p21-Activated Kinases and Therapeutical Responses

Subjects: Oncology

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Angiogenesis has been associated with numbers of solid tumours. Anti-angiogenesis drugs starve tumours of nutrients and oxygen but also make it difficult for a chemo reagent to distribute into a tumour, leading to aggressive tumour growth. Anti-angiogenesis drugs do not appear to improve the overall survival rate of pancreatic cancer. Vessel normalisation is merging as one of the new approaches for halting tumour progression by facilitating the tumour infiltration of immune cells and the delivery of chemo reagents. Targeting p21-activated kinases (PAKs) in cancer has been shown to inhibit cancer cell growth and improve the efficacy of chemotherapy. Inhibition of PAK enhances anti-tumour immunity and stimulates the efficacy of immune checkpoint blockades.

angiogenesis pancreatic cancer vessel normalisation p21-activated kinases (PAKs) immunotherapy chemotherapy

1. Introduction

The global incidence of pancreatic ductal adenocarcinoma (PDA) is increasing. Pancreatic cancer now ranks among the top ten most prevalent cancers [1]. The poor prognosis of PDA is mainly due to the lack of an early detection method and no effective systemic therapy for metastatic and locally advanced diseases [2]. Less than a third of patients receive surgical resection, of which 65% of patients experience tumour recurrence after surgical resection [3].

One of the main barriers to developing effective treatments for PDA is its unique characteristic of the tumour microenvironment (TME). The TME of PDA exhibits a dense stromal environment, containing cancer-associated fibroblasts and immune cells that interact with different subsets of cancerous cells, including cancer stem cells (CSCs) [4]. In addition, the blood vessels in the TME facilitate the exchange of nutrients, oxygen, and other essential factors and provide pathways for the dissemination of tumour cells to distant sites [5]. Interactions between blood vessels and other elements of the TME influence overall tumour behaviour and therapeutic response [6].

Inhibiting blood vessels may limit the expansion of the tumour and enhance the survival of cancer patients [7][8]. In 2004, FDA approved the clinical application of bevacizumab, an anti-VEGF antibody, to be used as an initial therapeutic approach for metastatic colorectal cancer (5-FU) [9]. Furthermore, FDA has approved multi-target tyrosine kinase inhibitors (TKIs) like sunitinib, sorafenib, and pazopanib. These TKIs are designed to specifically

target VEGF receptors, with a particular focus on VEGFR-2 receptor [10]. In pancreatic cancer treatment, various clinical trials have been conducted to assess the efficacy of anti-angiogenic agents. However, the outcomes have been disappointing across multiple studies [11]. Although a few clinical trials have shown improvements in progression-free survival (PFS) [12], none of them have indicated any significant extension in the overall survival (OS) of pancreatic cancer patients. The use of anti-angiogenic treatment for pancreatic cancer remains a subject of debate [13]. Nevertheless, an increasing body of clinical evidence suggests that a substantial number of tumours exhibit an initial lack of response to anti-angiogenic agents, termed de novo resistance, while others gradually acquire resistance over time, resulting in tumour progression even after several months of treatment [14]. Although high microvessel density (MVD) is generally considered to be a predictor of poor prognosis [15][16], several studies found no correlation between poor prognosis and high MVD [17][18]. PDA exhibits limited response to traditional chemotherapy treatments because of the low MVD. Instead of the inhibition of blood vessels, vascular normalisation holds potential promise as an innovative approach for cancer treatment [19], especially for pancreatic cancer.

Over 90% of PDA carry KRas mutations [20]. KRas is a member of the Ras family proteins that bind to guanosine triphosphate (GTP), becoming active. When mutated, KRas becomes constitutively active, triggering the activation of downstream pathways critical for cell survival and growth [21]. Through direct and indirect mechanisms, KRas activates p21-activated kinases (PAKs) [22]. PAKs have gained considerable attention for their roles in the tumorigenesis of PDA [23].

In pancreatic cancer, the extensive desmoplastic reaction leads to the formation of a dense stroma, causing inadequate vascularisation, inefficient drug delivery, and ineffective immune cell infiltration into the tumour sites, which eventually leads to therapeutical resistance and cancer progression [24]. PAKs have been recognised for their roles in the regulation of vasculature and therapeutical response. Here, the impact of PAK effects on tumour vasculature in chemotherapy and immune response will be discussed with a focus on pancreatic cancer.

2. PAK and Chemotherapy

PAKs are activated and play a crucial role in facilitating the resistance of cancer cells [25][26]. Modulating PAK activity holds the promise of sensitising cancer cells to chemotherapeutic agents, ultimately leading to an improved response to treatment. Shikonin, a natural inhibitor of PAK1, sensitises the pancreatic cancer cells to the chemotherapeutic drugs of gemcitabine and 5-FU [27]. A PAK1 inhibitor, FRAX597, when combined with gemcitabine, led to a synergistic inhibition of pancreatic cancer proliferation in vitro and a further reduced tumour growth in vivo [28]. The structural normalisation of blood vessels through PAK4 inhibition can enhance drug distribution and mitigate intra-tumoral hypoxia, ultimately resulting in enhanced tumour responses to molecular targeted therapy as well as radiotherapy and chemotherapy [29]. Inhibition of PAK4 not only reduces tumour growth but also enhances the efficacy of gemcitabine in mice carrying pancreatic cancer [30]. The combination of the PAK4 inhibitor, KPT-9274, and NAMPT modulators (KPT-9307) more effectively suppressed tumour growth in a xenografted mouse model [31]. The effects of PAK inhibition on the chemotherapy response of cancer cells are summarised in **Table 1**.

