Key Role of Combination Therapy and Nanodelivery in Triple Negative Breast Cancer

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Abstract. Cancer involves the effects of cancer cells with the tumor microenvironment, including non-cancer cells and cytokines, and the preferred treatment option is to target TME rather than directly targeting cancer cells. Triple-negative breast cancer has a unique tumor microenvironment, and immunotherapy targeting PD-1 can restore T cell immune function, but immunotherapy alone is ineffective in most patients and is often used in combination with other treatments. Since triple-negative breast cancer is sensitive to paclitaxel, combined chemotherapy can effectively regulate the tumor microenvironment and cause cancer cell death. By limiting the dose of paclitaxel, it can be used in combination with nanotechnology to regulate the combination therapy. This paper summarizes the potential of spatial transcriptome, single-cell RNA sequencing, and immunogenic cell immunogenic mortality estimation to investigate the efficacy of paclitaxel and anti-drug combinations in the treatment of various types of breast cancer in the third negative group, and analyzes examples of nanotechnology applications in the field of thiothiols to significantly improve the tumor microenvironment. Paclitaxel combined with anti-PD-1 therapy can regulate the tumor microenvironment, and drug transport and chemical repair are the main mechanisms.

Keywords: Anti-PD-1 therapy, Paclitaxel, Triple-negative breast cancer, Tumor microenvironment, Nanotechnology.

1. Introduction

Cancer progression is largely due to ongoing or evolving effects between malignant cells and the tumor microenvironment (TME). TME is a complex physiological system containing different types of cells, including non-immune cells such as fibroblasts and endothelial cells, as well as immune cells such as t lymphocytes and macrophages (TAM). Strong expression of VEGF and tumor-associated macrophages on TME contributes to tumor cell proliferation and invasion [1].

Direct targeting of tumor cells has significant disadvantages compared to targeting TME. Because of genomic instability, cancer cells are more sensitive to drugs. The corresponding non-tumor cells in TME showed higher genetic stability and greater susceptibility to regulatory interventions over time [2]. Unlike other breast cancer subtypes, breast cancer (TNBC) is characterized by an increased density of tumor-infiltrating lymphocytes (TILs), as well as specific populations of CD3+T cells and CD20+B cells in TME [3].

Tasuku Hongjo discovered PD-1, a T cell membrane protein involved in apoptosis, and later discovered a similar B7 homologous protein, now called PD-L1, as a response inhibitor of T lymphocytes, and Wood Freeman showed that PD-1 is a binding and functional companion of PD-L1. Previous experiments have confirmed that the interaction between PD-1 and PD-L1 plays a dominant role in inhibiting T lymphocytes in vivo [4].

Most patients with malignant tumors receive normal treatment interventions such as surgery, radiation, and chemotherapy. However, finding a way to minimize chemical toxicity and address the volatility and sustainability of chemotherapy remains a major challenge. With the goal of minimizing side effects, there is a class of technologies that encapsulate drugs in small rooms, produce the integration of substances in carefully designed pores or substrates, create a relatively stable biological microenvironment, produce positive results in the body with the help of bionic membranes, and release drugs after transport to the desired site, which is nanotechnology [5].

2. Anti-pd-1 and paclitaxel combination therapy

2.1. Anti-PD-1 immunotherapy

The purpose of cancer immunotherapy is to enhance TME immunity by regulating the host immune response, thereby reducing the impact of immunosuppression on tumors and improving the immune system's ability to eradicate tumor cells. Immunotherapies targeting cancer checkpoint proteins, specifically antibodies, have shown positive results for triple-negative breast cancer (TNBC). The effect of treatment depends on the degree of T lymphocyte infiltration. TNBC is characterized by increased expression of programmed protein death 1 (PD-1) and the ligand PD-L1. Cancer cells can produce PD-L1, which reacts with PD-1 on T cells, causing lymphocyte apoptosis. Unresponsive and abnormal cytokine secretory cytogenic secretion helps increase the likelihood that cancer cells will evade the T cell immune response, thereby contributing to immune escape [6]. Monoclonal antibodies against PD-L1 inhibit the signaling axis of PD-1/PD-L1, thereby restoring T cell immune function and destroying tumor cells.

