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Mechanisms of drug resistance in HER2-positive breast cancer cells and methods to overcome

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Abstract. Breast cancer (BC) has become one of the major malignant tumors affecting women's health in recent years, and the expression of human epidermal growth factor HER2 elevates the proliferation and migration of BC tumors. The HER2-targeted drug trastuzumab is initially effective, but resistance can develop after a period of time. There is currently no particularly effective approach to drug resistance in HER2-positive BC. In this paper, we have compiled and summarized several major drug resistance mechanisms of HER2-positive BC cells and proposed several coping strategies, suggesting the possibility of immunotherapy, as well as providing theoretical references for future immunotherapy for the remaining tumors. However, the safety of immunotherapy has not yet been demonstrated, and future studies could focus on ensuring the safety and prognosis of patient treatment.

Keywords: BC, HER2, NK cells, ADCC, ADC, KIR2DL4.

1. Introduction

BC is the main type of systemic malignant tumor, accounting for 7 to 10 percent of all cases. In recent years, its incidence has increased among young women, posing a serious threat to women's health. There are various methods of treating BC, ranging from surgery to chemotherapy and radiotherapy to endocrine and immunotherapy. Nonetheless, chemotherapy remains the mainstay of treatment for advanced BC or those who have developed metastases. However, chemotherapy is associated with a variety of side effects and may cause the resistance of tumors to the drug.

BC is classified based on the expression of its surface receptors, including estrogen receptor(ER), progesterone receptor(PR), and human epidermal growth factor receptor 2 (HER2). Differences in the expression of these receptors categorize BC into four subtypes: luminal A, luminal B, triple-negative, and HER2-positive. HER2 belongs to the ErbB receptor tyrosine kinase family, which may be altered in a variety of cancers. When HER2 dimerizes and is activated, it triggers a cascade of tyrosine kinase signaling. These signals affect cell growth and division through multiple signaling pathways such as MAPK and PI3K/AKT, and are involved in tumor development processes such as proliferation, infiltration and angiogenesis. Due to its role in promoting tumor growth and metastasis, HER2 has become a key point in many cancer treatment strategies. HER2 is lowly expressed in normal cells, and the expression of HER2 on the surface of HER2-positive tumor cells is 100 times higher than that of normal cells. more than 40% of HER2 mutations occur in BC patients, of which 20% are HER2 gene amplification, 15-20% HER2 overexpression, and 2% HER2 gene mutation [1].

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HER2-targeted therapy is an important treatment for patients with HER2-positive BC and has significantly improved the prognosis of these patients. By binding specifically to the HER2 receptor, anti-HER2 targeted therapies are able to block the production of the HER-2 receptor-ligand complex and inhibit the activation of the associated transduction pathway. This treatment can counteract a series of reactions such as over proliferation and vascular proliferation caused by HER-2 overexpression, thus inhibiting the transformation of tumor cells to a malignant phenotype, killing tumor cells, and effectively inhibiting the replication and spread of cancer cells.

Among the currently applied anti-HER2 receptor drugs, trastuzumab is the most widely used. This drug has been shown to have significant effects in the treatment of HER2-positive BC. In addition to trastuzumab, there are other anti-HER2 targeted drugs such as patuzumab and T-DM1, which also play an important role in the treatment of HER2-positive BC [2]. However, the efficacy of trastuzumab is obvious at the initial stage of treatment, and it will gradually lose its sensitivity with time delay. This paper will take the starting point of sorting out the mechanism of HER2 resistance and its corresponding solution treatment to demonstrate the feasibility of different therapeutic pathways and extend the thinking to the treatment of the rest of the tumors.

