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REVIEW

# Complex roles of the stroma in the intrinsic resistance to gemcitabine in pancreatic cancer: where we are and where we are going

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**Pancreatic ductal adenocarcinoma (PDAC) is among the most devastating human malignancies. The poor clinical outcome in PDAC is partly attributed to a growth-permissive tumor microenvironment. In the PDAC microenvironment, the stroma is characterized by the development of extensive fibrosis, with stromal components outnumbering pancreatic cancer cells. Each of the components within the stroma has a distinct role in conferring chemoresistance to PDAC, and intrinsic chemoresistance has further worsened this pessimistic prognosis. The nucleoside analog gemcitabine (GEM) is usually the recommended first-line chemotherapeutic agent for PDAC patients and is given alone or in combination with other agents. The mechanisms of intrinsic resistance to GEM are an active area of ongoing research. This review highlights the important role the complex structure of stroma in PDAC plays in the intrinsic resistance to GEM and discusses whether antistroma therapy improves the efficacy of GEM. The addition of antistroma therapy combined with GEM is expected to be a novel therapeutic strategy with significant survival benefits for PDAC patients.**
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## INTRODUCTION

Pancreatic cancer is among the most devastating of human malignancies and is currently the fourth leading cause of cancer-related deaths in the United States.[1,2] Pancreatic ductal adenocarcinoma (PDAC) is the most common form of pancreatic cancer and accounts for ~90% of all pancreatic tumors.[3] It is associated with an overall 5-year survival rate of <8%, exhibiting the poorest prognosis of all solid tumors.[2] One of the reasons for this poor prognosis is the high resistance of PDAC to conventional chemotherapy treatments.[4,5] Although intense research efforts have been made to develop chemotherapy options and patient-targeted therapeutic strategies, there has been no significant improvement in the overall survival (OS).[2] In addition to overcoming the challenges of chemoresistance, novel therapeutic strategies are desperately needed to improve patient outcomes.[2]

Following the initial success of gemcitabine (GEM) in advanced PDAC, combination therapies with GEM were administered to tackle locally advanced and metastatic disease with limited success.[6,7] This failure is attributable to many factors, including extrinsic or intrinsic resistance to GEM.[8–11] Notably, PDAC is tumor characterized by the development of extensive fibrosis termed desmoplasia, with stromal components outnumbering pancreatic cancer cells.[12] Thus, PDAC stroma is regarded as a determinant of GEM resistance. Abundant evidence indicates that the stroma plays an important role in extrinsic resistance by impairing GEM delivery (Figure 1); however, the stroma-mediated mechanisms of intrinsic resistance to GEM remain an active area of ongoing investigation. This review focuses on understanding how various components within the stroma are instrumental in mediating intrinsic resistance to GEM and whether antistroma therapies have positive effects on the efficacy of GEM. This research is expected to develop a novel strategy to increase the cytotoxic effects of GEM, eventually achieving a significant survival benefit. The addition of antistroma therapies is expected to increase the cytotoxic effects of GEM, increasing patient survival.

## STROMA CONFERS INTRINSIC RESISTANCE TO GEM IN PDAC

Compared with other malignancies, a cardinal histopathological feature of PDAC is the occurrence of prominent hyperplasia

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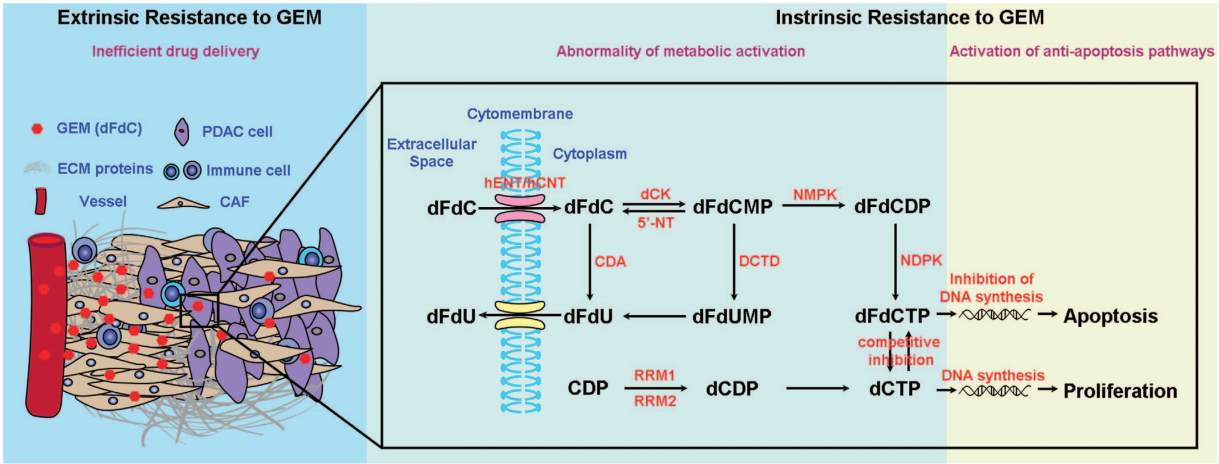


