Abstract. Pancreatic ductal adenocarcinoma (PDAC) is one of the most clinically challenging cancers to manage. An estimated 48,960 people will be diagnosed with pancreatic cancer in 2015, of that population, 94% are projected to perish within 5 years. These dismal survival rates can be attributed, in part, to an advanced diagnosis occurring in 80% of cases. The heterogeneous and dynamic microenvironment of pancreatic cancer, and the lack of both specific risk factors and efficacious screening tools contribute to the challenge of diagnosing pancreatic cancer in its early stages. These clinical challenges have directed research into the unique characteristics that define PDAC. Recently, there has been an increased focus on the interaction of tumor cells with their microenvironment in the hope of identifying new therapeutic targets. One of the most promising avenues in this new vein of research is targeting protein communication between the cancer cells and the extracellular matrix. The secreted protein acidic and rich in cysteine (SPARC) is one such extracellular matrix protein that has shown potential as a therapeutic target due to its influence on PDAC invasion and metastasis. In this review, we discuss the complex interaction of SPARC with PDAC cells and its potential to guide treatment and eventually improve the survival of patients diagnosed with this devastating disease.

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1. Introduction

Pancreatic cancer is projected to be the second leading cause of cancer-related deaths in the United States by the year 2030 (1). Pancreatic ductal adenocarcinoma (PDAC) is one of the most devastating malignancies worldwide, with a median survival rate of 3-5 months for metastatic and 6-10 months for locally advanced disease (2). These dismal median survival rates have remained consistent over the past three decades (3-5). The heterogeneous and multifactorial nature of pancreatic cancer, further clouded by a lack of specific risk factors, has hindered successful prevention strategies. Tobacco use and obesity are believed to increase a patient's risk of pancreatic cancer by 20-30% (6,7). Family history, race and gender also contribute to an increased risk of developing PDAC. Approximately 5-10% of pancreatic cancer patients report a history of pancreatic cancer in their family (8). African Americans are more likely to develop pancreatic cancer than Caucasians and men are 30% more likely to develop pancreatic cancer than women. Additional identified risk factors include: diets with excessive red meat consumption, alcohol abuse, and type II diabetes (9).

In addition to a wide range of risk factors, pancreatic cancer is exceedingly difficult to diagnose. Patients present with non-specific symptoms and anatomically the pancreas is not palpable on physical examination; leading to an advanced-stage PDAC diagnosis in 80% of cases (10). The lack of effective blood tests and other screening tools for early detection biomarkers has also contributed to the low rate of diagnosis of PDAC in pre-malignant stages. In order to confirm a diagnosis of pancreatic cancer, clinicians must use visualization technology, most commonly, computerized tomography (CT) and magnetic resonance imaging (MRI) scans (11). CT scans are used to identify the presence of pancreatic cancer lesions (12-14) while MRI scans are useful in assessing metastasis and local invasion (15). In addition to CT and MRI scans for diagnosing pancreatic cancer, endoscopic ultrasound provides information about vascular invasion (16), laparoscopy is used for more accurate staging (17), and fludeoxyglucose-positron emission tomography scanning is often employed for assessing
recurrent tumors (18). Despite the success of these technologies in diagnosing pancreatic cancer, none have proven effective as practical screening tools for patients in both general and high-risk populations.

Upon diagnosis with PDAC, a number of chemotherapy and radiation therapy options exist for the patient. However, the dynamic molecular composition of PDAC has nullified the effects of the majority of combination treatments that have proven successful in combating breast and hematologic cancers (19). Therefore, the immediate treatment strategy for PDAC includes surgical resection of the tumor followed by chemotherapy and radiation (20). While surgical resection of pancreatic tumors has been shown to increase survival rates by 10 months in case of stage I and II, the majority of patients that undergo these procedures experience recurrence or an associated co-morbidity (21) mainly due to late detection. In the past decade, pancreatic cancer research has shifted to investigate the unique tumor microenvironment as the source of PDAC therapy resistance (22-26). Decreased angiogenesis, cancer stem cells (CSCs), and dense stromal proliferation are all characteristics of the PDAC microenvironment that now serve as potential targets for future therapies.