2. Mechanisms of resistance to targeted HER2 therapy

2.1. Impaired binding of drugs to HER2

2.1.1. Heterogeneity of HER2 Expression. Twenty percent of BC cells exhibit overexpression of the HER2 receptor or amplification of the HER2 gene on their surface. However, the amount of expression or gene amplification varies greatly, which leads to heterogeneity of HER2 expression as well [3], namely BC in the same organism consists of HER2BC cells with different expression statuses, causing different tolerances to anti-HER2 drugs. Drug-sensitive, highly expressed HER2BC cells are cleared during treatment, while drug-insensitive, low-expressed HER2BC cells survive treatment, leading to BC recurrence [3]. A study by WU et al [4] showed that as the level of HER2 amplification increased, the tumor size increased, while at the same time the expression of estrogen receptor ER and progesterone receptor PR decreased, which was also accompanied by an increase in tumor migratory properties. OCANA et al [5] retrospectively investigated the relationship between the presence of intra-HER2 heterogeneity and the development of resistance to anti-HER2 therapy and analyzed the significance of anticancer drugs targeting HER2 heterogeneity on BC treatment and prognosis. In this way, the importance of HER2 heterogeneity for BC drug resistance can be seen.

2.1.2. Endogenous structural alterations of HER. On the HER2BC cell membrane, there exists a C-terminal fragment (CTF) that is about 90-95 kDa long and also has tyrosine kinase activity as a splice variant of HER2, which mainly includes p95HER2 and HER2Δ16, among others [1]. It is formed by the cleavage of HER2 in the presence of integrins, disintegrins or metalloproteinases 10. The most important difference between it and normal HER2 is that it lacks the extracellular ligand structural domain (ECD fragment), thus lacks the specific recognition site of trastuzumab or emtricituzumab, and thus is not recognized and binded, but at the same time, it can substitute for normal HER2 to play a physiological role in activating the downstream signaling pathway leading to drug resistance [3]. In a prospective clinical trial lasting 5 years, researchers found that the expression of p95HER2 in trastuzumab-treated patients was correlated with their overall tumor survival (OS) and progression-free survival (PFS), and the results showed that the p95HER2/HER2 ratio was greater, the shorter the OS and PFS of patients [6], which also proved that p95HER2 was closely related to the resistance to trastuzumab in HER2-positive BC patients.

When exon 16 of the HER2 gene is deleted, HER2 is transformed into a new oncogenic isoform called HER2Δ16. 50% of HER2-positive BC patients also express HER2Δ16, which can form HER2Δ16 dimers in a non-receptor tyrosine-protein kinase (SRC) dependent manner to form HER2Δ16

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dimers, which activates downstream signaling pathways in tumors, promotes tumor cell proliferation and migration, and induces tumor cell resistance to trastuzumab [3].

2.1.3. Specific protein expression masks drug binding sites. In 30% to 95% of trastuzumab-resistant BC patients, overexpression of cell-surface mucin 4 (Muc4) has been observed. Muc4 is able to mask trastuzumab binding sites on HER2, thereby preventing trastuzumab from binding to it, which may contribute to or exacerbate resistance. Furthermore, by binding to HER2, Muc4 can promote its phosphorylation and activate downstream signaling pathways, which may also contribute to resistance to HER2 drugs. [1].

2.2. Aberrant activation of downstream signaling pathways

2.2.1. Abnormal activation of PI3K/AKT/mTOR signaling system. PI3K/AKT/mTOR is an extremely important intracellular growth-regulating signaling pathway, which influences physiological functions such as cell growth, differentiation, infiltration, proliferation, and resistance to antitumor therapy, etc. Specific inhibition of PI3K/AKT/mTOR is the mechanism by which many anticancer drugs exert their anticancer effects. However, it has been shown that mutations in PI3K/AKT/mTOR-related somatic genes are present in BC cells of many HER2-positive patients, such as concurrent multiple single-nucleotide variants in the PI3K/AKT pathway in the BT-474 cell line detected by single-cell sequencing [1], which allows tumor cells to bypass the effect of trastuzumab on PI3K/AKT, and the PI3K/AKT/mTOR pathway is abnormally activated, leading to tumor resistance to trastuzumab.

