Title of the Contribution:
Analysis of the pancreatic tumor progression by a quantitative proteomic approach and immunohistochemical validation.

Abstract:
To increase the knowledge about the development of pancreatic ductal adenocarcinoma, (PDAC) detailed analysis of the tumor progression is required. To identify proteins differentially expressed in the pancreatic intraepithelial neoplasia (PanIN), the precursor lesions of PDAC, we conducted a quantitative proteome study on microdissected PanIN cells. Proteins from 1000 microdissected cells were subjected to a procedure combining fluorescence dye saturation labeling with high resolution two-dimensional gel electrophoresis (2-DE). Differentially regulated protein spots were identified using protein lysates from PDAC tissues as a reference proteome followed by nanoLC-ESI-MS/MS. Thirty-seven single lesions of different PanIN grade (PanIN 1A/B, PanIN 2, PanIN 3) from nine patients were analyzed. Their protein expression was compared with each other, with PDAC cells and with normal ductal cells. The differential expression of differentially regulated protein spots was validated by means of immunohistochemistry using tissue microarrays. Of 2500 protein spots, 86 were found to be significantly regulated (p ≤ 1.6) during PanIN progression. Thirty-one nonredundant proteins were identified by mass spectrometry. Immunohistochemistry
revealed that the differential expression of the selected candidate proteins major vault protein (MVP), anterior gradient 2 (AGR 2) and 14-3-3 sigma, annexin A4, and S100A10 could be successfully validated in PanIN lesions. The highly sensitive and robust proteome analysis revealed differentially regulated proteins involved in pancreatic tumor progression. The analysis of normal preneoplastic and neoplastic pancreatic tissue establishes a basis for identification of candidate biomarkers in PanIN progression that can be detected in pancreatic juice and in serum or are candidates for in vivo imaging approaches.