Tumor-associated neutrophils: potential therapeutic targets in pancreatic cancer immunotherapy

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Abstract

Pancreatic cancer (PC), a highly malignant tumor of the digestive system with poor therapeutic response and low survival rates. In recent years, immunotherapy have developed rapidly and achieved substantial results in many malignant neoplasms. However, responses to immunotherapy in PC are rare and its immunosuppressive and desmoplastic tumor microenvironment (TME) composes an important impediment to their efficacy in PC. Tumor-associated neutrophils (TANs) play a crucial role in the PC microenvironment, exerting a profound influence on PC immunotherapy through establishing a robust stromal shelter and restraining immune cells to assist PC cells in immune escape, which may subvert the current situation of immunotherapy for PC. The purpose of this review is to offer a thorough summary of the latest progress in comprehending the involvement of TANs in PC desmoplastic and immunosuppressive functions, as well as to emphasize the potential therapeutic consequences of focusing on TANs in the immunotherapy of this destructive ailment. Last but not least, we have provided an outlook for the future of TANs in PC immunotherapy.

Tumor-associated neutrophils: potential therapeutic targets

in pancreatic cancer immunotherapy

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Abbreviations:

TANs: tumor-associated neutrophils

PC: pancreatic cancer

TME: tumor microenvironment

ICIs: immune checkpoint inhibitors

PD-L1: programmed death ligand 1

CAR-T: chimeric antigen receptor T cell

MSI-H: microsatellite instability high

G-CSF: granulocyte colony-stimulating factor

IL-7: interleukin-7

IL-6: interleukin-6

MDSCs: Myeloid-derived suppressor cells

NF-ϰB: nuclear factor-k-gene binding

TGF-β: transforming growth factor-β

IFN: i interferon

NET: neutrophil extracellular trap

PSCs: pancreatic stellate cells

CAFs: cancer-associated fibroblasts

TAMs: tumor-associated macrophages

CTL: cytotoxic T lymphocyte

HIF-1α: hypoxia-Inducible factor 1-alpha

LDHA: lactate dehydrogenase A

ARG1: arginase1

Abstract:

Pancreatic cancer (PC), a highly malignant tumor of the digestive system with poor therapeutic response and low survival rates. In recent years, immunotherapy have developed rapidly and achieved substantial results in many malignant neoplasms. However, responses to immunotherapy in PC are rare and its immunosuppressive and desmoplastic tumor microenvironment (TME) composes an important impediment to their efficacy in PC. Tumor-associated neutrophils (TANs) play a crucial role in the PC microenvironment, exerting a profound influence on PC immunotherapy through establishing a robust stromal shelter and restraining immune cells to assist PC cells in immune escape, which may subvert the current situation of immunotherapy for PC. The purpose of this review is to offer a thorough summary of the latest progress in comprehending the involvement of TANs in PC desmoplastic and immunosuppressive functions, as well as to emphasize the potential therapeutic consequences of focusing on TANs in the immunotherapy of this destructive ailment. Last but not least, we have provided an outlook for the future of TANs in PC immunotherapy.

Keyword: Tumor-associated neutrophil, Neutrophil extracellular trap, Pancreatic cancer, Immunotherapy,Immune-checkpoint-inhibitor therapy

1: Introduction

Pancreatic ductal adenocarcinoma (PDAC) is regarded as one of the most perilous and demanding malignancies in clinical practice[1], which has surpassed breast cancer to become the third primary contributor to cancer-related fatalities, with projections indicating it will soon become the second most prevalent cause of cancer-related mortality[2, 3]. The 5-year survival rate of PDAC is only 11%[4], due to the subtle initial symptoms of the majority of PDAC cases, resulting in patients being diagnosed during advanced stages of the illness[5]. Currently, surgery and chemotherapy are the most effective clinical treatment for most PDAC

patients. Nevertheless, the surgical intervention is improbable to affect the result for individuals with advanced diseases in the late stages or in the local area. Additionally, over the last ten years, FOLFIRINOX and the pairing of gemcitabine and nab-paclitaxel (two new chemotherapy programs) have shown specific efficacy, but the anticipated improvements in survival and limitations on treatment-related toxicities are not as effective as expected[6-8]. In the face of pancreatic cancer (PC) treatment, immunotherapy may give patients hope.

Immunotherapy known as immune-checkpoint-inhibitors (ICIs), including anti-PD-1/anti-PD-L1, have significantly enhanced results for individuals diagnosed with melanoma and non-small cell lung cancer[9]. Certain subsets of B cell leukemia or lymphoma have shown remarkable clinical responses through the use of adoptive cell therapy, such as CAR-T therapy utilizing chimeric antigen receptor T cells[10]. Although immunotherapy has made impressive progress, the efficacy in the immunotherapy of PDAC is disappointing. Apart from the less than 1% of patients who have microsatellite instability high (MSI-H) tumors, PDAC is mostly resistant to immunotherapies approved by the FDA[11], largely because the fact that PDAC has an immunologically 'cold' tumor microenvironment (TME) where myeloid cells are abundant and CD8+ T cells are usually absent[12]. This helps the pancreatic tumor evade immune surveillance and anti-tumor disorders. Furthermore, PC is characterized by elevated desmoplasia and severe hypoxia, which are common pathological traits and major factors contributing to the limited efficacy of immunotherapy in treating PC. To overcome these challenges, there has been a growing interest in addressing the immunosuppressive TME and transforming it from a 'cold' to a 'hot' environment. In this particular scenario, myeloid cells, specifically neutrophils, are regarded as a potential focus for the future of PC immunotherapy.

