

The Tumor Microenvironment Mechanism and New Immunotherapy Strategies in Pancreatic Ductal Adenocarcinoma

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Abstract

Pancreatic ductal adenocarcinoma (PDAC) is a highly aggressive neoplasm associated with an unfavorable clinical outcome. The immunosuppressive microenvironment and physical barriers within the tumor microenvironment (TME) are major obstacles limiting therapeutic efficacy. Therefore, targeting TME components and developing novel immunotherapies, such as CAR-T cells and mRNA vaccines, have become important research directions. This article summarized the basic principles of immunosuppression and mechanical barrier caused by three kinds of cells in TME: pancreatic cancer cells (PCC), pancreatic stellate cells (PSC), and cancer associated fibroblasts (CAF). At the same time, the specific principles of CAR-T technology and mRNA vaccine technology were introduced, as well as the principles and clinical trial results of new technologies currently used in PDAC treatment. Understanding the complex network composed of TME molecules and cells provides a foundation and ideas for researching therapeutic techniques. Meanwhile, understanding the latest developments and principles of CAR-T technology and mRNA vaccine technology can also provide new ideas for treatment. Improving existing technologies or developing new technologies based on them is a crucial step in completing PDAC treatment.

Key words

Pancreatic ductal adenocarcinoma; Tumor microenvironment; CAR-T; mRNA vaccine

1. Introduction

PDAC, a malignancy derived from the epithelial cells lining the pancreatic ducts, is among the most aggressive tumors affecting the gastrointestinal tract.

Under the current standard of care, fewer than 5% of patients survive beyond 5 years post diagnosis, with a reported survival figure of just 8.2%. According to recent projections, PDAC is anticipated to emerge as the

second leading contributor to cancer related mortality by 2030 [1]. Compounding this poor outlook is the marked resistance of PDAC to conventional therapeutic strategies, encompassing chemotherapeutic agents, radiotherapy, and immunotherapies.

TME is the main cause of high drug resistance in PDAC. It is composed of cancer cell components and non-cancer cell components, with non-cancer cell components accounting for 90% of the total volume [2]. The core features of PDAC's tumor microenvironment (TME) are cellular fibrosis, heterogeneous stromal cells, and suppressed immune microenvironment. The immune microenvironment helps cancer cells escape immune surveillance and promotes cancer cell metastasis. Immunotherapy has shown promise in the treatment of various cancers, but the clinical trials of PDAC failed to meet the expectations [1]. The main reason is that under the effect of the core characteristics of TME, immune cells cannot enter the tumor and their activity is also suppressed. Another reason is that PDAC is a cold tumor, which means that the surface antigen of tumor cells is less, so the immune system is difficult to recognize or attack cancer cells, so even if the immune cells are activated, the effect is also limited. The current treatment methods have shifted from simply attacking tumor cells to reshaping the microenvironment, with the aim of transforming "cold tumors" into "hot tumors" and further improving treatment efficacy [3]. At the same time, other treatment methods for PDAC, including radiotherapy, chemotherapy, and surgical intervention, have not achieved ideal results, with one key challenge being TME. Therefore, research on TME may provide new pathways for treatment or improve the success rate of current treatment methods, prolonging the survival cycle of patients. At the same time, this article also discusses the basic mechanisms and latest research progress of CAR-T therapy and vaccine therapy, providing a foundation and reference for the development of new treatment plans.

2. Tumor Microenvironment

The tumor microenvironment (TME) constitutes a major obstacle to the effective treatment of PDAC. Pancreatic cancer cells, pancreatic stellate cells and cancer related fibroblasts are both important components of

TME and constitute the PDAC signature immune escape and immunosuppression microenvironment by secreting cytokines, ECM proteins and other substances. Understanding the specific working mechanisms of these three types of cells can be helpful for future drug development to a certain extent.

2.1 pancreatic Cancer Cells

The substances released by PCCs affect cell recruitment and immune cell function. Emerging evidence indicates that PCC releases interleukin 1 β (IL 1 β) at the early stages of neoplastic progression. This cytokine, which is conventionally classified as pro inflammatory, has also been linked to the chemoattraction and functional activation of immunosuppressive cell types [4]. In parallel, PCC derived chemokines—namely CSF 3, CSF 2, and CSF 1—have been found to exert a dual effect on the TME: they diminish the presence of dendritic cells that exert anti tumor activity, while concurrently fostering the expansion of immunosuppressive populations [5]. PCCs suppress immune components in TME by releasing exosomes. In terms of drug resistance, extracellular vesicles released by PCC deliver miR-155 and miR-31-5p, increasing PC resistance to gemcitabine (GEM) by downregulating the expression of drug metabolizing enzyme DCK and stimulating SPARC secretion [6]. In terms of cellular fibrosis, extracellular vesicles released by PCC deliver miRNA155 and miRNA1290 into CAFs, leading to highly fibrotic TME [7]. Fibrotic TME promotes the adaptation of PCC and contributes to the occurrence of large tumors [8]. PCCs induce immune suppression by secreting cytokines and promote the differentiation of CAFs through exosomes, increasing the degree of fibrosis in TME.