**Figure 1** The intrinsic and extrinsic mechanisms of gemcitabine (GEM) resistance. Intrinsic resistance refers to modification of transport mechanisms and the metabolism of the drug and activation of intracellular antiapoptosis pathways. Extrinsic resistance is primarily due to impairment of drug delivery.

of the stroma surrounding the local infiltrated tumor tissues that distorts the normal architecture of pancreatic tissue.[13] The primary cellular components of stroma are cancer-associated fibroblasts (CAFs), immune cells and endothelial cells, as well as acellular components such as collagens, laminin and cytokines that are stored in the extracellular matrix (ECM).[14,15] Interactions between the neoplastic and nonneoplastic cells and the acellular matrix have been proposed to stimulate the extensive desmoplastic reaction that is responsible for the main tumor bulk and accounts for up to 90% of the tumor volume.[14,16] One gene array analysis indicated that the gene expression pattern in GEM-resistant tumors was enriched in stroma-related pathways.[17] This experiment highlights the central role of stroma in GEM resistance in PDAC patients.[17] Stroma confers intrinsic resistance to GEM by mediating the innate or acquired modification of genes involved in GEM metabolism and activation of intracellular antiapoptotic signaling pathways.[18,19]

## Stroma affects GEM metabolism in PDAC

The metabolic availability and activity of GEM toward tumor cells is an important target of stromal interference that leads to intrinsic GEM resistance. Stromal interference of GEM availability and activity against tumor cells highlights the heterogeneous cell populations in the tumor. Certain transporters and metabolic enzymes that process GEM have often been related to GEM resistance in human pancreatic cancer and have therefore been proposed as predictive markers for the response to GEM in a clinical setting.[20] GEM metabolism is illustrated in Figure 1. Both human equilibrative nucleoside transporter and human concentrative nucleoside transporter transport GEM through the hydrophobic cell membrane.[21] Once in the cell, GEM (a prodrug; dFdC) is phosphorylated by deoxycytidine kinase to produce dFdC monophosphate (dFdCMP) and then phosphorylated again by pyrimidine kinases to its active diphosphate and triphosphate derivatives, dFdCDP and dFdCTP. The enzyme opposing deoxycytidine kinase, 5′-nucleotidase, catalyzes the conversion of nucleotides back to the monophosphate form. Moreover, the main mechanism of GEM inactivation is through deamination by cytidine deaminase to difluorodeoxyuridine. Because difluorodeoxyuridine is not a substrate for pyrimidine nucleoside phosphorylases, the drug is degraded and excreted out of the cell. Deamination of dFdCMP to difluorodeoxyuridine by deoxycytidylate deaminase is another inactivation pathway. Importantly, levels of dFdCTP must comprise a sufficient proportion of the cellular pool of dNTPs in order to be efficiently incorporated into DNA, and ribonucleotide reductase plays an essential role in the maintenance of the deoxyribonucleotide pool.[20] Eventually, the active form of GEM, dFdCTP, is integrated into DNA to impede the synthesis of DNA and exert antitumor effects (Figure 1). Various components within the PDAC stroma can affect the GEM transporters and metabolic enzymes, decreasing the accumulation of the active form of GEM in pancreatic cancer cells.[18,22,23] Stroma induces epithelial-to-mesenchymal transitions (EMTs) in neoplastic epithelial cells. Notably, EMT results in the suppression of human equilibrative nucleoside transporter/human concentrative nucleoside transporter,[24–26] inadvertently protecting $EMT^+$ cells from GEM. Thus, the stroma, by impairing GEM metabolism, is a key element in intrinsic GEM resistance.[27]

## Stroma activates antiapoptosis pathways to counteract the cytotoxicity of GEM

The final metabolite, dFdCTP, competes with endogenous dCTP to incorporate in the DNA, leading to the dislodgement of DNA polymerase from the DNA strand. GEM exerts its cytotoxic effects based on DNA damage at a magnitude that cannot be repaired at the cellular level. This masking of GEM by the extra nucleotide eventually induces apoptosis.[20,28] Resistance to apoptosis has been implicated in the moderate efficiency or failure of a number of anticancer treatments. Thus, another mechanism that contributes to intrinsic GEM resistance involves the activation of DNA damage repair

