

EGFR TKIs Combining the inhibition of RAS-ERK signaling in NSCLC Treatment

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ABSTRACT

Epidermal growth factor receptor (EGFR) mutations and EGFR overexpression have become the key factor to the occurrence of non-small cell lung cancer (NSCLC). EGFR tyrosine-protein kinase inhibitors (EGFR-Tyrosine Kinase Inhibitors, EGFR-TKIs) were used in clinic to treat NSCLC by blocking the EGFR signaling pathway. Due to the existence of rare EGFR mutations and secondary mutations under drug load, the first generation, second generation, and third generation of EGFR TKIs have been used in clinical practice. In addition, EGFR secondary mutation and abnormal activation of EGFR downstream signaling molecules or bypass signaling pathways are the reasons for NSCLC resistance to EGFR TKIs. The combination of EGFR TKIs and EGFR downstream signal molecule inhibitors can improve the efficiency of targeted therapy for NSCLC. Based on clarifying the mechanism of resistance of NSCLC to EGFR TKIs, this paper introduces new inhibitors targeting RAS-ERK signaling downstream of EGFR. This paper also reviews progresses in a combination of EGFR TKIs and downstream signal molecule inhibitors in the treatment of NSCLC.

Key words: EGFR mutation, EGFR-TKIs, non-small cell lung cancer, RAS-ERK, targeted therapy

INTRODUCTION

Cancer is a major public health problem worldwide.^[1] Non-small cell lung cancer (NSCLC) is one of the deadliest malignant tumors in humans and the main cause of cancer deaths worldwide, accounting for 80–85% of lung cancers.^[2]

Targeted EGFR mutation therapy has been widely used in the treatment of NSCLC. EGFR fulfills its function through the intracellular domain of its tyrosine protein kinase. Tyrosine kinase inhibitors (TKIs) are a class of compounds specifically designed to inhibit the activity of EGFR tyrosine protein kinase. Studies have shown that the PFS of EGFR-TKIs is more effective than chemotherapy (patients receiving erlotinib had significantly longer PFS: 13.1 months vs.

4.6 months).^[3,4] After using EGFR-TKIs for a period of time, patients will inevitably develop resistance to TKIs. Therefore, the discovery of new drugs targeting molecules in the downstream pathway of EGFR signaling is necessary for the treatment of lung cancer patients who are resistant to EGFR-TKIs.

RAS/RAF/MEK/ERK is one of the downstream signaling pathways of EGFR, and its abnormal activation is one of the reasons for EGFR-TKIs resistance.^[5] At present, there is no effective clinical drug for RAS/RAF/MEK/ERK. However, related scientific research has found that drugs that can inhibit tumor cell proliferation by inhibiting RAS/RAF/MEK/ERK.^[6] This article focuses on the mechanism of lung cancer patients' resistance to EGFR-TKIs and reviews the current research progress in targeting RAS/RAF/MEK/ERK

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signaling pathways to overcome EGFR-TKIs resistance, providing references for clinical treatment and research.

EGFR AND EGFR-TKIS

EGFR and NSCLC

EGFR belongs to the epidermal growth factor receptor (ErbB) family and plays an important role in cell proliferation and apoptosis. EGFR is the receptor of EGF, and its mutation or over-activation of EGF plays an important role in NSCLC angiogenesis, invasion, and metastasis. About 23% of lung cancers worldwide are closely related to EGFR signaling,^[7] up to 50% among Asians.^[8,9]

EGFR consists of three domains, extracellular ligand-binding domain, transmembrane domain, and intracellular tyrosine kinase domain.^[10] Ligands, including epidermal growth factor (EGF), can bind to EGFR, as shown in Figure 1. When there are too many ligands or abnormal activity of EGFR and overexpression or mutation of EGFR, the downstream signal transduction pathways, including PI3K/AKT, JAK/STAT, and RAS/RAF/MEK/ERK and other signal pathways are activated, resulting in abnormal cell proliferation, differentiation, adhesion, migration, and tumorigenesis.^[11,12] Many malignant tumors, including lung cancer, ovarian cancer, prostate cancer, colon cancer, breast cancer, and cervical cancer, are associated with abnormal expression of EGFR.^[13]

