**Reg3β contributes to the immunosuppressive tumor microenvironment in pancreatic cancer.**

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Abstract:  
Reg3β is up-regulated in serum and pancreatic juice from patients with pancreatic ductal adenocarcinoma. However, whether such expression is relevant to the development of tumors has not yet been carefully examined. We have recently demonstrated that silencing Reg3β in a pancreatic cancer model impaired tumor growth by skewing macrophage polarization.
Pancreatic ductal adenocarcinoma (PDAC) continues to be a leading cause of cancer-related deaths worldwide due to its late diagnosis and the high-intrinsic resistance to standard treatments for advanced disease. During the past decade, numerous promising agents - targeting some properties of cancerous cells such as angiogenesis, proliferation and metastasis - have been evaluated alone or in combination with gemcitabine. Unfortunately, most of them have so far failed to significantly increase the overall patient survival (1), indicating an urgent need for novel treatment approaches that provide improved patient outcomes.

Nonetheless, it’s not all about tumor cells: the functional roles and contributions made by recruited stromal cells to tumor biology have made remarkable progress in cancer research. Continuous interactions between tumor cells and host stroma cells are increasingly recognized as being fundamental for tumoral growth, invasion, and metastasis. One of the defining features of PDAC is the generation of a dense desmoplastic stroma which accounts for up to 80% of the tumor mass (2). Among the cellular components of the compartment, macrophages are present in early PDAC and persist throughout the evolution of tumors (3).

During cancer progression, macrophages are recruited into the tumor microenvironment, where they switch into tumor-associated macrophages (TAMs). TAMs have a phenotype and function similar to alternatively activated M2 macrophages being involved in the promotion of growth and angiogenesis, and suppression of adaptive immunity (4). In other situations, macrophages develop a classical M1 phenotype characterized by a pro-inflammatory cytokine profile and are potent killers of pathogens and tumor cells. Because of the high plasticity of macrophages, the M1 and M2 phenotypes are likely to represent the opposite ends of a functional spectrum.

Studies aimed at characterizing TAMs in PDAC have found up-regulated the expression of M2 markers in macrophages from patients with pancreatic cancer and in a murine metastatic pancreatic adenocarcinoma model. Interestingly, these M2-polarized macrophages were associated with larger tumor size and poor prognosis due to accelerated lymphatic metastasis (5).

Reg3β is a pancreatic secretory protein, rarely expressed in normal pancreas, but strongly over expressed in injured pancreatic tissue. Our group has previously reported Reg3β anti-inflammatory function during pancreatic inflammation, a potential risk for pancreatic cancer. Hence, targeted disruption of Reg3β gene in mice resulted in
enhanced inflammation of the pancreas (6). Reg3β has also been found up-regulated in serum and pancreatic juice from patients with PDAC, but its function in this context has not yet been carefully examined.

In a recent issue of Cancer Research (7), we demonstrated that Reg3β significantly contributes to immunosuppression during the progression of pancreatic cancer. Deletion of Reg3β in mice drastically impaired pancreatic tumor growth correlating with decreased angiogenesis and increased apoptosis of tumor cells. In a macrophage cell line, Reg3β inhibited the anti-tumor M1 phenotype in response to pro-inflammatory stimuli but, more interestingly, Reg3β enhanced the alternative differentiation of macrophages by inducing the expression of M2 target genes. Moreover, an analysis of angiogenesis-related factors in macrophages from deficient Reg3β mice bearing tumors revealed decreased marked reductions in MMP9 and VEGF, which is known to attract yet more macrophages to the tumor microenvironment. This situation provided an explanation for the poor vascularisation as well as the absence of metastasis found in these mice, suggesting the contribution of Reg3β to the angiogenic switch required for tumor progression during pancreatic carcinogenesis.

Since we observed the same extent of macrophage infiltration in tumors growing in wild type mice and those in Reg3β deficient mice, we hypothesized that Reg3β would act by skewing these macrophages toward an immunosuppressive and pro-tumorigenic M2 phenotype. Indeed, we confirmed this fact and we also found that the increased apoptosis of tumor cells in mice lacking Reg3β was not a direct consequence of the anti-apoptotic role described for this protein but was an indirect effect mediated by polarized macrophages.

Accumulating evidence has indicated that pharmacological skewing of macrophage polarization, from M2 to M1 phenotype, is able to maintain an anti-tumor activity. For instance, in a study with different tumor models, Guiducci et al. (8) tested the hypothesis whether the treatment of tumor-bearing mice with a macrophage chemoattractant would synergize with a treatment that would simultaneously switch the macrophage phenotype from M2 to M1. Administration of macrophage chemoattractant, in combination with a microbial stimulus and an anti-IL10 receptor antibody was shown to promptly skew the tumor-infiltrating macrophage phenotype and triggered an innate response regressing pre-established large tumors.

The importance of the polarization state of TAMs has also been highlighted in PDAC mice and human studies. An example has been recently provided using an agonist
antibody against the co-stimulatory molecule CD40 in a cohort of metastatic PDAC patients (9). CD40-activated macrophages switched M2-like TAMs into anti-tumor M1-like TAMs. These macrophages rapidly infiltrated pancreatic tumor, became tumoricidal and facilitated the depletion of tumor stroma, thus restoring tumor immune surveillance.

Finally, analysis of the mechanisms revealed that Reg3β-induced STAT3 activation in primary macrophages was associated with a phenotypic switch towards the M2 phenotype (Figure 1). In the pancreas, the transcription factor STAT3 is aberrantly activated in human PDAC and promotes PanIN progression through transcriptional regulation of anti-apoptotic and pro-proliferative genes (10).

With this in mind, and considering the central role of Reg3β in pancreatic disorders, inhibition of Reg3β would represent a promising and more effective specific approach in the treatment of pancreatic cancer, aimed at reprogramming TAMs in favour of an anti-tumor phenotype.


Figure 1: Pro-tumorigenic role of Reg3β in pancreatic adenocarcinoma. Reg3β secreted by pancreatic acinar cells surrounding tumors acts in a paracrine manner on peritumoral macrophages by skewing them towards the M2 phenotype. Mechanistically, Reg3β function is performed through STAT3 activation, a transcription factor required for the development and progression of pancreatic cancer.
Pancreatic pro-inflammatory microenvironment

M1 macrophage

Pancreatic acinar cell

Tumor cell

M2 macrophage

STAT3 signaling

Reg3β

Pancreatic tumor progression