P65. PREDICTION OF METASTASES AND LOCAL TUMOR INVASION IN PANCREATIC CANCER USING AN ORTHOTOPIC SCID MOUSE MODEL

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Background: Gene expression profiling in pancreatic cancer is complicated by the high amount of RNAses in human tissue and suitable models. In order to reflect early metastasizing, models should be constructed with respect to the anatomical environment. Using the orthotopic pancreatic tumor SCID mouse model these interactions are taken into account. In order to identify genes associated with local tumor invasion and metastases in ductal pancreatic cancer we investigated pancreatic tumor cell lines derived from an orthotopic pancreatic tumor model in SCID mice. Differential gene expression was performed based on cDNA microarray technique.
Methods: Human MiaPaca cell lines were orthotopically implanted in SCID mice ($n = 18$). Transcriptional profiling (Affymetrix, HGU133) was performed with tissue derived from the primary tumor, duodenal tumor invasion frontier, and liver metastases. Differentially expressed genes were identified after statistical analysis (ANOVA) and validated with external data bases (NCBI: PubMed, LokuslLink, Unigene, Swissprot, Geneontology).
Results: Of 22283 genes investigated, 1066 were high significantly altered ($p < 10^{-6}$). With respect to the primary tumor, in liver metastases 196 genes were differentially expressed, whereas in the duodenal tumor invasion frontier 964 genes were altered. Comparing current data bases we established a panel of 14 genes associated with liver metastases and local invasion for functional validation. These genes can be assigned to apoptosis (e.g. BNIP3L, GADD45A), angiogenesis (VEGF), or cell migration and -adhesion (SERPINE1).
Conclusion: The SCID mouse model is able to simulate the human metastasizing cascade in vivo and facilitate gene expression analyses. Using transcriptional profiling in the SCID mouse model marker genes for local invasion and liver metastases can be identified. These marker genes are associated with apoptosis, angiogenesis, and cell interactions.

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P66. THE THROMBIN RECEPTOR PAR-1 PLAYS AN IMPORTANT ROLE IN PANCREATIC CANCER CELL INVASIVENESS IN VITRO

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Background: Thrombosis and activation of coagulation are frequently observed in cancer patients. This phenomenon is reflected in the excess production of thrombin, which is thought to play an important role in the progression of different solid tumors. Cellular effects of thrombin are mediated by the thrombin receptor PAR-1, a member of the G-protein coupled receptor family. Aim of this study was to investigate the role of PAR-1 on pancreatic cancer cell invasion.
Material and Methods: The human pancreatic cancer cell line MIA PaCa-2, which expresses high levels of PAR-1, was transfected with an antisense construct against PAR-1. The expression level of PAR-1 after transfection was determined by RT-PCR and Western blot analysis. For invasion assays the respective transfected, control transfected (sense and mock) and untransfected cells were stimulated with thrombin (1.0 U/ml) and subjected to a standardized Matrigel invasion assay. After 24 h the number of invasive cells were counted.
Results: Antisense transfection resulted in 80% downregulation of PAR-1 expression compared to parental MIA PaCa-2. In the Matrigel invasion assay the number of invasive cells was significantly reduced for the antisense transfected cells. The control transfected cells showed no change of invasiveness compared to untransfected MIA PaCa-2.
Conclusion: The expression of the thrombin receptor PAR-1 was successfully reduced in pancreatic cancer cells by antisense transfection. Downregulation of the thrombin receptor lead to significantly reduced invasiveness in vitro. These results emphasize the crucial role of PAR-1 in pancreatic cancer progression in vitro. In vivo assays to confirm the impact of PAR-1 expression on pancreatic cancer cell invasiveness are prospected.

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P67. PRETHERAPEUTICAL GENE EXPRESSION PROFILING FOR RESPONSE PREDICTION OF RECTAL ADENOCARCINOMAS TO PREOPERATIVE CHEMORADIOTHERAPY AND ITS IMPACT ON DISEASE FREE SURVIVAL

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Background: There is a wide spectrum of tumor responsiveness of rectal adenocarcinomas to preoperative chemoradiotherapy (CT/RT) ranging from complete response to resistance. We therefore investigated whether gene expression profiling can assist in stratifying patients into responders or non-responders. Furthermore, we evaluated whether distinct gene expression signatures can be used for individualized prognostication (disease free survival).
Methods: Pretherapeutic biopsies from 30 locally advanced rectal carcinomas were analyzed using microarrays. Class comparison was used to identify a set of genes that were differentially expressed between responders and non-responders (as measured by T-level down-sizing). Leave-one-out cross-validation (LOOCV) was performed to explore the predictive value of the identified gene-set.
Results: Responders and non-responders showed significantly different expression levels for 54 genes ($p < 0.001$). When we

applied LOOCV, we were able to correctly predict the tumor behavior (T-level down-sizing) in 83% of patients ($p = 0.02$). We then observed that after a median follow-up of 34.5 months, all five patients with metastatic disease belonged to the group of non-responders. We again applied LOOCV and correctly predicted all five patients with recurrence. Furthermore, all 11 patients who were predicted to remain cancer free showed no evidence of recurrence.