Application of EGFR-TKIs in the treatment of NSCLC

Since EGFR mutations were found to be the main cause of NSCLC, tyrosine kinase inhibitors targeting EGFR mutations have been widely used in NSCLC treatment.^[14] EGFR-TKIs have become effective chemotherapy drugs for patients with EGFR mutations and have the advantages of fewer side effects and high progression-free survival.^[15] EGFR-TKIs can bind to the tyrosine kinase on the surface of EGFR to

inhibit its activity, thereby inhibiting the growth of tumor cells and promoting apoptosis.^[3,15]

There are two important and widely used first-generation EGFR-TKIs, including gefitinib and erlotinib. They were respectively approved by the FDA for the treatment of non-small cell lung cancer in May 2003 and November 2004.^[16-20] Prevent abnormal binding of mutant EGFR and its ligands, inhibit the activation of tyrosine kinase structural regions in cells, and prevent the cascade of downstream signaling pathways.^[21] The prognostic survival rate of erlotinib is higher than that of gefitinib, but the clinically common adverse reactions are relatively more.^[22]

Afatinib is the second-generation EGFR-TKIs, an irreversible EGFR blocker, and its inhibition principle is similar to the first-generation EGFR-TKIs.^[23,24] Studies have shown that the first and second-generation EGFR-TKIs have the same inhibitory effect on wild-type and mutant EGFR.

The third-generation TKIs include osimertinib and avelotinib. Osimertinib was officially approved by the FDA in 2015 for the treatment of non-small cell lung cancer. Due to its good therapeutic effect, osimertinib, as a first-line drug for the treatment of non-small cell lung cancer, is currently widely used in clinical practice, but it has developed drug resistance in lung cancer patients with T790M mutation.^[25] For NSCLC patients with EGFR T790M mutation, avelotinib has shown better efficacy as the third-generation EGFR-TKIs.^[26]

EAI045 belongs to the fourth generation of TKIs. Due to the rapid increase in resistance to osimertinib, usually after 9–13 months of use.^[27] The fourth-generation EGFR inhibitor EAI045 is the first allosteric inhibitor targeting the T790M and C797S EGFR mutants. This inhibitor is only effective in combination with cetuximab and has not yet undergone clinical trials.^[27]

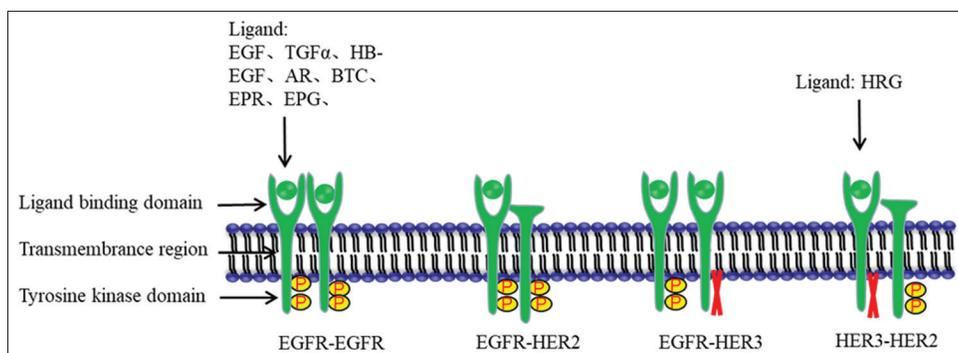


Figure 1: EGFR consists of three domains, extracellular ligand-binding domain, transmembrane domain, and intracellular tyrosine kinase domain. There are four ways of pairing between EGFR family receptors: EGFR-EGFR, EGFR-HER2, EGFR-HER3, HER3-HER2, and HER2 has no ligand-binding domain. The ligands of EGFR are EGF, TGF α , HB-EGF, AR, BTC, EPR, EPG, and the ligands of HER3 are only HRG

In short, patients with non-small cell lung cancer usually develop resistance to TKIs after using EGFR-TKIs for about 10 months. TKIs targeting mutant EGFR and inhibitors targeting downstream proteins are strategies for a new generation of chemotherapeutics for NSCLC. With the continuous maturity of targeted therapy and the continuous improvement of lung cancer treatment methods, tumor-targeted therapy has become the main treatment method.

