

Cancer-Associated Fibroblasts in Pancreatic Cancer: Should They Be Deleted or Reeducated?

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Abstract

Pancreatic ductal adenocarcinoma is characterized by an extensive stromal response called desmoplasia. Within the tumor stroma, cancer-associated fibroblasts (CAFs) are the primary cell type. CAFs have been shown to play a role in pancreatic cancer progression; they secrete growth factors, inflammatory cytokines, and chemokines that stimulate signaling pathways in cancer cells and modulate the cancer biology toward increased aggressiveness. Therefore, targeting CAFs may serve as a powerful weapon against pancreatic cancer and improve therapeutic effects. However, a previous study aiming to deplete CAFs by inhibiting sonic Hedgehog signaling failed to show any benefit in survival time of pancreatic cancer patients. We reported that the natural product curcumin reeducated CAFs in pancreatic cancer treatment. A low concentration of curcumin reversed the activation of fibroblasts without exhibiting growth suppression effects. In addition, curcumin suppressed CAF-induced pancreatic cancer cell migration and invasion in vitro and lung metastasis in vivo. The results of our study suggest that active CAFs can be inactivated by certain natural products such as curcumin. Reeducation of CAFs back to their normal state, rather than their indiscriminate depletion, may broaden our view in the development of therapeutic options for the treatment of pancreatic cancer.

Keywords

cancer-associated fibroblast, pancreatic cancer, curcumin

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Pancreatic cancer is the fourth leading cause of cancer-related death and has an overall 5-year survival rate of less than 5%. Despite advances in clinical management, the high mortality rate of pancreatic cancer, which is almost equal to its incidence, remains largely unchanged.¹ The mechanisms regulating pancreatic cancer cell growth, invasion, and resistance to chemotherapy are poorly understood. Pancreatic ductal adenocarcinoma (PDAC) is characterized by an extensive stromal response called desmoplasia. Cancer-associated fibroblasts (CAFs) are the primary cell type in the tumor stroma,² and the important role of CAFs in tumor progression is now well accepted.

The Role of CAFs in Pancreatic Cancer

Compared with other malignant tumors, one of the defining features of pancreatic cancer is the presence of extensive desmoplasia.² The host response to invading cancer cells results in

the generation of fibrous tissue, or tumor stroma, in the tumor microenvironment through the desmoplastic reaction. The desmoplasia in pancreatic cancer is a complex structure consisting of activated fibroblasts and other kinds of stromal cells, such as endothelial cells, pericytes, inflammatory cells, and macrophages. The predominant cellular component of the stroma in pancreatic cancer is activated fibroblasts, which are called CAFs.³ CAFs have been shown to be involved in cancer progression through the secretion of growth factors, inflammatory cytokines, exosomes containing miRNAs, and metabolites that

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stimulate signaling pathways in cancer cells and promote aggressive biology, including tumor growth, metastasis, metabolism under stress, and chemotherapy resistance.³ For example, interleukin-6 released by CAFs promotes epithelial-mesenchymal transition (EMT) of pancreatic cancer cells, which contributes to metastasis progression.⁴ GM-CSF (granulocyte-macrophage colony-stimulating factor) produced by CAFs is also a key regulator in promoting tumorigenesis of pancreatic cancer.⁵ CAF-derived insulin-like growth factor and interleukin-1 β are suggested to drive chemotherapy resistance in pancreatic cancer.⁶ CAFs also secrete excessive amounts of structural matrix components, including proteoglycans, collagens, and fibronectin, together with matrix metalloproteinases, which are involved in degradation and dynamic remodeling of the matrix to create a pro-cancerous microenvironment.⁷ Collective evidence has led to the prevailing paradigm that CAFs act as a “partners in crime” with tumor cells to promote the progression of pancreatic cancer.

Deleting CAFs in the Treatment of Human Pancreatic Cancer

Considering the roles of CAFs in pancreatic cancer, one might assume that targeting CAFs would be a powerful weapon against pancreatic cancer and improve therapeutic efficacy. However, targeting CAFs has not always been successful. One important potential target is smoothened (SMO), which regulates the sonic Hedgehog (SHH) pathway. The Hedgehog pathway is hyperactivated in PDAC and stimulates CAFs to regulate stromal production. PDAC cells produce SHH ligand, which binds to its receptor Patched-2 located on CAFs. This results in signaling that removes the inhibitory effects of SMO and allows translocation of the Gli1 transcription factor into the nucleus and expression of a host of genes including extracellular matrix proteins.⁸ Several strategies to interrupt SHH signaling and thereby create stromal depletion have been proposed. Inhibition of the SHH pathway by small-molecule antagonists, such as cyclopamine, vismodegib (GDC-0449), erismodegib, and saridegib (IPI-926, a cyclopamine derivative), has shown promising results in preclinical studies.^{9,10} However, in phase II clinical trials (NCT01130142, NCT01064622), a combination of vismodegib and gemcitabine showed no benefit in progression-free survival and overall survival.¹¹ The proposed explanation for this lack of success is that depletion of the pro-stromal SHH pathway leads to fewer myofibroblasts and less tumor stroma.