Compared to other types of cancer, tumor-associated neutrophils (TANs) are immune cells that infiltrate the immunosuppressive PC microenvironment in significant numbers. TANs can form NETs to promote pancreatic cancer progress[13]. They facilitate immunosuppression and desmoplastic function to interfere with immunotherapy through interaction with other TME cells, upgrading expression of PD-L1/PD-1 and metabolic reprogramming. Therefore, scientists have progressively redirected their attention towards targeting TANs in order to enhance the efficacy of PC immunotherapy, which holds immense promise.

2. Transition of neutrophils to TANs

2.1: Neutrophils: origin and recruitment to PC

Neutrophils are derived from precursor cells of the myeloid lineage that are situated in the bone marrow and other tissues outside the marrow, such as the spleen. Throughout the early stages of differentiation, the myeloid progenitors maintain their inclination to differentiate into both the monocyte/macrophage lineage and the neutrophil lineage, in addition to the eosinophils and basophils[14, 15]. Neutrophils undergo amplification and maturation in the bone marrow, proceed into the circulatory system, and then migrate to the pancreatic tumor, eventually infiltrating the PC micro-environment[16]. Although neutrophils are typically regarded as cells with a brief lifespan, they actually have a circulation half-life of around 8 hours. Nevertheless, certain research indicates that their lifespan in the circulation is approximately 5.4 days[17-19], which provides the time necessary for TANs to play an immunosuppressive role in pancreatic cancer.

The entry of neutrophils into the circulatory initiates through alterations in endothelial cells and proceeds through various stages: attachment to the blood vessel lining, rolling, adhesion, crawling, and ultimately transmigration[20]. Neutrophils initially rely on selectin for rolling near the vascular edge before transitioning to integrin-mediated adhesion, enabling a firm attachment to endothelial cells[21, 22]. Subsequently, neutrophils exit the vascular endothelium by attaching to platelet endothelial cell adhesion molecules, which can be found on both neutrophils and endothelial cells[23]. Neutrophils, upon arrival at the vascular basement membrane, utilize substances like collagenase to break it down, demonstrating a liking for areas with reduced expression of extracellular matrix components in order to penetrate the adjacent tissues. Neutrophils then navigate through intercellular signals between pericytes, maneuvering along the cell surface until they locate openings that allow them to exit the vasculature. Once extravasated, neutrophils display directed movement

aligned with the concentration gradient of chemical signals, ultimately gathering at PC. The chemokine of the CXC family, is one of the core chemical signals of attracting neutrophils to PC microenvironment, jointly regulated by granulocyte colony-stimulating factor(G-CSF) and interleukin-7(IL17). Neutrophils transitioning functions in humans are mediated by a minimum of seven chemokines (CXCL1, CXCL2, CXCL3, CXCL5, CXCL6, CXCL7, CXCL8 (IL8)) and two receptors (CXCR1, CXCR2). Specifically, neutrophils express CXC receptor CXCR1 and CXCR2, which interact with the CXC family chemokines released by tumor sources[24]. Monomers and dimers of chemokines from the CXC family are present, and their conformation can be altered reversibly upon receptor binding. These changes are associated with both G-protein and β-arrestin signaling pathways. Activation of G-protein signaling triggers the activation of different effectors, like calcium channels and phospholipase C. Conversely, β-arrestin functions as a versatile adapter and is linked to numerous signaling centers, such as MAP kinase and tyrosine kinase pathways. Neutrophil migration is facilitated by the dynamic remodeling of actin through both G-protein and β-arrestin signaling pathways. During the migration towards PC, neutrophils are induced by the concentration gradient of CXCR2 ligand, leading them from lower to higher concentrations[25]. The regulation of neutrophils proliferation and maturation is significantly influenced by G-CSF. Simultaneously, the presence of G-CSF diminishes the presence of CXCR4 and its corresponding ligand CXCL12, consequently controlling the movement of neutrophils[26]. Subsequently, G-CSF collaborates with CXCR2 and CXCL2 to facilitate the mobilization of neutrophils. Moreover, IL-17, highly effective in attracting neutrophils, enhances the production of different chemokines, such as G-CSF, IL6, CCL2, and CXCR2, ultimately impacting the movement of neutrophils[27-29].

During the recruitment and infiltration of neutrophils, PC cells play a key role which is mainly derived from the PC cells producing cytokines. Tumor cells from PDAC are able to attract neutrophils through the release of CXCL-16, CXCL-8 and CXCL5[30, 31]. The immersion of neutrophils is aided by the release of IL-1β from PC cells[32]. The control of these chemokines is highly reliant on the binding of the nuclear factor-k-gene (NF-ϰB). In PC cells, the inhibition of KRAS/MEK triggers the activation of NF-ϰB signaling, resulting in the release of CXCL5. Consequently, this leads to an enhanced recruitment of neutrophils[33]. Conversely, the downregulation of NF-ϰB signaling leads to reduced infiltration of MDSCs through the action of CXCL2 and CXCL5 at low levels[34]. The production of cytokines can be attributed to various factors, including the impact of inherent genes and proteins originating from PC cells. The gain-of-function mutation of the Trp53 gene which suppresses tumors, in PC cells enhances the chemokines CXCL2 and CXCL5 production, resulting in the recruitment and infiltration of neutrophils[35]. PDAC cells upregulated SPRY1 gene expression to control protein derivatives that interacted with ubiquitin carboxy-terminal hydrolase L1, leading to the activation of the NF-ϰB signaling pathway and ultimately enhancing the expression of CXCL12[36]. For inherent proteins, the deficiency of PC cell-intrinsic SETD2, a histone H3K36 trimethyltransferase, which is an inherent protein, enhanced the PI3K-AKT pathway and led to the overexpression of CXCL1 and GM-CSF, thus contributing to the recruitment of neutrophils[37]. Reg3g, a soluble small protein expressed in PC cells, can increase the secretion of IL-10 and transforming growth factor-β (TGF-β) to facilitate the recruitment of MDSCs[38]. In addition to influencing the production of chemokines, PC cells enhance the formation of channels between PDAC cells and neutrophils by upregulating the gap junction protein beta 3, thereby facilitating the transfer of cAMP from cancer cells to neutrophils, which promotes the infiltration[39]. Overall, these discoveries emphasize the intricate connections between PC cells and neutrophils, offering understanding into the mechanisms that drive the transformation of neutrophils into TANs.