The mechanism of NSCLC resistance to EGFR-TKIs

Patients with lung cancer who use EGFR-TKIs for a period of time will develop drug resistance, which is usually divided into primary drug resistance and secondary drug resistance, and secondary drug resistance is divided into adaptive drug resistance and acquired drug resistance.^[28]

The reasons why lung cancer patients develop resistance to EGFR-TKIs can be divided into the following three [Figure 2].

Activation of EGFR downstream signaling pathways

The downstream pathways of EGFR such as PI3K/AKT/mTOR, RAS/RAF/MEK/ERK, and JAK/STAT pathways are activated, Figure 2. These signal transduction pathways,

as the main cell signal pathways, play an important role in the proliferation, differentiation, and migration of body cells. In addition, these signaling pathways can also regulate gene mutations, deletions, amplifications, insertions, and methylation caused by physical, chemical, and biological factors.^[29] When EGFR-TKIs act on non-small cell lung cancer, EGFR can be blocked, but its downstream signal transduction pathway can be activated by other genes. These genes may be dysregulated. For example, loss of PTEN and ALK fusion can abnormally activate the PI3K/AKT pathway, and KRAS mutation and BRAF fusion can abnormally regulate the RAS/RAF pathway. Therefore, the effect of EGFR-TKIs in inhibiting tumors will be weakened or disappeared.^[5,30] Other downstream pathways, such as epithelial-mesenchymal transition (EMT) or the activation of other genes that are beneficial to the growth and differentiation of lung cancer, are also the cause of human resistance to drugs.^[31]

Activation of the EGFR alternative pathway

Mutation or activation of the EGFR bypass is also an important reason for tumor cell resistance to EGFR-TKIs, as shown in Figure 2. First, the activation of other members of the EGFR family can lead to resistance to EGFR-TKIs. The heterodimer formed by HER2 and HER3 binds to modulin to form an ErbB receptor complex, which activates downstream signaling pathways, leading to resistance to gefitinib.^[32-34]