TME regulation is the key to determine the effect of anti-PD-1 immunotherapy. The role of immune cells, basic components and cytokines in TNBC is significant. Tumor-derived cytokines attract more immunosuppressive cells into TME, which affects the effectiveness of anti-PD-1/PD-L1 therapy that inhibits T cell function. The therapeutic effect of PD-1/PD-L1 inhibitors is significantly enhanced in malignant tumors where CD8+T cells are strongly present, so in the context of anti-PD-1 therapy, CD8+T lymphocytes become a key biological marker for prognosis and treatment. In contrast, resistance to PD-1/PD-L1 inhibitors is often observed in advanced TNBC cases when CD8 +T lymphocytes are not infiltrated [7].

2.2. Paclitaxel (PTX) chemotherapy

Independent immunotherapy targeting PD-1/PD-L1 immune checkpoint inhibitors is ineffective in more than 80% of patients, so many immunotherapy studies targeting anti-PD-1 have been conducted in combination with other treatments such as chemotherapy in recent years. A combination of immunotherapy and chemotherapy regimens enhances anti-tumor immunity through drug-induced immunogenic cell death (ICD). ICD is a specific form of cell death that targets tumor cells for immune activation. This process relies on the simultaneous expression and secretion of several molecular patterns associated with immune stimulation damage [8].

A classic example of chemotherapy is PTX, to which TNBC is very sensitive. PTX chemotherapy mainly enhances the systemic immune response to tumors, increases the release and diffusion of neoantigens, stimulates the production and maturation of dendritic cells (DC), and causes macrophages to secrete pro-inflammatory cytokines [9]. At the same time, the lack of immunosuppressive myelopathic cells (MDSCs) and regulatory T lymphocytes (TREGs) can enhance tumor susceptibility to cytotoxic T lymphocytes, thereby improving the effectiveness of immunotherapy.

Dkk1, a regulator of secreted proteins and WNT signaling channels, has shown significant improvement in many human malignancies. Immunohistochemical analysis showed a significant increase in DKK1 in breast cancer tissue after chemotherapy, and it may be an excellent candidate for cancer immunotherapy. To further investigate, Shi et al. used breast cancer cells from 4T1 mice to create a mouse xenograft model. Considering that DKK1 is a secreted glycoprotein, Shi quantitatively analyzed serum DKK1 of tumor-bearing mice, and the results showed that DKK1 increased significantly after PTX treatment. Shi and others believe that paclitaxel enhances DKK1 expression by activating EGFR signaling in TNBC cells and dorsal root ganglion (DRG) neurons. The increase of DKK1 expression in TNBC cells may affect the therapeutic effect of TNBC by stimulating the polarization of immunosuppressive macrophages, stimulating the accumulation of myeloid suppressor cells (MDSC), or enhancing the expression of PD-L1 in tumor cells. In addition, the expression of DKK1 up-regulates PTX-induced peripheral neurotoxicity. Notably, the use of anti-DKK1 antibodies not only increased the effectiveness of PTX and TNBC models combined with anti-

PD-1 therapy in mice, but also reduced the toxicity of peripheral neurons associated with PTX therapy [10].

3. Efficacy evaluation of combination therapy

3.1. Spatial transcriptomics (ST-Seq) and single-cell RNA sequencing (SCRNA-SEQ) were used to evaluate the efficacy

RNA sequencing (RNA-SEQ) is a powerful technique for studying the transcription profile of RNA molecules within a single cell. This method has been widely used in cancer research, helping to reveal tumor heterogeneity, TME, and intercellular communication mechanisms. For immune cells over time, RNA sequencing (RNA-SEQ) focuses on various novel functional subtypes of T cells and conventional T cells. Some T cells, such as those with cytotoxic and immunosuppressive properties, have been classified according to their gene expression models [11]. Obtaining the results of SCRNA-SEQ requires several steps, including sample preparation, sequencing, and data analysis, to produce the sequencing results. The cells must be separated from the tissue, a process that may result in the loss of the cell's original spatial orientation. ST-Seq reduces this limitation by receiving RNA data and sequencing it in situ [12].

