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Heterogeneity of Acquired Resistance Mechanisms to PD-1/PD-L1 Inhibitors in Non-Small Cell Lung Cancer and Clinical Strategies

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Abstract. Immune checkpoint inhibitors targeting PD-1/PD-L1 have changed the treatment of non-small cell lung cancer (NSCLC), but some patients who are effective at first will develop acquired drug resistance (AR), which has become a big problem. The causes of AR are very complicated, and some tumors change, for example, antigen presentation problems, IFN-γ signal pathway mutation, or carcinogenesis pathway activation. In addition, the tumor microenvironment (TME) is disturbing, which suppresses the immune system by infiltrating cells, metabolic reprogramming and physical barriers. This review systematically classifies these causes, and also evaluates some advanced diagnostic tools, such as single-cell omics, liquid biopsy and AI-based imaging omics. The article further analyzes the emerging clinical strategies, such as combined immunotherapy, targeted TME and biomarker-guided therapy. Combining mechanism understanding with diagnosis progress, a multi-step scheme can be formed, including clarifying the mechanism, stratifying patients and then carrying out targeted intervention. Current research shows that personalized combination therapy under the guidance of dynamic monitoring can reverse specific drug resistance types and improve the results. In order to overcome AR, it is necessary to transform the understanding of the mechanism into a clinically feasible strategy. The future work should focus on real-time tracking of tumor evolution and adaptive therapy, and at the same time solve the cost and ethical challenges.

Keywords: Tumor immunotherapy, Acquired resistance, PD-1/PD-L1 inhibitors, Non-small cell lung cancer, Tumor heterogeneity.

1. Introduction

There is a drug called Immune Checkpoint Inhibitors (ICIS), which specifically deals with PD-1 and PD-L1. It changes the way people treat advanced non-small cell lung cancer (NSCLC), and enables some patients to control their disease for a long time [1]. However, the later appearance of acquired resistance (AR) became a big problem. New data show that after ICI treatment, NSCLC patients will develop AR in about 8 to 12 months on average, and patients with AR usually have less than 30% overall survival(OS) in 5 years [2]. AR is not a single case, it is very complicated and will change, which reflects that cancer will change itself under the pressure of immunotherapy [3]. Only by

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systematically understanding the complex reasons behind AR can people come up with a good way to restore patients' sensitivity to immunotherapy and help them live longer. This article mainly talks about the reasons why patients with NSCLC have acquired resistance to PD-1/PD-L1 inhibitors, advanced detection methods and some new treatment strategies. By carefully analyzing the changes of the tumor itself and the dynamic changes in tumor microenvironment (TME), and exploring cutting-edge detection technologies such as single-cell multi-omics and liquid biopsy, this paper analyzes the existing and possible ways to overcome drug resistance in the future. It hopes to fully demonstrate the complexity of AR and point out the direction for developing personalized precision medical solutions.

2. Manuscript preparation

Acquired drug resistance is a complex evolutionary process, and tumor cells and their microenvironment will avoid the surveillance of the immune system in this way. To understand how complicated it is, people need to study it from many angles. Table 1 summarizes the main mechanisms of acquired drug resistance, and divides them into two categories: tumor-induced and tumor microenvironment-mediated, and lists specific examples and related references.

Table 1. Summary of key acquired resistance mechanisms

Mechanism Category	Specific Mechanism	Key Details/Examples	References
Tumor-Intrinsic Mechanisms	Antigen Presentation Defects	Mutations/deletions in B2M prevent MHC-I assembly; TAP2 downregulation impairs antigen presentation.	[4,5]
	IFN-γ Signaling Pathway Alterations	Loss-of-function mutations inJAK1/JAK2 lead to IFN-γinsensitivity and an immune-coldphenotype.	[6,7]
	Oncogenic Pathway Activation	Co-mutations (e.g.,STK11/KEAP1), EGFR mutations, PTEN loss, or KRAS-driven COX-2 signaling mediate resistance.	[8-10]
	Immunosuppressive Cell Infiltration	MDSCs, M2-like TAMs, and Tregs suppress effector T-cellfunction via inhibitory cytokines, nutrient consumption, or physical interference.	[9,11,12]
TME-Mediated Mechanisms	Metabolic Reprogramming	High glycolysis leads to lactate accumulation, inhibiting T/NKcells; IDO activation depletes tryptophan.	[13,14]
	Physical Barriers &Hypoxia	CAF activation and ECM (e.g.,collagen) deposition formphysical barriers; aberrant vasculature and hypoxia directly inhibit T-cell function.	[15-18]

2.1. The trick of the tumor itself

Tumor cells will find their own way to avoid the discovery and attack of T cells.

Failure of antigen presentation is a basic method that makes tumor cells invisible to CD8+ T cells. For example, a problem with the B2M gene will make MHC-I molecules poorly assembled, and the number of antigen processing assistants like TAP2 will decrease, which will also make it impossible to display antigen peptides properly [4,5].