2.2: Polarization of neutrophils to TANs

Neutrophils, the initial cells to reach locations of emerging inflammation, have a crucial function in inflammation associated with cancer, often referred to as the "wound that never heals". However, continuous infiltration of neutrophils is a distinctive feature of long-lasting inflammation and adds to the damage of tissues. Consequently, due to the inflammatory environment in the tumor cells, neutrophils undergo stimulation and polarization into various epithelial types. Based on the anti-tumorigenic and pro-tumorigenic function of neutrophils, it is widely classified in N1 and N2 types by the academic community, including the proinflammatory protumor, anti-inflammatory protumor, proinflammatory antitumor, anti-inflammatory

antitumor[40]. Specific surface markers have been identified in order to distinguish between the two types. N1 neutrophils exhibit elevated levels of ICAM-1 and CD95 expression, while displaying reduced CD182 expression[25]. Conversely, N2 neutrophils demonstrate diminished levels of ICAM-1 and CD95 expression, while displaying elevated CD182 expression[25]. The drivers of neutrophils polarization are complex, involving many cytokines and signal pathways. TGF-β functions as an immunosuppressive compound within the tumor microenvironment, causing neutrophils to adopt a protumorigenic phenotype (N2) type[41]. Blocking TGF-β with the TGF-β receptor inhibitors SM16, LY2157299, and AGEN1423 result in the accumulation of neutrophils exhibiting an antitumor phenotype (N1). I interferon (IFN) induced the phenotype changes in both mouse and human neutrophils from N2 type to N1 type [42]. There is a high probability that the polarization of N1 and N2 represents an antagonistic signaling pathway involving TGF-β and type 1 IFN cytokines. Nevertheless, the precise system has yet to be validated. In addition, In recent years, HIF-1 ∝, BHLHE40, A2A adenosine receptor, S100A9, TLR4, Lipocalin-2(LCN2), adenosine triphosphate(ATP) and adenosine(ADO) have been shown to correlate with the polarization of N1 and N2[43-49]. S100A9 disrupts the NF-kB signaling pathway, reducing the activity of the pro-inflammatory N1 phenotype[47]. A2AAR together with A2BAR promotes N2 at the expense of N1 activation[49].

The current limitations of TANs studies are due to the absence of technical proficiency and the intricacy of the populations involved. The primary emphasis is on the subsequent domains: (1) The categorization of TANs primarily relies on the M1 and M2 classification of tumor associated macrophages (TAMs). However, this simple binary partitioning is far from meeting the current research on TANs. Reassuringly, MDSC has been recently found as an immunosuppressive neutrophil to broaden the polarization family of TANs. Furthermore, the rapid progress in single-cell sequencing techniques has enhanced our comprehension of TAN polarization. (2) TANs have the ability to assume either the anti-tumorigenic 'N1' phenotype or the pro-tumor 'N2' phenotype depending on their function, and extensive studies have been conducted to understand the mechanisms behind promoting N2 polarization. Nevertheless, the N1 characteristics of TANs exhibit their capacity to combat tumors, a trait that we must not disregard. Sadly, few studies related to promoting neutrophils polarization to N1.Targeting the polarization of TANs to inhibit the transition to N2 and promote it to N1 is the future of PC immunotherapy. Therefore, studies about N1 need to be studied further.

Fig.1 The process of neutrophils transforming to TANs

Neutrophils undergo amplification and maturation in the bone marrow, enter the circulatory system, and then migrate to the PC, infiltrate the micro-environment, eventually polarize to N1 and N2. The figure was created using Figdraw (*www.figdraw.com*).

3: Neutrophil extracellular trap(NET)

NET, which is a net-shaped structure composed of nucleic or granular DNA fibers, polymorphic and chromosome-binding cytoproteins, and particulate proteins, is formed by neutrophils in the extracellular space. NET formation follows a regulated and specialized cell death process called NETosis[50]. NET formation comprises key steps such as nuclear enlargement, breakdown of the nuclear envelope, merging of nucleic acids and granule proteins within a spacious intracellular vacuole, discharge of nuclear content into the cytoplasm, and ultimately, cell membrane disintegration[51]. Recently, Tumor-derived protein tissue inhibitor of metalloproteinases-1 (TIMP1) has been studied and found to be a new mechanism that forms NET in PDAC[52], binding to CD63 on neutrophils thereby triggering ERK phosphorylation to form NET, which is important because TIMP1 is overexpressed and secreted in PDAC development[53]. Another mechanism involves the loss of KDM6A and the activation of the receptor for advanced glycation end products (RAGE), both of which induce the generation of NET in PDAC[54, 55]. The failure of immunotherapy in PC can be attributed to various factors, and one such factor is the involvement of NETs in tumor progression, which includes PC immunoevasion and crosstalk in the immune system and TME cells[56] (this will be discussed in the next paragraph). Therefore, metformin and chloroquine as emerging inhibitors of NET have been seen as the future of optimizing immunotherapy [57, 58]

