Angiogenesis and Pancreatic Cancer: novel approaches to overcome treatment resistance

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Abstract: Pancreatic cancer (PCa) is acknowledged as a significant contributor to global cancer-related mortality and is widely recognised as one of the most challenging malignant diseases to treat. Pancreatic ductal adenocarcinoma (PDAC), which is the most common type of PCa, is highly aggressive and is mostly incurable. The poor prognosis of this neoplasm is exacerbated by the prevalence of angiogenic molecules which contribute to stromal stiffness and immune escape. PDAC overexpresses various proangiogenic proteins including vascular endothelial growth factor (VEGF)-A, and the levels of these molecules correlate with poor prognosis and treatment resistance. Moreover, VEGF-targeting anti-angiogenesis treatments are associated with the onset of resistance due to the development of hypoxia, which in turn induces the production of angiogenic molecules. Furthermore, excessive angiogenesis is one of the hallmarks of the second most common form of PCa, namely, pancreatic neuroendocrine tumor (PNET). In this review, the role of angiogenesis regulators in promoting disease progression in PCa, and the impact of these molecules on resistance to gemcitabine and various therapies against PCa are discussed. Finally, the use of anti-angiogenic agents in combination with chemotherapy and other targeted therapeutic molecules are discussed as novel solutions to overcome current treatment limitations in PCa.

Keywords: pancreatic ductal adenocarcinoma, angiogenesis modulators, immunotherapy, hypoxia

1. INTRODUCTION

Pancreatic cancer (PCa) is one of the leading causes of mortality in the world, and the Global Cancer Observatory (GLOBOCAN) 2020 data estimates a total of 495,773 new pancreatic cancer cases with a worldwide mortality of 466,003 [1]. The American Cancer Society estimates that the relative 5-year survival rate of this cancer is 11% at the time of diagnosis, with upwards of 80% presenting with unresectable or distant disease in 2020 [2]. According to the World Health Organization International Agency for Research on Cancer (WHO - IARC), pancreatic cancer is considered the 12th most commonly diagnosed cancer in both men and women worldwide, with data estimating a higher incidence and mortality in men compared to women [3]. These figures highlight the alarmingly high incidence and death from pancreatic cancer in both high-income and low-to-middle-income nations. According to recent projections, pancreatic cancer will likely be the 2nd most common cause of cancer-related mortality in the United States of America (USA) within a few decades, due to an annual growth of just over 1% since the 1970s [1,4]. Glaringly, age-standardized incidence rates (ASRs) remain relatively high in Asian countries, with a total incidence and mortality of almost 50% of all known cancer cases [3]. The rising incidence of pancreatic cancer diagnosis in all countries is offset by a high mortality rate primarily as a result of late diagnosis [5]. The reasons for regional differences in incidence and mortality remain largely unknown but could be explained by possible exposure to certain risk factors, access to and variation in diagnostic modalities, as well as the varying degree of completeness of cancer registries.

Approximately 90% of PCa's are pancreatic ductal adenocarcinomas (PDAC), while cancers of the endocrine pancreas, also known as pancreatic neuroendocrine tumors (PNETs), occur at a much lower frequency [6,7]. The most effective treatment for pancreatic cancer is still early surgical resection, but late clinical presentation compromises successful treatment [5]. Kamisawa *et al.* maintain that even after undergoing early, complete surgical resection, the 5-year survival rate is no higher than 25% [5]. Treatment resistance is largely influenced by the fibrotic landscape of the tumor and a dysregulated immune response, both of which are fueled by high levels of pro-angiogenic molecules [6,7]. Therefore, an understanding of how angiogenesis regulators drive disease progression and influence drug perfusion in PCa is pivotal for effective clinical management.

