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Secretome as a promising source for biomarker discovery in pancreatic cancer

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Until now pancreatic carcinoma is almost exclusively diagnosed in the late stage. The average survival time after diagnosis is only 5-6 months and less than 1% of patients survive 5 years. Therefore, the goal of this project was the development of a blood-based assay for the diagnosis of pancreatic cancer in high risk patients.
We delineated the secretome of pancreatic carcinoma cell cultures using one-dimensional polyacrylamide gel electrophoresis coupled with liquid chromatography electrospray tandem mass spectrometry (LC-ESI-MS/MS). We utilised an LTQ-Orbitrap mass spectrometer combined with nanoUPLC to identify proteins from the secretome of the pancreatic cell lines.
In total, we identified 2859 unique proteins, which were released from the six cell lines (BxPc3, A818, Panc1, MiaPaca2 and Paca44). We chose three potential biomarkers from these catalogues, namely the protocadherin Fat1, the Amyloid Precursor Protein (APP) and EGFR for further validation. Release of the Fat1 ectodomain was shown in secretomes from human pancreatic cell lines and colorectal carcinoma cell lines (SW948, SW620, CaCo2) via Western blotting with antibodies directed against an intracellular and an extracellular epitope. Using mass spectrometric analysis of gel slices we showed that the Fat1 ectodomain is clearly present in the secretome of pancreatic cancer cell lines. The ectodomain also appears to be produced in vivo, as it was detected in the serum of a patient with ovarian cancer.
Another potential biomarker is the Amyloid Precursor Protein (APP). Our mass spectrometric data showed that APP undergoes proteolytic processing to release a soluble N-terminal ectodomain fragment (sAPP), which is found in secretomes from pancreatic cancer cells (Panc1, Paca44, MiaPaca2 and BxPC3). We confirmed these data by Western blot analysis using antibodies directed against the ectodomain and cytosolic epitope of APP. In addition we performed immunohistochemical labelling using the same antibodies. This indicated the overexpression of the APP protein in small and large ductal epithelium in pancreatic cancer tissue, whereas these cells in donor pancreas tissue indicated no immunolabelling.
We also detected diverse forms of the EGFR released into the conditioned medium of cultured pancreatic cancer cells, namely a soluble fragment and two membrane forms residing on exosomes. The 110 kDa soluble fragment consists of the highly glycosylated extracellular ligand binding domain of EGFR. Released on exosomes we found the 170 kDa intact full-length transmembrane EGFR. In addition we detected a 65 kDa form on exosomes, which consists of the intracellular kinase domain and presumably retains the transmembrane domain; this form most probably represents the C-terminal remnant fragment corresponding to the 110 kDa N-terminal soluble fragment of the EGFR.