Despite improved understanding of pancreatic cancer on a molecular level, overall patient survival rates have remained relatively stagnant (27). In order to advance treatment options and median survival rates of patients, we must first increase our understanding of the therapeutic resistant nature of pancreatic cancer.

2. Therapeutic resistance in pancreatic cancer

Cellular mechanisms. The majority of current research into the therapeutic resistance of pancreatic ductal adenocarcinoma (PDAC) is centered on the molecular composition of the pancreatic cancer cells (PCCs) and the surrounding stromal cells. Majority of PDAC cases show accumulation of highly penetrant genetic aberrations at four common genetic loci: the oncogene KRAS (95% of cases), and the tumor suppressor genes p53 (80%), CDKN2A (85%) and SMAD4/DCP4 (50%) (28). Point mutations in KRAS oncogene result in the constitutive expression of the Ras protein. The Ras protein initiates a signaling cascade that activates proliferative and cell survival pathways thus, increasing the tumor cell's invasive capacity (29-32). TP53 is a tumor-suppressing gene that is inactivated in ~80% of PDAC cases (28). The inactivation of TP53 leads to an impaired response to DNA damage due to a loss of cell cycle control and the lack of cellular apoptotic signals (33,34). CDKN2A is another tumor suppressor loci and is inactivated in 85% of PDAC (28). The genes p16\(^{INK4a}\) and p15\(^{ARF}\) are encoded at the CDKN2A locus and confer similar cellular malfunction seen with inactivation of TP53 (35,36). DCP4 gene mutations are seen in ~50% of PDAC and confer a metastatic phenotype (28). While these genetic mutations are responsible for the initiation and invasion of PDAC, the proliferation of the lesion is fostered by the unique microenvironment surrounding the tumor.

The stromal microenvironment. The stroma describes the interstitial tissue surrounding the malignant lesion in pancreatic ductal adenocarcinoma (PDAC) (32). Several stromal components contribute to a dense desmoplastic reaction seen in many epithelial tumors (breast, prostate, ovarian, colorectal) with pancreatic cancer exhibiting some of the most extensive stromal development (37). The desmoplastic reaction encourages tumor invasion, metastasis, and chemoresistance (37-39). In addition to its role in cancer invasiveness, the desmoplastic reaction also contributes to 90% of the tumor volume by promoting the deposition of extracellular matrix (ECM) (40). Initially, the desmoplastic reaction was thought to serve a protective function (41). However, a more recent study has shown that once the stromal components are activated, they can take on malignant phenotypes and may even contribute to carcinogenesis (38). The negative characteristics of the desmoplastic reaction further complicate treatment options. For example, any ‘activated’ stroma left behind after resection may encourage proliferation of small numbers of tumor cells missed by the procedure (42). In addition, this inflamed area can act as a barrier to chemotherapy and decrease the efficacy of adjuvant therapy following resection (42). These stromal reactions have also been observed to precede cancer development (43); further highlighting the importance of understanding the composition and molecular biology of the stromal microenvironment.

3. Pancreatic stellate cells and SPARC

Pancreatic stellate cells (PaSCs). Since their isolation in 1998 (44), pancreatic stellate cells (PaSCs) have been implicated in the maintenance of tissue architecture through regulation of ECM protein synthesis and degradation. PaSCs have been identified in a variety of organs including liver, kidney, intestine, and spleen (45). In the normal pancreas, PaSCs exist in a quiescent state and are activated in response to damage. Activated PaSCs allow the repair process to occur by triggering fibrosis through various stromal interactions (42). PaSCs are implicated in tumor progression because they secrete proteins that increase cancer cell invasiveness and migration, notably SPARC (42) and periostin (46).