2. ANGIOGENESIS AND PANCREATIC CANCER

Angiogenesis is the formation of blood vessels from pre-existing microvessels and in the normal physiological setting, the balance between inhibitors and stimulators of this process is controlled carefully [8]. On the other hand, the loss of balance between pro- and anti-angiogenic molecules underlies the transition from tumor dormancy to malignancy in many solid tumors [9]. Pancreatic neuroendocrine tumors exhibit the same loss of balance between pro- and anti-angiogenic molecules as most solid tumors and are characterized by excessive angiogenesis [8]. In contrast, PDAC is considered hypovascularized due to collapsed blood vessels Fig. **(1)**, although this cancer develops endothelial cell (EC) projections or vessel microvilli which contribute to vessel density. These vessel microvilli enable blood flow and nutrient uptake but limit drug extravasation [6,10].

The notion that because PDAC is hypovascular, angiogenesis and its regulators play no role in this neoplasm is incorrect. One of the most potent angiogenic regulators, vascular endothelial growth factor-A (VEGF), was characterized in six human PCa cells, including MIA-Pac-2 and PANC-1, which are PDAC cell lines [11]. Also, there is more than a five-fold increase in VEGF levels in these cell lines compared to non-cancerous pancreatic cells. Similarly, patients with PDAC overexpress VEGF, and a positive VEGF immunoreactivity correlates with a lower survival rate [12]. Moreover, the canonical receptor for VEGF, VEGFR-2 was found to be a predictor of survival in patients with PDAC [13]. Interestingly, VEGF is considered a more accurate predictor of liver metastasis and poor outcome in resected PCa than the tumor, node, and metastasis (TNM) staging [14].

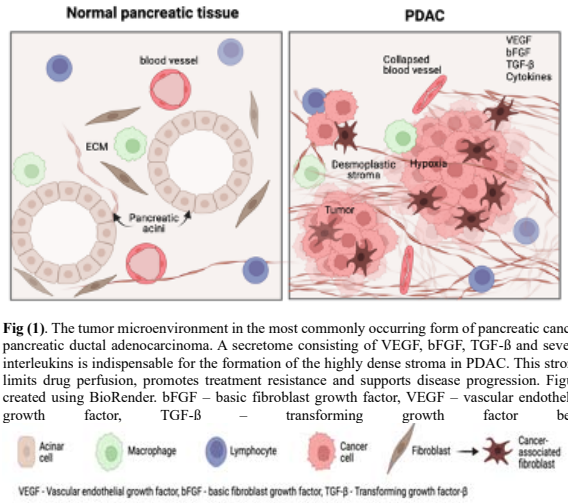

Fig (1). The tumor microenvironment in the most commonly occurring form of pancreatic cancer, pancreatic ductal adenocarcinoma. A secretome consisting of VEGF, bFGF, TGF-ß and several interleukins is indispensable for the formation of the highly dense stroma in PDAC. This stroma limits drug perfusion, promotes treatment resistance and supports disease progression. Figure created using BioRender. bFGF – basic fibroblast growth factor, VEGF – vascular endothelial growth factor, TGF-ß – transforming growth factor beta.

The evaluation of the PDAC secretome has revealed that several proangiogenic factors including VEGF, basic fibroblast growth factor (bFGF), and transforming growth factor (TGF)-ß Fig. **(1)** were overexpressed. Another angiogenic factor that has been detected in PDAC is platelet derived growth factor (PDGF)-A Fig. **(1)** [15]. The PDAC stroma is composed of fibroblasts, various immune cells including macrophages and lymphocytes, as well as a thick extracellular matrix Fig. **(1)**. In a pre-clinical model of pancreatic cancer, VEGFR-2 inhibition correlated with an upregulation of bFGF in both endothelial and PCa cells. The study further revealed that bFGF levels rose in tandem with the onset of hypoxia [16]. Taken together, the study signifies that the inhibition of the VEGF/VEGFR-2 signaling may activate other angiogenic factors, leading to the development of resistance against VEGF/VEGFR-2 targeted anti-angiogenic treatments. In PNET, high levels of angiogenic cytokines, VEGF and IL-8 were detected in patient samples and these molecules correlated with poor outcome [17]. Additionally, over-expression of VEGF and PDGF-A are associated with poor prognosis as well as

resistance to anti-angiogenic treatments [17]. The presence of these angiogenic molecules is further associated with a disturbance in immune responses to cancer.