4: TANs lead to failure of immunotherapy for pancreatic cancer

Immune cell entry into the tumour microenvironment is crucial in anti-tumour immunity for recognizing and eliminating PC cells. However, the existence of TANs promotes the development of a robust stromal barrier by interaction with TME cells thereby hindering this process. Consequently, immune cell infiltration is impeded, resulting in the failure of immunotherapy. Moreover, even when immune cells manage to enter the PC microenvironment, the immunosuppressive factors and hypoxic microenvironment present suppress their activity[59]. TANs contribute to this immune evasion mechanism by enhancing PD-L1 and PD-1 expression, and inducing metabolic reprogramming in hypoxic conditions. These factors collectively contribute to the overall failure of PC immunotherapy.

4.1: Interaction with other TME cells

Pancreatic cancer exhibits a substantial and compact desmoplastic stroma, which makes up 70–90% of the tumor's overall volume[60]. The network is intricate and comprises different types of extracellular matrix (ECM) and stromal cells, like cancer-associated fibroblasts (CAFs) and pancreatic stellate cells (PSCs), along with vascular-associated smooth muscle cells, pericytes, endothelial cells, mesenchymal stem cells, immune cells such as tumor-associated macrophages (TAMs), neurons, and blood vessels[61, 62].This review focuses on the interaction between TANs and PSCs, CAFs, TAMs in PC microenvironment.

Pancreatic stellate cells (PSCs)

Pancreatic stellate cells (PSCs), which are distinct to the pancreas, can usually be found in a dormant condition on the outer surface of the acinus[63]. The differentiation of PSC into myofibroblastic and inflammatory subtypes is greatly affected by soluble cytokines released by PC cells[64]. In the desmoplasia, myofibroblastic PSCs play a significant role, forming a physically protective barrier around PC cells, providing immunological protection against recognition[65]. Likewise, inflammatory PSCs generate hyaluronic acid (HA), which forms a physical barrier by inducing external pressure, thereby decreasing the infiltration of immune cells into the PC microenvironment[66, 67].

PSCs in the orthotopic PDAC model caused the enlargement of TANs, resulting in heightened infiltration of TANs within the tumor[68]. The combination of PSCs and PC cells greatly amplified this impact. In contrast, the release of TANs DNA can stimulate the proliferation of PSCs by activating the signal from the receptor for advanced glycation end products (RAGE), and this role of DNA in promoting stellate cell proliferation is eliminated when the receptor is absent[69]. IL-1β plays a crucial part in the clash between the PSCs and the TANs. In PDAC, TANs secrete IL-1β to induce PSCs activation enhancements and then PSCs can also further secrete IL-1β to recruit/activate TANs, as demonstrated by a study conducted by Joao Incio and his colleagues[70]. In addition, TANs can alter the micro-structures of the base of pancreatic cancer by reprogramming stellar cells[71], and the related mechanism is that TANs regulate the expression of α-SMA in stellar cells.

Cancer-associated fibroblasts (CAFs)

CAF, like PSCs, can also generate a stromal barrier to hinder the infiltration of immune cells, but the diffidence is that there is limited investigation on the interaction between TANs and CAFs in PC, primarily concentrating on NETs and CAFs. TANs generating NET adjust the activity of CAFs and also driven by CAFs. Shin Takesue team applied treatment with DNase I, a NET inhibitor, suppressed the activation of CAFs in PDAC mice[72]. Conversely, CAFs released Amyloid β in the vicinity through CD11b on TANs within the PDAC, thereby triggering NET formation in a ROS and PDA4-dependent manner[73]. According to the same study, NET stimulates CAF expansion, contractility, and matrix component deposition supportive of PDAC growth.

Tumor-associated macrophages (TAMs)

The previous article described the recruitment of TANs, and the recruitment of TAM is also inseparable from the G-CSF and CXC families, and the related channels also seem to be intervention. For example, the

activated neutrophils that release IL-8 (CXCL8) and TNF-α activate in the inflammation site and recruit macrophage cells [74]. In terms of polarization, TGF-β not only promotes N2 polarization but also promotes M2 polarization, where neutrophils recruit macrophages prior to their N2 polarization. It suggests that TAMs are closely associated with TANs in a TME. TAMs and TANs are also noteworthy in relation to the development of stromal barrier in PC. In co-cultivation, TANs and TAMs generated elevated amounts of oncostatin M (OSM) and IL-11, respectively, compared to single cultivation[75]. Reprogramming OSM-OSMR signals can induce fibrous cell formation, thereby enhancing PC growth[76]. Not only TAMs, but also TANs, secrete OSM to stimulate tumors[77]. However, TANs and TAMs may be mutual inhibiting relationships in PC. Timothy's team conducted therapeutic responses and related studies in mice with established PDAC tumors with small molecular CCR2 inhibitors and CXCR2, respectively, in combination with chemotherapy. CXCR2 neutrophils were found to be elevated in the blood, bone marrow and tumor of human PDAC patients and associated with low overall survival rates, while hormone mice showed an increase in CXCR2 neutrophils after treatment targeting CXCR2 TAMs. Instead, preventing CXCR2 granulocyte mobilization in PDAC may result in an increase in TAMs [30].