The change of IFN-γ signaling pathway is very important, because IFN-γ plays a central role in activating anti-tumor immunity and up-regulating PD-L1 expression. If the key components in this pathway, such as JAK1 or JAK2, fail to function, the tumor cells will not respond to IFN-γ, thus becoming an immune "cold" tumor [6,7].

Activation of the carcinogenic pathway means that some specific carcinogenesis-driven mutations or signal pathway changes can directly or indirectly make tumors resistant. Mutations such as STK11/KEAP1, sensitive EGFR mutation, PTEN loss, or COX-2 signal caused by KRAS

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can all help tumor resistance therapy by transforming immunosuppressive TME or changing tumor immunogenicity [8-10].

2.2. Mechanisms mediated by the tumor microenvironment

The tumor microenvironment (TME) will actively form a "microenvironment" of immunosuppression, which will make the drug resistance problem more serious.

Infiltration of immunosuppressed cells means that bone marrow-derived suppressor cells (MDSCs), M2-like tumor-associated macrophages (TAMs) and regulatory T cells (Tregs) gather in TME. These cells will suppress their functions by secreting inhibitory cytokines, consuming key nutrients, or directly blocking effector T cells [9,11,12].

Metabolic reprogramming in TME is an important promoter of immunosuppression. The high glycolytic activity of tumor cells leads to lactic acid accumulation, which inhibits the functions of T cells and NK cells. In addition, the key enzyme in tryptophan metabolism-indoleamine 2,3-dioxygenase (IDO), is activated, which will consume tryptophan needed by T cell proliferation and further weaken the immune response [13,14].

Physical barrier and hypoxia are caused by the overactivity of cancer-related fibroblasts and massive deposition of extracellular matrix components such as collagen. These physical barriers will keep T cells out of the tumor core area [15,16]. At the same time, abnormal tumor blood vessels and the resulting hypoxia will not only hinder the delivery of drugs but also directly inhibit the function of T cells and promote their death [17,18].

3. Diagnostic methods and biomarkers

Traditional biomarkers such as PD-L1 expression and tumor mutation load (TMB) can give us some preliminary hints, but they are not good enough to fully grasp the dynamic and changeable characteristics of AR [19,20].

3.1. Basic research diagnostic tools

These tools can help us to understand the mechanism of drug resistance with unprecedented clarity.

Single-cell omics combines single-cell transcriptomics, genomics and protein omics, so that people can accurately see how cells interact in tumor microenvironment, and also find out the main drug-resistant cell subsets and their unique immune escape methods [21,22].

Spatial transcriptomics can preserve the spatial information of tissue structure while analyzing gene expression profiles, which helps us to understand the heterogeneity and spatial relationship within tumors, such as finding those areas where T cells can't enter [21].

Tumor organs are 3D organs cultured from patients' tumor cells, which can simulate the tumor microenvironment in vivo, and can also be used to screen Qualcomm drugs, providing a pre-clinical reference for us to choose personalized treatment plans [23].

3.2. Clinically feasible diagnostic methods

These methods are easier to integrate into clinical work for dynamic monitoring.

Liquid biopsy includes circulating tumor DNA(ctDNA), which can non-invasively monitor mutations related to drug resistance, and can almost reflect the evolution and cloning dynamics of the tumor genome in real time, such as finding new NOTCH1/3 or STK11 mutations [24]. Exosome

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PD-L1 exists on the surface of exosomes from tumors, which can inhibit the activation of T cells systematically, and its level may be related to clinical drug resistance [25,26].

AI-based imageology can extract many high-dimensional features from conventional CT or PET-CT images. When it is combined with a machine learning algorithm, it can non-invasively predict tumor immunophenotype, tumor microenvironment and treatment response, which has great prospects in clinical application [27,28].

3.3. Diagnostic methods for monitoring acquired resistance and their link to resistance mechanisms

Table 2 compares diagnostic methods for monitoring acquired resistance, highlighting their core principles, advantages, current clinical availability, and crucially, their capability to identify specific resistance mechanisms outlined in Section 2 through the detection of corresponding biomarkers.

Table 2. Diagnostic methods for monitoring acquired resistance and their link to resistance mechanisms