3. IMMUNE REGULATION-ANGIOGENESIS CROSSTALK AFFECTS PCA TREATMENT

The immune system regulates early tumor growth, and the dysfunction of its innate and adaptive responses favors cancer progression [18]. The immune system is capable of protecting against tumor growth as well as shaping the immunogenicity of cancer *via* immunoediting [19,20]. Three phases are involved in this dynamic process of immunoediting and are present in PCa [21,22]. In the first phase, known as the elimination phase, the immune system regulates early tumor growth, thus preventing disease progression. In the second phase, cancer cells develop resistance to immune responses, and while tumor growth is halted temporarily, the resistant cancer cells survive elimination, this is known as the equilibrium phase. The third or escape phase is underpinned by uncontrolled tumor growth due to the proliferation of cancer cells that have evaded immune control, and there is subsequent progression to clinical disease [18]. Various angiogenesis regulators such as VEGF have been implicated in this phase Fig. (**2**) [24, 25].

Effective immune response against PCa progression requires cytokine-activation of cytotoxic T lymphocytes (CTL), T-helper cells (Th1), mature dendritic cells (DCs) that primarily act as antigen-presenting cells (APCs), pro-inflammatory macrophages (M1) and natural killer cells (NKC) to promote an "immune-stimulatory environment" [18,23]. This 'immune-stimulatory environment' is hampered by the presence of angiogenic factors which promote the escape phase through its effects on immune cells. VEGF impairs the maturation of DCs Fig. (**2**) and induces their differentiation into EC-like cells [24]. DCs also secrete VEGF and transforming growth factor (TGF)-β which induce tumor vascularization [X]. Additionally, VEGF promotes the presence and proliferation of regulatory T cells (Tregs) in the tumor microenvironment Fig. (**2**). Regulatory T cells in turn secrete angiogenic cytokines such as IL-10 and TGF-β, which promote tumor vascularization. Noteworthy is that these Tregs denote both advanced disease and poor post-resection survival [23]. These angiogenesis modulators further impair T lymphocyte development, and lead to a reduction in CD4/CD8 cells, thus possibly contributing to cancer-associated immune-deficiency [25]. VEGF also promotes macrophage infiltration in the tumor site and these tumor-associated macrophages (TAMs) Fig. (**2**) confer a poor prognosis in various malignancies and are notably elevated in pancreatic cancer [26]. When the protective mechanism provided by the body's immune system is disabled, progression to clinical disease is apparent. This 'immune-inhibition environment', triggered by the activation of angiogenic factors such as VEGF and TGF-β, as well as several cytokines (IL-4, IL-5, IL-6, IL-10, and IL-13), renders NKCs ineffective [24]. Of note is that TGF-β is an angiogenesis modulator and a potent immune suppressor that is associated with PCa immune evasion [24]. Together with other angiogenesis regulators, namely bFGF and TGF-β is involved in the remodeling of the tumor microenvironment (TME) in both PDAC and PNET. Importantly, TAMs, TREGs and MDSCs secrete VEGF [X]. This ligand acts in a paracrine manner to stimulate tumor angiogenesis. Therefore, this cross-talk between modulators of angiogenesis and immune cells promotes disease progression and negatively affects disease response to therapy. Noteworthy is that hypoxia is an important stimulus for the production of the of VEGF and other proangiogenic cytokines such as IL-6 and TGF-β [X] [27,28].