Potential of the Natural Product Curcumin for Reeducating CAFs in Pancreatic Cancer Treatment

Most studies have shown that depletion of desmoplasia and CAFs improves the efficacy of gemcitabine in mouse models; however, clinical trials did not achieve equally satisfactory

results.¹¹ Results of recent studies support the idea that the desmoplastic stroma forms a barrier that reduces the invasion and metastasis of cancer cells.^{12,13} Hence, the roles of desmoplastic stroma seem to be context dependent, and induction of quiescence of CAFs might be a more promising approach than complete ablation of desmoplastic stroma in future development of therapies targeting tumor desmoplasia of pancreatic cancer.

Curcumin, commonly known as turmeric, is a natural polyphenol present in the *Curcuma longa* plant. As curcumin is nontoxic and has various therapeutic properties containing antioxidant, anti-inflammatory, analgesic, and antiseptic activities, it has been used prevalently in ayurvedic medicine for centuries.¹⁴ Recently, curcumin has been shown to have anticancer activities through its effect on different biological pathways involved in oncogene expression, cell cycle regulation, apoptosis, tumorigenesis, and metastasis.¹⁵ We recently showed that a high concentration of curcumin exhibited cytotoxic effects on CAFs. In contrast, a low concentration of curcumin minimally affected the proliferation of CAFs but decreased their expression of α -smooth muscle actin (α -SMA) and vimentin.¹⁶ This observation suggested that a low concentration of curcumin reversed the activation of fibroblasts. To further evaluate the effect of these inactivated fibroblasts on pancreatic cancer cells, we treated CAFs with a low concentration of curcumin and collected the conditioned media (CM). CM from nontreated CAFs increased the cell migration capacity of pancreatic cancer cells. However, CM from curcumin-treated CAFs had little effect on pancreatic cancer cell migration, suggesting that inactivating CAFs, instead of deleting them, suppressed pancreatic cancer migration. To further explore the effects of curcumin treatment of CAFs on metastasis in vivo, we examined metastatic nodules formed in the lungs of nude mice. Both the control and CAF-treated pancreatic cancer cells formed tumors in the lungs following tail vein injection. However, pancreatic cancer cells exposed to curcumin-treated CAFs produced significantly fewer and smaller lung tumors. Taken together, our findings indicated that curcumin might inhibit pancreatic cancer metastasis through targeting CAFs.

Compared with normal fibroblasts, CAFs acquire a myofibroblast-like phenotype accompanied by excessive secretion of extracellular matrix proteins including laminins, fibronectins, and collagens.¹⁷ In addition, CAFs express high levels of α -SMA, a marker of activated fibroblasts that is not expressed in normal quiescent fibroblasts. In our study, curcumin attenuated the EMT-promoting capacity of CAFs without exhibiting cytotoxicity. Moreover, it significantly suppressed the expression of α -SMA, fibronectin, and vimentin, suggesting that after treatment with curcumin the CAFs lose their mesenchymal features and are almost transformed back into normal fibroblasts.

In fact, our study is not the first to report that curcumin can target the dynamic mutual interaction of CAFs and tumor cells in cancer. Dudás et al previously reported that curcumin treatment of a coculture of oral squamous cell carcinoma cells and fibroblasts resulted in a decrease in tumor cell migration and invasiveness, reversal of EMT in tumor cells, and synthesis of invasion associated protein such as SDF-1 α and BDNF in fibroblasts.¹⁸ Modification of the interaction between CAFs and tumor cells is achieved at low curcumin concentrations, which do not have growth suppression effects. Similarly, Hendrayani et al also tested the effect of low concentrations of curcumin on patient-derived primary breast CAF cells¹⁹ and showed that curcumin treatment suppressed the paracrine proinvasive/migratory effects of CAFs on breast cancer cells. These results, combined with ours, demonstrate that active stromal fibroblasts can be inactivated (reeducated to a normal state) and provide proof of principle that the application of natural products such as curcumin might be used to normalize CAFs and suppress their procarcinogenic effects. In this regard, curcumin could be an efficient fibroblast-directed therapeutic approach.

Perspective

When considering the failure to prevent pancreatic cancer, the use of anti-stroma drugs for advanced pancreatic cancer is worthy of attention. Targeting stroma to prevent pancreatic cancer may provide new insights to understand the biological behavior of tumors and surrounding tissues.

As positive regulators of cancer progression, CAFs showed more resistance than tumor cells to chemotherapy treatment. To eradicate stroma, we often need to use a much higher concentration of cytotoxic drugs, and the side effects of these high concentrations may limit the efficiency of anti-tumor activity. The need to balance the side effects and pharmacological effects is frustrating. In addition, these findings indicate that the desmoplastic stroma might form a barrier that reduces invasion and metastasis. Our study provides the hypothesis that the mesenchymal features maintain the cancer promotion capacity of CAFs and pharmaceutical intervention to attenuate these features may abrogate CAF function. In addition, as conversion of quiescent to activated fibroblasts on tumor education drives the severe stromal reaction that characterizes pancreatic cancer, drugs that target CAFs have the potential to reverse CAFs to an inactive state and thus prevent cancer development. Although the crosstalk between stroma and pancreatic cancer cells makes the tumor response to chemotherapy more complicated, we need to understand these correlations in detail and should not simply regard targeting CAFs as indiscriminate depletion of the stroma. An attempt to reeducate the CAFs back to a normal state may broaden our view with regard to developing therapeutic options for the treatment of pancreatic cancer.

Declaration of Conflicting Interests

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