Analyzing the TNBC spatial transcriptome, geneticists found that TAMs were the dominant cell population for TLR4 expression in gene 4T1 and EO771 tumor models, as well as in transgenic tumor models. The results suggest that PTX, as an agonist of TLR4, may be the most susceptible to TAM, given the high expression of TLR4. Scanning the RNA sequence data set using gene set enrichment analysis (GSEA), it was observed that PTX-positive TNBC patients had significantly higher levels of the TLR4 signaling pathway in TME than non-responsive patients. Given the critical role of bone marrow cell cross-presentation in tumor infiltration of CD8+T lymphocytes, which is the target of PD-1 blockade, CD8+T cell infiltration plays a key role in anti-tumor immunity. Studies by Cheng et al. using GSEA showed that the median TAM cross-presentation signaling pathway that responds to PTX is also more abundant in TNBC patients compared to those who do not respond. In addition, interferon (IFN) -associated signaling pathways (IFN-α) and IFN-γ were significantly increased in TAM in TNBC patients who responded to PTX or assembly treatment compared to non-responders. Finally, the data provided by Cheng in this study showed that TLR4 overexpression and TLR4 signaling regulation, cross-presentation ability in TAM, significantly improved the immunotherapy effect of PTX on TNBC. This study explains the immunomodulatory function of PTX in TNBC and highlights the promise of immunotherapy strategies represented by targeted antigen-presenting TAM antigens [13]. It is speculated that PTX has a synergistic effect on PTX/PD-1 antibody and enhances the immune efficacy of PTX/PD-1 antibody against tumor.

3.2. Evaluation of efficacy of 3D co-culture combination therapy

Chimeric antigen receptor CAR-T immunotherapy mainly targets tumor specificity. Immunosuppressants can block the infiltration of CAR T cells and release various cytokines and immune checkpoint molecules, thus enabling tumor growth. Microthree-dimensional immunoassays using microfluidic and lab-on-a-chip techniques significantly improved the effectiveness of T cells. Microfluidic technology can help improve the complexity of three-dimensional tumor models in the field of CAR-T cell screening. Three-dimensional co-culture can reduce the effects of cytotoxicity by inhibiting the recognition of EGFR by CAR T cells [14]. When TNBC cells showed elevated levels of EGFR and PD-L1 expression, the cytotoxicity of EGFR-targeted CAR T cells became very pronounced. Compared with immunotherapy alone, PTX-related combination therapies showed excellent anti-tumor efficacy in oncolytic viruses, especially in the MDA-MB-231 and MCF-7 cell lines that introduced the virus after infection. Based on the available studies, it is reasonable to hypothesize that pembrolizumab is a drug that stimulates and inhibits T cell prophylactic activity, helping to prevent malignant degeneration in MDA-MB-231 cells. In all of the cell systems tested, the co-expression of PD-1 on the surface of T cells led to overexpression of PD-L1 [15]. PTX

combined with inhibition of PD-1 checkpoint integration can cause severe antitumor response of primary pathological metastasis and distant metastasis. Cancer immunotherapy (such as immune checkpoint inhibitors and immune cell therapy) combined with activators and PTX strategies are more effective than immunotherapy alone.

4. Mechanisms of nanotechnology in combination therapy

4.1. Effects of nanotechnology on PTX

The clinical application of PTX has great limitations. First, the manifestation of multi-drug resistance (MDR) is often mainly due to the overexpression of MDR transporters or microtubule changes, while in TNBC therapy, the extracellular matrix (ECM) forms a complex biometric barrier on the TME, limiting the distribution and penetration of PTX. This can interfere with treatment [16]. Nanotechnology overcomes the disadvantage of poor water solubility of PTX and has a more closed and comprehensive drug delivery system. PTX packaged with nano glue has less toxicity and better water solubility.

Polyglycerol carbonate has excellent biological cooperation, is a component of nanoparticles, and can be further degraded into glycerol and carbon dioxide in combination with chemotherapy drugs. RC et al. designed nanoparticles composed of polymeric acid-paclitaxel. PTX loading of about 80% NPs significantly inhibited tumor growth in mice with 4T1 metastatic breast cancer. The presence of adipose tissue regions at the tumor site reduces lung metastasis by reducing tissue networks and increasing NK activity [17].

Protein nanoparticles are immunogenic and dissolved well, which is the research direction of nanoparticles. The biologists developed two natural proteins that self-form protein nanoparticles to deliver PTX through topological expression. All three treatment groups significantly slowed tumor growth in mice with 4T1 heterogeneous tumors, with TIR growth reaching about 80%.

