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.