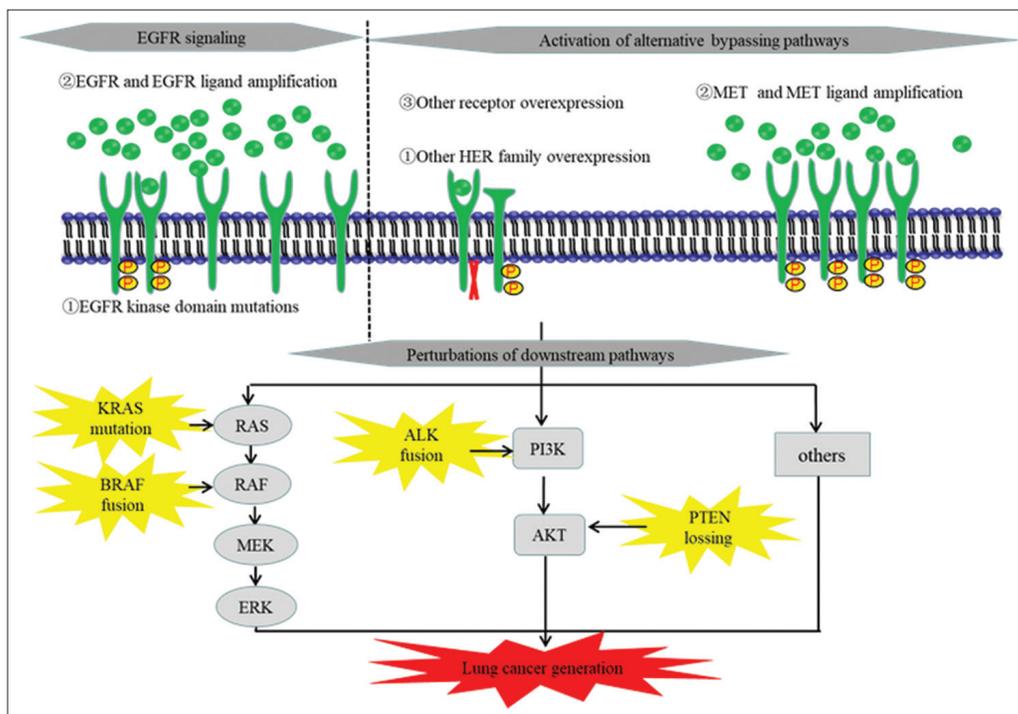


Figure 2: The main reason for the resistance of EGFR-TKIs is the abnormal signal of EGFR (including the amplification of EGFR and EGFR ligands, the mutation of the structural region of EGFR tyrosine kinase), alternative pathway (overexpression of other receptors in the HER family and overexpression of genes that can replace EGFR to activate EGFR downstream signaling pathways), and downstream pathways (due to KRAS mutation, BRAF fusion, ALK fusion, and PTEN loss can abnormally activate the classic cell signaling pathway, leading to abnormal cell proliferation) activate abnormally

Other receptors, such as mesenchymal to epithelial transition factor (MET), whose amplification (found in 61% of NSCLC) and mutations in exon 14 lead to activation of the resistance signaling pathway.^[35,36]

EGFR kinase structural region mutation

At present, the 18–21 exon mutation of EGFR is the main basis for clinical judgment of EGFR mutation, and it is also the main reference index for doctors to judge the efficacy of EGFR-TKIs.^[37] The most common EGFR mutations in clinical patients are exon 21 L858R mutation (41% of EGFR mutations) and exon 19 deletion (accounting for 45% of EGFR mutations). Patients with this mutation are sensitive to EGFR TKIs.^[38,39] In addition, some mutations are also sensitive to EGFR-TKIs, such as G719S, G719C, G719A, and S720F in exon 18, L861Q, and L861R in exon 21, and V765A, T783A, and S768I in exon 20.^[38,39] However, certain EGFR mutations can cause the body to develop resistance to EGFR-TKIs.^[40-44] Small insertion or duplication of EGFR exon 20 (accounting for 5% of EGFR mutations) is the cause of primary drug resistance.^[38,40,41] EGFR T790M is the main reason for clinical patients' acquired resistance to EGFR-TKIs, and the C797S mutation in exon 20 is also an important reason for patients' resistance to third-generation EGFR-TKIs. Therefore, not all non-small cell lung cancer patients with EGFR mutations are sensitive to EGFR-TKIs and how to use the drug depends on the type of EGFR mutation.^[42,43]

TARGETING RAF/RAS/MEK/ERK SIGNALING PATHWAY TO OVERCOME EGFR-TKIS RESISTANCE STRATEGY

In general, anti-tumor drugs can inhibit the growth of cancer cells by regulating cell apoptosis or proliferation.^[44-47] Since EGFR-TKIs are resistant to tumor cells during use, it is important to develop different medication strategies for different reasons of resistance for the prognosis of patients. Combination targeted therapy is currently a common clinical method to resist drug resistance. The type of combination method is to use EGFR-TKIs (such as gefitinib, erlotinib, afatinib, and osimertinib) in combination with PI3K-AKT, MET, HSP90, PTEN, or other signaling pathway inhibitors.^[5,48,49] Most of them have passed clinical trials, and there are many reports in the literature. There is no comprehensive clinical trial on the combined use of EGFR-TKIs and RAS-RAF-MEK-ERK signaling pathway inhibitors to overcome the resistance of EGFR-TKIs, but there are related academic studies that have proved the effectiveness of the combination of the two. In the following, this article mainly focuses on

EGFR-TKIs combined targeting RAF/RAS/MEK/ERK, a cell signaling pathway inhibitor.

