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Disrupting the Balance between Tumor Epithelia and Stroma is a Possible Therapeutic Approach for Pancreatic Cancer

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Pancreatic ductal adenocarcinoma (PDAC) is a type of highly lethal malignant tumor. PDAC is locally invasive and is surrounded by a dense desmoplasia or fibrosis, which can involve adjacent vital structures. Previously, the effect of pancreatic stellate cells (PSCs) of stroma in the progression of PDAC has received more attention, and most *in vitro* and *in vivo* studies revealed that PSCs appear to confer biological aggressiveness. However, clinical trials targeting desmoplasia or PSCs showed disappointing results. Recent studies found that stromal components, especially activated PSCs, are able to inhibit the occurrence and progression of PDAC. Inhibition of the stroma or desmoplasia through genetic regulations or drugs accelerates the formation and progression of PDAC. Thus, we hypothesized that in various times and spaces, there is a balance between the tumor epithelia and stroma; once the balance is upset, the tumor traits may undergo certain changes. Therefore, finding the key changing points of this relationship to corrupt or influence it, instead of blindly inhibiting the stroma motivation or simply maintaining stroma activation, will destroy the cooperation or promote the competition and antagonism among cells. This approach may render tumors more vulnerable and thus unable to resist anti-cancer therapies.

MeSH Keywords: **Hedgehog Proteins • Pancreatic Neoplasms • Pancreatic Stellate Cells • Stromal Cells • Tumor Microenvironment**

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Background

Pancreatic cancer (pancreatic ductal adenocarcinoma, [PDAC]) is a type of highly lethal malignant tumor, with a median survival time of less than 6 months. Early pancreatic cancer is difficult to diagnose, and when diagnosed, the cancer is often at the progression stage. Pancreatic cancer is usually accompanied by infiltration and metastasis, causing a low surgical resection rate and poor treatment effect. Most patients will relapse after radical surgery, with a 5-year survival rate of less than 5% [1]. Moreover, the incidence rate of pancreatic cancer is still increasing and it is expected to become the second leading cause of cancer-related death within 15 years, ahead of breast, prostate, and colorectal cancer [2]. Therefore, investigating the biological mechanisms involved in the development of pancreatic cancer is still an urgent need in order to develop effective strategies for pancreatic cancer treatment [3].

Tumor Epithelia – Stroma Interactions in PDAC

The tumor microenvironment is composed of tumor epithelial cells, stromal cells, and other cellular components as well as extracellular matrix and various molecules that mediate intercellular interactions [4]. Compared to other malignancies, a typical histopathological feature of pancreatic cancer is the occurrence of a significant hyperplasia of the mesenchyme surrounding the local infiltrated tumor tissues, which is called “desmoplasia” [5]. Stromal components in fibrous tissue hyperplasia include pancreatic stellate cells (PSCs) or myofibroblasts, blood vessels, lymphatic endothelial cells, immune cells, and abundant extracellular matrix. Together, these stromal components form a complex tumor microenvironment that prominently affects the occurrence, growth, invasion, metastasis, and drug resistance of pancreatic cancer [6,7]. Clinical studies suggest [8] that the degree of stromal activation is an independent prognostic factor for pancreatic cancer. However, the molecular mechanism of pancreatic epithelia – stroma interactions is extremely complicated. Its causes and impact on tumor growth and invasion require further investigation.

PSC is a major component involved in the facilitation of desmoplasia in pancreatic cancer [9]. In normal tissues, PSCs only account for 4% of total pancreatic cells and are in a resting phase, whereas in pathological states (due to the influence of various growth factors, cytokines, and oxidative stress) PSCs are activated and transformed into myofibroblast-like phenotype cells that express α -smooth muscle actin (α -SMA), undergo active proliferation, and enhance migration. Simultaneously, PSCs also express cytokines, chemokines, and cell adhesion molecules, and secrete an excess of extracellular matrix and matrix-degrading enzymes [10]. Conversely, PSCs also affect the

biological behaviors of pancreatic cancer cells [11]. However, an in-depth understanding of how PSCs and desmoplasia affect the biological behaviors of tumor cells in pancreatic cancer is currently lacking.

A large Number of *in vivo* Experiments Suggest that Activated PSCs and Desmoplasia in the Pancreas Promote Pancreatic Cancer Progression

It is currently believed that, in the event of malignant transformation of tumor cells, many cytokines secreted by tumor cells will change the microenvironment surrounding the tumor cells, resulting in abundant stroma formation around the tumor tissue in PDAC. The stroma includes PSCs or cancer-associated fibroblasts (CAFs), white blood cells, and extracellular matrix. Through the secretion of cytokines, tumor cells transform the surrounding normal fibroblasts to CAFs [12]. These transformed CAFs impose feedback effects on the tumor cells through alteration in gene expression and cytokine secretion. *In vitro* [13] and *in vivo* models [6, 14] demonstrated that PSCs or CAFs significantly enhanced the proliferation and invasion of tumor cells. Moreover, CAFs can also suppress the immune response [15] and indirectly promote the survival and growth of tumors. Therefore, tumor cells and fibroblasts build a microenvironment suitable for malignant proliferation and tumor cell metastasis.

