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PD-1 Immunotherapy Resistance Mechanism and Combination Therapy Strategy

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Abstract:

Programmed death receptor-1 (PD-1) immunotherapy has made breakthroughs in the treatment of a variety of malignancies (such as non-small cell lung cancer, melanoma, gastric cancer) by blocking the PD-1/PD-L1 signaling pathway and reactivating the antitumor activity of tumor-infiltrating lymphocytes. However, in clinical practice, about 60%-80% of patients develop primary or acquired resistance, which usually occurs 6-12 months after treatment initiation. This severely limits its efficacy and further clinical application. This paper systematically reviewd the drug resistance mechanisms of PD-1 immunotherapy around the regulatory axis of "tumor microenvironment-immune escape-PD-1 receptor", focusing on analyzing key factors such as tumor microenvironment disorders, abnormal activation of immune escape pathways, and abnormal regulation of PD-1 receptors. It also proposed combination treatment strategies based on different resistance mechanisms (e.g., combined blockade of multiple immune checkpoints, combination with anti-angiogenic drugs, PI3K/mTOR inhibitors, radiotherapy, and chemotherapy), aiming to provide a theoretical reference for overcoming PD-1 immunotherapy resistance.

Keywords: Immunotherapy; Resistance; Combination therapy.

1. Introduction

The occurrence and development of malignant tumors are closely related to the dynamic imbalance of the immune system, and immune escape is one of the core mechanisms by which tumors evade immune surveillance [1]. As an immune checkpoint molecule, the PD-1 receptor is mainly expressed on the surface of activated T cells, B cells, and natural killer cells, while its ligands (PD-L1/PD-L2) are highly expressed on the surface of tumor cells and immunosuppressive cells in the tumor microenvironment (TME) [2]. After PD-1 binds to its ligands, it can inhibit the T cell activation signaling pathway by re-

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cruiting phosphatases, leading to T cell function depletion and ultimately achieving tumor immune escape [3].

PD-1 inhibition therapy has been approved for clinical application in various tumors (e.g., melanoma, non-small cell lung cancer, gastric cancer) by blocking the PD-1/PD-L1 interaction via specific antibodies [2]. For example, pembrolizumab monotherapy yields a 5-year survival rate of 34% in advanced melanoma, which is significantly better than conventional chemotherapy [4]. However, clinical data show that only 20%-40% of patients benefit from PD-1 immunotherapy, and some initially responsive patients develop acquired resistance 6-12 months after treatment [5]. Therefore, elucidating the mechanisms of PD-1 resistance and developing combination therapy strategies have become the focus of current research in the field of tumor immunotherapy [6].

This paper aims to systematically dissect the molecular and cellular mechanisms underlying PD-1 immunotherapy resistance from the perspective of the „tumor microenvironment-immune escape-PD-1 receptor“ regulatory axis, clarify the role of key factors (e.g., immunosuppressive cells in TME, alternative immune checkpoints, PD-1 receptor dysregulation) in resistance development, and propose evidence-based combination therapy strategies targeting different resistance mechanisms (including immune checkpoint dual blockade, targeted therapy combination, chemo-radiotherapy combination, and emerging metabolic/microbiome intervention) to provide theoretical support for improving the response rate and durability of PD-1 immunotherapy in clinical practice.

2. PD-1 Immunotherapy Resistance Mechanism

2.1 Drug Resistance Mediated by Tumor Microenvironment Disorders

The tumor microenvironment is a complex ecosystem composed of tumor cells, immune cells, stromal cells, and cytokines, and its immunosuppressive state is a key trigger for PD-1 resistance [1].

2.1.1 Immunosuppressive cell infiltration

Regulatory T cells (Tregs), tumor-associated macrophages (TAMs), and myeloid-derived suppressor cells (MDSCs) are abundantly enriched in the TME of drug-resistant patients. Tregs inhibit the proliferation and activation of effector T cells by secreting cytokines such as IL-10 and TGF-β; M2-type TAMs weaken the immune response by expressing PD-L1 and ARG1; MDSCs induce T cell apoptosis by producing reactive oxygen species (ROS) and indoleamine 2,3-dioxygenase (IDO) [7]. A study found that the proportion of MDSCs in tumor tissues of non-small cell lung cancer patients with PD-1 resistance was 2.3 times higher than that in responsive patients, and this proportion was negatively correlated with the efficacy of PD-1 therapy [5].