2.2.2. ER pathway. The estrogen receptor (ER) is a nuclear receptor that functions primarily as a ligand-dependent transcription factor that regulates cellular gene expression. There is bidirectional crosstalk between ER and HER2. Trastuzumab was found to inhibit HER2 signaling while increasing ER gene expression. It has also been shown that ER can directly activate the Ras/MAPK pathway or the PI3K/AkT pathway, thereby activating tumor cell proliferation, growth and anti-tumor resistance. It has also been shown that ER can reduce the expression of HER2, thus reducing the anchor site of trastuzumab, weakening the recognition of trastuzumab and inducing drug resistance.

2.3. Cell cycle regulation

The cyclin dependent kinase (CDK) 4/6 signaling axis, especially binding to cyclinD1, plays a key role in promoting cells into the proliferation cycle. When overexpression of cyclinD1 and CDK4 occurs or when CDK4 is mutated, this may lead to aberrant activation of the cyclinD1-CDK4/6 signaling axis, which in turn causes over proliferation of tumor cells. This over proliferation may be the cause of secondary resistance of tumors to therapeutic agents such as trastuzumab or lapatinib. [2].

2.4. Fatty acid synthase pathway

Fatty acid synthase (FASN) and HER2 are cross-regulated. Clinical studies have shown that HER2 promotes the expression of FASN, and that the localization of HER2 on cell membranes and signal transduction can also be mediated and enhanced by FASN, which can lead to drug resistance secondary to HER2 [7].

2.5. Antibody-dependent cell-mediated cytotoxicity (ADCC) escape

NK cells, as an important part of the body's intrinsic immunity, can kill tumor cells in three main ways: 1. release perforin and granzyme to act on the surface of tumor cells to make the tumor cells lysed, playing a cytotoxic role; 2. through the surface ligand FasL and the surface of the tumor cell Fas binding to induce the initiation of apoptosis program of the tumor cells; 3. the surface of the FabR and the combination of Fab, to initiate ADCC to kill tumor cells. Initiate ADCC to kill tumor cells. In addition, NK cells also secrete a large number of cytokines, such as IFN-γ, TNF and other cytokines to play a regulatory role and inhibit the growth of tumor cells.

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The resistance of tumor cells to ADCC is mainly reflected in two broad categories. The first category is that the tumor itself generates immune escape. the surface of NK cells expresses inhibitory receptor PD-1, and PD-L1 on the surface of tumor cells can recognize and bind to NK cells leading to a decrease in the activity of NK cells, decreasing the degranulation function of NK cells, and generating tumors with a greater ability to grow in vivo [8]. Secondly, NKG2D, the specific activating receptor on the surface of NK cells, should bind to the NKG2D ligand on the surface of tumors to generate specific immune responses, but the MHCI homologs MICA and MICB on the surface of tumors can act as special ligands of NKG2D and bind to it, leading to the internalization and degradation of NKG2D, thus inhibiting the activation of NK cells. At the same time BC cells also secrete the transforming growth factor TGF-β, which also inhibits NKG2D activity [9]. In addition to this there is the KIR2DL4/HLA-G pathway. Killer cell Ig-like receptor 2DL4 (killer cell Ig-like receptor 2DL4, KIR2DL4, CD158d) is a member of the killer cell immunoglobulins, and is a surface receptor for NK cells, which possesses the structures of both activating and inhibiting receptors. It can promote the secretion of IFN-γ from NK cells under normal conditions, and IFN-γ can also promote the expression of KIR2DL4 through the JAK2/STAT pathway, which in turn promotes the killing function of NK cells and exhibits an activating effect. However, human leukocyte antigen G (HLA-G) is expressed on the surface of tumors, and when it binds to KIR2DL4, the immunoreceptor tyrosine inhibitory motif (ITIM) in KIR2DL4 undergoes phosphorylation and recruits SRC-homology domain 2-containing protein tyrosine phosphatase 1(SHP-1)or SHP-2 to transmit inhibitory signals [1]. IFN-γ also promotes the combination of the two, thereby inhibiting NK cell activity [10]. The second category is the combined regulatory effect of trastuzumab mediating cytokine release aggravating immune escape. Some studies surface that trastuzumab can promote the release of IFN-γ and TGF-β from NK cells and BC cells, respectively, and the two can act simultaneously to promote the expression and binding of PD-1 and PD-L1, thus inhibiting NK cell function [10].

