

Pancreatic Stellate Cells and Pancreatic Cancer Cells: An Unholy Alliance

Alain Vonlaufen,^{1,2} Phoebe A. Phillips,^{1,2} Zhihong Xu,^{1,2} David Goldstein,¹ Romano C. Pirola,¹ Jeremy S. Wilson,^{1,2} and Minoti V. Apte^{1,2}

¹Pancreatic Research Group, South Western Sydney Clinical School and ²School of Medical Sciences/Pathology, The University of New South Wales and Sydney, Australia

Abstract

Pancreatic cancer—a tumor displaying a particularly abundant stromal reaction—is notorious for its poor prognosis. Recent studies, via newly developed orthotopic models, provide compelling evidence of an important role for pancreatic stellate cells (PSC) in pancreatic cancer progression. Characterization of the mechanisms mediating PSC-cancer interactions will lead to the development of much needed alternative therapeutic approaches to improve disease outcome. [Cancer Res 2008;68(19):7707–10]

Introduction

It has long been recognized that malignant tumors do not represent a homogeneous mass of a single cell type but a highly heterogeneous mixture of cells of different lineages such as cancer cells, stromal cells, endothelial, and inflammatory cells. Hence, after decades of concerted research into the genomic instability of cancer cells, attention has gradually shifted to include the tumor microenvironment, in particular, the stromal cells. Many epithelial malignancies such as breast, prostate, ovarian, and pancreatic cancers often exhibit a significant stromal reaction around tumor cells.

Early studies in breast cancer have shown that the mitotic activity of stromal cells is significantly increased in the vicinity of epithelial components (1), suggesting a potential interaction between the two cell types. Human studies describing genetic alterations in breast cancer-associated stromal cells—some of which preceded genotypic changes in the epithelial component—suggested an active role for fibroblasts in the neoplastic transformation of epithelial cells (2). Subsequent studies have reported that breast cancer-associated fibroblasts (in contrast to normal fibroblasts) may initiate morphologic changes and proliferation of normal and preneoplastic breast cancer cells (3). Similar associations have been described for other cancer cell types, in particular, prostate cancer (4). The possible effects of genotypic changes in stroma on tumor behavior have also been recently examined. Patocs and colleagues (5) have shown that p53 mutations or allelic imbalance of stromal markers in stromal cells correlate with nodal metastasis, highlighting a central role for stromal cells in tumor progression. In contrast to breast and prostate cancer, research into the epithelial-stromal interaction in pancreatic cancer has been initiated only recently and has

proceeded relatively slowly, possibly due to limited access to tumor tissue and the lack of a suitable animal model.

Pancreatic cancer—a tumor with a propensity for early local and distant growth—represents the fourth leading cause of cancer-related death in the USA. In spite of advances in surgical techniques and adjuvant chemotherapeutic and radiotherapeutic regimens, the overall prognosis of pancreatic cancer has remained poor and virtually unchanged over the last three decades (6). Newer therapeutic regimens aimed at specific targets such as vascular endothelial growth factor (VEGF) and epidermal growth factor (EGF) in pancreatic cancer have yielded conflicting findings with little improvement in disease outcome (7).

From a histologic standpoint, pancreatic cancers display perhaps the most prominent stromal reaction of all epithelial tumors, the extent of which is often greater than the epithelial component. Studies with human pancreatic cancer sections have shown that increased expression of cytokines such as insulin-like growth factor-I (IGF-I; ref. 8) and fibroblast growth factor (FGF; ref. 9)—factors mainly secreted by mesenchymal cells—are correlated with disease prognosis. Improved insight into the mechanisms mediating epithelial-stromal interactions in pancreatic cancer has now been made possible by the ability to isolate and culture pancreatic stellate cells (PSC) *in vitro* (10, 11) and the knowledge that these cells are responsible for producing the stromal reaction in pancreatic cancer (12).