4.2: The immunosuppressive functions of TANs in PC

Observations of interactions between TANs and T cells are evident in cases of pancreatic cancer. CD17 and γδT cells can enhance the recruitment of neutrophils by secreting IL-4[78]. Moreover, the rise in the ratio of cells generating IFNγ within the activated subsets of CD4 and CD8 T cells might play a role in the transformation of TAN from N2 to N1[79].On the contrary, TANs suppress immune cells. The removal of TANs from PC can additionally hinder the growth of CD8 T cells and promote the growth and demise of the CD56 T cell[80]. The powerful immunosuppressive function of TANs is the most direct factor for PC immunotherapy failure. Our main emphasis is on presenting the expression of PD-L1/PD-1 and the metabolic reprogramming of TANs in PC.

PD-1,PD-L1 on TANs

The role of PD-L1/PD-1 in tumor immune evasion is important as it hinders the activation of RAS-ERK1/2 and PI3K/AKT signaling pathways. This inhibition leads to the suppression of downstream molecules PI3K, PLCγ2, and ERK, which in turn inhibits the proliferation of T lymphocytes[81, 82]. Additionally, it reduces IL-2 production and glucose metabolism[83], resulting in long-term growth arrest[84]. In general, PD-L1 is found on cells that belong to the hematopoietic lineage, which encompasses activated T cells, B cells, monocytes[85, 86], dendritic cells[87], and macrophages[88]. Lymphocytes and myeloid cells naturally express PD-1 on their cell membranes. It should be emphasized that neutrophils and NETs have the ability to express PD-L1 and PD-1 as well[89]. TANs and NETs contribute to immune evasion by interacting in a PD-L1/PD-1-dependent way, a phenomenon well acknowledged in PC. Xu Wang groups conducted in vitro experiments to assess the proliferation of cytotoxic T lymphocytes (CTLs). The findings suggested that the existence of P2RX1-deficient neutrophils significantly suppressed the proliferation of CTLs. Nevertheless, the suppressive impact was counteracted upon the introduction of an anti-PD-1 neutralizing antibody. In addition, neutrophils lacking P2RX1 were observed to effectively suppress the cytotoxic activity of OVA-specific CTL. Similarly, suppression was also subsequently reversed upon the introduction of Anti-PD-1 antibody[90]. Importantly, PDAC liver metastases systematically attract neutrophils that lack P2RX1, leading to the development of an immunosuppressive microenvironment in PDAC liver metastases through the involvement of PD-L1/PD-1[91]. NET is no exception, NETs in PDAC contain the immunosuppressive ligand PD-L1, which exerts an exhausting and dysfunctional effect on T cells[92, 93]. Mechanistically, tumor cells exhibiting elevated glycolysis rates mechanistically discharged lactic acid into their vicinity, potentially leading to heightened PD-1 expression on TANs[94]. The mechanisms of TANs expressing PD-L1 are diverse. Nuclear factor-erythroid 2 p45-related factor 2 (Nrf2) is a crucial transcription factor that controls multiple cellular reactions to environmental stresses[95]. It plays a significant role in regulating the metabolism of monoxide, purines, fatty acids, and the TCA cycle[96]. In PDAC, TANs increase reactive oxygen species (ROS) production to boost Nrf2 function and inhibiting Nrf2 with a chemical antagonist can increase the expression of PD-L1 on TANs[90]. Furthermore, autophagy is expected to enhance the expression of PD-L1

on TANs in PDAC. The impact of autophagy on the expression of PD-L1 on neutrophils has been examined in numerous studies[97, 98], yet the specific mechanism in PDAC remains uncertain. The research team led by Shenghong Yang discovered that the absence of autophagy due to the removal of ATG5 resulted in an increase in the expression of PD-L1 in PDAC[99]. This suggests that the deletion of ATG5 can activate TBK1, leading to elevated levels of CCL5[100], that recruited T cells to produce IFNγ, thereby fueling PD-L1 upregulation.

Currently, the only information available is that TANs facilitate immune evasion of PC cells by performing immunosuppressive activities via the PD-L1/PD-1 pathway, while the specific mechanisms behind this process are still unknown. However, TANs can hinder the activity of T-cells through a PD-L1-PD-1 reliant mechanism[101-104] in other certain malignancies. Moreover, they can impede the NK cells' immune response against tumors by interacting with the PD-L1 / PD-1 axis[105] and even potentially modulating the self- toxicity of tumor cells through binding with PD-1[89]. In conclusion, relevant research in PC is urgently needed. Contrary to popular belief, a recent investigation carried out by Keyu Li proposed a contrasting point of view. Multi-omic analyses were conducted in the research, examining paired pre- and post-treatment PDAC samples obtained from a platform neoadjuvant study involving GM-CSF-secreting allogeneic PDAC vaccine (GVAX) vaccine with or without nivolumab (PD-1).The findings indicated that while TANs have a greater influence on immune regulation in PDAC TMEs, there was no observed correlation between PD-L1 TANs and OS in the treatment arm[106].This implies that in the future, we may need to shift our focus towards PD-1 TANs; however, there is currently limited research on this topic.

Metabolic reprogramming

Metabolic reprogramming refers to the changes in energy metabolism of cells to enable their survival in harsh environments. TANs' function is influenced by glucose metabolism, lipid metabolism, tricarboxylic acid cycle, and amino acid metabolism[107]. This review primarily concentrates on the regulation of immune cell function by TANs, specifically emphasizing metabolic reprogramming in glucose and amino acid metabolism.

Glucose metabolism

The strong fibroinflammatory tissue contributes to making tumors deprived of oxygen in PC, changing metabolic pathways within the tumor and resulting in unfavorable survival. TANs have a glycolytic character and demonstrate a robust commitment to anaerobic glycolysis for energy production and to support their effector functions[108]. TANs are capable of performing their immunosuppressive role due to the storage and buildup of glycogen in neutrophils[109], allowing them to function effectively in a hypoxic PC microenvironment with restricted oxygen and glucose availability.