2.1.2 Cytokine imbalance

The imbalance between pro-inflammatory factors (e.g., IL-2, IFN-γ) and anti-inflammatory factors (e.g., IL-10, TGF-β) in the TME directly affects the efficacy of PD-1 therapy. IFN-γ can induce tumor cells to express PD-L1, enhance the binding efficiency of PD-1 antibodies, and promote the activation of effector T cells [3]. In contrast, IL-10 inhibits dendritic cell (DC) maturation and reduces antigen presentation [7]. The concentration of IFN-γ in the tumor tissues of drug-resistant patients was 40% lower than that in responsive patients, while the concentration of IL-10 increased by 1.8 times, making it difficult to maintain a sustained immune response [5].

2.2 Abnormal Activation of Immune Escape Pathways

In addition to the PD-1/PD-L1 pathway, abnormal activation of other immune checkpoint pathways and intracellular signaling pathways can bypass PD-1 blockade and lead to immune escape [6].

2.2.1 Upregulation of alternative immune checkpoints

Alternative immune checkpoints (e.g., CTLA-4, LAG-3, TIM-3) are highly expressed on the surface of T cells in drug-resistant patients. CTLA-4 inhibits the initial activation of T cells by competitively binding to CD80/CD86; after binding to MHCII molecules, LAG-3 can inhibit T cell proliferation and cytokine secretion. The expression rate of LAG-3 in peripheral blood T cells of melanoma-resistant patients reached 35%, which was significantly higher than that in responsive patients (12%), and blocking LAG-3 could restore T cell function [8].

2.2.2 Abnormal intracellular signaling pathways

Aberrant activation of the PI3K/AKT/mTOR pathway and Wnt/β-catenin pathway can mediate PD-1 resistance by regulating tumor cell proliferation and the expression of immunosuppressive molecules [1]. After activation, the PI3K/AKT pathway promotes tumor cells to secrete VEGF and inhibits DC maturation [7]; activation of the Wnt/β-catenin pathway reduces CD8+ T cell infiltration and impairs immune response efficiency [1]. Among colorectal cancer patients, the objective response rate (ORR) of PD-1 therapy in those with activated Wnt/β-catenin pathway was only 8%, which was significantly lower than the 32% in patients with normal pathway activity [5].

2.3 Abnormal Regulation of PD-1 Receptors

Abnormalities in PD-1 receptor expression, phosphorylation status, and endocytosis directly affect the binding and blocking effects of PD-1 antibodies [2].

2.3.1 PD-1 receptor overexpression

Long-term antigen stimulation leads to overexpression of PD-1 receptors on the surface of T cells, forming a „super-inhibited" state. Even if PD-1 antibodies block some receptors, the remaining receptors can still inhibit T cell function by enhancing signaling [3]. The expression level of PD-1 in tumor-infiltrating T cells of lung cancer patients with PD-1 resistance was 1.6 times higher than that in responsive patients, and the co-expression rate with the T cell depletion marker TIM-3 reached 60% [8].

2.3.2 Abnormal PD-1 receptor phosphorylation

Phosphorylation of the ITIM/ITSM domain in the intracellular segment of the PD-1 receptor is a key step in signal transduction [2]. In drug-resistant patients, the activity of Src family kinases (e.g., Lck) is enhanced, which promotes PD-1 receptor phosphorylation and accelerates the activation of downstream signaling pathways [3]. In vitro experiments confirmed that inhibiting Lck activity increased IFN-γ secretion in PD-1-resistant T cells by 2.1 times [7].

3. PD-1 Immunotherapy Combination Treatment Strategy

In response to the above resistance mechanisms, a variety of combination therapy regimens have been developed to improve treatment efficacy by reshaping the TME, blocking alternative pathways, and enhancing PD-1 blockade effects [6].

3.1 Combined with Immune Checkpoint Inhibitors

3.1.1 PD-1/CTLA-4 dual blocking

CTLA-4 inhibitors can deplete Tregs in the TME, enhance T cell activation, and exert a synergistic effect with PD-1 inhibitors [6]. Ipilimumab (CTLA-4 inhibitor) combined with Nivolumab (PD-1 inhibitor) in the treatment of advanced melanoma has a 5-year survival rate of 52%, which is significantly higher than the 34% of PD-1 monotherapy. The mechanism lies in that CTLA-4 blockade increases effector T cell infiltration, while PD-1 blockade relieves T cell depletion, and the two jointly reshape the immune microenvironment [4].

