Title: Targeting the cancer (stem) cells – tumor microenvironment crosstalk to improve pancreatic cancer prognosis

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Introduction: To date, most efforts to identify new therapeutic targets for pancreatic cancer (PC) have focused on tumor cells themselves or on the tumor as a whole. Histologically, however, pancreatic cancer consists of a heterogeneous population of tumor cells including cancer stem cells and desmoplastic stromal tissue, which account for up to 90% of the tumor area and is mainly constituted by pancreatic stellate cells (PSC). The formation of distant metastases is the deadliest phase of cancer progression and the stromatic compartment play a critical role during this process. Current molecular diagnosis of PC is based on the analysis of epithelial cells and very little is known about the stromal compartment. Interestingly, a broad subset of PCs is characterized by high levels of TGFβ1 and by the prominent TGFβ signaling in tumor stromal cells in the primary tumors and in the metastatic site. We hypothesize two models on how this may occur (i) cells-autonomous: the cells holding metastasis initiating capacity are those capable of activating the TGFβ1-driven stromal response by themselves at the metastatic site. (ii) non cell-autonomous: High levels of TGFβ1 or other cytokines secreted by the primary tumor may act systemically to activate stromal cells residing at distant organs and to prepare the metastatic niche prior to the seeding of tumor cells. The main goal of this project is to unravel how the microenvironment of the primary tumor helps tumor cells to colonize a distant organ.

Results: By RNAseq, we compared the expression profile of four human primary cell lines cultured as anchorage independent spheres at low passages (enriched in cancer stem cells/CSCs) versus the culture in monolayer (enriched in differentiated cells). We found 20 common up-regulated genes in CSC; among them, we selected SCA-1. We showed that SCA-1+ (positive) cells defines a pure CSCs subpopulation able to promote tumor formation more efficiently than the SCA-1- (negative) population. Then, by RNAseq we compared the expression profile of SCA-1+ cells versus SCA-1- cells. We focused our attention on L1CAM (L1, strongly downregulated, >20 fold, in SCA-1+). We found that the majority of pancreatic tumor cells has reduced L1 expression, that involves a more mesenchymal phenotypes and an augmented resistance to chemotherapy. By sorting different patient derived-xenograft (PDX) cells for the L1 expression and by genetically downregulating L1 through the use of shRNA vectors, we found that the L1- cells (both sorted and silenced) were more poorly differentiated and had enhanced CSC phenotypes, including self-renewal, migration, invasion and chemoresistance. In vivo the L1- cells display enhanced early tumorigenic potential and were able to recapitulate the tumor heterogeneity compared to their L1+ counterpart. Conversely, ectopic overexpression of L1 resulted in a consistent decrease in cell proliferation and reduction in the stem property of tumor cells, thus indicating that restoration of L1 expression counteracts the malignant behavior of the tumor cells, thus leading to a less aggressive phenotype. Mechanistically we found that the PSC-derived TGFβ1 increased in vitro the migratory potential and the chemoresistance to gemcitabine of the PC cells through the reduction of L1 expression, while the in vivo co-injection with tumor cells enhanced their tumorigenic potential. PSC silenced for TGFβ1 were not able to modulate the L1 expression, and in vivo these cells completed failed to form visible tumors when co-injected with PC cells. Altogether these dates demonstrate that PSCs represent a supportive niche for PC cells promoting their aggressiveness and stemness through the downregulation of L1 mediated by TGFβ1 (2).

Future prospectives: Our findings will pave the way to 1) identifying additional factors/genes involved in the metastatic spreading and expressed by the different tumor
cell subpopulations in vivo and in an organ specific manner, 2) improving the patient staging system, and 3) identifying ways to interfere with the formation of metastatic niche.

References:


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