4. SPARC and pancreatic cancer

Role of SPARC in PDAC. The secreted protein acidic and rich in cysteine (SPARC), also known as osteonectin or basement-membrane protein 40 (BM-40), is an extracellular matrix glycoprotein that is implicated in the metastatic potential of several cancer types (47). Normal SPARC expression occurs during mammalian development and is ultimately limited to tissues with high ECM turnover, such as gut, epithelia and bone. Wound healing, angiogenesis, and the stroma during tumorigenesis, induce SPARC activity (48). These processes suggest SPARC functions as a regulator of tissue remodeling in healthy cells. The role of SPARC in cancer has been shown to both promote and inhibit tumor progression, depending on the initiating cell type, tumor, stage, and context of the microenvironment (48). For example, in neuroblastoma tumors, SPARC significantly impaired tumor growth by acting as an anti-angiogenic factor produced by the Schwann cells (49). However, in pancreatic ductal adenocarcinoma (PDAC), pancreatic cancer cells (PCCs) induced overexpression of SPARC in PaSCs located in close proximity to malignant...
tumor cells (50). While at the same time, the PCCs themselves were unable to express SPARC due to aberrant hypermethylation of the CpG island on their promoter (51). The altered methylation patterns on the SPARC gene were then suggested as a potential biomarker for early PDAC detection (52). A small study evaluating 40 cases of pancreatic cancer, 6 chronic pancreatitis tissues, and 6 acute pancreatitis tissues were analyzed for these methylation patterns (52). The study concluded that aberrant methylation of CpG region 2 might be an early indication of PDAC development and progression and differentiate malignant tissues from healthy and chronic pancreatitis tissues (52).

SPARC influences growth and invasion of PCCs through a variety of pathways. In primary PDAC, SPARC was expressed in tumor cells and in the surrounding ECM components including fibroblast and endothelial cells (47). This indicates that SPARC may promote tumor infiltration into adjacent pancreatic tissue by affecting tumor-ECM interactions (47). PCCs change the composition of the ECM by increasing inflammatory cell recruitment, and promoting fibroblast proliferation (53), more specifically, increasing pancreatic stellate cell (PaSC) populations (53), which, in turn, secrete SPARC (42). In addition to influencing tumor-ECM interactions, SPARC is known to interact with growth factors, including VEGF and TGF-β (47). Overexpression of SPARC directly inhibits VEGF expression in PDAC cells (47). In addition, Notch signaling is suppressed by SPARC (54). Notch and VEGF are involved in numerous aspects of vascular development and angiogenesis (54). The combination of decreased angiogenesis and increased metabolic activity of the surrounding stroma results in a hypoxic environment (55). In PDAC, hypoxia is correlated with both increased tumor growth and metastasis (56). In addition to downregulating VEGF and Notch, SPARC also regulates MMP-2 expression and cytoskeleton architecture in PDAC (47). MMP-2 is a matrix metalloproteinase that has been associated with tumor invasion, metastasis, and early recurrence after PDAC resection (47). MMP-2 is also directly linked to the development of the desmoplastic reaction in PDAC (47), which hinders drug delivery, as discussed earlier (42). While the influence of SPARC on PCCs and the surrounding stroma is being teased out, more research is necessary to understand its contribution to metastasis in PDAC.

In contrast to primary PDAC, metastatic PDAC expression of SPARC was present predominantly in the stroma surrounding and adjacent to the metastatic tumor cells (47). Moreover, SPARC expression in the metastatic tumor cells themselves was below the level of detection (47). This highlights an area of potential future research to investigate whether enhanced expression of SPARC in the ECM of pancreatic tumors act to promote tumor cell invasion or block tumor growth and spread. Mantoni et al showed that patients with resected ampullary cancer and overexpression of SPARC in the stroma lived significantly shorter and had more nodal metastases than those with lower SPARC expression (57). Additionally, the study results showed that SPARC has no influence on radiation survival. The researchers could not confirm any influence of SPARC on clonogenic radiation survival neither exogenous SPARC or after siRNA knockdown of SPARC in hPSC co-cultured with pancreatic tumor cells. SPARC’s ability to promote invasiveness and migration in pancreatic cancer monoculture via paracrine signaling from PaSCs enhanced the tumor cell invasiveness. Therefore, the
study postulates that due to the lack of influence on radiation sensitivity in vitro, it is likely the detrimental effect of SPARC on overall survival is related to the protein's anti-adhesive and invasion-promoting properties (57).