3.1.2 PD-1/LAG-3 dual blocking

LAG-3 inhibitors restore the proliferation and cytokine secretion of depleted T cells. Relatlimab (LAG-3 inhibitor) combined with Nivolumab in the treatment of melanoma has an objective response rate of 43%, an increase of 18% compared with PD-1 monotherapy, and still has an objective response rate of 27% for PD-1 monodrug-resistant patients. This regimen is particularly suitable for resistant patients with high LAG-3 expression [8].

3.2 Combination with Targeted Therapy

3.2.1 Combination with anti-angiogenic drugs

Antiangiogenic drugs (e.g., bevacizumab) improve vascular disturbances in the TME and increase effector T cell infiltration [3]. Bevacizumab combined with Pembrolizumab in the treatment of non-small cell lung cancer has an objective response rate of 55%, which is 23% higher than that of PD-1 alone, and can reduce the proportion of MDSCs and increase IFN-γ secretion. The mechanism lies in that anti-angiogenic therapy reduces VEGF-mediated immunosuppression while normalizing vascular structure and promoting T cell entry into tumor tissue [5].

3.2.2 Combination with PI3K/mTOR inhibitors

PI3K/mTOR inhibitors can inhibit tumor cell proliferation and down-regulate PD-L1 expression. Alpelisib (PI3K inhibitor) combined with Pembrolizumab in the treatment of triple-negative breast cancer has an objective response rate of 38% and an objective response rate of 22% in PD-1 monodrug-resistant patients. Studies have confirmed that PI3K inhibition can reduce the translational expression of PD-L1 and enhance the blockade effect of PD-1 antibodies [7].

3.3 Combination with Chemotherapy and Radiotherapy

3.3.1 Combination with chemotherapy

Chemotherapy drugs (such as paclitaxel, cisplatin) can enhance the immune response by inducing apoptosis of tumor cells and releasing tumor-associated antigens. Paclitaxel combined with PD-1 inhibitors in the treatment of gastric cancer had an objective response rate of 42%, an increase of 19% compared with PD-1 monotherapy. The mechanism is that chemotherapy can increase the antigen-presenting ability of DCs while reducing the proportion of Tregs and enhancing the efficacy of PD-1 blockade [6].

3.3.2 Combination with radiotherapy

Radiotherapy can activate the systemic immune response through the „remote effect" (abscopal effect) and synergize with PD-1 inhibitors. Stereotactic radiotherapy combined with PD-1 inhibitors in the treatment of melanoma

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brain metastasis had an intracranial objective response rate of 58%, an increase of 32% compared with PD-1 monotherapy. Radiotherapy can induce tumor cells to express calreticulin, promote DC maturation, and increase effector T cell infiltration, enhancing the systemic efficacy of PD-1 blockade [1].

3.4 Combined with Metabolic Regulation and Microbiome Intervention Therapy

Metabolic reprogramming of tumor cells and metabolite accumulation in the tumor microenvironment are important drivers of PD-1 resistance, and recent studies have confirmed that targeting metabolic abnormalities or modulating the tumor microbiome can significantly enhance immunotherapy efficacy [9,10].

3.4.1 Combined strategies to target adenosine metabolism

High concentrations of adenosine in the tumor microenvironment inhibit T cell function by activating A2A and A2B receptors on the surface of immune cells, forming an immunosuppressive metabolic barrier [9]. AB928 acts as a dual adenosine receptor antagonist that blocks both A2A and A2B receptors, reversing adenosine-mediated immunosuppression [9,11]. In the phase II PANTHER trial(patients with rectal adenocarcinoma who had not received pelvic radiotherapy or chemotherapy), AB928 combined with the PD-1 inhibitor AB122 and short-course radiotherapy for rectal cancer was well tolerated; preliminary results showed that the combination strategy achieved disease stability for more than 6 months in some patients, with mechanisms involving reducing intratumoral MDSCs infiltration and increasing the proportion of effector T cells and inducing immunogenic cell death through concurrent radiotherapy, which was synergistic with AB928 to relieve metabolic inhibition, and ultimately enhanced the PD-1 blockade effect of AB122 [11]. This protocol is particularly suitable for patients with „cold tumors“ who do not respond to PD-1 monotherapy, providing a new idea for metabolic-immune combination therapy [9].