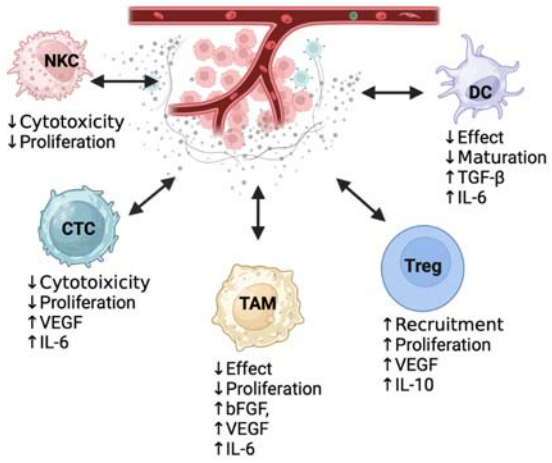

Fig. (**2**). Cross-talk between immune cells and the tumor vasculature. VEGF, which is secreted by endothelial and tumor cells, sets up an immunosuppressive environment. Immune cells secrete angiogenic molecules, VEGF, bFGF, IL-6 and IL-10 and TGF-β, which in turn promote angiogenesis. IL – interleukin, VEGF – vascular endothelial growth factor, bFGF – basic fibroblast growth factor, TGF-β – transforming growth factor-β, DC – Dendritic cell, NKC – Natural killer cell, CTC – Cytotoxic T-cell, TAM – tumor associated macrophage, Treg – regulatory T-cell

4. HYPOXIA IS ASSOCIATED WITH RELAPSE AND POOR RESPONSE TO TREATMENT

The pancreatic tumor microenvironment is endowed with a fibrotic extracellular matrix, proliferating tumor cells, and in PDAC, is characterized by poor vascularization which limits the effectiveness of immunotherapies [27,28]. This deficient tumor vascularity predisposes the underlying malignant tissue to hypoxia. Besides the loss of conventional tissue vascularity, aberrant growth of tumorigenic cells increases oxygen demand in tissues beyond that which can be supplied. Also, expanding tumors increase the distance of neoplastic cells to the inherent tissue blood supply, further reducing diffusion and worsening hypoxia [29]. Hypoxia in pancreatic tissue promotes the induction of hypoxia-inducible factors (HIFs), particularly HIF-1α. In PNET hypoxia induces rapid and aberrant blood vessel formation, creating leaky, immature, and wholly abnormal new blood vessels [27-29]. HIF-1α also plays a role in the pathogenesis of PDAC through binding to fascin actin-bundling protein 1 and Lim and SRC Homology 3 (SH3) protein 1 [32]. These proteins promote metastasis. Also, there is a significant correlation between HIF-1α and proangiogenic factors VEGF, bFGF and PDGF-A in patients with PDAC [15]. Furthermore, HIF-1α leads to the activation of the Phosphatidylinositol-3-kinase/Protein kinase B (PI3K/PKB) pathway *via* the CXC3CLI/CXCR1 axis, thus promoting tumor cell proliferation, migration and the development of resistance to radiation therapy and chemotherapy in PDAC [30,31].

Hypoxia, in addition to affecting the cellular mechanisms that influence treatment resistance, results in the upregulation of various proangiogenic molecules and contributes to processes that limit drug delivery to the tumor [29,32].

5. APPROACHES TO OVERCOME RESISTANCE TO THERAPY

The management of PCa, including limiting the possibility of resistance and refractoriness, will improve with an effective screening of high-risk individuals, an accurate early diagnosis, and the employment of efficacious targeted therapies.

Early detection, screening, and disease staging

Studies exploring the benefits of population-based screening for pancreatic cancer have concluded that widespread screening is not appropriate [33,34]. However, according to the International Cancer of the Pancreas Screening Consortium (CAPS) individuals meeting the criteria for familial pancreatic cancer (FPC) should be the major targets for screening, with a focus on screening for high-risk individuals [33,34]. Early screening aims to ensure early diagnosis as this contributes to early surgical resection- the only potential cure for pancreatic cancer. However, the asymptomatic nature of early disease implies that the majority of those diagnosed with pancreatic cancer present with unresectable or metastatic disease [2,4]. Clinicians rely on imaging tools such as computed tomography (CT), magnetic resonance imaging (MRI), endoscopic ultrasonography (EUS), endoscopic retrograde cholangiopancreatography (ERCP), and magnetic cholangiopancreatography (MRCP)[35] to make an early diagnosis as a clinical evaluation of patients in the early stages of the disease remains elusive due to non-specific clinical presentation. Biomarkers can thus play a major role in the detection of early disease in high-risk individuals. Commonly used biomarkers include carbohydrate antigen (CA) 19-9, carcinoembryonic antigen (CEA), and CA12-5 and the downside to the use of these biomarkers is their poor specificity [36,37]. There is, therefore, a need to

include additional biomarkers for early diagnosis in PCa, including select markers found in body fluids [38].