Table 1. Inhibition of PAKs enhances cancer cells' response to chemotherapy drugs.

Cancer Type	PAK Inhibitors	Target	Treatment	Mechanism	Ref.
Melanoma	PF-3758309	PAK1 PAK4	BRAFi inhibitor MEKi inhibitor	Enhance apoptosis and reduce cellular resistance to combined therapy via regulation of multiple pathways including JNK, ERK, β-catenin, and mTOR.	[26]
Pancreatic	Shikonin	PAK1	Gemcitabine 5-FU	Increase apoptosis in cancer cells.	[27]
Pancreatic	FRAX597	PAK1	Gemcitabine	Suppress HIF1α and AKT.	[28]
Pancreatic	PF-3758309	PAK1 PAK4	Gemcitabine 5-FU Abraxane	Reduce cell proliferation and enhance apoptosis. Decrease the expression of αSMA, Phalloidin, and HIF1α in vivo.	[32]
Pancreatic	PAKib	PAK4	Gemcitabine	Induce cell cycle arrest and cell death and regulate cell junction and adhesion.	[30]
Pancreatic	KPT-9274 KPT-9307	PAK4	Gemcitabine	Downregulate p-Bad-microRNA drug resistance axis and upregulate tumour-suppressive miRNAs.	[31]

3. PAK in Tumour Immune Responses and Immunotherapy

Tumour vasculature normalisation would potentially facilitate immune cell infiltration and increase the efficacy of immunotherapy. Immune checkpoint inhibitors have been found to enhance vessel normalisation by engaging CD4+ T lymphocytes [32]. PD-L1 expression is upregulated via the activation of the PI3K/AKT and interferon-γ pathways, both of which have close interactions with PAK1 [33][34][35]. We have reported that PAK1 inhibition increased levels of intratumoral CD3+, CD4+, and CD8+ T cells and decreased PD-L1 expression of cancer cells, thereby stimulating anti-tumour immunity [30]. The observed diminished protein expression levels of αSMA and Desmin in the PAK1KO tumours suggest a potential impact on the deactivation of cancer-associated fibroblasts, which in turn may lead to the anticipated reduction in angiogenesis within the PAK1KO tumours [4][36].

PAK4 expression is negatively correlated with immune cell infiltration in other cancers. High PAK4 expression is associated with limited infiltration of T cells and dendritic cells in immune checkpoint inhibitor-resistant melanoma patients. However, genetic deletion of PAK4 reversed PD-1 blockade resistance by increasing CD8 T cell infiltration in mice carrying flank tumours of melanoma. A combination of anti-PD-1 treatment and a PAK4 inhibitor enhanced anti-tumour responses compared to anti-PD-1 treatment alone. The effects of PAK4 KO were mediated via inhibition of the WNT pathway [37]. Wnt/β-catenin signalling is involved in fundamental vascularisation processes, including sprouting and non-sprouting angiogenesis, vasculogenic mimicry, and the formation of mosaic vessels [38].

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Inhibition of PAK4 enhanced the infiltration of CD103+ dendritic cells and CD8 T cells, and its expression correlates with CD8 T cells in the melanoma biopsies. Notably, PAK4 KO cells exhibited increased expression of genes associated with blood vessel formation, including Cercam, Enpep, Itga, and Lgals. CD31, a primary blood vessel marker, was increased in tumours treated with anti-PD-1 in PAK4 KO mice [39]. In glioblastoma (GBM), inhibition of PAK4 has induced intra-tumoral CD3+ T-cell infiltration, rendering GBM more susceptible to the immunotherapy of CAR-T cells. It has been demonstrated that PAK4 inhibition transformed the disordered morphology of the tumour-associated vasculature, characterised by tortuous and disjointed vessels with spatial heterogeneity, into a well-organised structure marked by continuous vessels [29]. A similar effect has been observed in prostate cancer, where targeting PAK4 increases the infiltration of CD8 T cells and B cells into the tumour, reversing PD-1 blockade resistance. Inhibiting PAK4 also leads to increased angiogenic activities and increased expression of adhesion molecules in the TME, attracting CD8+ lymphocytes [40]. The effects of PAK1 and PAK4 on the tumour infiltration of immune cells and the associated changes in tumour vasculature have been summarised in **Table 2**.

Table 2. Inhibition of PAKs causes the infiltration of immune cells into the tumour area.

Type of Cancer	Treatment	Techniques	Type of Immune Cell Activation	Possible Effect on Tumour Vasculature	Ref.
Pancreatic	↓ PAK1	Western blot and IHC	T cells (CD3+, CD4+, and CD8+)	↓ αSMA and Desmin, which indicates cancer associated fibroblast deactivation, in turn anticipating the reduced angiogenesis.	[36]
Melanoma	↓ PAK4	Mass cytometry and IHC	T cells (CD8+) and dendritic cells	↑ Activation of WNT signalling pathway, anticipating the increasing angiogenesis.	[37]
Melanoma	↓ PAK4	Transcriptomic, IHC, and flow cytometry	T cells (CD8+) and dendritic cells (CD103+)	↑ Alterations in genes associated with blood vessel formation, specifically, Cercam, Enpep, Itga, and Lgals.	[39]
Glioblastoma	↓ PAK4	IHC and bioluminescence imaging	T cells (CD3+)	↑ Vascular normality with reduced hypoxia.	[29]
Prostate	↓ PAK4	Flow cytometry and IHC	T cells (CD8+) and B cells	↑ ICAM1 and VCAM1. ↑ Inflammatory signals (CCR7, CCL19 and CCL21, CXCL13, and CXCR5).	[40]

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