3. Drug resistance solutions

3.1. Antibody-coupled drugs

Classical targeted anti-HER2 drugs rely on the HER2 signaling pathway, namely they can only act on HER2-positive BC cells. However, due to the highly heterogeneous nature of tumors, HER2-positive tumor cells may change from HER2-positive to HER2-negative when they are treated with trastuzumab, resulting in tumor resistance. However, a study [11] found that the efficacy of therapeutic measures against HER2 would be greatly increased if a combination of chemotherapy and trastuzumab was taken, so antibody-drug conjugate (ADC) was developed. The new generation ADC trastuzumab deruxtecan (T-Dxd) is an antibody-drug conjugate consisting of trastuzumab and cytotoxic topoisomerase I. The new generation ADC T-Dxd is a combination of trastuzumab and cytotoxic topoisomerase I. Compared with traditional trastuzumab and emtricitumomab, it has a broader range of anti-tumor activity. Its most significant advantage is the "bystander killing" effect in the tumor microenvironment due to the medium membrane permeability and high drug-antibody ratio, namely not only on the target tumor, but also on some cells around the target point, whose mechanism is that the drug can be released from the target cells to the extracellular space after phagocytosis and degradation of the target cells by the target HER2-positive cells. The mechanism is that when the drug is phagocytosed by the target HER2-positive cells and released from the target cells to the extracellular space after degradation, the drug can be absorbed by the surrounding cells for a second time, thus resulting in a killing effect [12]. This can attenuate drug resistance due to the heterogeneity of BCs, namely targeting and killing HER2-positive BCs while also killing the surrounding HER2-negative cells, and avoiding immune escape of BC cells.

However, the safety and efficacy of T-Dxd still need to be evaluated. Study [13] found that compared with the rest of antitumor drugs, HER2-positive BC patients treated with T-Dxd had better benefits in terms of OS, PFS, and objective response rate (ORR). Meanwhile, a study [14] proved that about 50% of HER2-positive BC would develop brain metastasis. Compared to BC without brain turn, it has lower OS and worse anticancer drug benefit. However, in a phase II clinical trial, brain-transformed patients

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treated with T-Dxd had a PFS of 18.1 months, which was higher than that of the overall population of 16.4 months, proving that T-Dxd has central system activity and can enter the blood-brain barrier to act with better efficacy.

In terms of safety, one study [13] found a significantly higher incidence of grade 3 and higher immune-related adverse events (irAEs) in patients treated with T-Dxd compared to patients treated with the remaining antineoplastic agents, which may suggest that T-Dxd may cause autoinjury. Another study [15] found that patients treated with T-Dxd may have an increased risk of developing interstitial lung disease (ILD). Also trastuzumab, a component of T-Dxd, may have a toxic effect on the heart to some extent. Measurements of the heart's ejection fraction have shown that T-Dxd increases the risk of developing adverse effects of reduced ejection fraction.

3.2. Blocking KIR2DL4 enhances the killing function of NK cells

NK cells, as an important member of the components of the intrinsic immune system, are not specific and MHC-restricted per se. Under normal conditions, NK cells are in a latent state. When they come into contact with autoantigens in the body, the contact between autoantigens and inhibitory receptors on the surface of NK cells reduces the toxic effect of NK cells. When NK cells come into contact with foreign antigens, the foreign antigens bind to the activation receptors on the surface of NK cells and activate the degranulation and cytokine release functions of NK cells. Therefore, altering the activation state of NK cells or modifying the expression of activating and inhibitory receptors on their surface can be an important way to alter the toxicity of NK cells and thus regulate autoimmunity. Studies [16] showed that HLA-G expression was high in human primary BC cells and was the only ligand for KIR2DL4. The binding of KIR2DL4 to HLA-G on the surface of BC cells led to the phosphorylation of ITIM in vivo, transmitting inhibitory signals, reducing the release of granzymes such as granzyme and IFN-γ, and attenuating cytotoxic effects. In contrast, when KIR2DL4 was blocked, NK cells released significantly more IFN-γ and increased the expression of CD107a on the surface, which reflected a significant increase in the degranulation function of NK cells, thus enhancing the killing function of BC cells.