3.4.2 Tryptophan metabolic pathway targeted intervention

Indoleamine 2,3-dioxygenase (IDO)-mediated tryptophan degradation is a key metabolic mechanism for tumors to evade immune surveillance, and its product, kynurenine, inhibits T cell proliferation and promotes Treg differentiation. The combination of IDO inhibitors and PD-1 inhibitors synergistically restores T cell viability. A Phase Ib clinical trial in melanoma showed a 15% improvement in objective response rates with the IDO inhibitor Epacadostat combined with pembrolizumab compared to monotherapy, and sustained responses were still observed in patients with low PD-L1 expression Mechanistic studies have confirmed that this combination strategy can increase the concentration of IFN-γ in tumors by 2.3-fold while reducing the levels of inhibitory cytokines such as IL-10, which is a targeted reversal effect on the resistance mechanism of MDSCs inducing T cell apoptosis through IDO [12].

3.4.3 Tumor microbiome regulation strategies

Emerging evidence suggests that the intratumoral microbiota modulates the local immune microenvironment through metabolites. In non-small cell lung cancer models, intratumoral bifidobacteria promote dendritic cell maturation through the production of short-chain fatty acids (SCFAs), enhancing the efficacy of PD-1 inhibitors [10]. A prospective study of 48 patients with advanced lung cancer showed that the objective response rate (41.7%) of oral probiotics (including Lactobacillus reuteri and Lactobacillus rhamnosus) combined with PD-1 inhibitors was significantly higher than that of the monotherapy group (27.1%), and the diversity of gut microbiota was positively correlated with treatment response [13]. This strategy provides a non-invasive intervention to improve the response of „cold tumors“ to PD-1 inhibitors by modulating the microbiome-immune axis [10,13].

4. Future Prospects

The resistance mechanisms of PD-1 immunotherapy are complex and diverse, involving multiple levels such as tumor microenvironment, immune escape pathway, and PD-1 receptor regulation [6]. At present, although some progress has been made in combination therapy, there are still some problems: first, there is a lack of accurate biomarkers, making it difficult to screen the optimal combination regimen [5]; second, the toxicity of some combination regimens increased, such as the incidence of grade 3-4 adverse reactions of PD-1/CTLA-4 dual blockade reached 35%, which was higher than that of monotherapy [4]; third, the mechanism of acquired drug resistance is not fully studied, and there is a lack of targeted reversal strategies [3].

Future research should focus on the following directions: first, develop multi-omics joint detection technology to screen compound biomarkers based on „tumor microenvironment-immune escape-PD-1 receptor“ to achieve individualized combination therapy [1]; second, explore low-toxicity combination regimens, such as PD-1 inhibitors combined with low-dose chemotherapy or local radiotherapy, to improve efficacy and reduce adverse reactions [6]; finally, the dynamic mechanism of acquired drug resistance is studied in depth, and targeted drugs for drug resistance clones are developed to achieve precise reversal

of drug resistance to PD-1 [7].

5. Conclusion

PD-1 immunotherapy has brought revolutionary breakthroughs in the treatment of malignant tumors, but the resistance rate is still a key issue that needs to be solved in clinical practice . In this study, we systematically elucidated the triple mechanism of PD-1 resistance: the physical barrier posed by immunosuppressive cell infiltration and cytokine imbalance in the tumor microenvironment; the abnormal activation of alternative immune escape pathways (e.g., LAG-3, PI3K/AKT) other than the PD-1/PD-L1 pathway; and the abnormal regulation of PD-1 receptor expression and signaling. These mechanisms are intertwined and collectively contribute to T cell depletion and immunotherapy failure.

Combined treatment strategies targeting the above mechanisms have shown significant clinical value: PD-1/CTLA-4 dual blocking increases the 5-year survival rate of advanced melanoma; the combination of anti-angiogenic drugs with PD-1 inhibitors increased the objective response rate of non-small cell lung cancer ; the synergistic effect of radiotherapy with PD-1 inhibitors enables a systemic anti-tumor response through the „remote effect“ ; the combination of adenosine receptor antagonists and PD-1 inhibitors provides a new paradigm for metabolic-immune cross-regulation ; and IDO inhibitors or probiotic interventions further expand the efficacy of PD-1 therapy in „cold tumors“ .

Despite progress, PD-1 immunotherapy faces challenges such as lack of biomarkers , increased toxicity in combination therapy , and unclear mechanisms of acquired resistance . Future research needs to rely on multi-omics technology to construct a „tumor microenvironment-immune escape-PD-1 receptor“ composite biomarker system , develop a low-toxicity combination scheme , and deeply explore the dynamic evolution of drug-resistant clones . Only through precise targeted and multi-dimensional synergistic treatment strategies can we break through the bottleneck of current PD-1 immunotherapy and achieve long-term control and even cure of malignant tumors.

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