The progression of PC and immunosuppression are promoted by HIF-1α, a crucial factor that regulates cell adaptation to hypoxia[110, 111]. LDHA, also known as lactate dehydrogenase A, is an enzyme composed of four subunits. It plays a crucial role in controlling the last stage of aerobic glycolysis and has the ability to generate L-2 hydroxyglutarate in low oxygen conditions. This compound hinders the proliferation and movement of T cells, thereby aiding in the immune evasion of PC cells[59, 112]. In PC, glucose metabolism helps TANs restrain immune cells through improving HIF-1α and LDHA expression. The BHLHE40 gene functions as a link between HIF-1α and LDHA, leading to a notable increase in the expression of HIF-1α and LDHA, having a suppressive impact on the production of pro-inflammatory cytokines by CD8 T cells and the proliferation capacity of lymphocytes[43, 113]. Among these, by increasing the level of ARG1 in TANs, HIF-1α carries out immunosuppressive functions[44]. In the meantime, neutrophils lacking HIF-1α enhanced the anti-cancer function of CTLs and NK cells, thereby contradicting the immunosuppressive impact of HIF-1α[44]. Ιτ ις ηιγηλψ προβαβλε τηατ τηε ραπαμψιν (μTOP) σιγναλινγ πατηωαψ ις ρεσπονσιβλε φορ τηε ιντριςατε προςεσσες, ας τηε αβσενςε οφ ΗΙΦ-1α in mice could lead to heightened mTOR activity in tumor infiltrating CTLs, resulting in hindered proliferation, cytotoxicity, and granzyme B expression due to the presence of mTOR inhibitors[114]. Similarly, TANs exhibit increased LDHA expression, leading to decreased IFNγ and TNFα expression in CD13 T cells. LDHA is also upregulated in response to CD3/CD28 activation, resulting in a slight inhibition of T cell proliferation[43].

Amino acid metabolism

Arginase1 (ARG1), an important enzyme in the urea cycle, breaks down L-arginine into urea and L-ornithine. This enzyme can limit the immune response of T cells by depleting arginine, thereby controlling the immune evasion of PC cells[115], owing to arginine is crucial for the survival, growth, differentiation, production of cytokines, and functioning of T cells[116, 117]. Recent research has closely linked ARG1 to TANs. In PDAC, Jing Zhang and colleagues discovered that a specific inhibitor of ARG1 could eliminate the inhibition of T cell proliferation caused by TANs[118]. This indicates that the potent immunosuppressive effects are mediated through mechanisms related to ARG1. It is interesting to note that NET has the ability to not only produce ARG1 but also boost its functionality. The NETs released by TANs derived from individuals with PDAC establish a microenvironment where cathepsin S (CTSS) breaks down human ARG1 into various molecular forms that possess heightened enzymatic activity under normal pH conditions[119]. Consequently, these enzymatically active aggregates are formed, leading to the inhibition of T cells.

Fig.2 Interaction between TANs with other TME cells and the immunosuppressive functions of TANs in PC

TANs promote the development of a robust stromal barrier by interaction with PSCs, CAFs and TAMs. In addition, TANs enhancing PD-L1 and PD-1 expression, and utilizing metabolic reprogramming in hypoxic conditions suppress immune cells thereby lead to PC immunoevasion. These are why PC immunotherapy has failed. The figure was created using Figdraw (*www.figdraw.com*).

5: TANs and PC immunotherapy

Numerous experiments have been carried out to assess the effectiveness of immunotherapeutic approaches in PDAC, such as immune checkpoint inhibitors (ICI), adoptive cell transfer, oncolytic virus (OV) therapy, and agonistic anti-CD40 monoclonal antibody (mAb) therapy, resulting in notable outcomes. The role of the TANs is closely associated with these strategies, which is quite fascinating. Studies have shown that individuals receiving CAR-T cell treatment often encounter decreased toxicity caused by neutrophils[120], and G-CSF is frequently employed to handle associated negative responses[121]. Furthermore, OV treatment enhanced the maturation of DCs and activation of cytotoxic T cells, resulting in a notable increase in the survival duration of mice afflicted with PDAC. After the administration of OV-mOX40L, the combination of OV and OX40L resulted in a synergistic or additive effect, leading to a decrease in the proportion of N2 neutrophils, a significant increase in pro-inflammatory N1 neutrophils and an enhancement of anti-tumor immune responses[122]. Another promising immunotherapy in PDAC is anti-CD40 mAb. When activated, CD40 is a cell-surface member of the TNF receptor superfamily that promotes the activation of antitumor T cells. Consistent with other treatments, TANs have been linked to reduced survival in patients with PDAC who receive a combination of an anti-CD40 monoclonal antibody and gemcitabine[123]. As a result, the focused therapy of TANs has become increasingly popular in the realm of PC immunotherapy.

5.1: Targeting TANs optimizes PC immunotherapy

Despite the widespread use of immunotherapy in clinical practice, it is insufficient on its own to fully impede the advancement and spread of pancreatic tumors. Consequently, there is a focus on directing attention towards TANs, as they have the potential to enhance the effectiveness of immunotherapy treatments, specifically ICI. The primary approaches for treating PC ICI that focus on TANs involve suppressing the recruitment and polarization of TANs, as well as disrupting the immunosuppressive capabilities of TANs. Currently, the most prevalent treatment is blocking recruitment.

