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**Background:** Pdcd4 (programmed cell death protein4) is a potential tumor suppressor, expression of which is downregulated in various human tumor types. Additionally, Pdcd4 is able to inhibit the neoplastic transformation in the JB6 mouse model. Pdcd4 has been shown to inhibit translation of diverse regulatory factors important for neoplastic transformation. Moreover, it was shown that AKT phosphorylates Pdcd4, causing nuclear translocation and inactivation of the latter. This suggests that Pdcd4 activity is dependent on its cellular localisation. Up to now, Pdcd4 protein expression and cellular localisation has not been analysed in a large series of patients with colorectal cancer (CRC).
**Methods:** We investigated the expression pattern and localisation of Pdcd4 tumor suppressor protein in resected tumor and corresponding normal tissue in a series of 41 CRC patients (32 R0-resected) who did not receive neoadjuvant treatment, by Western blotting (WB) and immunhistochemistry (IHC). A separate semiquantitative score for ICH staining of the cytoplasm and nuclei was established. Preliminary analysis of Pdcd4 expression and localisation was correlated with patient's clinical tumor stage (UICC) and with recurrence-free survival.
**Results:** In WB high overall Pdcd4 amounts were detected in normal tissue in comparison to the tumor samples where the signal was significantly decreased ($p = 0.025$, Wilcoxon). IHC analysis revealed strong nuclear presence of Pdcd4 in the apical cryptal epithelium of normal tissue, as opposed to the complete loss of nuclear expression in tumor tissue ($p = 0.001$, Wilcoxon). In normal tissue, loss of Pdcd4 nuclear expression/increase of cytoplasmic Pdcd4-staining was significantly associated with advanced UICC stages ($p = 0.027$, $\chi^2$). Preliminary Kaplan–Meier-analysis (median recurrence-free survival time: 38 months, range: 1–74 months) showed a trend for loss of Pdcd4 expression in the nuclei of the normal tissue to be associated with poor recurrence-free survival ($p = 0.09$, Breslow log rank).
**Conclusion:** This is the first clinical study that demonstrates a potential relevance of Pdcd4 expression and localisation in resected colorectal tumors and corresponding normal tissue, for tumor diagnosis and progression. Further analysis of colorectal adenomas will be performed to study the role of Pdcd4 localisation as a potential clinical marker for carcinogenesis in CRC.

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## P6. PROTEINASES ACTIVATED RECEPTORS: EXPRESSION AND QUANTIFICATION IN COLON AND PROSTATE HUMAN CANCER

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**Background:** Proteinases activated receptors (PARs) are important members of the G protein-coupled receptors family. The proteolytic cleavage of their extracellular domain by serine proteases such as trypsin or thrombin, generates an autoactivating tethered-ligand. Thus, the corresponding activated G proteins trigger a cascade of downstream events leading to diverse cellular outcomes such as calcium signalling, cell adhesion, cell migration and mitogenesis. The role of proteases in promoting invasion of cancer cells and tumorigenesis have prompted us to study the expression level and localisation of the four known members of the PARs family and explore their role in cell proliferation.
**Methods:** First, by using quantitative RT-PCR, we have quantified: (i) the expression of each PAR in colon and prostate cancer cell lines, (ii) the PARs expression in 40 patients samples (colon or prostate normal tissue and cancer). Second, we have studied PARs tissue localisation in colon and prostate, by using immunohistochemistry staining on TMA samples. Finally, we investigated the role of the four members of PAR family (PAR1-4) in cell proliferation by stimulating cell lines in different conditions (various thrombin concentrations).
**Results:** Our results show that the expression profile of the PARs is different for each PAR and depend mostly of the cell lines characterisation (origin and stage). However, we demonstrate a constant overexpression of two PARs in every tested cell line, that was also found in tumor samples.

Immunohistochemichal analysis shows PAR1 endothelial expression which was stronger in malignant tissues (prostate and colon samples), and weaker (prostate) or absent (colon) in normal tissues.
**Conclusion:** These results confirm the importance of PARs in cancer, whose expression pattern is likely to influence cancer cell behaviour in tumors.

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## P7. COMPLETE COMPILATION OF MATRIX METALLO-PROTEINASE EXPRESSION IN HUMAN MALIGNANT GLIOMAS

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**Background:** Glioblastomas are the most common malignant brain tumors in adults characterized by very aggressive local growth and invasiveness. Tumor invasion into surrounding brain tissue is facilitated by increased expression and activity of matrix metalloproteinases (MMPs), which may be marker for tumor aggressiveness. However, for several of the 23 human MMPs there are no or only very limited literature data available concerning expression by glioblastomas. Therefore, we screened an extensive panel of 15 low-grade astrocytomas and 15 glioblastomas in order to fill the gaps in our knowledge about MMP expression by these tumors.
**Methods:** Expression of MMPs was analysed by semiquantitative RT-PCR and immunostaining. Total RNA was used as template for RT-PCR. Immunostaining was performed on cryosections.

