P16. CHEMORESISTANCE OF PANCREATIC TUMORS – A PROTEOME ANALYSIS

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Background: Tumors of the pancreas are characterized by a high potency to develop chemoresistance towards cytostatic drugs, which is the main cause of ineffective treatment. The biological mechanisms of this resistance are still unknown. We used a proteomic approach to analyse protein regulation of pancreatic cell lines treated with cytostatic drugs.
Methods: Three human pancreatic cancer cell lines (PANC-1, Paca44, CAPAN-1) were treated with 5-FU, Gemcitabine and Mafosfamide for 24 h. A 5-FU resistant CAPAN-1 cell line was developed through exposure to increasing concentrations of 5-FU. High-resolution 2D-gels were produced (IEF, SDS–PAGE) and the resulting gels were stained and digitalized. Image analysis was performed (PDQuest) and differentially regulated proteins were excised from the gel, digested, and submitted to mass spectrometry (MALDI-TOF-MS). Proteins were identified by Peptide Mass Fingerprint (PMF).
Results: We identified more than 80 cell line protein spots to date. Image analysis showed that more than 10 protein spots are differentially regulated – one of them identified as annexin IV. Work is ongoing to identify all differentially expressed proteins.
Conclusions: The proteomic approach is a solid and reproducible method for identifying differentially regulated proteins. Identification of chemoresistance-related proteins will further our understanding of the biological mechanism of chemoresistance and may lead to novel cyctostatic drugs.

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P17. EFFECTS OF TUMOR–STROMA INTERACTION ON GLOBAL GENE EXPRESSION IN BREAST CANCER

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Background: Perturbation in intercellular communication are a key feature of cancer. However, the systematic effects of cell–cell interaction on global gene expression in cancer are largely unexplored.
Methods: We simulated tumor–stroma interaction in vitro by systematically co-cultivating each of seven different breast cancer cell lines with stromal fibroblasts from three different sites, and determined associated gene expression changes with cDNA microarrays. A dataset of pretreatment gene expression profiles from 295 early stage breast cancers (stages 1 and 2) with a follow up of 12.6 years allowed us to evaluate the prognostic significance of the gene expression signatures of specific cell–cell interactions derived from our ex vivo models.
Results: Co-culturing normal human breast epithelial cells and breast cancer cells with stromal fibroblasts revealed multiple effects on gene expression. The most prominent was an up-regulation of interferon-response genes (IRG), which was detected in about half of the breast cancer co-cultures, but not with normal mammary epithelial cells. In vivo, expression of the IRG was remarkably coherent, providing a basis for segregation of the 295 early-stage breast cancers into two groups. Tumors with high expression levels ($n = 161$) of IRG were associated with significantly shorter overall survival; 59% at 10 years versus 80% at 10 years for tumors with low expression levels ($n = 134$) (log-rank $p = 0.001$).
Conclusion: This suggests that an interaction between some breast cancer cells and stromal fibroblasts can induce an interferon response, and that this response may be associated with a greater propensity for tumor progression.

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P18. QUANTITATIVE MULTIGENE EXPRESSION PROFILING OF PRIMARY PROSTATE CANCER

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Background: This study describes the evaluation of the expression pattern of prostate-specific transcripts in 106 matched prostate tissues as predictors for prostate cancer (PCa). RNA was prepared from cryo-preserved paired malignant and non-malignant prostate specimens, which had been removed during radical prostatectomy and examined by a trained pathologist.
Methods: Quantitative PCR (QPCR) assays with site-specific hybridization probes were established for four housekeeping genes and nine prostate-specific genes (AibZIP, DD3/PCA3, D-GPCR, EZH2, PDEF, prostein, PSA, PSCA, TRPM8). In the analyzed patient cohort, statistical differences for the commonly used housekeeping genes GAPDH ($p = 0.038$), HPRT ($p = 0.036$) and PBGD ($p = 0.00003$) were observed.
Results: The only housekeeping gene being not differentially expressed between malignant and non-malignant prostate tissues was TBP ($p = 0.531$). Therefore, all expression was normalized to TBP. The logarithmized relative mRNA expression of AibZIP, DD3/PCA3, D-GPCR, EZH2, PDEF (all $p < 0.001$), prostein ($p = 0.019$), PSA ($p < 0.001$) and TRPM8 ($p < 0.001$) were significantly higher in malignant vs. non-malignant prostate tissues. Receiver operating characteristic (ROC) curves were generated, and their areas under the curve (AUC) were calculated for all single parameters. DD3/PCA3 is the marker with the highest AUC (0.85), i.e. the best single tumor marker. A logit model was developed which employs the logarithmized relative expression levels of DD3/PCA3, EZH2, prostein and TRPM8 and yields an AUC of 0.90.
Conclusion: It can be concluded that DD3/PCA3 is a powerful predictor of PCa but the addition of EZH2, prostein and TRPM8 adds even more to the predictive power.

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