Targeting RAS to overcome EGFR-TKIs resistance

There are three genes related to human tumors in the RAS gene family: H-RAS, K-RAS, and N-RAS, which are located on chromosomes 11, 12, and 1. Among them, KRAS has the greatest impact on human cancers. KRAS mutations are found in 25% of lung adenocarcinomas and KRAS mutations are the main reason for lung cancer patients' resistance to EGFR-TKIs.^[5] Although RAS is one of the more important targets in non-small cell lung cancer, there is no effective RAS anti-tumor targeted drug in clinical practice.^[50]

KYA1797K is an inhibitor developed by Park *et al.*^[6] and was first confirmed in colon cancer, which can inhibit RAS through the Wnt/catenin signaling pathway. To verify that KYA1797K is caused by KRAS mutations in lung cancer cells that are resistant to EGFR-TKIs, Park *et al.* used KRAS wild-type and mutant cell lines. After 72 h of treatment with erlotinib, the proliferation of KRAS wild-type cell models was affected and KRAS mutant cell model has no inhibitory effect. The cell proliferation of both models was inhibited after treatment with KYA1797K. On the other hand, it was also confirmed in animal experiments that KYA1797K had a tumor-suppressive effect on EGFR-TKIs resistant mice caused by KRAS mutation.

Targeting RAF to overcome the resistance of EGFR-TKIs

In non-small cell lung cancer resistant to EGFR-TKI, BRAF fusion accounts for 2%.^[51] Vojnic *et al.*^[51] have recruited 374 patients with metastatic EGFR-mutant lung cancer, 200 of which have not been treated with EGFR-TKIs. The study found that patients who were not treated with EGFR-TKIs did not have BRAF fusion. Only patients treated with EGFR-TKIs had BRAF fusion. Although the probability of BRAF fusion is very small, the cell (HCC827 and H1975) experiments proved that BRAF fusion is a cell One of the mechanisms of acquired resistance. BRAF fusion can activate the downstream MEK-ERK signaling pathway and then make patients resistant to EGFR-TKIs. Combined inhibition of EGFR and MEK may be effective in resisting acquired drug resistance caused by BRAF fusion. As an inhibitor of RAF, LY3009120 can effectively inhibit EGFR-TKIs resistance caused by BRAF fusion. Vojnic *et al.* used anti-erlotinib cells to be confirmed. Therefore, the effect of EGFR-TKIs and combined RAF inhibitors against EGFR-TKIs resistance needs further clinical research.

Sorafenib^[52] is an oral multikinase inhibitor that targets tumor growth, survival, and angiogenesis. It is reported to have effective anti-tumor effects on many types of tumors

(including non-small cell lung cancer). Sorafenib inhibits the growth of tumor cells by inhibiting RAF kinase and blocking the RAF/MEK/ERK signaling pathway. Gemcitabine is a new type of multikinase inhibitor that targets tumor proliferation and tumor angiogenesis and is a cell cycle-specific anti-metabolic and anti-tumor drug. It mainly acts on tumor cells in the DNA synthesis stage (S stage). It can damage DNA and cause cell death which is suitable for non-small cell lung cancer.^[53] Li *et al.* established lung cancer cell lines against EGFR-TKIs: A549, H1666, and H1975 and treated them with sorafenib and gemcitabine as a single agent for 72 h. In A549 and H1666 cells, sorafenib and gemcitabine have a synergistic effect, and the expression levels of p-AKT, p-ERK, and Bcl-2 are reduced, effectively inhibiting tumors.

Targeting MEK to overcome the resistance of EGFR-TKIs

MEK is a key gene in the downstream signaling pathway of EGFR. Inhibition of MEK can block the activation of its downstream and ERK/MAPK, which can effectively inhibit cell proliferation and promote cell apoptosis and decline. Huang *et al.*^[54] found that MEK inhibitors, CI-1040, and smetinib (AZD6244), can reverse resistance to EGFR-TKIs *in vitro* and *in vivo*. They established gefitinib-sensitive and drug-resistant cell lines and found that among gefitinib-resistant cell lines, AZD6244 or CI-1040 alone could not induce cell death, but gefitinib plus AZD6244 or CI-1040 can completely eliminate the phosphorylation of ERK and AKT, completely inhibit the growth of tumor cells, and increase the cytotoxic and anti-tumor effects of gefitinib. Li *et al.*^[55] studied the synergistic effect of selumetinib and gefitinib, and also established PC-9 lung cancer cells that are sensitive to EGFR-TKIs and A549 that have KRAS mutations that are not sensitive to EGFR-TKIs. Gefitinib can completely inhibit the expression of pEGFR in PC-9 lung cancer cells, and the inhibitory effect of gefitinib on pEGFR in A549 cells is lower than that on PC9 cells. They also tested the expression of its downstream signaling pathways and found that treatment of A549 cells with gefitinib alone can reduce the expression level of pAKT, and the combination of the two drugs has a stronger inhibitory effect on ERK and

