RAGE-S100A4 interaction in promoting a metastatic phenotype of human melanoma.

464 The impact of hypoxia on gene expression and protein synthesis of Eph receptors and ephrin ligands in human melanoma cells

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Background: The transmembrane Eph receptors (Eph) and their ephrin ligands represent the largest subfamily of receptor tyrosine kinases. Eph/ephrins are key players in cell-cell communication due to their capability of bidirectional signaling. There is evidence that Eph/ephrins also play an important role in tumour progression and metastasis. Since hypoxia is an important elicitor for metastatic behaviour of tumour cells, the aim of our study was to investigate the influence of hypoxia on Eph and ephrin expression in primary and metastatic melanoma cell lines.
Materials and Methods: The influence of experimental hypoxia (6 to 72 h) on viability and metabolism of three melanoma cell lines (Mel-Juso, A375, and A2058) was characterized using MTT tests and cellular uptake of both ^{18}F-fluoromisonidazole (FMISO) and ^{18}F-fluorodeoxyglucose (FDG). The mRNA expression of EphA2, EphB4, ephrinA1 and ephrinB2 was analyzed with quantitative RT-PCR. Protein synthesis was determined by flow cytometry.
Results: The uptake of FMISO increased in all three melanoma cell lines after incubation under hypoxic conditions. The FDG uptake under hypoxic conditions decreased in all three cell lines. The MTT test demonstrated that viability of A375 cells decreased to 29±3% after 72 h of hypoxia. A2058 cells showed only a weak decrease of viability by approximately 30%, whereas viability of Mel-Juso cells under hypoxia was not influenced. In all cells Eph/ephrin gene expression under hypoxic and normoxic conditions showed only minor differences, except for EphA2 expression in A375 cells, which increased by >40% after 12 h hypoxia. Flow cytometry showed no alteration in ephrin ligands under hypoxic conditions. In contrast, after 72 h hypoxia we detected a slight increase in EphB4 protein in all melanoma cell lines, and enhanced EphA2 protein only in metastatic cell lines A375 and A2058.
Conclusion: The metastatic melanoma cell lines A375 and A2058 react more sensitive to hypoxic conditions than the primary melanoma cell line Mel-Juso. Experimental hypoxia increases Eph receptor gene expression and protein synthesis, particularly, in metastatic melanoma cell lines, which could be indicative for a further mechanism by which hypoxia affects tumour metastasis.

465 The effects of a selective cyclooxygenase-2 inhibitor on canine mammary carcinoma cell line

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Mammary cancer is the second leading cause of cancer death in women, and mammary gland tumours are the second most common type of neoplasm in both male and female dogs. Canine mammary tumours have been proposed to be a good animal model for human breast cancer due to similarities in morphology, histopathology and patterns of malignancy. Therefore, determination of treatment or prevention modalities for the dog population not only is beneficial to the pet population but also may prove useful to humans. Recent epidemiological studies in humans and studies in spontaneous canine tumours and experimentally induced rodent tumours have shown that cyclooxygenase (COX)-2 or COX-1/COX-2 inhibitors have antitumour and chemopreventive effects in several different forms of cancer. The mechanisms by which COX inhibitors exert their antitumour effects are not completely defined but studies have shown that COX-2 derived prostaglandins contribute to tumour cell resistance to apoptosis, new blood vessel formation, and tumour cell proliferation. The purpose of this study was to confirm the antitumour effects of COX-2 inhibitors and determine the effects of the COX-2 inhibitor, deracoxib, on apoptosis, proliferation, PGE_2 concentration in CMT-U27 canine mammary carcinoma cell line.
The cells were seeded and exposed to deracoxib 50, 100, 250, 500 and 1000 μM concentrations for 24, 48 and 72 h. The viability of the cells (% of control) was measured using the colorimetric 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay. Apoptosis was assessed by flow cytometry and PGE_2 levels were measured by using (a PGE_2 monoclonal enzyme) an immunoassay kit.
CMT-U27 Cells treated with deracoxib at various concentrations exhibited a profound dose-and time-dependent reduction in the proliferation rate over the 72 h test period with the IC_{50} value of 974.481 μM. Cytotoxic effect typically seen in apoptosis, as well as early apoptotic changes by Annexin V tests. The apoptotic index at the end of 72 h period was increased to 53.52% and 68.49% in CMT-U27 cells treated with deracoxib at high concentrations (500 μM and 1000 μM), respectively, in comparison to control cells. No significant difference was found on endogeneous and exogenous PGE_2 release in canine mammary cancer cells between deracoxib treated cells and control cells.
High doses of deracoxib exerts tumouricidal activity via induction of apoptosis over mammary cancer cell. In this cell line, deracoxib would not directly target COX-2 or PGE_2 activity, thus suggesting, the involvement of COX-independent mechanisms in deracoxib induced cytotoxicity. This compound may be useful in the prevention and treatment of canine mammary cancer.

466 Activation of Wnt/β-catenin signaling pathway in HNSCC

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Background: The Wnt/β-catenin signaling pathway affects different processes such as cell proliferation, differentiation, migration or embryonic development and plays an important role in oncogenesis. Dysregulation of Wnt activity and the aberrant expression of β-catenin are often linked to cancer including head and neck squamous cell carcinoma (HNSCC).
Methods: HNSCC cell lines and primary tumour cells were analyzed using immunofluorescence and western blot. Antibodies against total versus active β-catenin, cytokeratin8 and cyclin D1 were used to evaluate the level of β-catenin expressed in the cell nucleus and in other compartments. Cells were stimulated with lithium chloride (LiCl) and the dynamics of β-catenin and cyclin D1 levels were detected.
Results: Using immunofluorescence the presence or absence of β-catenin in HNSCC cell lines and primary tumour cells was quite diverse and heterogeneous.
Stimulating HNSCC cells with LiCl resulted in accumulation of β-catenin and had an effect on typical Wnt target genes.
Conclusions: These results indicate the presence of a β-catenin positive subpopulation in solid HNSCCs and corresponding metastases which strongly suggest an important role of the canonical Wnt signaling pathway in HNSCC.

467 Caspase mediated apoptosis regulation in HNSCC

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Background: Dysregulation of apoptosis interrupts the balance between cell growth and cell death and is associated with cancer including HNSCC (head and neck squamous cell carcinoma), whereas the molecular processes are mostly unknown.
Material and Methods: To induce apoptosis in cancer cells, the HNSCC cell lines were treated with the conventional cytotoxic drug Paclitaxel. The effects of Paclitaxel treatment on apoptosis induction and cell cycle arrest were elucidated in several HNSCC cell lines as well as in solid HNSCC tumours and the corresponding metastases at different times. The apoptosis induction was quantified by Annexin-V-APC FACS analysis. The processing and activation of caspases during apoptosis were determined by Western blot analysis and caspase-substrate-assays. The flow cytometric cell cycle arrest analysis and DNA content were measured by Propidium Iodide (PI) staining.
Results: Paclitaxel induced cell cycle arrest in each treated HNSCC cell line following programmed cell death. However, the efficiency on apoptosis induction as well as caspase processing and activation under Paclitaxel treatment was quite diverse in HNSCC tumours and the corresponding metastases.
Conclusion: According to our findings, Paclitaxel treatment is likely to effect the mediated apoptosis regulation in HNSCC. Paclitaxel induced apoptosis in all treated HNSCC cell lines; nevertheless in every experiment at least some cells avoided Paclitaxel induced programmed cell death, overcame cell cycle arrest and finally survived.