Along the same logic, another experiment showed that downregulation of SPARC expression in PANC-1 cells, which overexpress SPARC, resulted in the decreased invasiveness (47). Therefore, SPARC expression in PCCs, when present, appears to selectively enhance their invasiveness and migratory properties (58). Thus, PCCs that express SPARC appear to have enhanced metastatic virulence. In addition to SPARC expression by PCCs, high levels of SPARC expression are evident in virtually all pancreatic circulating tumor cells (CTCs) (58). This raises the possibility that SPARC significantly contributes to the metastatic spread of PDAC. The same study by Ting et al showed that the most highly enriched CTC-specific transcripts shared by almost all 'classical CTCs' encode ECM proteins, like SPARC (58). When the experimenters knocked down SPARC expression, CTC migration was attenuated (58).

In summary, SPARC overexpression is a hallmark of PDAC with low expression in the cancer cells themselves and high expression in stromal fibroblasts (PaSCs). SPARC expression in the stromal cells is associated with poorer prognosis with median overall survival of 15 vs. 30 months (p<0.001) (57). Whereas SPARC expression in the cancer cells was not associated with prognosis. What remains to be understood is the mechanism for induction of SPARC overexpression in these cell types and what the implications are for treatment.

**SPARC's influence on cancer therapy.** The implications of SPARC and its influence on metastasis and invasion of PDAC make it a promising biomarker for guiding cancer therapy. Gundewar et al identified SPARC expression as predictive of PDAC's response to gemcitabine (51). The overexpression of SPARC was associated with prolonged survival in the subgroup of patients receiving nab-paclitaxel + gemcitabine (51). A phase I/II trial of 36 patients with previously untreated advanced pancreatic cancer being treated with nab-paclitaxel + gemcitabine exposed a potential link between SPARC expression and treatment efficacy (59). The results of this study showed that higher SPARC expression in the stroma was associated with longer overall survival (17.8 vs. 8.1 months). The molecular results of the study showed that nab-paclitaxel alone and with gemcitabine caused a depletion of stromal stiffness with resultant vasodilation (59). It should be noted that all the patients in this study were treated with nab-paclitaxel + gemcitabine. These results led to a phase III metastatic pancreatic adenocarcinoma clinical trial (MPACT), which compared nab-paclitaxel + gemcitabine vs. gemcitabine alone for treatment of metastatic PDAC (60). The trial involved a SPARC assay to examine samples from metastatic lesions. The results of the study showed no significant association between stromal SPARC level and efficacy in either treatment group (60). In addition, no correlation existed between plasma SPARC levels and efficacy of the adjuvant therapy (60). A preclinical experiment performed on mouse models of PDAC with overexpression and diminished SPARC expression also showed a lack of association between SPARC expression and treatment efficacy (61). However, metastatic lesions are frequently expected to be SPARC negative since they have less SPARC: a target for stromal depletion. SPARC has a high affinity for albumin and, therefore, enhances the uptake of an albumin-based formulation of the taxane, nab-paclitaxel (61). Nab-paclitaxel is transported into the tumor lesion piggy-backed on albumin, which is sequestered by SPARC. The mechanism of delivery is particularly active in cancer tissues, which exhibit high perfusion and metabolic activity (63). By exploiting SPARC's high affinity for albumin, nab-paclitaxel accumulates in SPARC-positive areas. In addition, SPARC expression is correlated with tumor response in both preclinical and clinical models of breast, head, and neck cancers. Therefore, it was hypothesized that the enhanced delivery of nab-paclitaxel would cause 'stromal collapse', a process of stromal depletion bringing tumor cells closer together and increasing vascularity, which would increase delivery and efficacy of chemotherapeutic drugs (63). A phase III study in which 861 metastatic PDAC patients were randomized to receive nab-paclitaxel + gemcitabine weekly for 7 weeks for cycle 1 and then on days 1, 8, and 15 every 4 weeks (cycle 2). The results showed a median overall survival of 8.5 vs. 6.7 months (60).