Pancreatic cancer is staged according to tumor extent (T), lymph node involvement (N), and presence of metastases (M), referred hereto as TNM staging I – IV; with stage I referring to cancer confined to the pancreas and stage IV indicating the spread of cancer to other organs. This staging considers the examination of resected pancreatic tissue and is classified as the pathological or surgical stage. When a tumor is too large to be resected or has metastasized and therefore cannot be surgically removed, it has reached the clinical stage and chemotherapy and radiotherapy may be required as initial treatment in this stage[39]. Furthermore, criteria which considers the degree of resectability of the tumor and the possibility of recurrence is required primarily to plan treatment [40,41].

Treatment

Early surgical resection remains the only potentially curative treatment for pancreatic cancer, however, despite total resection, the 5-year survival rate remains over 20% due to local recurrence. Oftentimes, postoperative chemotherapy and radiotherapy are provided in order to limit recurrence and improve overall survival. Moreover, radiotherapy has not been used as a single modality in recent times with locally advanced pancreatic cancer patients largely receiving combination chemotherapy with a select cohort undergoing concurrent chemoradiotherapy. Gemcitabine and 5-Fluorouracil-based treatments are the most commonly used chemotherapies in PCa, and they've been ineffective in the treatment of both early and advanced stage disease [33,34]. The targeting of pathways that contribute to resistance, such as the inhibition of angiogenic regulators, is necessary to enhance the efficacy of PCa treatments.

Targeting the vasculature in PCa

Several angiogenesis inhibitors have received approval from regulatory agencies in various continents including the U.S. Food and Drug Administration (FDA) to treat solid tumors.

Some of the treatments have been studied in pre-clinical models of pancreatic cancer and others have undergone clinical evaluation (Table I) in patients with PCa. The key proangiogenic molecule which also functions as an immune modulator, VEGF, has been targeted using bevacizumab in phase I/II clinical trials of patients with unresectable PDAC, but there was a low success rate and a considerable increase in toxicity [42,43]. In a phase II trial of bevacizumab and gemcitabine in patients with advanced PCa the median survival was 9.2 months [44]. However, a phase III randomized trial investigating the combination of gemcitabine and bevacizumab did not improve OS. In contrast, a randomized phase II trial in unresectable, locally advanced or metastatic PCa revealed that axitinib, a multi-targeting VEGFR inhibitor resulted in a slight improvement in OS compared to gemcitabine alone, and the observations were confirmed in a double-blind randomized phase III study in patients with advanced PCa [45,46]. In vitro studies have shown the effectiveness of sunitinib malate in PCa [47,48]. However, the combination of erlotinib, bevacizumab and gemcitabine had a significant improvement on PFS, although but not in OS. In a separate phase III study, erlotinib and gemcitabine resulted in an improvement in both OS (23%) and PFS (hazard ration 0.77%) [44].

Drugs that target multiple angiogenic molecules such as sorafenib (Table 1) seem to be more effective than single molecule targeting angiogenesis inhibitors.

Table 1. Selected angiogenesis inhibitors that have been evaluated in pancreatic cancer.