A wide range of studies [16–18] have shown that paracrine sonic hedgehog (SHH) protein, which is an Hh pathway ligand and derived from PDAC epithelial cells, is the pivotal factor in both the regulation of the pancreatic tumor microenvironment and the promotion of tumor development and metastasis. Tumor-derived SHH protein acts on PSCs [19], whose activation in turn promotes the malignant behavior of pancreatic cancer cells, including reduced patient survival rates, uncontrolled growth, invasion, and therapeutic resistance. In addition, we also reported [20] that, after activation by surrounding SHH signals, PSCs in pancreatic stroma secrete cytokines to promote tumor cell proliferation and invasion, which enhance tumor infiltration and metastasis. Furthermore, these stromal cells over-express nerve growth factors, leading to aberrant nerve growth. Our study confirmed the hypothesis previously proposed by other researchers [21] that activated PSCs regulate the tumor microenvironment to promote pancreatic cancer perineural invasion.

Clinical Trials Targeting Pancreatic Stroma and Desmoplasia Showed no Success

On the basis of previous studies on the pancreatic stroma and activated PSCs functions [22], researchers have proposed a new cancer treatment strategy, the anti-stroma therapy [23].

However, despite the fact that the suppression of PSCs or CAFs functions *in vitro* and in animal models achieved significant anti-tumor effects [24], the clinical trials had disappointing results and sometime the opposite results [25]. Specifically blocking fibroblast function in pancreatic cancer has even led to accelerated tumor progression, ultimately resulting in a failed clinical trial. For example, in a phase II, randomized, double-blind, controlled clinical study on saridegib (IPI926, a cyclopamine derivative), 122 patients with previously untreated metastatic PDAC received saridegib plus gemcitabine or placebo plus gemcitabine treatment. The overall survival (OS) was the primary endpoint. Unfortunately, the interim data analysis showed that the median OS of the saridegib plus gemcitabine group was less than 6 months and the median OS of placebo plus gemcitabine group was more than 6 months. Thus, this clinical trial had to be terminated (NCT01130142; <http://www.clinicaltrials.gov/>). Although saridegib has been successful in the treatment of basal cell carcinoma [26], the unfavorable results of this SMO inhibitor in pancreatic cancer clinical trials have sparked suspicion towards anti-stroma therapy.

Loss of Function of PSCs Results in Higher Invasiveness of PDAC

What leads to the constant failure of current clinical trials targeting the tumor stroma? Several very recent studies may be able to provide some clues. Ozdemir [27], Rhim [28], Lee [29], and other research teams found that stromal components, especially activated PSCs, are able to inhibit the occurrence and progression of pancreatic cancer. Inhibition of the stroma or desmoplasia through genetic regulations or drugs accelerates the formation and progression of PDAC.

Ozdemir et al. [27] constructed a mouse model of pancreatic cancer with α -SMA+ fibroblast loss of function, through a gene knockout approach. They found that, after specific knockout for α -SMA+ fibroblast, pancreatic cancer cells in mice became more invasive. The epithelial-mesenchymal transition of cancer cells was also more prominent. Moreover, the survival rate of the mice was also significantly reduced. In mice with fibroblast loss of function, the number of regulatory CD4+ Foxp3+ T cells significantly increased, suggesting that the immunologic surveillance function was also significantly inhibited. In samples from patients with PDCA there was a positive correlation between the number of fibroblasts and patient survival time.

Rhim et al. [28] provided another clue from the SHH signaling pathway. SHH is a soluble protein secreted by pancreatic ductal adenocarcinoma cells, which can promote the formation of fibroblast-enriched matrix and microenvironment around the tumor. Similarly, they knocked out SHH in a mouse model of pancreatic cancer. Originally, they speculated that,

by knocking out SHH to inhibit fibroblast function, the malignant tumor phenotype could be suppressed. But unexpectedly, the results were the opposite of their original hypothesis. These SHH-knockout tumors became more metastatic and displayed more morphologic de-differentiation features; they observed a more extensive capillary network in these tumors and the cell proliferation capacity was also significantly elevated. Therefore, the tumor matrix does not promote tumor growth; instead, it plays a role in the inhibition of tumor proliferation.

Lee et al. [29] inhibited Hedgehog pathway through genetic and pharmacological approaches to affect the tumor epithelial-stromal balance in 3 different lines of genetically engineered mice and found that inhibition of the Hedgehog pathway led to the suppression of desmoplasia, accelerating rather than delaying tumor epithelium growth in pancreatic intraepithelial neoplasia lesion driven by Kras and tumor progression. Conversely, pharmacological-activated Hedgehog pathway induced mesenchymal hyperplasia that relieved tumorigenesis. Thus, they believed that desmoplasia constrained pancreatic cancer progression.

