(PDAC) were analyzed by QRT-PCR, laser capture microdissection, DNA microarray analysis, immunoblotting, radioimmunoassay, immunohistochemistry, cell growth, invasion, scattering, and adhesion assays.

Results: BSP mRNA was detected in 40.7% of normal, in 80% of CP and in 86.4% of PDAC samples. The median BSP mRNA levels were 6.1 and 0.9 and zero copies/μl cDNA in PDAC, CP and normal pancreatic tissues, respectively. BSP was localized in the cytoplasm of the tubular complexes of CP and PDAC, and in pancreatic cancer cells. Five out of eight pancreatic cancer cell lines expressed BSP mRNA. Recombinant BSP (rBSP) inhibited Capan-1 and SU8686 pancreatic cancer cell growth, with a maximal effect of $-46.4 \pm 12.0\%$ in Capan-1 cells and $-45.7 \pm 14.5\%$ in SU8686 cells. rBSP decreased the invasion of SU8686 cells by $-59.1 \pm 11.2\%$ and of Capan-1 cells by $-13.3 \pm 3.8\%$ ($p < 0.05$), whereas it did not affect scattering or adhesion of both cell lines.

Conclusion: Endogenous BSP expression levels in pancreatic cancer cells and low to absent BSP expression in the surrounding stromal tissue elements may indirectly enhance the proliferation and invasion of pancreatic cancer cells.

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P11. INFLUENCE OF NEUROENDOCRINE TUMOR DIFFERENTIATION ON CELL ADHESION MOLECULE EXPRESSION IN PROSTATIC CARCINOMA

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Background: Neuroendocrine (NE) differentiated tumor cells can be recurrently found in prostatic carcinoma (PCa). NE tumor are involved in the proliferation of the surrounding tumor cells by paracrine mechanisms. The loss of E-cadherin and β-catenin is a central factor for local invasion and metastasis. To evaluate the relationship between NE tumor differentiation and the expression of E-cadherin and β-catenin was correlated with NE differentiation.

Methods: This study included 102 previously untreated PCa tissue specimens with a low (LNE) or high (HNE) NE differentiation. The intensity and cellular localization of E-cadherin and β-catenin was evaluated by immunohistochemistry. A homogeneous membranous staining in >70% of the tumor cells was regarded as normal, whereas an altered cellular distribution or heterogeneous staining in >30% of the tumor cells was regarded as aberrant. The expression of E-cadherin and β-catenin was correlated with the NE differentiation.

Results: Aberrant expression of E-cadherin and β-catenin was found in 72.7% and 90.2% of the tumors, respectively. In HNE tumors aberrant expression was significantly increased compared to LNE tumors ($p = 0.010$ for E-cadherin and $p = 0.016$ for β-catenin). In addition, NE cells of which 78.2% were located at the invasion front did not express E-cadherin or β-catenin as demonstrated by comparing serial sections.

Conclusions: Tumors with a HNE differentiation have a significantly decreased expression of the cell adhesion molecules E-cadherin and β-catenin which were absent in the NE tumor cells. These results indicate that NE tumor cells might influence the cell–cell adhesion by paracrine mechanisms and play an important role in tumor progression and invasiveness.

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P12. EXPRESSION OF MUC18 (CD146) IN HUMAN CHOROIDAL MELANOMAS

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Background: Choroidal melanoma is the primary eye cancer in adults, and displays some features in common with cutaneous melanoma. MUC18 is an important diagnostic marker of cutaneous melanoma, with increased expression in tumors associated with metastatic potential. However, MUC18 expression in primary choroidal melanoma and melanocytes remains to be fully investigated. We examined a series of choroidal melanoma cell lines, melanocytes, and primary choroidal melanomas, for a possible association between MUC18 expression and more aggressive forms of choroidal melanoma.

Methods: MUC18 expression (protein and mRNA) was assessed in choroidal and metastatic melanoma cell lines using immunoblotting and RT-PCR. Sections of whole eyes with mixed spindle/epithelioid choroidal melanomas ($n = 18$) were immunolabelled using a polyclonal antibody to the extracellular domain of MUC18 (R&D), and visualised using peroxidase and VectorRed.

Results: Immunoblotting of lysates from melanoma cells showed a positive band ~113 kDa, and MUC18 mRNA was detected in all cell lines. Moderate/strong cytoplasmic MUC18 immunolabelling was seen in 5/18 primary tumors (mixed spindle/epithelioid, <18 months detection to enucleation). MUC18 immunolabelling was seen on tumor vasculature, and in some cases, on networks/channels, characteristic of more aggressive tumors. Tumor extracellular matrix showed MUC18 immunolabelling in some cases.

Conclusions: Melanoma cell lines all expressed MUC18, however, only some primary choroidal melanomas, mostly with features suggesting more aggressive histopathology, expressed moderate/strong MUC18 immunolabelling. These observations suggest that MUC18 may play a role in tumor progression in some cases, and may be an appropriate marker for more aggressive choroidal melanomas.