In health, PSCs exist in a quiescent state. In diseased states, under the influence of growth factors, cytokines, and oxidant stress, PSCs transform into a myofibroblast-like phenotype secreting excess amounts of extracellular matrix (ECM) as well as matrix-degrading enzymes (10). Growth factors such as transforming growth factor- β 1 (TGF- β 1), platelet-derived growth factor (PDGF), and VEGF, all known to induce PSC activation, are secreted by pancreatic cancer cells. Proof that pancreatic cancer cell secretions induce PSC activation has been provided by Apte and colleagues and Bachem and colleagues (12, 13). Notably, this activation was inhibited in the presence of neutralizing antibodies against TGF- β 1 and basic FGF (13). Recent studies have reported that cancer cells also have the capacity to secrete the ECM metalloproteinase inducer EMMPRIN (11). This leads to increased matrix metalloproteinase 2 secretion by PSCs, a factor that has been associated with the invasive phenotype of pancreatic cancer cell lines (14).

In vivo support for the interactions observed *in vitro* was first provided by Bachem and colleagues (13) who reported that s.c. coinjection of PSCs and cancer cells into the flanks of nude mice resulted in larger tumors with a significant stromal reaction than those produced by injection of cancer cells alone. In another study by the same group, coinjection of PSCs with cancer cells overexpressing the serine protease inhibitor SERPINE2 into nude mice was reported to result in increased tumor growth (15). These findings suggest that increased ECM (in particular, fibrillar

Requests for reprints: Minoti V. Apte, South Western Sydney Clinical School, Room 505, Level 5, Wallace Wurth Building, The University of New South Wales, Sydney, NSW 2052 Australia. Phone: 61-2-93858273; Fax: 61-2-93851389; E-mail: m.apte@unsw.edu.au.

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collagen) deposition as a consequence of protease inhibition might facilitate cancer progression, a concept supported by the observation that ECM proteins protect pancreatic cancer cells from apoptosis (16). Although the above studies provided important evidence of stromal-tumor interactions, it must be acknowledged that subcutaneous models are not an ideal choice for studies of abdominal malignancy, given that they do not allow the assessment of tumor behavior within the appropriate microenvironment and they do not have the appropriate (if any) metastatic pathways.

To overcome the limitations of subcutaneous models, we have developed an orthotopic mouse model of pancreatic cancer, as described in our recently published article in this journal (17). Using human pancreatic cancer-associated PSCs from several individual patients and injecting them in combination with an equal proportion of pancreatic cancer cells, we have shown a significant enhancement of both local cancer growth and distant metastasis (17). Importantly, no tumors developed in mice injected with PSCs alone. The observed facilitatory effect of PSCs on pancreatic cancer growth has also been reported by Hwang and colleagues (18) in a similar model of pancreatic cancer induced by coinjection of immortalized human PSCs from a single patient and the cancer cell line BxPC3 into the pancreas of athymic mice. In this study, too, immortalized PSCs alone were not reported to form tumors. However, the reported rate of

growth of these cells in culture (a doubling time of 12 hours) is significantly dissimilar from primary cultures of human PSCs. Thus, although the study by Hwang and colleagues (18) lends support to the concept of epithelial-stromal cross-talk in pancreatic cancer, the use of immortalized PSCs with possible significantly altered growth characteristics has to be analyzed with caution.

The observed growth advantage of pancreatic cancer cells in the presence of PSCs may be mediated by two mechanisms—increased mitosis and decreased programmed cell death (apoptosis). As shown in our model (17), animals injected with both cell types displayed a higher mitotic index than mice injected with cancer cells alone. PSC secretions significantly increased cancer cell proliferation, and this effect was partially abolished by neutralizing antibodies directed against the mitogenic factor PDGF. It is highly likely that other factors such as stromal-derived factor-1, EGF, IGF-1, or FGF might also exert mitogenic effects on cancer cells, and studies examining the role of these and other factors are currently under way. Figure 1 summarizes current concepts regarding the interaction between pancreatic cancer cells and pancreatic stellate cells.