**Table 1 Clinical trials for antistroma therapy combined with GEM therapy clinical trials that are mentioned in this review**

| *Target* | *Agent* | *Trial ID* | *Combinations* | *Trial status* | *Trial phase* | *PDAC stage* |
|---|---|---|---|---|---|---|
| Hypoxia | TH-302 | NCT01746979 | GEM | Completed | Phase 3 | Locally advanced, unresectable pancreatic cancer |
| CTGF | FG-3019 | NCT02210559 | GEM+n-P | Recruiting | Phase 1/2 | Locally advanced, unresectable pancreatic cancer |
| CTGF | FG-3019 | NCT01181245 | GEM+erlotinib | Completed | Phase 1 | Locally advanced or metastatic pancreatic cancer |
| PDGFR | Imatinib | NCT00161213 | GEM | Completed | Phase 2 | Locally advanced or metastatic pancreatic cancer |
| VEGFR | Sorafenib | NCT00114244 | GEM | Completed | Phase 2 | Stage IV pancreatic cancer |
| VEGFR | Axitinib | NCT00471146 | GEM | Completed | Phase 3 | Advanced pancreatic cancer |
| SMO | saridegib | NCT01130142 | GEM | Completed | Phase 1/2 | Metastatic pancreatic cancer |
| SMO | Vismodegib | NCT01064622 | GEM | Completed | Phase 1/2 | Stage IV pancreatic cancer |
| SMO | Vismodegib | NCT01088815 | GEM+n-P | Active, not recruiting | Phase 2 | Metastatic pancreatic cancer |
| SMO | Vismodegib | NCT01195415 | GEM | Active, not recruiting | Phase 2 | Stage IV pancreatic cancer |
| SMO | Vismodegib | NCT00878163 | GEM+erlotinib | Active, not recruiting | Phase 1 | Metastatic pancreatic cancer |
| HA | PEPGH20 | NCT01453153 | GEM | Completed | Phase 1/2 | Stage IV pancreatic cancer |
| HA | PEPGH20 | NCT01839487 | GEM+n-P | Active, not recruiting | Phase 2 | Metastatic pancreatic cancer |
| HA | PEPGH20 | NCT02715804 | GEM+n-P | Recruiting | Phase 3 | Stage IV previously, untreated PDAC |

Abbreviations: CTGF, connective tissue growth factor; GEM, gemcitabine; HA, hyaluronan; n-P, nanoparticle albumin-bound-paclitaxel; PDAC, pancreatic ductal adenocarcinoma; PDGFR, platelet-derived growth factor receptor; SMO, smoothened; VEGFR, vascular endothelial growth factor receptor.

pathways and antiapoptosis pathways. This mechanism is deeply rooted in the genetic makeup of PDAC. PDAC harbors high-frequency mutations in major driver genes of cancer, including *Kras*, *CDKN2A*, *TP53* and *SMAD4*, numerous low-frequency driver mutations and regions of hypermutations.[29,30] These mutations dominate the complex genetic landscape of PDAC and independently hamper the apoptotic process. However, the formation of a complex genetic landscape cannot exist without the influence of the tumor microenvironment.[30] The stroma affects the expression of various genes and alters the signaling pathways in PDAC cells, including the extracellular signal-regulated kinase (ERK), AKT and signal transducer and activator of transcription 3 (STAT3) pathways.[18,31–34] Thus, PDAC cells are inherently resistant to GEM because the stroma is the same force that drives malignant transformation as well as GEM resistance[20] (Figure 1).

