

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 cytostatic 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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