Effect of extracellular matrix components on the expression of epithelial-to-mesenchymal transition markers in cultured human pancreatic ductal adenocarcinoma cells

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Epithelial-to-mesenchymal transition (EMT) is a step-wise process leading to the phenotypic switch of epithelial to mesenchymal cells, providing these cells with a metastatic phenotype. During EMT epithelial cells lose adhesion by down regulation of E-cadherin and express N-cadherin, display cytoskeleton reorganization by expressing vimentin and α-smooth muscle actin (αSMA), acquire motile properties and become invasive by secretion of matrix metalloproteinases (MMPs).

Cancer cell phenotype is influenced by the tumor microenvironment in relation to tumor progression, as well as to cell proliferation and invasion.

The role of the extracellular matrix (ECM) in the microenvironment is particularly relevant in pancreatic ductal adenocarcinoma (PDAC) since this carcinoma is characterized by an intense desmoplastic reaction, representing the environment where the complex interplay between tumor cells, stromal fibroblasts and ECM components occurs.

We aimed at analyzing in vitro the effect of the crosstalk between PDAC cells and their microenvironment by characterizing PDAC cell phenotype in cells cultured on different ECM proteins used as a substrate, in order to better understand the relationship between cancer cell behaviour and the proteins occurring in the desmoplastic tissue.

We analyzed by immunofluorescence the expression of the main EMT markers such as E-cadherin, N-cadherin, β-catenin, αSMA, vimentin and collagen type I (COL-I) in PDAC cells cultured on laminin, fibronectin, COL-I and without coating (NC). Moreover, we investigated cell proliferation and MMPs activity in cell culture supernatants by SDS-zymography.

Cell morphology was similar in PDAC cells cultured on laminin, fibronectin, COL-I, and in NC, as well as the E-cadherin/β-catenin complex, αSMA and COL-I expression; by contrast, vimentin was undetectable in all the experimental conditions. N-cadherin was slightly detectable in cells cultured on fibronectin, COL-I, and laminin, and at lower extent in NC cells. Cell proliferation resulted similar in NC and in cells cultured on fibronectin, decreased on laminin and increased on COL-I. MMP-9 activity exhibited a similar trend, resulting similar on fibronectin, decreased on laminin and stimulated on COL-I.

These preliminary results provide new insights in the characterization of the mutual effects elicited by the tumor-stroma interplay on the cancer cell, and will contribute to better understand the influence of the stroma on PDAC cancer cell phenotype, in order to develop new therapeutic strategies.

Keywords
Epithelial-to-mesenchymal transition, tumor microenvironment, pancreatic ductal adenocarcinoma, matrix metalloproteinases.