Fig.3 The strategies of treatment on PC ICI targeting to TANs. (1) Inhibiting neutrophils recruitment to PC. (2) Inhibiting the formation of NET. (3) Inhibiting neutrophils polarizing to N2 and promoting them to N1. (4) Inhibiting TANs immunosuppression-related targets. The figure was created using Figdraw (*www.figdraw.com*).

Blocking the recruitment

The receptor CXCR2 is extensively researched as a site of action for TANs-targeted PC ICI therapy due to its crucial role in the recruitment and activation of neutrophils. To mobilize and recruit neutrophils, this G-protein-coupled receptor interacts with different human CXC chemokines, such as CXCL1, CXCL2, CXCL3, CXCL5, CXCL6, CXCL7, and CXCL8. Consequently, targeting CXCR2 can be beneficial in reducing TANs in the PC microenvironment. Indeed, blocking CXCR2 has been demonstrated to significantly improve the responsiveness to PC anti-PD1 immunotherapy[124]. Blocking CXCR2 enhanced the quantity of CD3, CD4, CD8 T lymphocytes and decreased suppressive regulatory T cells, resulting from the impediment of TANs recruitment[33]. Furthermore, the suppression of CXCR1[125] and CXCR8[126] further enhances the efficacy of PC ICI treatment.

Similarly, blocking G-CSF and GM-CSF can optimize PC ICI therapy. By inhibiting the impact of G-CSF and GM-CSF on neutrophil development and decreasing the recruitment of TANs to the PC microenvironment, Lorlatinib effectively hinders the progression of PDAC [127]. By combining with immune checkpoint inhibition, lorlatinib enhances the effectiveness of PD-1 inhibition, resulting in increased activation of CD8+ T cells within PDAC tumors[127]. Furthermore, the combination of Gemcitabine with anti-CSF1 receptor, anti-PD-1, and anti-PD-1 not only decreased the recruitment and infiltration of TANs but also enhanced the antitumor efficacy[128].

Blocking the polarization

By function, TANs have the ability to transition into either the anti-tumorigenic 'N1' phenotype or the pro-tumorigenic 'N2' phenotype. The current treatment strategy is to target the polarization of TANs to inhibit the transition to N2 and promote it to N1. TGF-β, a widely studied polarization target for TANs, can convert TANs to N2 specimens. The growth of PC is significantly inhibited and the immersion of anti-tumor M1 macrophage cells in TME is enhanced by testing the TGFβ receptor I kinase inhibitor in combination with the anti-PD-L1 antibody to treat PC patients[129, 130]. Furthermore, in comparison to ICI alone, Irreversible electroporation (IRE) has emerged as a new ablative method for the clinical management of pancreatic cancer[131]. the combination of IRE with ICI exhibits encouraging therapeutic efficacy in both pre-clinical and clinical experiments[132]. TANs regulation with TGF inhibitors can enhance PC response to IRE and immunotherapy combined[133]. IFN can promote the polarization of TANs to N1 epithelium, and has been used in PC ICI. TMAO is a metabolite produced by microorganisms in the gut. A recent study found that TMAO seems to enhance the type-I IFN pathway, thereby increasing the effectiveness of checkpoint immunotherapy and improving the survival of mice with tumors[134].

Blocking the Immunosuppression-related targets

Until recent years, some immunosuppressive mechanisms of TANs in PC have been explained. Although, significant researches have been conducted on targeting immunosuppression-related targets, implementation of these findings in the clinical setting is still lacking. In the PC setting, Methotrexate-induced microparticles derived from tumor cells have been discovered to boost T-cell antitumor reactions by reducing the expression of PD-1 in TANs. The enhancement of the curative impact of individual anti-PD-L1 therapy has been proven[94]. Furthermore, when it comes to NETs, IL17 can recruit neutrophils, induce NET formation, and eradicate cytotoxic CD8 T cells from the tumor[78]. By inhibiting IL17 in this process, the sensitivity of immune system blockage (PD-1, CTLA4) can be enhanced, leading to an improvement in PC immunotherapy[78]. NETs contain non-cellular ARG1 released by activated bone marrow cells, which is another significant aspect of PC. When an ARG1 inhibitor is combined with ICIs, it can reverse the inhibition of T lymphocytes and restore the function of CD8+T cells in PDAC tumors within the extracellular milieu[119].

Table 1 Clinical study on targeting TANs optimizing PC ICI

Strategy	Target	Drug	Immune checkpoint inhibitor	Status
Block the recruitment	CXCR1	LY2510924	Durvalumab	Terminated
	CXCR1/CXCR2	SX-682	Nivolumab	Recruiting

Strategy	Target	Drug	Immune checkpoint inhibitor	Status
			Tislelizumab	Recruiting
	CXCR4	BL-8040	Pembrolizumab	Active, not Recruiting
				Completed
			Cemiplimab	Recruiting
			Atezolizumab	Recruiting
		AMD3100	Cemiplimab	Completed
	CXCR2/CXCR5	BMS-813160	Nivolumab	Completed
				Recruiting
	CXCL12	NOX-A12	Pembrolizumab	Not yet Recruiting
				Completed
	IL-8	BMS-986253	Nivolumab	Recruiting
	IL-6	Tocilizumab	Ipilimumab, Nivolumab	Terminated
Block polarization	TGF-β	LY2157299	Durvalumab	Completed
		AGEN1423	Balstilimab	Not yet recruiting
		TGFβ peptide vaccine	Ipilimumab	Recruiting