## MICROENVIRONMENT SUPPORTS PDAC AND COUNTERACTS THE CYTOTOXICITY OF GEM

### A hypoxic microenvironment mediates intrinsic GEM resistance

Desmoplastic stroma exerts pressure on blood vessels that impairs perfusion and frustrates the excessive metabolic demand for growth, resulting in hypoxic niches with insufficient nutrients.[30,35] Hypoxia stabilizes hypoxia-inducible factor-1α, a central node that mediates the activation of multiple signaling pathways that may contribute to GEM resistance.[24,36] Hypoxia-inducible factor-1α is overexpressed in GEM-resistant PDAC cells and is critically involved in EMT.[24,37] EMT leads to decreased expression of nucleoside transporters, contributing to decreased sensitivity of PDAC to GEM treatment.[27] In addition, hypoxia has been shown to maintain stemness that is also associated with GEM resistance.[38,39] Therefore, areas of hypoxic tumor tissue are more resistant to treatment.[40] Recently, a hypoxia-activated prodrug, evofosfamide (TH-302), was used to target cancer cells under hypoxic conditions.[41] It was tested in combination with GEM in a phase III clinical trial for treating patients with advanced pancreatic cancer (MAESTRO, NCT01746979; Table 1). Although there was a trend toward improved OS in patients treated with a combination of the two drugs, it was not a statistically significant improvement. The failure questions the new horizon in the treatment of PDAC: is the role of hypoxia as a potential therapeutic target for the treatment of PDAC?[42,43] However, PDAC is heterogeneous and harbors a heterogeneous microenvironment. Some pancreatic cancers may have a low level of hypoxia, and the inclusion of patients with these tumors might have weakened the statistical power. Thus, patients should be stratified based on the levels of hypoxia in their tumors to reassess the efficacy of combination therapy that is expected to increase OS in patients with high levels of hypoxia.

### Microenvironment-induced redox status affects intrinsic GEM resistance

Microenvironmental stress is involved in cellular metabolism reprogramming, from oxidative phosphorylation to aerobic glycolysis, a remarkable phenomenon called the Warburg effect.[35] Importantly, glycolytic PDAC cells tend to suppress mitochondrial function.[44,45] The mitochondrion is an organelle that regulates cell death pathways, not only by controlling intrinsic apoptotic pathways but also by generating reactive oxygen species (ROS).[46] Excessive production of ROS can cause cellular damage that ultimately leads to cell death. A redox-mediated pathway contributes to the intrinsic resistance of PDAC to GEM.[8,47] GEM treatment causes intracellular water ionization that produces ROS.[8] However, the generation of ROS is suppressed in tumor cells by metabolic reprogramming, and the cytotoxic effects are thereby directly reduced, conferring resistance to tumor cells.[48,49] Thus, cellular redox homeostasis is important to the intrinsic resistance to GEM, and

modulating ROS generation contributes to the design of drug combinations to overcome the resistance.[8]

### An acidic microenvironment mediates the intrinsic resistance to GEM

The Warburg effect is also an obviously beneficial tradeoff for cancer cells to increase chemoresistance by acidification.[50] The increase in glycolytic metabolism results in the production of lactate that acidifies the tumor microenvironment. The resulting extracellular acidification, coupled with hypoxia-inducible factor-1α-induced expression of carbonic anhydrases, causes a significant change in the pH ratio between the intracellular and extracellular environments. This pH shift decreases the passive absorption of many drugs that would otherwise accumulate at a greater concentration within the cell.[51,52] An acidic microenvironment can induce EMT that negatively affects GEM uptake. Moreover, acidification also mitigates oxidative stress,[53] likely providing a defense against GEM-induced ROS that contributes to chemoresistance.

## ACELLULAR, STROMAL COMPONENTS INVOLVED IN GEM RESISTANCE

### Type I collagen

PDAC is characterized by a pronounced fibrotic reaction composed primarily of type I collagen.[39] Type I collagen can affect various behaviors of PDAC, especially metastasis, by maintaining the invasive phenotype of cancer cells or by generating a barrier to invasion.[54,55] Opposing functions might be performed by different isoforms of type I collagen.[54] The normal isoform of type I collagen is a heterotrimer that is degraded by CAF-derived collagenases. However, a homotrimeric isoform was found in carcinomas and is normally not present in healthy tissues, suggesting that the homotrimeric protein might enhance the proliferation and migration of cancer cells.[56] These homotrimers are resistant to all collagenolytic matrix metalloproteinases (MMPs) and are produced by all invasive cancer cell lines, but not by CAFs, thereby comprising a specialized fraction of tumor collagen. Thus, invasive cancer cells may use homotrimers for building MMP-resistant invasion highways, facilitating metastasis by directing cancer cells to the vasculature needed for local proliferation and distant spread, whereas surrounding normal heterotrimeric collagens are cleaved.[54,56]