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P13. RalA AND RalB PROTEINS CONTRIBUTE TO METASTASIS STIMULATION THROUGH DISTINCT PATHWAYS

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Background: Previously we showed that oncogene Ha-Ras stimulates the metastatic activity of both spontaneously transformed and v-src-transformed Syrian hamster fibroblasts in vivo. We revealed that Ha-Ras/RalGDS/Ral-associated signalling pathway was the most important for metastasis among the variety of Ha-Ras associated signaling cascades.
Methods: Retroviral infection, molecular cloning, spontaneous metastatic activity, gelatine zymography, RT-PCR, cell viability assay, peroxide decomposition activity assay.
Results: We showed that both RalA and RalB stimulate the metastatic potential of cells, but RalB is less efficient than RalA. To understand what Ral interacting proteins contribute to this phenomenon we compared the level of metastatic potential of the cell lines expressing RalA and RalB effecter domain mutants. The search revealed that among the three best-studied Ral downstream partners RalA-PLD1 and RalB-RalBP1/RalB-Sec5 interactions were essential. The introduction of active form of RalA (RalA+ cells), unlike RalB, downregulates the secretion of MMP1, MMP2 and MMP9 in comparison to parental cells, although the cocultivation of RalA+ cells with hamster embryo fibroblasts leaded to stimulation of MMPs secretion suggesting that RalA+ cells can it in stromal cells. The highly metastatic cells acquired the peroxide resistance as well as peroxide decomposition activity and this viability may serve as a protection against the host immune system.
Conclusion: RalB can stimulate metastatic ability of transformed cells, but less than RalA. These two closely-related proteins probably stimulate metastasis through distinct downstream signaling pathways. MMPs secretion is involved in Ral-dependent metastasis, but RalA and RalB utilize different cellular mechanisms.

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P14. ADAM-17: A MEDIATOR OF BREAST CANCER PROGRESSION?

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Background: The ADAMs are a family of transmembrane, multidomain proteins, involved in both cellular adhesion and proteolysis. Multiple studies from model systems suggest that specific ADAMs are involved in cancer progression. The aim of this investigation was to see if ADAM-17 was involved in the progression of human breast cancer.
Materials, Methods and Results: ADAM-17 protein was measured by both Western blotting and ELISA. ADAM-17 protein was found to exist in two main forms in human breast tissue, a 120 kDa precursor and 100 kDa active protein. Both forms of ADAM-17 protein were found to be upregulated in primary breast carcinomas compared to normal breast tissue (Mann–Whitney *U*-test: $p = 0.005$, $p = 0.0003$, respectively). The ratio of 100 kDa protein to 120 kDa protein increased with disease progression from normal breast tissue to axillary node metastases (Kruskal–Wallis statistical test: $p = 0.002$), indicating an increase in processing from precursor to active protein in malignant breast tissue. A moderate, but significant correlation was found between the 100 kDa form of ADAM-17 (measured by Western blotting) and ADAM-17 levels determined by ELISA (Spearman Rank: $p = 0.0006$, $r = 0.405$). No significant relationship was found between the 120 kDa protein form and levels measured by ELISA. ADAM-17 protein levels, as measured by ELISA, were significantly higher in grade 3 tumours as compared to tumours classed as grades 1 and 2 (Mann–Whitney *U*-test: $p = 0.03$), and in node-positive compared to node-negative cancers (Mann–Whitney *U*-test: $p = 0.04$). Furthermore, both forms of ADAM-17 correlated with cell proliferation, as measured by PCNA (Spearman Rank: $r = 0.524$, $p < 0.0001$; $r = 0.365$, $p = 0.002$, respectively) and with metastatic potential, as measured by uPA (Spearman Rank: $r = 0.246$, $p = 0.032$; $r = 0.428$, $p = 0.0001$, respectively).
Conclusion: We conclude that ADAM-17 protein exists in two main forms in breast cancer. Furthermore, our results suggest that ADAM-17 is involved in breast cancer progression.

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P15. PROFILING OF NEOPLASMS OF THE PANCREAS USING MICROARRAY-TECHNOLOGY

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Background: Pancreatic cancer is the fifth most frequent cause of cancer-related deaths in industrialized countries. The diagnosis of ductal adenocarcinoma of the pancreas is associated with a poor prognosis, an increasing incidence and no or only ineffective means of treatment. Cystic pancreatic neoplasms account only approximately 5% of primary malignancies of the pancreas and may be benign, pre-malignant or malignant. In this study, we are developing and evaluating a DNA-diagnostic-chip with about 3500 human genes known to be differentially transcribed in pancreatic cancer cells and thus expected to show a representative expression pattern in both ductal adenocarcinoma and cystic lesions.
Methods: cDNAs representing different human genes were PCR-amplified, purified and robotically arrayed onto slides with an epoxy surface. Fluorescently labelled cDNA samples were prepared from total RNA isolated from cells of human pancreas tissue by incorporation of labelled dCTPs during reverse transcription.
Results: Microarray experiments on various samples are being performed and analysed allowing classification of different types of pancreas lesions and the identification of potential targets as a means of eventually developing new modes of treatment.
Conclusion: The resulting diagnostic DNA-chip will be of significant clinical utility to detect cancer cells in tissues from patients with different types of pancreatic carcinoma and to draw prognostic conclusions based on their molecular appearance. Furthermore, comparative studies on transcriptional profiling and actual protein expression by means of complex DNA- and antibody microarrays are under way.

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