2.2 Pancreatic Stellate Cells

When not activated, PSC is characterized by higher levels of vitamin A and less migration or proliferation [9]. In PDAC, cancer cells secrete Sonic Hedgehog (SHH) and other signal molecules to induce its activation. At the same time, the hardening of tissue fibers in TME, as well as physical and chemical conditions such as hypoxia and acidosis, can also activate PSC.

Activated PSC is a major contributor to the fibrotic environment. The function of PSC is to produce bone

proteins, collagen, matrix metalloproteinases (MMPs), and fibronectin [10]. Under normal circumstances, protein production and recovery reach equilibrium in the pancreatic interstitium. However, when PSC is overactivated, these proteins are overexpressed and unable to recover, ultimately leading to increased fluid pressure within the tumor, which is 2 to 3 times higher than the normal pancreatic tissue surrounding it, and inadequate vascular supply perfusion [11]. At the same time, these two results will further increase the drug resistance of TME. PSC also contributes to the immunoregulatory landscape of the TME. The IL 6 secreted by PSC facilitates both the activation and trafficking of bone marrow derived suppressor cells (MDSCs) to the tumor microenvironment, in addition to promoting immunosuppressive functions. In addition, PSC inhibits the intrinsic anti-tumor activity of CD8+T cells by releasing CXCL12, promoting immune regulation [8].

PSCs undergo activation in response to the unique milieu of the TME and neighboring cancer cells. Upon activation, these cells reshape the physical architecture of the TME through the abundant secretion of ECM constituents, including collagen and fibronectin, which collectively generate a physical barrier that impedes the penetration of therapeutic agents. Meanwhile, they also regulate the infiltration and function of immune cells by secreting cytokines.

2.3 Cancer Associated Fibroblasts

Cancer associated fibroblasts (CAFs) are also an important component of TME, producing extracellular matrix proteins within the TME. CAF is differentiated from other cells, among which PSC occupies an important source. Meanwhile, PCC will induce different cell lines to exhibit fibroblast like phenotypes based on the location of the tumor. According to the structure of CAFs, they can be divided into multiple subgroups, each with different inducing factors, distribution, and functions. Complement secreting CAFs (csCAFs) only exist in PDAC TME and are typically located adjacent to PCC. The complement proteins expressed by it can regulate complement activity and promote inflammation, and their concentration is highest in the early stages [12] Fibroblast associated fibroblasts (myCAFs) are mainly induced by transforming growth factor beta (TGF - β), which is distributed around cancer cells. Its main func-

tion is to construct a mechanical barrier by generating a dense extracellular matrix (ECM) in the TME, making it difficult for drugs to reach the matrix [8] Inflammatory CAFs (iCAFs), mainly induced by IL-1, are mainly distributed in the stromal regions far from cancer cells. Tumor proliferation is promoted by PSC primarily via its release of pro inflammatory cytokines and chemokines, with IL 6 being a notable example.

CAFs form a physical barrier by producing EMC proteins that hinder drug penetration and cause immune escape by inhibiting T cells through cytokines.

3. Immunotherapy

3.1 CART

Chimeric antigen receptor T (CAR T) cell therapy represents an innovative immunotherapeutic approach that, via genetic engineering, confers upon autologous or allogeneic T cells the capacity to identify specific molecular targets.

Three functionally distinct regions constitute the basic framework of a CAR: the extracellular antigen-binding portion, the transmembrane anchor, and the intracellular signaling apparatus. The outermost domain features a single-chain variable fragment (scFv) for target recognition, connected via a hinge region to the membrane-spanning segment. In the cytoplasmic compartment, the presence of both a costimulatory domain and an activation motif ensure full T-cell stimulation [13].

ScFv, or single chain variable fragment, is a recombinant antibody construct in which the heavy chain and light chain variable domains are joined via a peptide linker. This design retains the antigen binding specificity and affinity of the parent full length antibody. The principal function of scFv is to equip T cells with the capacity for specific recognition and binding to target antigens. In comparison with unmodified natural TCR T cells, scFv engineered CAR T cells exhibit markedly enhanced affinity for their target antigens. Furthermore, scFv enables CAR T cells to identify and engage target antigens independently of MHC mediated antigen presentation. On one hand, this prevents tumor cells from evading immune surveillance through modulation of MHC molecules; on the other hand, the scFv domain

confers upon CAR T cells the capacity to recognize non peptide antigens. These two features collectively constitute the core advantage of CAR T therapy in overcoming tumor immune escape [14].