Collagen also contributes to GEM resistance. PDAC cells grown in a three-dimensional collagen microenvironment continue to proliferate in the presence of GEM.[18] Mechanistically, collagen increases membrane type 1-MMP (MT1-MMP) expression in PDAC.[25] Overexpression of MT1-MMP in the collagen microenvironment increases ERK1/2 phosphorylation and high-mobility group AT-hook 2 (HMGA2) expression and thereby further attenuates the GEM-induced checkpoint arrest to limit the effect of GEM *in vitro* and *in vivo*.[18] Through HMGA2 expression, a three-dimensional collagen microenvironment promotes histone H3K9 and H3K27 acetylation and increases histone acetyltransferase expression in PDAC cells to mediate GEM resistance *in vitro* and *in vivo*.[19] Notably, MT1-MMP potentiates integrin signaling only in the three-dimensional microenvironment, thus enhancing PDAC resistance to GEM.[57,58] Type I collagen induction of EMT is another mechanism of resistance in which pancreatic cancer cells respond to type I collagen by becoming more motile and invasive. Type I collagen binding to receptors upregulates the expression of two mesenchymal markers, Snail and N-cadherin, to further increase the expression of MT1-MMP, leading to EMT in PDAC.[25,26] It is not hard to see the central role of MT1-MMP in collagen-induced GEM resistance, suggesting that targeting MT1-MMP could be a novel approach to sensitizing pancreatic tumors to GEM.

### Hyaluronan

Hyaluronan (HA) is a nonsulfated glycosaminoglycan ECM component produced by HA synthases and degraded by hyaluronidases.[59] In normal physiological conditions, the amounts of HA in tissues are tightly regulated by a balance between synthesis and degradation. Compared with other malignant tumors in humans, HA content is highest in the desmoplastic stroma of PDAC[60] and plays a critical role in a variety of cellular processes, depending on its size and the cell type.[59,61] A HA-rich microenvironment may promote tumor progression by enhancing cell proliferation, metastasis and angiogenesis.[62–64] Importantly, strong HA expression is an independent prognostic factor in patients with PDAC.[65]

HA not only affects the malignant behaviors of cancer cells but also protects cancer cells against chemotherapy. HA bound to CD44 increases the phosphorylation of the stem cell marker Nanog. This contributes to an upregulation of the inhibitors of apoptosis proteins and multidrug-resistant protein-1 (MDR1), resulting in antiapoptosis and chemotherapy resistance.[66] Nevertheless, there is little experimental evidence to support the role of HA in the intrinsic GEM resistance of PDAC. HA induces EMT in PDAC through loss of epithelial E-cadherin and accumulation of cytoplasmic β-catenin.[67] However, binding of HA to CD44 activates the Ras/mitogen-activated protein kinase/ERK and phosphatidylinositide 3-kinase–Akt pathways[61,68,69] that are important for mediating GEM resistance and ensuring cell survival.[70,71]

After taking into account these critical roles of HA in PDAC progression, there has been great interest in developing therapeutic approaches targeting HA. Three different therapeutic approaches have been identified: (1) inhibiting HA synthesis, (2) blocking HA signaling and (3) depleting stromal HA in PDAC to improve chemosensitivity.[63] PEGPH20 is a pegylated hyaluronidase that effectively ablates stromal HA.[60,72] In addition to being effective in increasing GEM efficacy in a murine PDAC model,[72,73] PEGPH20 offers more insights for PDAC treatment in future clinical trials.[72] Some exciting results have been obtained in clinical trials of PEGPH20 (NCT01453153; NCT01839487; NCT02715804),[74] suggesting that HA ablation may be a promising therapeutic strategy for PDAC with high levels of HA (Table 1).

### Laminin

Laminin (LN) is another key component of the pancreatic ECM. Cytoplasmic expression of LN is correlated with a poor prognosis in pancreatic cancer.[75] In addition, the intrinsic chemoresistance of tumor cells has been shown to be induced by ECM–integrin interactions and is called cell adhesion-mediated drug resistance.[76] LN is one of the most effective ECM proteins for inducing cell adhesion-mediated drug resistance.[77,78] Moreover, PDAC cell adhesion to LN with the subsequent activation of signaling pathways contributes to the protection of cancer cells from the cytotoxicity of GEM.[10,77,79]

Focal adhesion kinase (FAK) functions as a critical intracellular molecule in transducing signals from the ECM to cells, and the level of constitutive phosphorylation of FAK at Tyr397 is correlated with the extent of intrinsic GEM resistance in PDAC.[34] LN-induced FAK phosphorylation is an important event for LN-mediated intrinsic chemoresistance to GEM in PDAC.[10] LN-induced FAK and AKT phosphorylation increase the levels of survivin expression and Bad phosphorylation at Ser136 that decrease GEM-induced cytotoxicity and apoptosis in PDAC.[10] FAK is a promising therapeutic target in pancreatic cancer. Targeted therapy against FAK could potentially be used to inhibit the cell–ECM interaction and thus suppress cell adhesion-mediated drug resistance.