Method Category	Specific Technology	Core Principle & Advantages	Clinical Availability	Detectable Biomarkers & Linked Resistance Mechanisms	References
Basic Research Tools	Single-Cell Multi-Omics	Decodes cellular interactions within TME at high resolution.	Low (Primarily Research)	Identifies subclones with B2M mutations (Antigen Presentation Defects), JAK1/2 mutations (IFN-γSignaling Alterations), and characterizes immunosuppressive cell populations (e.g., Tregs, MDSCs).	[21,22]
	Spatial Transcriptomics	Analyzes gene expression while preserving tissue architecture.	Low (Primarily Research)	Maps the spatial distribution of T-cell exclusion zones (Physical Barriers), hypoxic regions, and localized immunosuppressive cell aggregates.	[21]
	Tumor Organoids	3D models from patient cells mimic the TME for drug screening.	Low (Pre-clinical)	Functional testing of resistance linked to oncogenic pathway activation (e.g., KRAS/STK11) and TME-mediated immunosuppression.	[23]
Clinically Feasible Methods	Liquid Biopsy (ctDNA)	Non-invasive monitoring of tumor DNA in blood for real-time tracking.	High (Gradually entering clinic)	Detects emerging mutations: B2M (Antigen Presentation), JAK1/2 (IFN-γSignaling), STK11/KEAP1, PTEN, EGFR (Oncogenic Pathways).	[24]
	Liquid Biopsy (Exosomal PD-L1)	Detects PD-L1 on circulating exosomes.	Medium (Under clinical validation)	Measures exosomal PD-L1 levels, indicative of systemic immune suppression potential (TME-mediated).	[25,26]
	AI Radiomics	Extracts features from medical images to non-invasively predict response.	High (Easily Integrable)	Infers TME status ("cold" vs. "hot" tumors, fibrosis/barriers, hypoxia) and potentially overall tumor mutational burden related to resistance.	[27,28]

4. Clinical methods to defeat acquired drug resistance

To overcome AR, people need creative methods, often combining different methods to deal with the specific causes of drug resistance.

Combination immunotherapy is to block multiple inhibitory pathways at the same time, so as to deal with the duplication of immune checkpoints. For example, PD-1/CTLA-4 bispecific antibody,

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or inhibiting TIGIT and PD-1/PD-L1 at the same time, has been shown to be promising to overcome some types of AR in clinical trials [29-31].

Targeting the tumor microenvironment includes some strategies to turn "cold" tumors into "hot" tumors. This method uses some drugs to reprogram or clear immunosuppressive cells, and uses collagenase or TGF-β inhibitor to break the physical barrier, which can help T cells enter the tumor better [11,15,16].

Personalized combination therapy represents a method under the guidance of precise medical care. For example, for patients with KRAS mutation and STK11 loss, people can consider combining ICIs with a COX-2 inhibitor; For those patients with defective IFN-γ signaling pathway, it is necessary to explore the combination of ICIs and PARP14 inhibitors [6,10].

Table 3 summarizes the clinical methods to overcome acquired drug resistance, and lists the core strategies, action mechanisms and representative examples in detail.

Table 3. Overview of clinical strategies to overcome acquired resistance

Strategy Category	Core Approach	Mechanism of Action	Key Examples	References
Combination Immunotherapy	Simultaneous blockade of multiple immune checkpoints	Overcomes checkpoint redundancy, amplifies T-cell response.	PD-1/CTLA-4 bispecific antibodies; Co-inhibition of PD-1/PD-L1 and TIGIT.	[29-31]
Targeting the Tumor Microenvironment	Reprogramming/depleting immunosuppressive cells; Disrupting physical barriers	Converts "cold" tumors to "hot," enhances immune cell infiltration.	Targeting TIM-3+VISTA+ TAMs, MDSCs; Using anti-TGF-β therapy, collagenase.	[11,15,16]
Personalized Combination Therapies	Biomarker-guided combination of agents with different mechanisms	Precisely targets specific resistance pathways for synergistic effects.	For KRAS-mutant with STK11 loss: ICI + COX-2 inhibitor; For IFN-γ signaling defects: ICI + PARP14 inhibitor.	[6,10]

5. Challenges and future perspectives

Although people have made great progress in understanding and overcoming AR, there are still some unsolved problems.

Technical and Economic Challenges, including advanced detection methods such as single-cell sequencing, are too expensive and data analysis is very complicated, which makes it difficult for hospitals to use them on a large scale. People must develop cheaper and more convenient detection methods.

Biological Complexity Challenges come from the differences of tumors and clonal evolution, which means that the mechanism of drug resistance will change over time or exist at the same time, so people need to formulate flexible treatment plans.

Ethical and accessibility challenges mean that precision medicine may make the allocation of medical resources more unfair. How to ensure that all patients can use the latest tests and treatments fairly is an important social and ethical issue that people need to face.

6. Conclusion

In NSCLC, acquired resistance to PD-1/PD-L1 inhibitors is a highly complicated process, which is influenced by both the changes of the tumor itself and the surrounding environment of the tumor. To solve this problem, people need to understand several key mechanisms: for example, antigen presents problems, IFN-γ signaling pathway changes, the carcinogenic pathway is activated, immunosuppressed cells enter the tumor area, and the tumor metabolism mode is reconstructed.

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With the help of advanced technologies such as single cell omics, liquid biopsy and AI, people can accurately classify and dynamically monitor drug resistance, which is an important bridge to transform laboratory findings into clinical results. Looking forward to the future, if people can integrate various kinds of data to formulate personalized and flexible combination treatment schemes and actively respond to the subsequent technical, economic and ethical challenges, people will hopefully finally break through the obstacles of acquired drug resistance and bring more lasting survival benefits to NSCLC patients.

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