**Results:** Our data in combination with the literature data showed that MMP-2, -7, -9, -12, -14, -15, and -25 are expressionally correlated with the tumor grade. The data for MMP-1, -11, -19 and -24 are contradictory, since some studies, including our own, suggest involvement during brain tumor development, whereas the results of other groups deny such connection. The remaining MMPs do not seem to play a major role during glioblastoma development, because they are either constitutively expressed or not expressed at all.
**Conclusion:** This is the first complete compilation of expression of all 23 human MMPs by astrocytic tumors. Specific MMP expression patterns may mark tumor aggressiveness. Expression studies will extend our understanding of tumor invasiveness and help us to find more effective therapeutic means for treatment of glioblastomas.

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### P8. THE SERINE PROTEASE Bssp REGULATES KERATINOCYTE PROLIFERATION AND MIGRATION BY MODULATION OF E-CADHERIN/β-CATENIN SIGNALLING

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**Background:** Carcinogenesis is a multistage process driven by genetic and epigenetic alterations that are associated with changes in the expression or function of oncogenes and tumor-suppressor genes.
**Methods:** In order to identify novel tumor-associated genes, we performed global gene expression profiling with specimens of a well-established multistage tumor model of mouse skin resulting in a comprehensive list of differentially expressed genes.
**Results:** One of these genes encodes for the secreted serine protease Bssp and is characterized by elevated expression in advanced stages of mouse skin tumors. Moreover, we found enhanced levels of kallikrein 6, the human homolog, in malignant squamous skin tumors of human patients as well as in tumors of other epithelial tissues suggesting a common role in carcinogenesis. To unravel the consequence of elevated Bssp expression in epithelial cells, we established stable transfected keratinocytes expressing exogenous Bssp. These cells displayed increased proliferation and advanced migration accompanied by impaired E-cadherin-mediated cell–cell adhesion and increased nuclear β-catenin localization. Enhanced proliferation of keratinocytes was confirmed in a wound healing experiment using a transgenic mouse model with exogenous Bssp expression in skin.
**Conclusion:** In summary our data imply that elevated Bssp/Klk6 levels support epithelial neoplasia due to modulation of E-cadherin/β-catenin signalling and thereby facilitate proliferation and migration of tumor cells.

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### P9. Eps8 ASSOCIATES WITH ACTIN AT THE LEADING EDGE AND INDICATES MIGRATORY POTENTIAL IN PANCREATIC CANCER CELL LINES

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**Background:** Pancreatic carcinoma is characterized by early metastasis. Increased cell motility is critical for metastasis of tumor cells. Cell motility is based on reorganization of the actin cytoskeleton in lamellipodia at the leading cell edge. Rac is a Rho-family small GTPase, which regulates rearrangement of the actin network and is also involved in tumor metastasis. Eps8, originally identified as a substrate of the epidermal growth factor receptor (EGFR), serves as a Rac guanine nucleotide exchange factor thus activating Rac. Eps8 is involved in cancer cell invasiveness and motility in fibrosarcoma cells. Eps8 further binds actin directly and possess barbed end capping activity.
**Methods:** Pancreatic tissue was obtained from patients that underwent pancreatic resection at the University of Heidelberg. Eight pancreatic cancer cell lines were used: AsPc-1, BxPC3, Capan1, Colo357, Miapaca, Panc1, Su8686, and T3M4. QRT-PCR was performed with the Light Cycler Fast Start DNA SYBR Green Kit. Immunofluorescence of EGFR-GFP, actin and Eps8 was analysed by confocal microscopy. Cell motility was quantified by the phagokinetic assay.
**Results:** QRT-PCR reveals that Eps8 expression is 4-fold increased in pancreatic carcinoma compared to normal pancreatic tissue. In pancreatic tissue, Eps8 is located apically in ductal epithelial cells. In all eight pancreatic cell lines analysed, Eps8-expression correlates with staging of the original tumors: cells derived from ascites exhibit the strongest Eps8 expression, followed by cells from metastases and primary tumors. In parallel, Eps8 expression in cell lines is associated with increased cancer cell motility. Subcellulary, Eps8 does not colocalize with the EGFR but is located at the tip of actin filaments and at the leading cell edge.
**Conclusion:** Eps8 is increased in pancreatic cancer. Association with dynamic actin at the leading cell edge in pancreatic cancer cell lines seems to be an important mechanism for pancreatic cancer cell migration promoting metastasis.

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### P10. EFFECTS OF BONE SIALOPROTEIN ON PANCREATIC CANCER CELL GROWTH, INVASION AND METASTASIS

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**Background:** Bone sialoprotein (BSP); an acidic glycoprotein that plays a role in cancer cell growth, migration and invasion.
**Methods:** The expression, localisation and function of BSP in chronic pancreatitis (CP) and pancreatic ductal adeno-carcinoma