Drug	Target	Type of pancreatic cancer	Preclinical/ Clinical evaluation	Study Outcome	Reference
Bevacizumab	VEGF	PDAC	Clinical	No improvement in OS, Significant toxicity	[43, 44, 50]
Axitinib	VEGFR-1,-2,-3,	PDAC	Clinical	Slight but insignificant improvement in OS	[47]
Nintedanib	VEGFR-1,-2,-3, FGFR, PDGFR	PDAC, PNET	Preclinical	Inhibited cell proliferation and tumor angiogenesis	[51] [52]
Sorafenib	VEGFR-2,-3, EGFR, PDGFR, c-Kit	PDAC	Clinical	Marginal insignificant effect	[53]
Sunitinib malate	VEGFR-1,-2,-3, PDGFRβ, FLT3, c-KIT	PDAC, PNET	Clinical	PFS 1.31 and 10.4 mnths; OS 3.6 mnths Significant toxicity	[54, 55]
Pazopanib	VEGFR-1,-2,-3, FGFR -1,-3, KIT, PDGFR-β	PNET	Clinical	Partial response, OS 24.6 mnths	[56]
Pertuzumab	VEGFR-1,-2,-3, PDGFRs, FGFRs	PDAC, PNET	Clinical	Well tolerated, OS 24.6 mnths, PFS 6.5 mnths	[57]

OS- overall survival, PFS- progression free survival, VEGF- vascular endothelial growth factor, VEGFR- vascular endothelial growth factor receptor, PDAC- pancreatic ductal adenocarcinoma, FGFR- fibroblast growth factor receptor, PNET- pancreatic neuroendocrine tumor, PDGFR- platelet-derived growth factor receptor, FLT- Fms related receptor tyrosine kinase.

Preclinical studies revealed that nintedanib inhibited angiogenesis and reduced pancreatic cancer growth in vivo, while in vitro the drug suppressed the proliferation of a PDAC cell line and induced apoptosis in these cells [49]. Slight improvement was observed in a phase II randomized trial of post-operative sorafenib + gemcitabine treatment, although promising results were observed when treatment was administered for more than 6 months [49]. A previous study reported a median OS of 1.3 months (Table 1), with moderate toxicity [49]. When employed in combination with evofosfamide in advanced progressive PNETs (Table 1), sunitinib malate resulted in a median progression-free survival of 10.4 months, although there was considerable toxicity [49]. The co-administration of hypoxia-targeting drugs may alleviate the side-effects associated with sunitinib in PNET since the drug is associated with the development of hypoxia. On the other hand sunitinib malate was shown to be effective as maintenance therapy in a randomized phase II trial in metastatic PDAC patients and resulted in a significantly high OS of 22.9% after 2 years while stable disease was 51.9% [50]. Pazopanib, another tyrosine kinase inhibitor, had a mean progression free survival of 11,6 months and a mean OS of 24.6 months from various trials [50]. These observations highlight the possible involvement of various angiogenesis modulators and the need to profile these markers in PCa to enable effective targeting of angiogenic pathways. Hypoxia-induced alterations which include increased secretion of VEGF-independent growth factors, mobilization of bone marrow-derived endothelial cells, the induction of epithelial-to-mesenchymal transition and vessel co-option can promote resistance to anti-angiogenic drugs [10,51,52]. Moreover, clinical trials have revealed that blocking VEGF/VEGFR signaling can aggravate tumor hypoxia, which results in tumor cells secreting proteins such as basic fibroblast growth factor and platelet-derived growth factor [53].

Hypoxia has been shown to be promoted by histone deacetylase (HDAC) through the induction of HIF-dependent gene expression [54]. The drug Abexinostat which suppresses hypoxia by inhibiting HDAC could thus be useful in overcoming hypoxia-associated resistance to anti-angiogenics [55]. In pre-clinical studies the Abexinostat was found to have a significant effect on the suppression of tumor angiogenesis [55]. Another drug which targets hypoxia, panobinostat was evaluated in Phase II clinical trials for the treatment of B-cell lymphoma and has also showed promise in solid tumors when used as a part of combination treatments [54,56]. It is possible that these hypoxia-suppressing drugs could contribute to the reduction of resistance to angiogenic inhibitors and be of benefit as part of combination anti-angiogenic strategies. Moreover, since several angiogenic molecules suppress immune function in cancer, modulating immune system responses ought to be considered in treatment approaches.

Immunotherapy

To overcome the barrier of a compromised immune response promoted largely by angiogenic cytokines and growth factors, various immunotherapies have been employed (Table 2). Important to note is that while immune-targeting therapies have yielded successful results in the treatment of many cancers, results have been disappointing in PCa owing largely to the unique histopathological features of the cancer [57].