The success of nab-paclitaxel + gemcitabine leads to the next step of determining if any other potential agents will be successful in combination with the drug. In addition, more drugs targeting the stroma, such as the hedgehog inhibitor GDC-0049 are currently under clinical trials for PDAC (63). A large cohort of studies is underway and a review by Neuzillet et al provides a comprehensive Table of clinical trials evaluating stromal depletion in PDAC as well as a Table citing abstracts for nab-paclitaxel use in PDAC (63).

**Does attenuation of SPARC overexpression in cancer cell lines decrease cancer cell migration and invasion?** SPARC is highly expressed in multiple cancer phenotypes, including astrocytic gliomas. In fact, SPARC is a central player in the invasive activity of glioma cells under hypoxic conditions (64). Seno et al attenuated SPARC expression by transfecting glioma cells with small interfering RNA (siRNA), a synthetic RNA molecule designed to target mRNA for degradation, and confirmed the downregulation of the proteins with western blot analysis (64). Their results indicated that decreasing SPARC expression inhibits glioma cell migration and invasion in vitro. In addition, suppression of SPARC inhibited glioma cell invasion in a rat brain slice model. Another interesting
result from the study was the upregulation of SPARC expression following hypoxic stress in glioma cells (64). This suggests that the proteins expressed under hypoxic conditions influence SPARC expression thereby promoting the invasion of hypoxic cells into the normoxic tissue surrounding the tumor. This study is promising for PDAC research because, as previously discussed, hypoxia in PDAC is associated with both an increase in tumor growth and metastasis. Therefore, more PDAC research is needed to evaluate any correlation between SPARC, hypoxia, and metastasis.

Another strategy for attenuating SPARC expression is by employing microRNAs (miRNAs), which are small, non-coding RNA molecules that regulate gene expression. In a study evaluating hepatocellular carcinoma (HCC), SPARC expression was knocked down using miR-29a (65). The proposed target pathway of the miR-29a decrease in SPARC expression was the SPARC-AKT pathway, an important aspect of cell proliferation and survival (65). The researchers confirmed that SPARC siRNA also inhibited HCC cell growth.

In addition to using RNA to target SPARC’s contribution to metastasis and invasion, siRNAs have been used to alleviate SPARC’s influence on collagen deposition. Zhou et al demonstrated reduced collagen I expression in patients with scleroderma by targeting siRNA for SPARC present in fibroblasts (66). Camino et al also knocked down collagen deposition by using anti-sense RNA against SPARC delivered by adenovirus (67). These studies suggest that attenuating SPARC is sufficient to decrease collagen accumulation in tissues and point to a new direction of research into therapies that alleviate fibrosis in disease. PDAC has a dense, fibrotic microenvironment surrounding the tumor cells and SPARC’s role in collagen deposition makes it an attractive target to penetrate this stroma and increase the delivery and efficacy of chemotherapy drugs to the lesion.

5. Conclusion

Pancreatic ductal adenocarcinoma (PDAC) is a devastating, molecularly complex disease. The dynamic nature of the microenvironment of the tumor and the mechanism of interaction between the extracellular matrix and the tumor cells poses a daunting task for management of the disease. However, this attribute of PDAC presents an exciting frontier of research into novel therapeutic cancer targets. A better understanding of the molecular composition of PDAC and the surrounding microenvironment has placed SPARC at the forefront of research into innovative PDAC therapies and predictive biomarkers of progression and metastasis. The increase in clinical and preclinical studies on the mechanisms of communication between the tumor cells and surrounding stromal cells is providing a solid foundation for future treatments of PDAC. While certain nuances of SPARC and its efficacy as a therapeutic target are not yet completely understood, the preliminary studies show promise for it contributing to improvement in survival of PDAC patients.

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