### Cytokines and chemokines

Other acellular components of the tumor stroma, soluble cytokines and chemokines, are also central mediators of tumor–stroma crosstalk and are often described as GEM resistance modulators (Table 1). Connective tissue growth factor is overexpressed in PDAC and is currently a target for new therapies. Reportedly, antagonism of connective tissue growth factor with FG-3019, a monoclonal antibody against connective tissue growth factor, enhances GEM sensitivity without altering drug delivery in murine ductal pancreatic cancer.[80] Furthermore, the response to FG-3019 correlates with decreased expression of a previously described promoter of PDAC chemotherapy resistance, the X-linked inhibitor of apoptosis protein. Therefore, alterations in survival cues following targeting of tumor microenvironmental factors may play an important role in PDAC responses to treatment.[80] FG-3019 has been studied with GEM/erlotinib in localized or metastatic pancreatic cancer and conferred preferable OS rates.[81] This agent is currently being tested as a neoadjuvant therapy with GEM/nanoparticle albumin-bound paclitaxel (nab-paclitaxel) for locally advanced pancreatic cancer in an attempt to reduce the fibrotic stroma and enhance chemotherapeutic efficacy (NCT02210559). Human PDAC has been reported to overexpress transforming growth factor-β that activates the CAFs that are responsible for the formation of the ECM and acts as an immunosuppressor. Therapeutic approaches targeting the transforming growth factor-β pathway in PDAC are undergoing clinical trials.[14]

PDAC cells secrete a variety of CXC chemokines into their environment. A highly active axis in the cancer–stroma crosstalk is CXCL12/CXCR4 in PDAC. *In vitro*, treatment of PDAC cells with CXCL12 activates ERK and AKT signaling and promotes cancer cell resistance to GEM through inhibition of apoptosis.[82] PDAC cells do not necessarily exploit CXC receptor signaling in an autocrine manner. CXCL1 secretion by PDAC cells induces stromal fibroblasts in a CXCR2-dependent manner. Inhibition of CXCR2 also acts synergistically with GEM, resulting in extended survival of animals treated by both a CXCR2 inhibitor and GEM.[20,83,84] CXCL10, which mediates the crosstalk between pancreatic cancer cells and stellate cells through CXCR2, was recently shown to be correlated with reduced OS. This paracrine signaling pathway confers GEM resistance.[85]

Platelet-derived growth factor is a powerful chemoattractant for pancreatic stellate cells. Unfortunately, although imatinib, an inhibitor of platelet-derived growth factor receptors, improved the efficacy of GEM in preclinical trials,[86] a recently completed phase II study (NCT00161213) showed no difference in progression-free survival or OS between the imatinib plus GEM and GEM-alone arms of the study.[87] Targeting stromal-related angiogenic factors such as vascular endothelial growth factor and its receptor also demonstrated no activity when combined with GEM.[88–90] Poor perfusion and a deficient, nonangiogenic vasculature limits drug delivery and may also help to explain the recent failures of anti-vascular endothelial growth factor strategies in pancreatic cancer.

## CELLULAR COMPONENTS WITHIN STROMA INVOLVED IN GEM RESISTANCE

### Cancer-associated fibroblasts

In pathological states, CAFs are activated to transform from the usual quiescent cells to cells with a myofibroblast-like phenotype that express α-smooth muscle actin (α-SMA), undergo active proliferation, exhibit enhanced migration and secrete excessive amounts of ECM proteins.[24,91,92] These α-SMA-positive (α-SMA+) CAFs are also called activated pancreatic stellate cells in the pancreas. In addition to secreting insoluble ECM components, CAFs secrete soluble growth, angiogenic and inflammatory factors that engage in cancer and other stromal cell survival and metastatic signaling that promote tumor growth and invasion.[91,93,94]

Importantly, CAFs are innately chemoresistant; they stimulate the same signals in cancer cells as those targeted by therapies, conferring innate resistance. CAFs inhibit cancer cell apoptosis and induce resistance both *in vitro* and *in vivo*.[91] Furthermore, this antiapoptosis mechanism might be involved in the activated status of CAFs. Once the CAFs are induced into quiescence, they play negative roles in cancer cell proliferation and translocation of β-catenin to the nucleus and can increase cancer cell apoptosis.[32,33] Activated CAFs can alter tumor morphology,[33] suggesting that CAFs induce GEM resistance partly through promotion of EMT. CAFs promote EMT in cancer cells by reducing the expression of epithelial markers such as E-cadherin and increasing the expression of mesenchymal markers such as vimentin and Snail.[24] Moreover, increased Snail expression in cancer cells is a result of CAF-derived exosomes. CAFs exposed to GEM dramatically increase

the release of exosomes that support survival in recipient epithelial cancer cells, and the expression of Snail increases in the exosomes of GEM-treated CAFs.[95]