Resistance to apoptosis is a common trait of many tumors. We and others have shown that PSCs reduce basal as well as induced apoptosis in various pancreatic cancer cell lines *in vitro* (19, 20). Importantly, this translates into reduced apoptosis *in vivo* in mice

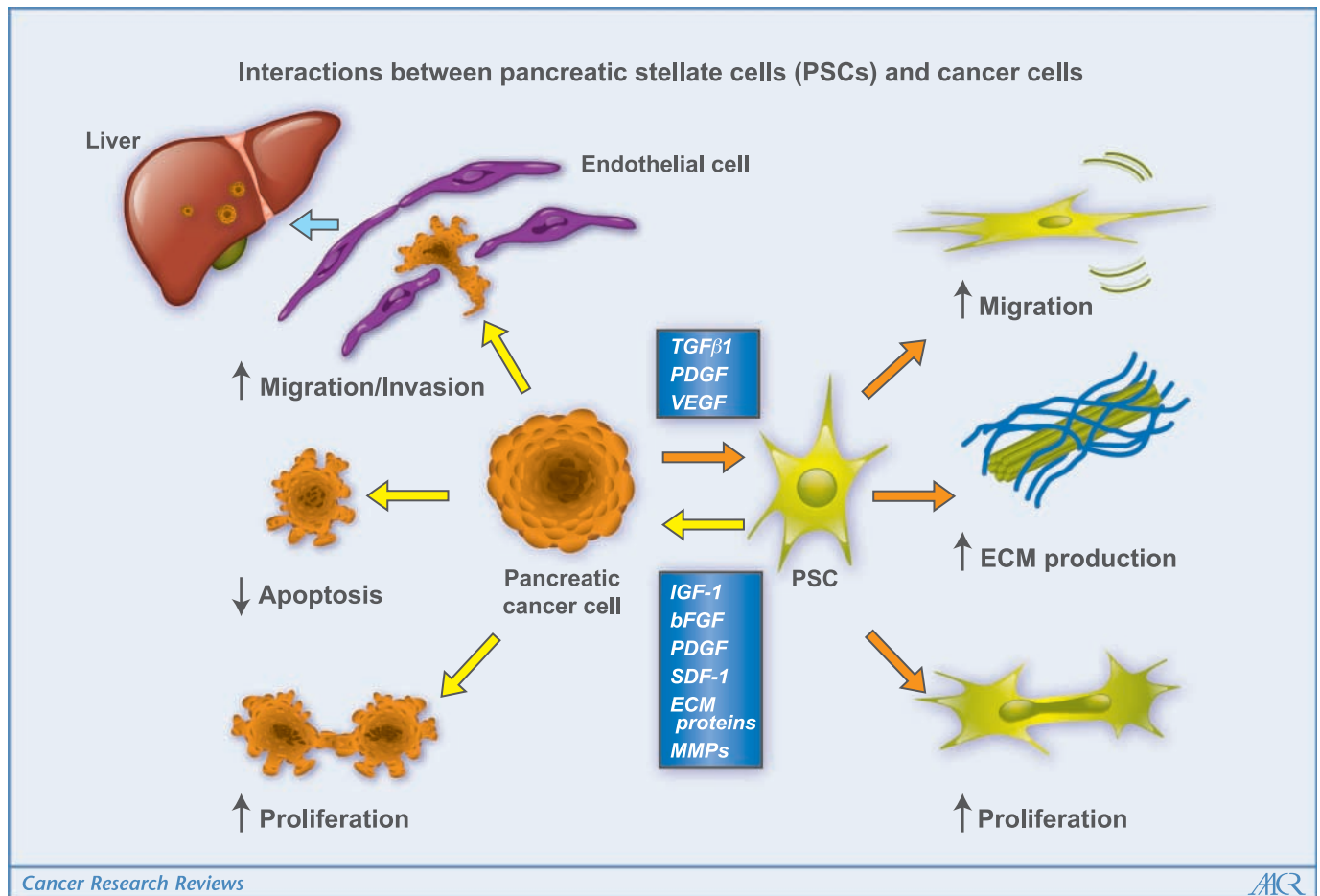


Figure 1. Schematic representation of the interactions between PSCs and cancer cells. Potential mediators are listed in the boxes. *bFGF*, basic fibroblast growth factor.

injected with PSCs and cancer cells, as opposed to animals injected with cancer cells alone. Inhibition of apoptosis might be related to up-regulation of anti-apoptotic proteins such as Bcl-2 and Bcl-xL. In addition, growth factors such as IGF-1 and basic fibroblast growth factor may exert anti-apoptotic effects on pancreatic cancer cells. A novel apoptosis regulating pathway has been described involving the “inhibitor of apoptosis” protein family. One of its representatives—survivin—has recently been shown to be up-regulated in pancreatic cancer (21). The role of these postulated mechanisms in mediating the PSC-induced reduction of cancer cell apoptosis is currently being studied in our laboratory.