Table 2. Potential immune targets in pancreatic cancer.

Target	Role in PCa	Targeting therapy	Reference
PDL-1	Upregulated in pancreatic cancer cells and inhibits T cell activity by binding to PD-1.	Immune checkpoint blockade	[69]
CTLA-4	Inhibits T cell activity.	Immune checkpoint blockade	[70]
PD-1	Inhibits T cell activity; expression associated with poor prognosis and reduced survival.	Immune checkpoint blockade	[69]
LAG3	Expressed on T cells and inhibits T cell activity	Immune checkpoint blockade	[71]
TIM3	Expressed on T cells and other immune cells and inhibits T cell activity.	Immune checkpoint blockade	[72, 73]
VISTA	Suppress T cell activation, proliferation, and cytokine production	Immune checkpoint blockade	[74]
CD137	Regulates T cell activation and proliferation	Checkpoint activation	[75]
CD27	Essential for T-cell and B-cell co-stimulation	Checkpoint activation	[76, 77]
CD40	Alter macrophage differentiation to M1 phenotype, reduce cancer proliferation	Checkpoint activation	[78]
GITR	Promotes growth and spread of cancer cells.	Checkpoint activation	[79]
OX40	Activates T cells and promote their survival and proliferation.	Checkpoint activation	[80]
ICOS	Enhances T cell ability to recognize and attack cancer cells in the pancreas.	Checkpoint activation	[71]
CCR2/CCL2	Stimulates release of proinflammatory cytokines	Myeloid suppression	[81, 82]
IDO1	Enzyme expressed by tumor cells and immune cells that can suppress T cell activity and promote immune tolerance in pancreatic tumors	Myeloid suppression	[83]
TGFB1	Promotes apoptosis and inhibits epithelial cell cycle progression; induces late-stage genomic instability, neoangiogenesis, immune evasion.	Myeloid suppression	[84]
TNF	Tumor promoting and suppressive: promote inflammation, cell survival, and proliferation; induce cell death, inhibit angiogenesis	Myeloid suppression	[85]
CSF1R	Promotes differentiation of myeloid progenitors.	Myeloid suppression	[86]
ADORA2A/CD39/CD73	Promotes development, differentiation, migration, and invasion of cancer cells	Metabolic suppression	[87]
NPR1/2	Mediates proliferation, survival, tissue invasion and metastasis, angiogenesis, energy metabolism, and cellular transformation.	Neuropillins	[88]

PD-1- Programmed cell death protein 1; PD-L1- Programmed death-ligand 1; CTLA-4- Cytotoxic T-lymphocyte-associated protein 4; LAG3- Lymphocyte-activation gene 3; TIM3- T-cell immunoglobulin and mucin domain-containing protein 3; VISTA- V-domain immunoglobulin suppressor of T cell activation; CD137- Cluster of differentiation 137; CD27- Cluster of differentiation 27; CD40- Cluster of differentiation 40; GITR- Glucocorticoid-induced TNF receptor-related protein; OX40- Tumor necrosis factor receptor superfamily member 4; ICOS- Inducible T cell co-stimulator; CCR2/CCL2- C-C chemokine receptor type 2/ C-C motif chemokine ligand 2; IDO1- Indoleamine 2,3-dioxygenase 1; TGFB1- Transforming growth factor beta 1; TNF- Tumor necrosis factor; CSF1R- Colony-stimulating factor 1 receptor; ADORA2A/CD39/CD73- Adenosine A2A receptor/Cluster of differentiation 39/Cluster of differentiation 73; NRP1/2- Neuropilin-1,-2.

Amedei *et al*. highlight a few ways in which the immune system may be targeted in pancreatic cancer through the use of vaccine-based immunotherapy would require the administration of tumor antigens in the form of DNA or peptides to stimulate tumor-specific immunity [23]. This implies that the expression of the antigen must be restricted to the tumor and not induce a systemic response. Vaccine-mediated immunotherapy strategies employed to date include whole-cell vaccines, peptide-based vaccines, dendritic cell vaccines, and DNA and mRNA vaccines [58,59]. While there has been little success with this treatment strategy in pancreatic cancer, recently a phase II study using vaccine OCV-C01 with gemcitabine showed superior efficacy to the use of gemcitabine alone [60].