In addition to the increased release of exosomes, CAFs also secrete proteins that activate survival to promote chemoresistance of pancreatic cancer cells. Conditioned medium from CAFs promotes chemoresistance in PDAC cells and activates the ERK, AKT and STAT3 pathways.[31] Moreover, CAFs treated with GEM upregulate various inflammatory mediators. This GEM-induced, senescence-associated secretory phenotype is mediated by stress-associated mitogen-activated protein kinase signaling to play tumor-supportive roles in chemotherapy-treated CAFs *in vitro* and *in vivo*.[96] This supportive effect is also mediated by paracrine CXCL12α/CXCR4 signaling-induced activation of the intracellular FAK/AKT and ERK1/2 signaling pathways in PDAC.[97] Moreover, CAFs directly support the chemoresistance of PDAC cells by secreting insulin-like growth factor.[98] As mentioned above, CAFs synthesize abundant insoluble ECM proteins. These ECM proteins provide an ideal microenvironment for pancreatic cancer cells and decrease the cytotoxicity of GEM.[77] Thus, inhibition of the protein synthesis regulatory pathway represents a promising new therapeutic strategy for improving the chemoresistance triggered by the CAF secretome.[99]

Given the critical roles of CAFs in GEM resistance in PDAC, a therapeutic strategy of inactivation of CAFs, to impair their function, was developed (Table 1). The sonic hedgehog (SHH) pathway is a potent regulator of CAF activation. The SHH pathway is perhaps the most debated mediator of stroma-induced chemoresistance in PDAC. In GEM-resistant cell lines, blocking the SHH pathway improves GEM sensitivity.[100] Moreover, 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 multiple preclinical studies.[11,101–103] Encouraged by these promising results, several clinical trials have been launched using SHH pathway antagonists combined with GEM for PDAC treatment.[104] Unfortunately, the results have been either negative or equivocal. A phase II placebo-controlled study was conducted in patients with metastatic PDAC to assess the combination treatment of GEM and saridegib (NCT01130142). The clinical trial had to be terminated after the interim data analysis that showed a difference in survival favoring the placebo plus GEM arm due to a higher rate of progressive disease in the saridegib plus GEM arm. The median OS for the saridegib plus GEM arm was <6 months, whereas the median OS for the placebo plus GEM arm was >6 months.[105] Similarly, another phase II study did not observe any significant progression-free survival or OS benefit after adding vismodegib to GEM treatment in patients with metastatic PDAC (NCT01064622).[106] A single-arm phase I/II study using vismodegib in combination with nab-paclitaxel and GEM is currently underway (NCT01088815). An interim analysis estimates a median OS of 10 months for vismodegib in combination with nab-paclitaxel and GEM that is greater than the published historic controls of 8.5 months for GEM plus nab-paclitaxel.[107] The final results are still awaited and will need to be interpreted further.

The main reason for the conflicting preclinical and clinical results may be the heterogeneity and complex roles of stroma in PDAC. Because the tumor stroma contains many cell types, the number of these cells in the tumor microenvironment and the signal transduction pathways are complex issues. Different components of the stroma have potentially diverse roles. This is better understood in light of new preclinical evidence indicating that inhibition of the SHH pathway promotes tumor progression in PDAC and at least some stromal constituents can act to restrain tumor progression.[108,109] Activated stroma with high α-SMA expression and low collagen deposition was associated with inferior survival after surgical resection of PDAC.[110] Interestingly, this activated stroma was dependent on gemcitabine-induced changes in CAFs that were modulated by metronomic chemotherapy.[111] Moreover, the content of stroma and CAFs may change with different cancer stages. In an attempt to explain these confusing results, SHH pathways were inhibited in three distinct mouse models of Kras-driven PDAC. These preclinical models might only partially recapitulate the complex composition of the PDAC microenvironment, while the stromal desmoplasia was indeed suppressed by SHH signaling interventions.[11,108,109] Other reasons for the confusion are the chronic versus acute abrogation of SHH signaling, or the off-target effects of the SHH pathway inhibitors. Thus, inhibition of the SHH pathway has been suggested to act in a synergistic manner with more potent cytotoxic agents, such as nab-paclitaxel.[108] We need to gain a greater understanding of the involvement of the SHH pathway in PDAC and to determine how this signaling cascade should be targeted.