p-ERK. Therefore, it is concluded that the MEK inhibitor simetinib and gefitinib have a synergistic inhibitory effect on tumor growth.

Miller *et al.*^[56] used 12 cell lines, including lung cancer cells, to test the MEK inhibitors U0126 and PD325901 (the inhibitory effect on ERK1 and ERK2 phosphorylation is about 500 times stronger than CI-1040) on AXL and MET. Whether *in vivo* or *in vitro* experiments, the levels of AXL and MET were significantly reduced.

El-Chaar *et al.*^[57] tested the combination of erlotinib (EGFR-TKI) and trametinib (MEK1/2-TKI) in 16 NSCLC cell lines with different RAS activation pathways. It was found that the combined inhibitory effect of EGFR and MEK can effectively block the growth of NSCLC tumor cells. Vojnic *et al.*^[51] also found in lung cancer cells resistant to osimertinib due to BRAF fusion, the combination of trametinib (MEK1/2-TKI) and osimertinib (third-generation EGFR-TKI) effect can effectively improve the inhibitory effect of both. They have a synergistic effect on inhibiting the growth of tumor cells.

Targeting ERK to overcome EGFR-TKIs resistance

ERK, as the last gene of the RAS pathway, its effective expression is essential for cell proliferation and apoptosis. Therefore, the combined targeting of ERK and EGFR is an effective method to treat drug resistance caused by RAS pathway signal transduction. Li *et al.*^[58] used two ERK inhibitors GDC0994 and VRT752271 in combination with osimertinib to effectively reduce the survival rate of the three cell lines resistant to osimertinib, and these cell lines had limited response to each inhibitor. The combination of ERK inhibitor and osimertinib can enhance cell apoptosis, and the reduction of Mcl-1 is also a key mechanism for enhancing the induction of cell apoptosis. They also conducted tumor-bearing experiments on nude mice. The study found that osimertinib and ERK inhibitors alone had no obvious inhibitory effect on the tumor size of nude mice, but the combination of the two had a significant inhibitory effect on tumor growth [Table 1].

Table 1: Combination of drugs targeting the RAS-RAF-MEK-ERK pathway to resist EGFR-TKIs resistance

Inhibitors	Targeting	In combination	Test phase	References
/	RAS	KYA1797K	Cells, Mouse	[6]
/	RAF	LY3009120	Cells	[51]
Gemcitabine	RAF	Solafini	Cells	[53]
Gefitinib (EGFR)	MEK	Smetinib, CI-1040	Cells	[54,59]
Erlotinib (EGFR)	MEK	Trametinib	Cells	[57]
Osimertinib (EGFR)	MEK	Trametinib	Cells	[51]
Osimertinib (EGFR)	ERK	GDC0994 、 VRT752271	Cells, Mouse	[58]