Meanwhile, due to the non-specificity of NK cells, they can kill various types of tumors in the body. In a study on acute lymphoblastic leukemia (ALL) [17], the relationship between KIR2DL4 and NK cells was also explored, and it was found that when KIR2DL4 was blocked, NK cell degranulation and cytokine secretion were enhanced, and their antigen-killing function was stronger, with a higher recovery rate for ALL patients. In a clinical study of colorectal cancer patients [18], it was noted that the three-year survival rate of colorectal cancer patients was related to the expression of HLA-G in the patient's cancerous tissue, with a three-year survival rate of 53.6% in patients with low HLA-G expression and 91.7% in patients with low HLA-G expression. At the same time, the study also pointed out that the effect of cancer cell invasion into the neuropil and survival time were related to the expression of KIR2DL4, and when HLA-G and KIR2DL4 were co-expressed at a high level, the prognosis of patients tended to be worse. This shows that specific blockade of KIR2DL4 can block NK cells from binding to HLA-G on the surface of a variety of cancer cells thereby inhibiting cytotoxic effects and avoiding immune escape from tumors. This may serve as one of the therapeutic tools to counteract the growth of multiple tumors.

In terms of safety, KIR2DL4 is responsible for regulating immune tolerance and signaling pathways. KIR2DL4 intracellular segment contains only ITIM, which exerts an inhibitory effect upon activation; however, its transmembrane region contains a positively charged amino acid residue, which can synergistically stimulate activation signaling upon binding to certain specific concomitant molecules. The integrity of the transmembrane region is necessary for KIR2DL4 to function, and when the amino acid residues in the transmembrane region are damaged, both its activation and inhibition cannot function; whereas, when only its ITIM is damaged, the activation function can still function normally, indicating that the activation signaling does not require the transmission of ITIM [19]. When the inhibitory function of KIR2DL4 is blocked and the activation function is normal, it may lead to a large increase in NK cell activity due to the loss of the inhibitory signal, and it is not MHC-restricted, which

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may cause killing of normal cells in the organism and suffer from autoimmune disease. If the function of intact KIR2DL4 is blocked, for example, by destroying the structure of its transmembrane region, blocking KIR2DL4 may lead to a part of immunodeficiency because KIR2DL4 can in turn regulate the inflammatory response in vivo through the secretion of cytokines, such as IFN-γ, by MAPK as well as inhibit the growth of tumors, and so on.

4. Conclusion

The mechanisms of drug resistance in HER2-positive BC mainly include impaired drug-tumor binding, abnormal activation of downstream pathways, cell cycle regulation, fatty acid synthase, and abnormal NK cell function, etc. Currently, the main methods of dealing with HER2 resistance include the development of antibody-coupled drugs and the use of KIR2DL4 pathway blockers. Both of these methods are emerging technologies and are currently less commonly used in clinical practice. Focusing on HER2, the NK cell pathway is the most important mechanism of drug resistance. Immunotherapy, as the main research direction of tumor treatment, is more on T-cell-mediated cellular immunity and less on NK cells, but NK cells, as non-specific immune cells with cytotoxicity, also have profound research value, and the regulation of the autoimmune system may be able to reduce the damage to the patient's body more than chemotherapy. This paper provides an idea for regulating the enhancement of NK cell function by blocking the KIR2DL4 receptor to kill tumors, but the experimental results of the clinical harm and effectiveness are still insufficient. If the harmlessness or low harm to human body is proved in the subsequent research, the next step can be carried out, and this technology can be used not only to deal with BC, but also can be used to deal with metastatic tumors, the rest of the primary tumors or to remove the pathogens in the body, which is of broad research significance.

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