4.2. Application of novel nanotechnology in combination therapy

4.2.1 Novel Puerarin Nanoemulsion (nanoPue)

The emulsifier is puerarin nanoemulsion with soybean biological capacity. Tumor-associated fibroblasts (TAFs) are key basal cells in connective tissue development, contributing to the formation of drug-resistant microenvironments and TNBC immunosuppressive factors. Reactive oxygen species (ROS) are central nodes in many fibrotic pathways and can activate TAFS. The main emulsifier is bioaccumulative follicular phospholipid extracted from soybeans, which can produce puerarin nanoemulsion. TAF, as a kind of stromal cell, plays a crucial role in the regeneration of connective tissue. In addition, they have been associated with the development of TNBC resistance and the formation of microenvironments that inhibit immunosuppressive processes. ROS plays a key role as a mediator in various fibrotic pathways and has the ability to activate TAFS. Xu et al. showed that compared with the PBS group, TAFs in the mouse nano treatment group was reduced by about six times, chemical exposure of nano paclitaxel to TNBC breeding tissue was increased, and the infiltration level of cytotoxic T lymphocytes was increased by one-third. Alpha-pd-l1 or nanoPue alone significantly slowed tumor growth compared with the simultaneous administration of both. Xu's TUNEL analysis showed that co-treatment of nanoparticles with α-PD-L1 significantly increased apoptosis compared with α-PD-L1 alone. This may be due to the increase of T lymphocyte infiltration caused by nanoparticles. In addition, compared with the α-PD-L1 group [18], the level of cellular plasticity in the nano + α-PD-L1 group was significantly higher than that in the α-PD-L1 individual treatment group. The expression level of Ki67 protein in cancer cells was significantly decreased in nano-scale and α-PD-L1 group ($p< <0.05$). Nanoparticle induced immune microenvironment significantly improves the therapeutic efficacy of monoclonal antibody α-PD-L1, which helps to change the immune microenvironment and promote the joint interaction of nanoparticles with PD-L1 in the TNBC model.

4.2.2 Albumin nanoparticle (nano-PI)

Tumor-associated macrophages can differentiate into inflammatory macrophages M1 or immunosuppressive macrophages M2 via microenvironmental signals. In the vicinity of tumors, M2 macrophages promote tumor progression and metastasis, hinder the effectiveness of commonly used treatments for alpha-PD1 / alpha-PDL1, and are often associated with poor prognosis in patients with breast cancer. Phosphatidyl octyl inositol 3-kinase γ (PI3Kγ) is considered a promising target for controlling M1/M2 polarization transformation in macrophages and modulating immune responses to cancer therapy. Clinical studies of a PI3K-γ inhibitor in combination with PTX and alpha-PDL1 antibodies for breast cancer were recently evaluated.

A complex formulation containing the PI3K-gamma inhibitor and PTX, which together produce stable nanoparticles. These nanoparticles contribute to the repolarization of macrophages from M2 to M1, thereby enhancing their function. In two mouse models of breast cancer, the nanoparticles interacted with alpha-PD1 to alter the lymph nodes and microenvironment within the tumors, successfully causing tumor regression and eradicating lung metastasis in the mice. Immune cell analysis showed that the combination of nanoparticles with α-pd1 helped to transform M2 into macrophages M1, enhance CD4+ and CD8+T, B and B lymphocytes, regulate the reduction of T cells, prevent the destruction of T cells, and thus reshape the immune microenvironment [19].

5. Conclusion

PTX and anti-PD-1 combination therapy, as a combination therapy in various TNBC environments, have shown interaction compared with single anti-PD-1 immunotherapy, which can affect TLR4 signaling pathway and T cell infiltration rate in TME.

The application of nanotechnology to PTX combination therapy showed synergistic anti-tumor effects, overcame the limitations of PTX drug delivery, and significantly improved the tumor immune microenvironment of α-PD-L1 in the TNBC model. Nanotechnology can be used as an adjunct to chemotherapy drugs, and checkpoint blocking immunotherapy can be performed in tumors.

When PTX and anti-PD-1 combination therapy are used to regulate TNBC, the chemical efficacy of PTX can be regulated by nano, liposome and other drug delivery methods, and chemical modification schemes can also be used to regulate the combination therapy.

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