These solid data suggest to us that: (1) in pancreatic cancer tissues, PSCs or stroma play a protective role to suppress tumor progression; (2) clinical treatments targeting the stroma will promote a tumor malignant phenotype; (3) although these data could not overturn all the biological functions of PSCs or CAFs in stroma, the functions of stroma and stroma cells will need to be re-examined. The main reason for the above confusion is the complexity of the tumor stromal microenvironment. Because the tumor stroma contains many cell types, the number of these cells in the tumor microenvironment and the signal transduction pathways are very complex issues.

Hypothesis and Application: The Balance Between Pancreatic Tumor Epithelia and Stroma is Indispensable for PDAC Development

Do PSCs in the pancreatic cancer stroma promote or prevent cancer progression? The 2 opposing views discussed above were both supported by reliable data and rigorous experiments, which seems to constitute a contradictory and puzzling paradox that is difficult to explain. However, after careful scrutiny of a series of studies, we found that these 2 theories can coexist. Because the former theory has focused on advanced tumor stage when the tumor has already been formed, abundant PSCs promote tumor metastasis rather than accelerating growth (Figure 1B1) and the latter theory has concentrated on the early phase of tumorigenesis (Figure 1A1). When the stroma is completely removed, the tumor cells are more likely to be undifferentiated and show highly malignant behavior.

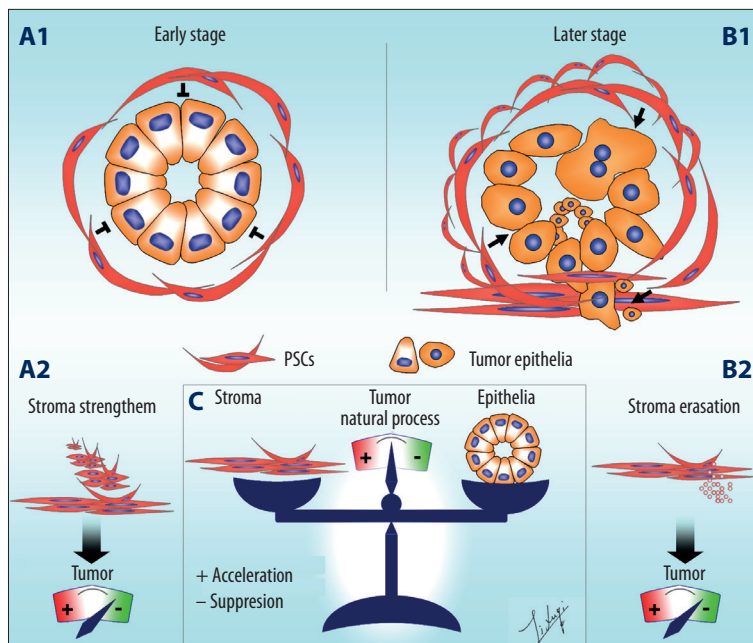


Figure 1. Disruption of balance between tumor epithelia and stroma is a possible therapeutic approach for pancreatic cancer. **(A1)** Stromal components, especially activated pancreatic stellate cells (PSCs), are able to inhibit the occurrence and progression of pancreatic cancer. **(B1)** Abundant PSCs promote tumor metastasis. **(C)** There seem to be a balance between the tumor epithelia and stroma in the tumor development process; once the balance is undermined, the tumor traits may undergo certain changes. **(A2, B2)** Corrupting of balance between tumor epithelial and stroma, instead of blindly inhibiting the stroma or simply maintaining stroma activation, will destroy the cooperation or promote the competition and antagonism among cells.

In various times and spaces, there seems to be a balance between the tumor epithelia and stroma; once the balance is undermined, the tumor traits may undergo certain changes (Figure 1C). Ardeshir Kianercy from Johns Hopkins University [30] tried to investigate relationships among cells, especially between cell growth and energy metabolism from a perspective of evolutionary game theory and he predicted the intercellular interaction from an ecological perspective. Different mesenchymal and epithelial cells inside tumor tissues are also in similar states of competition and cooperation, and can switch between the 2 states. Researchers used mathematical tools and computers to establish game parameters. According to their calculations, when the mutation rate stays within a particular range, if tumors suddenly change their energy metabolism strategy, the tumors have then undergone a key change. Such changes usually occur when the tumor is undergoing progression or formation. Similarly, Kadaba et al. [31] analyzed the effect on the biological behavior of cancer cells of various proportions of PSCs and cancer cells in the cell population, using a bio-engineered tissue culture model. They found that when

the cells were mainly PSCs (0.66 to 0.83) there was a maximum effect of increasing cancer cell invasion and reducing apoptosis. In addition, these cells also showed a maximum contractile effect on the surrounding extracellular matrix.

Thus, the stroma plays a dual role in tumors. In various time and space dimensions, the stroma or the relationship between the tumor and stromal cells can affect tumor formation and progression. Therefore, finding the key changing points of this relationship to corrupt or influence it, instead of blindly inhibiting the stroma or simply maintaining stroma activation, will destroy the cooperation or promote the competition and antagonism among cells (Figure 1A2, B2). This approach may render tumors more vulnerable and thus unable to resist anti-cancer therapies.

Conflict of interest

The authors declare that there is no conflict of interest related to his work.

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