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.

doi:10.1016/j.ejcsup.2006.04.067

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

Britta Klucky[a], Regina Mueller[a], Bettina Hartenstein[a], Kai Breuhahn[b], Peter Schirmacher[b], Jochen Hess[a], Peter Angel[a]. [a]*Division of Signal Transduction and Growth Control, Deutsches Krebsforschungszentrum, Im Neuenheimer Feld 280, D-69120 Heidelberg, Germany;* [b]*Institut of Pathology, University Hospital, Im Neuenheimer Feld 220/221, 69120 Heidelberg, Germany.*

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.

doi:10.1016/j.ejcsup.2006.04.068

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

T. Welsch[a], K. Endlich[b], T. Giese[c], A. Märten[a], M.W. Büchle[a], J. Schmidt[a]. [a]*Department of Surgery, University of Heidelberg, Germany;* [b]*Department of Anatomy, University of Greifswald, Germany;* [c]*Department of Immunology, University of Heidelberg, Germany.*

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.

doi:10.1016/j.ejcsup.2006.04.069

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

Hany Kayed[a], Jörg Kleeff[a], Shereen Keleg[a], Klaus Felix[a], Thomas Giese[b], Martin R. Berger[c], Markus W. Büchler[a], Helmut Friess[a]. [a]*Department of General Surgery, University of Heidelberg, Heidelberg, Germany;* [b]*Institute of Immunology, University of Heidelberg, Heidelberg, Germany;* [c]*Unit of Toxicology and Chemotherapy, German Cancer Research Center, Heidelberg, Germany.*

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

(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.

doi:10.1016/j.ejcsup.2006.04.070

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

Ö. Cuhadaroglu, C.G. Sauer, R. Grobholz. *Pathologisches Institute, Universitätsklinikum Mannheim der Universität Heidelberg, Germany.*

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.

doi:10.1016/j.ejcsup.2006.04.071

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

M.C. Madigan[a], V. Sharma[a], K. Lai[a], M.J. Jager[b], R.M. Conway[a]. [a]*Clinical Ophthalmology, Save Sight Institute, University of Sydney, Sydney, Australia;* [b]*Leiden Medical Center, Leiden University, Leiden, The Netherlands.*

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.

doi:10.1016/j.ejcsup.2006.04.072

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

V. Rybko, K. Ukraintsev, A. Martinjuk, N. Dyakova, A. Komelkov, O. Kovaleva, E. Tchevkina. *Oncogenes Regulation Laboratory, Institute of Carcinogenesis, Cancer Research Center, Moscow, Russia.*