Conclusion: Our results suggest that pretherapeutical gene expression profiling may assist in response prediction of rectal adenocarcinomas to preoperative CT/RT and in prediction of disease free survival if validated in larger independent studies.

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P68. GENE EXPRESSION SIGNATURE OF COLORECTAL CARCINOGENESIS

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Background: Colorectal carcinomas develop through the sequential stages of increasing morphological and molecular alterations. While the correlation of tumor phenotype with associated genomic alterations has been firmly established, the correlation with global gene expression profiles is less.

Methods: We analyzed tissue samples from 36 patients to identify sequential alterations of the genome and transcriptome that define the transformation of normal epithelium and the progression from adenomas to invasive disease.

Results: Comparative genomic hybridization (CGH) revealed patterns of stage specific, recurrent genomic imbalances. Gene expression analysis on 9K cDNA arrays identified 58 genes to be differentially expressed between normal mucosa and adenoma, 116 genes between adenoma and carcinoma, and 158 genes between primary carcinoma and liver metastasis ($p < 0.001$). Our analysis revealed a direct correlation of chromosomal copy number changes with chromosome-specific average gene expression levels.

Conclusion: Increasing genomic instability, a recurrent pattern of chromosomal aberrations and a specific gene expression pattern correlate with distinct stages of colorectal cancer progression. Chromosomal aneuploidies exert a direct effect on average expression levels of the genes residing on the aneuploid chromosomes, thereby contributing to a massive deregulation of the cellular transcriptome. The identification of novel genes and proteins might deliver relevant molecular targets for diagnostic and therapeutic interventions.

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P69. Ki-67 AUTO-ANTIBODIES IN COLORECTAL CANCER PATIENTS

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Background: Antibodies against the human nuclear antigen pKi-67 (Ki-67, MIB-1) are routinely used in oncology as immune-histological proliferation marker. PKi-67 is exclusively expressed in all active phases of the cell cycle (G1,S, G2, Mitosis). The Ki-67 index (relative number of positive stained nuclei) serves as an independent prognostic marker for certain tumor entities. We investigated whether colorectal cancer patients express auto-antibodies against pKi-67 and whether this has a prognostic relevance.

Methods: Auto-antibodies were detected by Western blot stainings from SW480 nuclear extracts with 36 pre- and 65 post-operative sera of colorectal cancer patients' sera. Sera of 20 voluntary healthy donors served as negative control. The same samples were simultaneously tested for p53 auto-antibodies.

Results: Thirteen percent of the sera proved to be positive for pKi-67 auto-antibodies while the control sera were completely negative. p53 auto-antibodies could be found in 53% of the patient sera. 75% anti-pKi-67 positive samples were also anti-p53 positive. For both antigens we found less positive antibodies in post-operative sera (pKi-67: 9%; p53: 42%) than in pre-operative sera (pKi-67: 19%, p53: 61%). There was, however, no significant correlation between pKi-67 positive sera and tumor stage (I: 13%, II: 4%; III: 23%; IV: 13%), grading or patient's prognosis. Remarkably, there is a significant ($p = 0{,}023$) correlation of pKi-67 positive sera of colon cancer patients (77%) in comparison to rectal cancer patients (60%).

Conclusion: PKi-67 auto-antibodies could be diagnostically valuable in the early detection of neoplasia and could be used as potential markers for recurrent or metastatic disease.

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P70. TRANSCRIPTIONAL AND MOLECULAR REGULATORS OF THE UROKINASE-RECEPTOR-(u-PAR)-GENE: FIRST ANALYSIS OF INDEPENDENT PROGNOSTIC RELEVANCE IN RESECTED COLORECTAL CANCER

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Purpose: Prognostic studies on transcription factors acting at specific promoter elements have never been performed so far. In previous studies we showed that the invasion-related gene *u-PAR* is regulated especially via an AP-2/Sp1(-152/-135)-, and an AP-1-promoter motif(-190/-171), mediating *u-PAR*-induction by