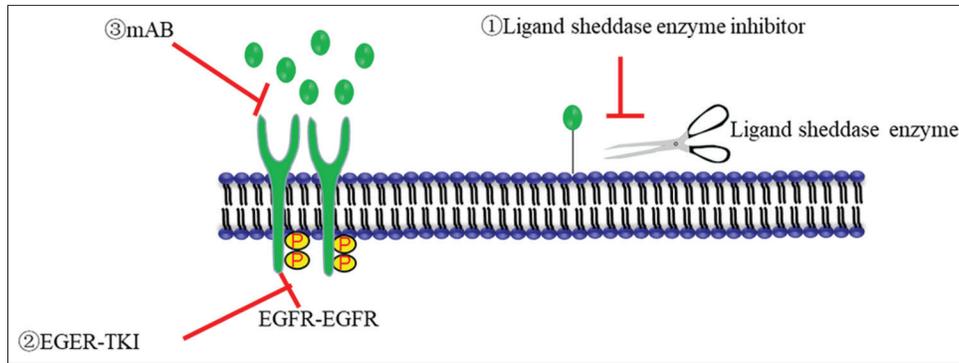


Figure 3: The following methods can be used to prevent the expression of EGFR. The binding of ligand and receptor can be directly inhibited. On the one hand, a ligand-cleaving enzyme inhibitor can be used, and on the other hand, EGFR monoclonal antibody can be used to compete with EGFR ligand to bind to EGFR. At present, the combination of mAB and EGFR-TKIs has been used clinically to resist patients' resistance to EGFR-TKIs

CONCLUSION

EGFR is of great significance in cancer treatment. The discovery of EGFR mutations and the good therapeutic effect of EGFR-TKIs in non-small cell lung cancer have changed the treatment of non-small cell lung cancer from chemotherapy to targeted therapy or a combination of both. EGFR-TKIs treatment has become the standard treatment for patients with EGFR mutations and has become the first-line treatment. Unfortunately, resistance to EGFR-TKIs has emerged. To overcome drug resistance, researchers have studied drug combination methods. Some PI3K/AKT signaling pathway inhibitors combined with EGFR-TKIs have entered clinical trials and have obtained good resistance to EGFR-TKIs. Although KRAS mutation is one of the important mechanisms of lung cancer and EGFR-TKIs resistance, the current inhibitors targeting the RAS-RAF-MEK-ERK signaling pathway have been studied in related basic scientific research, and most of the inhibitory effects have been verified at the cellular level. However, there is still a long period of time to verify before the clinical trial stage, which will be one of the research directions of future scientific researchers.

In the treatment of lung cancer, in addition to EGFR-TKIs targeting the kinase binding region, drugs targeting EGFR also have antibodies targeting the ligand-receptor binding region.^[15] [Figure 3]. In addition, the activation of the receptor is due to the binding of the ligand to the receptor, which activates the receptor. Therefore, if the binding of the ligand to the receptor is prevented, the effectiveness of EGFR-TKIs can be improved [Figure 3]. Aiming at the EGFR target, EGFR-TKIs combined with EGFR monoclonal antibodies have been used clinically to treat tumor cell resistance to EGFR-TKIs. On the other hand, from the perspective of ligand and receptor binding, blocking the shedding of ligand can also inhibit ligand-receptor binding, thereby resisting EGFR-TKIs resistance. A disintegrin and metalloproteinase (ADAM) ADAM10 and ADAM17 are widely considered to be the main “shedding enzymes”

on the cell surface, responsible for shedding hundreds of ectodermal domains of the transmembrane matrix. ADAM10 and ADAM17 are overexpressed in many cancers, and their activity is abnormally regulated by MAPK signaling.^[60,61] In addition, ADAM10 and ADAM17 are considered promising drug targets because they are involved in the shedding of EGFR family growth factor ligands from the surface of cancer cells, which is a process that mediates mitotic signals for EGFR family receptors in an autocrine manner.^[62-64] Therefore, in terms of pEGFR expression conditions, blocking the binding of EGFR ligand and receptor can control the expression of pEGFR from the root.

Since 2015, immunotherapy has become a hot topic in cancer treatment and has an important role in the treatment of lung cancer. At present, many hospitals have developed immunotherapy methods and achieved good results. Many scholars have begun to study the relationship between EGFR and immunotherapy. According to related research, EGFR mutation status is related to PD-L1 expression, which may eliminate the immune escape of EGFR-driven NSCLC.^[65] However, the relationship between EGFR and PD1 is not yet perfect. It is believed that in the future, immunotherapy can be effectively applied to the clinic to help resist EGFR-TKIs resistance, thereby solving the main treatment problem of lung cancer.

AUTHORS' CONTRIBUTIONS

The manuscript writing was instructed: MQ, ZG. Literature reading and writing the manuscript: WW. All co-authors commented on the manuscript and agreed with the conclusions of the manuscript.

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CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

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