Another form of immunotherapy, namely, immune checkpoint inhibitors (ICI) has led to improved clinical results in several cancers. The role of immune checkpoints lies in their inherent ability to modulate T-cell responses through regulatory mechanisms preventing an exacerbated immune response [61,62]. However, tumor cells exploit this inhibition in order to escape immune detection, promoting growth and progression of tumors. Cytotoxic T lymphocyte protein 4 (CTLA-4) is known to inhibit T-cell activation, whilst the binding of programmed death-ligand 1 (PDL-1) to its cognate receptor, programmed death-1 (PD-1), also has an inhibitory effect on T-cell activation, effectively "putting the brakes" on the anti-tumor immune response. CTLA-4, PDL-1 and PD-1 are the most studied immune checkpoint modulators and therefore remain viable targets for blockade therapy [62-65]. Pembrolizumab, is the only FDA-approved immunotherapy for patients with advanced PDAC [66,67]. Humanized monoclonal antibodies (atezolizumab, avelumab, durvalumab) which target PDL-1 ligand activity, induce T-cell activation and may be of benefit when used in combination treatments. PD-1 antibodies (pembrolizumab, nivolumab, cemiplimab) enhance the immune response against tumor cells, and may thus have a role in enhancing PCa therapies.

Ipilimumab and tremelimumab are two CTLA-4 human monoclonal antibodies designed to block the suppressive action of CTLA-4 on the T-cell and induce a CTL anti-tumor response. CTLA-4 is also involved in modulating the immune suppressive Treg activity, since Tregs in tumor microenvironments express higher levels of surface CTLA-4. This attribute underpins the putative role of CTLA-4 antibodies in blocking the action or reducing Treg activity [66]. Combination ICI therapies targeting both CTLA-4 and PD-1, primarily using ipilimumab and nivolumab, have shown promise in some tumors [64,68]. Chimeric antigen receptor (CAR) T cell immunotherapy, form of adoptive T cell transfer therapy, has shown some promise in solid tumor treatment and is currently being evaluated with checkpoint inhibitors as a promising combination therapy[69,70].

Despite the potential of ICI therapy, the response has not been entirely promising in PCa due to low bioavailability and immune-related adverse events [68,71]. Indeed, the dysfunctional vasculature in PCa's limits the effectiveness of ICIs. This conundrum can potentially be circumvented by combination strategies which target various cancer promoters in the TME, including angiogenesis markers [72].

CONCLUSION

Conventional chemotherapy has had little impact on PCa. The hypovascularization in PDAC which is characterized by collapsed blood vessels limits drug perfusion. Similarly, the hypervascularization that characterizes neuroendocrine

pancreatic tumors affects drug infiltration as the vessels are structurally and functionally defective. Proangiogenic molecules such as VEGF, bFGF and TGF-ß are overexpressed in the two most common forms of PCa, PDAC and NET, and these growth factors are potent immune modulators linked to gemcitabine and immunotherapy resistance. As a result, the targeting of the angiogenic molecules is an important therapeutic imperative for PCa. Moreover, targeting VEGF or VEGFR-2 is associated with an increase in hypoxia, which in turn stimulates several factors including bFGF and PDGF-A. As such, bFGF represents an important novel target in PCa and drugs such as nintedanib and Lenvatinib which inhibit this potent angiogenic factor may be of value as part of multi-targeting approaches. However, the efficacy of multi-targeting angiogenesis inhibitors is off-set by the development of hypoxia. Therefore, combination approaches that include anti-cancer chemotherapy, anti-angiogenics targeting multiple pathways including hypoxia, as well as the inclusion of immunotherapy may overcome resistance and thus enable more effective management of PCa. Drug delivery systems such as nano carriers could reduce the toxicity profile of such combination strategies and improve treatment efficacy.

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