The co stimulatory domain is typically derived from the CD28 receptor family (including CD28 and ICOS) or the tumor necrosis factor receptor (TNFR) superfamily (comprising 4 1BB, OX40, and CD27). This domain facilitates dual signaling via co stimulatory molecules and intracellular pathways, which enables sustained T cell proliferation and cytokine secretion, thereby augmenting their antitumor activity. The signal transduction domains are typically T cell receptor TCR/CD3 Zeta chains or immunoglobulin Fc receptor Fc ϵ RI γ chains, containing immune receptor tyrosine activation motifs (ITAMs) [13]. It is the first signal for T cell activation, initiating the killing function.

In the treatment of pancreatic cancer, in order to weaken the barrier effect of TME and the immunosuppressive environment, a new combined treatment strategy has been developed, which blocks antibodies and oncolytic virus (OV) through mA20CART, TGF - β . The data shows that compared to CAR T cell monotherapy, this novel therapy enhances the infiltration rate of CAR T cells and exhibits superior anti-tumor efficacy and anti-metastatic properties in a normal immune function positive PDAC model.

A20FMDV2 (A20) is a 20 amino acid peptide derived from the foot and mouth disease virus, which exhibits a binding specificity for $\alpha\beta 6$ that is more than 1,000 fold higher than that of the reference peptide [15]. Therefore, a mouse A20 CAR T cell (mA20CART) was developed. Overexpression of integrin $\alpha\beta 6$ has been detected in the majority of PDAC patients, and elevated expression levels of this integrin correlate with reduced survival rates among these patients.

The transforming growth factor beta (TGF β) cytokine is a key factor in immunosuppressive TME. TGF - β stimulates CAFs to synthesize and secrete a large amount of ECM components such as type I and type III collagen, promoting collagen cross-linking and increasing the hardness and density of ECM in tumor extracellular matrix. Simultaneously, TGF β markedly down-regulates the expression of critical activating receptors (NKG2D) and cytotoxic mediators on the surface of NK cells, thereby impairing their capacity to effectively

recognize and eliminate tumor cells and ultimately facilitating immune evasion [16].

Oncolytic virus (OV) is a cancer immunotherapy that utilizes genetic modification technology to acquire tumor regulated immune cell structures and immune regulatory molecules, allowing the virus to selectively lyse cancer cells [17]. Under appropriate conditions, Ovs can activate endogenous cytotoxic T cells, increase tumor permeability, and transform cold tumors into hot tumors [18]. In this study, an oncolytic vaccinia virus engineered to express murine interleukin 21 (mIL 21) was employed to enhance CAR T cell infiltration and therapeutic efficacy in PDAC. After a series of protocol improvements, the optimal protocol showed the best tumor suppression in mice, with a median survival of 7 weeks, a metastasis score of 2.5, a primary lesion CD3⁺ density of 760/mm², and a peak weight loss of approximately 14% [17].

At present, the CART technology used to treat pancreatic cancer is not mature enough, and the successful cases are not enough. The main problem is still the immune suppression caused by TME, which leads to off target T cells and physical obstruction, preventing drugs from entering TME. At the same time, the toxicity of drugs to cells also limits the development of therapeutic techniques. How to solve these problems is the key to completing the treatment of PDAC. In addition, most current Carter treatment experiments are still in the clinical or animal stage, so it will take some time to discover new efficient and safe treatment methods. From an economic perspective, CART requires personalized customization for patients, which leads to high manufacturing costs and requires a certain amount of time to complete.

3.2 mRNA Vaccine

The main principle of mRNA vaccines is to deliver mRNA encoding tumor specific antigens or tumor associated antigens into the body and encode corresponding antigen proteins. These antigens are targets associated with cancer cells and can be accurately recognized and cleared by T cells after uptake. These target molecules can be broadly classified into two groups: tumor associated antigens (TAAs) and tumor specific antigens (TSAs). TAA is a protein that is abnormally present in cancer but expressed in healthy tissues [18]. Because

TAAS is a normal human protein, most T cells develop central tolerance, which limits the effectiveness of vaccines. However, TSAs are molecules produced by cancer variant cells and are not expressed in healthy cells, so these targets can stimulate high affinity T cell responses [18].