5.2: Prognostic value after PC immunotherapy

Pancreatic cancer is notorious for its poverty in prognosis, and in recent years, there has been a lot of researches on TANs for the prognosis of pancreas cancer. The ratio of neutrophils to lymphocytes (NLR), which is regarded as one of the indicators of the systemic inflammatory response caused by PC cells, facilitating the infiltration of neutrophils and lymphocytes, is a useful predictor of PC prognosis following surgery[135, 136]. The relationship between NLR and the phenotypes of immune cells in patients with PDAC is closely connected. Patients with NLR [?] 2.5 exhibited a notable rise in the CD3+ and CD8+/CD28+ T cell subsets, whereas a significant decline was observed in the CD8+/CD28- and CD4+/CD25+ cell subsets[135]. Based on these, the NLR also having prognostic value for PC immunotherapy is not unexpected. After undergoing radiotherapy, the NLR can serve as a predictive indicator for pancreatic cancer patients who are treated with anti-PD-1 antibodies and stereo-directed radiotherapy[137]. Patients with PDAC who experience a substantial alteration in NLR following two doses of immune checkpoint blockade are at an increased likelihood of mortality[138]. Furthermore, the outcome in patients with PDAC treated with PD-1 inhibitors is associated with the combined indicators of NLR and LADH[139]. However, NLR, which predicts PC immunotherapy results, has not obtained a recognized critical value currently. Many research results are different and there are problems with less research samples. Additionally, N1/N2 is likely to be a future prediction target[140].

6. The future of PC immunotherapy based on TANs

Increasing evidence links the TANs to the immunosuppressive microenvironment of PC. TANs, as a novel area of study, have significant potential for PC immunotherapy. However, PC immunotherapy often leads to immune-related adverse events (irAEs) such as skin inflammation, inflammation of the thyroid gland, lung inflammation, inflammation of the colon, liver inflammation, kidney inflammation, inflammation of the pituitary gland, inflammation of the adrenal glands, and muscle inflammation[141]. IrAEs are believed to occur due to a disruption in self-tolerance, which is influenced by T-cell responses specific to antigens, autoantibodies, B cells, and cytokines. Interestingly, neutropenia is a common occurrence in almost all immunotherapies, warranting investigation into whether TANs play a role in the mechanisms underlying these adverse events and whether targeting TANs can mitigate them. Additionally, recent research has highlighted the importance of the interaction between TANs and the microbiota[142]. Modulating the microbiome has been shown to enhance the anti-cancer immune response and facilitate successful PC immunotherapy[143]. Given these findings, it is worth exploring the potential for targeting TANs and their interaction with the microbiota in PC immunotherapy. Hence, exploring the impact of focusing on TANs in medical immunotherapy for PC

shows great potential, yet additional investigation is necessary to accelerate its clinical application. These researches would offer valuable direction for the management of medication, specifically concentrating on the subsequent encouraging domains.

1) An encouraging strategy for utilizing TANs in the treatment of PC involves employing neutrophils in nanomedicine applications. Nanomedicine is an effective means of targeted tumor treatment. Neutrophils have become a viable option for drug transportation[144] and the utilization of neutrophilic membrane-derived nanocouples for delivering medication [145] is anticipated for clinical use in the near future. Nanoparticles (NPs) have the ability to selectively target activated neutrophils, potentially utilizing them for drug delivery[146]. Moreover, it is crucial to take into account that therapies on personal computers like radiation treatment and immunizations can inherently trigger the migration and infiltration of neutrophils, which can be advantageous for the implementation of this method. However, PC chemotherapy with drugs like gemcitabine and immunotherapy such as CAR-T cell therapy may result in neutrophils deficiency, posing challenges for the implementation of this technique.

2) For immunotherapy of PC, perhaps TANs are expected to become the main force in the future, and we will place our hope on chimeric antigen receptor neutrophils (CAR- neutrophils). Yun Chang's group utilizes CRISPR/Cas9 to modify human pluripotent stem cells, introducing different anti-Glioblastoma CAR constructs that contain either T-specific CD3ζ or neutrophil-specific γ-signaling domains. CAR-neutrophils are produced by selecting the CAR constructs that yield the most efficient anti-cancer effects. The main goal is to deliver nanodrugs that respond to the tumor microenvironment in order to target Glioblastoma, while avoiding any extra inflammation at the tumor locations[147, 148]. The non-specific and non-invasive nature of this method makes it an attractive option for treating pancreatic tumors. Therefore, TANs, specifically CAR- neutrophils, are expected to have a crucial part in the forthcoming immunotherapy of PC.

3) The combination of multi-immunotherapy is anticipated to be the key to the future of PC treatment, with TANs playing a crucial role as effective aides. Through iNOS-dependent mechanisms, Daniel Hirschhorn's team showcased the ability of TANs to eliminate tumor antigens with evasive mutations, thereby exerting anti-tumor effects. The results of this study have important consequences for the use of CD4+ T cell treatment, especially when combined with co-stimulated therapy that includes OX40 or CTLA-4 blockade[149]. Notably, this approach shows promise in eradicating tumors hat harbor antigen escape variants. Therefore, the role of TANs in mediating the anti-tumor function offers valuable insights into the future directions of PC immunotherapy treatment.

Declarations

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Availability of data and materials

The datasets generated and/or analysed during the current study are available in the Home-ClinicalTrials.gov repository,*https://clinicaltrials.gov*.

Authors' contributions

Qihang Wu and Han Mao drafted the manuscript. Qihang Wu researched the literature and drafted figures. Qihang Wu counted and plotted the tables. DongTang critically revised the article for important intellectual content. All authors read and approved the final manuscript.

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Competing interests

The authors declare that they have no competing interests

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