In our hands, tumors produced by injection of a mixture of PSCs and cancer cells into the pancreas of nude mice displayed large bands of fibrous tissue, comprising ECM, blood vessels, and human PSCs, thus mimicking the situation in human pancreatic cancer. Interestingly, a very small proportion of PSCs seemed to be derived from the host (mouse) as evidenced by negative immunostaining for the human nuclear antigen (marker for human cells) but positive α -smooth muscle actin (α SMA) staining within the stromal bands. Tumors in mice injected with pancreatic cancer cells alone also displayed some activated PSCs, suggesting that cancer cells may recruit host stromal cells (PSC) to their vicinity by inducing PSC migration. The ability of activated PSCs to migrate is well-established (10). More recently, we have shown that coculture with cancer cells significantly enhances the migratory capacity of PSCs (17). It is also pertinent that not only do cancer cells recruit PSCs to their vicinity, they also increase PSC proliferation (12), thereby increasing the potential for a growth permissive environment.

Given the above, the following question arises: are the changes observed in PSCs reversible or do cancer cells irreversibly prime PSCs in their vicinity? Genotypic comparisons of cultured normal human PSCs with chronic pancreatitis- and cancer-associated PSCs would be expected to shed light on this issue. This notion is supported by studies highlighting differential regulation of genes (encoding growth factors and signaling pathways) in micro-dissected pancreatic cancer-associated stroma versus chronic pancreatitis-associated stroma (19). However, studies with whole stromal tissue cannot rule out confounding effects of other stromal components, such as endothelial cells and inflammatory cells. Therefore, studies with pure preparations of PSCs are needed to provide more specific results.

Early distant metastasis is one of the major factors responsible for the poor outcome of pancreatic cancer. Using an orthotopic model of pancreatic cancer produced by injection of PSCs and cancer cells, we have shown that the presence of PSCs increases distant spread. This is likely due to increased migratory capacity as well as invasiveness of cancer cells (17). Another mechanism of

PSC-induced increased metastasis may be stimulation of angiogenesis by PSCs, a hypothesis that remains to be shown. An interesting observation in our study was the presence of human PSCs in metastatic nodules in the liver of nude mice, suggesting that PSCs might comigrate with cancer cells to distant sites and perhaps aid in the implantation of tumor in the new microenvironment. This observation—based on serial sections of liver metastatic nodules stained with antibodies directed against human nuclear antigen and α SMA—implies significant cell-cell interactions between the two cell types. Pancreatic cancer cells have been shown to express integrins of the β_1 family, and integrin expression seems to enhance their invasive phenotype (20). Although integrins facilitate interactions with components of the ECM such as collagen I, no direct interaction of tumor integrins with adhesion molecules on the surface of PSCs has been reported thus far. To address the question of whether PSCs cometastasize with cancer cells, the former would have to be tagged and followed *in vivo*.

In conclusion, the study of epithelial-stromal interactions in pancreatic cancer is at an early stage and many questions remain to be answered. Factors regulating proliferation and apoptosis of stromal cells need to be identified and characterized. In particular, the differences between normal, inflammation, and cancer-associated PSCs have to be highlighted and pathways of genetic alterations identified. The origin of activated PSCs also deserves close scrutiny. Although it is commonly thought that most cancer-associated PSCs arise from the existing pool of quiescent PSCs, there is increasing evidence that there might be alternative sources. Zeisberg and colleagues (22) have shown that endothelial cells have the capacity to transform into activated myofibroblasts and that this transition may depend upon exposure to TGF- β 1. Pericytes and vascular smooth muscle cells might also be capable of mesenchymal transition. Finally, mesenchymal stem cells (MSC) of bone marrow origin seem to preferentially localize to regions of pancreatic tumor growth (23). Importantly, MSCs have been shown to convert into tumor-associated myofibroblasts in experimental insulinoma (24). With burgeoning interest in the field, a better understanding of the cross-talk between the different components of this highly aggressive malignancy is anticipated and should lead to the development of novel therapeutic strategies and an improvement in disease outcome.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Acknowledgments

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