Cancer vaccines based on mRNA technology can be divided into two categories: traditional mRNA vaccines and self amplifying mRNA (saRNA) vaccines. Traditional mRNA only encodes target antigens and has short sequences. Therefore, the process of design, synthesis, and production is direct and fast. However, due to poor stability, high requirements for transportation and storage, and the need for large doses to elicit an immune response. In contrast, self amplifying mRNA is a larger construct that encodes both the target antigen and the viral replicase gene. Upon cellular entry, it undergoes self replication, enabling the production of substantial antigen quantities even at reduced doses.

Currently, no mRNA based vaccine has been approved for the treatment of pancreatic cancer. Autogene cevumeran (BNT122) is a vaccine in clinical trials, which contains two independent mRNA chains, each chain can encode up to 10 new antigens [19]. A chain encoding mainly targets MHC class I neoantigens, with the aim of activating CD8+cytotoxic T cells to kill cancer cells. The other chain encodes mainly for MHC class II neoantigens, with the aim of activating CD4+helper T cells to assist in enhancing immune memory. At the same time, the vaccine uses uridine-based mRNA, which activates TLR7 and TLR8 proteins inside immune cells. Due to the strong affinity of TLR7/8 receptors for uridine, this experimental design has a stronger immunostimulatory ability, which is crucial for activating innate immunity and subsequent adaptive T cell responses [20].

The experiment first involves tumor resection of the patient and the production of a specific vaccine using the tumor. Six weeks following surgery, treatment with atezolizumab (an anti PD L1 antibody) was initiated, with the objective of binding to PD L1 expressed on tumor cells and tumor infiltrating immune cells, thereby disrupting the interaction between PD L1 and the PD 1 receptor on T cells. Inject 1200mg atezolizumab intravenously. The administration regimen of Autogene cevumeran includes a total of 9 doses of vaccine. These

9 doses are divided into two completely different stages in terms of time, namely the initial immunization stage and the enhancement stage. The mRNA dose for each dose is fixed at 25 micrograms and is not adjusted based on the patient's weight or body surface area. The administration route is uniformly intravenous infusion. The initial immunization stage starts from 9 weeks after surgery, with an interval of one week between each session and lasts until 15 weeks after surgery[20]. The first booster vaccine was administered 17 weeks after surgery, and the second was administered at 46 weeks. The purpose of the initial immunization stage is to activate a large number of T cells to expand. The purpose of the strengthening phase is to consolidate the immune response established by primary immunity and prolong the lifespan of T cells, while maintaining long-term memory. Finally, the improved chemotherapy regimen mFOLFIRINOX was used. The experimental data shows that among the 16 people who received the vaccine, 8 did not respond to the vaccine, while 8 responded to the vaccine. The median lifespan of T cells in the 8 individuals who responded reached 7.7 years after undergoing the enhancement phase[20].

The problem with mRNA vaccines is similar to that of cart technology, as both lack clinical experimental data. Cannot be put into large-scale use. Meanwhile, TME also poses many obstacles to mRNA vaccines, such as the formation of immune rejection reactions. Therefore, combination therapy is equally important in mRNA expression. The most suitable carrier for vaccine transportation currently is lipid nanoparticles (LNPs). But LNPs also has hidden dangers, such as aggregation in the liver or triggering immune reactions that affect efficiency. Therefore, developing new carriers or modifying LNPs is also one of the challenges.

4. Conclusion

The cells in TME suppress the activity of immune cells by releasing cytokines, ultimately leading to immune escape. At the same time, cytokines also promote the production of EMC proteins by cells, changing the physical structure of TME to form a mechanical barrier that hinders drug entry, thereby affecting treatment. Therefore, combination therapy is currently the basis of PDAC treatment, which aims to break down the me-

chanical barrier of TME to allow drugs to enter TME or activate immune cells to prevent immune escape. The latest CAR-T technology improves the penetration rate of CAR T cells in PDAC through triple therapy (OVV+anti TGF β +CAR T), further enhancing the therapeutic effect. The mRNA vaccine technology ensures vaccine efficacy through early treatment with atezolizumab (anti-PD-L1). Based on the principles of different combination therapies, they may also be used in other treatment methods, such as chemotherapy and radiotherapy, to improve treatment efficacy. Unfortunately, CAR-T technology and mRNA vaccine technology are still immature. Triple therapy and CAR-T technology are still in the mouse experimental stage, and the cytotoxicity caused by combination therapy is also one of the challenges that need to be overcome. Although mRNA vaccine technology has entered the clinical trial stage, the sample size of the experiment is limited and due to time constraints, the data is not sufficient. Therefore, some patience is needed to wait for the final experimental data. Therefore, future experimental directions should focus on discovering new and efficient therapeutic targets, researching low toxicity combination therapy regimens, and continuously advancing clinical research for large-scale applications.

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