In addition to inhibiting the activation of CAFs, specifically depleting CAFs is another therapeutic approach to improving the efficiency of GEM in PDAC. In some studies, α-SMA-positive CAFs and type I collagen were associated with a worse prognosis in patients with PDAC, highlighting the impact of the stromal microenvironment on disease progression and patient survival.[112,113] However, in the PDAC model with depletion of α-SMA+ CAFs, depletion starting at both the early and late stages of pancreatic cancer led to the acceleration of PDAC progression with diminished animal survival, and α-SMA+ CAF-depleted tumors suppressed immune surveillance and did not respond to GEM. In PDAC patients, the number of α-SMA+ CAFs in their tumors was correlated with survival; specifically, fewer α-SMA+ CAFs were correlated with a reduced patient survival.[114] These results suggest that fibrosis associated with α-SMA+ CAFs and type I collagen might constitute a protective response from the host rather than offering an oncogenic supportive role, as speculated.[114–117] A minority of proliferating CAFs in PDAC tumors could aid survival by an α-SMA thymidine kinase strategy. An explanation for these inconsistencies is that the origin of CAFs in PDAC is diverse,[39,118,119] transforming into different subgroups that display various genetic contexts and biofunctions. Together, these studies underscore the need for caution in targeting CAFs in PDAC.[39] To devise effective treatment

strategies, instead of merely depleting CAFs, an in-depth understanding of how CAFs react to chemotherapy and how they may contribute to drug resistance is necessary.

**Immune cells**

Tumor-infiltrating immune cells can promote chemoresistance and metastatic spread in aggressive tumors. Consequently, the type and quality of immune responses present in the neoplastic stroma are highly predictive of patient outcome in several cancer types. PDAC is characterized as an inflammatory malignancy and an immune suppressive milieu is its best-described hallmark. Cancer immunotherapy generally offers limited clinical benefits without coordinated strategies to mitigate the immunosuppressive nature of the tumor microenvironment. Critical drivers of immune escape in the tumor microenvironment include tumor-associated macrophages and myeloid-derived suppressor cells that not only mediate immune suppression but also promote metastatic dissemination and impart resistance to cytotoxic therapies. Thus, strategies to ablate the effects of these myeloid cell populations may offer great therapeutic potential.

There is abundant intratumoral infiltration of macrophages and most of these tumor-associated macrophages (TAMs) are induced to a M2 phenotype by tumor cell-derived cytokines. Notably, M2-polarized TAMs induce GEM resistance in pancreatic cancer by inhibiting the activation of the caspase-3 pathway during GEM treatment, reducing GEM-induced apoptosis.[22,23,120] In PDAC models of mice, GEM is more effective in macrophage-depleted mice than in their wild-type counterparts. Similarly, inhibition of monocyte/macrophage trafficking in a transgenic mouse PDAC model that was resistant to GEM alone led to a better response to GEM.[121] Furthermore, analysis of multiple proteins involved in GEM metabolism revealed that TAMs enhanced the GEM resistance of PDAC by stimulating upregulation of cytidine deaminase.[22,23] The paracrine effects of TAMs might be mediated by several cytokines released by TAMs. These cytokines, including insulin-like growth factor, interleukin-4 and interleukin-27, activate the relevant pathways in PDAC cells to support acquired resistance to GEM.[23,98,122,123] TAMs also directly activate the transcription factor STAT3 to enhance the tumor-initiating capacity of pancreatic tumor cells[121] and STAT3 is involved in GEM sensitivity by downregulation of cytidine deaminase.[124]

## CONCLUSIONS

PDAC is a highly aggressive tumor with poor prognosis that also lacks effective therapeutic regimens compared with other human malignancies. This has encouraged us to improve our understanding of PDAC pathogenesis and biologic function, contributing to the development of a promising strategy for the treatment of PDAC. Desmoplastic stroma is one of the most important histopathological features of PDAC, making it a potential target for PDAC treatment. However, the complex roles of stroma and the failures of clinical trials using antistroma therapy make its future as a PDAC therapy unclear. Although the complex biochemical cancer–stroma crosstalk within the tumor microenvironment has a critical correlation with intrinsic resistance to GEM, heterogeneity within PDAC contributes significantly to the tumor response to chemotherapy, making this process more complex. Thus, we need to reassess the necessity of antistroma therapy and we should not simply regard it as an indiscriminate depletion of the stroma. More importantly, antistroma therapy combined with GEM should be administered to patients who have been stratified by stromal heterogeneity. An improved resistance to GEM is expected to extend the survival time of PDAC patients given antistroma therapy.

## CONFLICT OF INTEREST

The authors declare no conflict of interest.

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