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REVIEW – CLINICAL ONCOLOGY

Drug resistance of targeted therapy for advanced non-small cell lung cancer harbored EGFR mutation: from mechanism analysis to clinical strategy

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Abstract

Purpose Non-small cell lung cancer (NSCLC) accounts for about 85% in all cases of lung cancer. In recent years, molecular targeting drugs for NSCLC have been developed rapidly. The epidermal growth factor receptor-tyrosine kinase inhibitors (EGFR-TKIs) have changed the paradigm of cancer therapy from empirical cytotoxic chemotherapy to molecular-targeted cancer therapy. Currently, there are three generations of EGFR-TKIs, all of which have achieved good efficacy in clinical therapy. However, most patients developed drug resistance after 6–13 months EGFR-TKIs treatment. Therefore, a comprehensive understanding of EGFR-TKIs resistance mechanisms is of vital importance for clinical management of NSCLC.

Methods Relevant data and information about the topic were obtained by searching PubMed (Medline), Web of Science and Google Scholar using the subject headings, such as "NSCLC", "EGFR-TKIs resistance", "EGFR mutations", "human epidermal growth factor receptor-2 (HER2/erbB-2)", "hepatocyte growth factor (HGF)", "vascular endothelial growth factor (VEGF)", "insulin-like growth factor 1 (IGF-1)", "epithelial–mesenchymal transition (EMT)", "phosphatase and tensin homolog (PTEN)", "RAS mutation", "BRAF mutation", "signal transducer and activator of transcription 3 (STAT3)", and "tumor microenvironment", etc.

Results The mechanisms for EGFR-TKIs resistance include EGFR mutations, upregulation of HER2, HGF/c-MET, VEGF IGF1, EMT and STAT3 pathways, mutations of PTEN, RAS and BRAF genes, and activation of other by-pass pathways. These mechanisms are interconnected and can be potential targets for the treatment of NSCLC.

Conclusion In this review, we discuss the mechanisms of EGFR-TKIs drug resistance and the clinical strategies to overcome drug resistance from the perspective of EGFR-TKIs combined treatment.

Keywords Non-small cell lung cancer · Targeted therapy · Epidermal growth factor receptor-tyrosine kinase inhibitor · Drug resistance

Introduction

The most predominant pathological type of lung cancer is non-small cell lung cancer (NSCLC), which accounts for about 85% in all cases (Molina et al. 2008; Zappa and Mousa 2016). Fortunately, there is an increasing number of oncogenic driver mutations discovered in NSCLC, accompanied with an expanding spectrum of clinical signal transduction pathway inhibitors targeting these mutations, offers a considerable opportunity to improve the prognosis of patients. The discovery of epidermal growth factor receptor (EGFR) mutations is one of the most striking findings in NSCLC. Until now, the EGFR tyrosine kinases inhibitors (TKIs) has successfully changed the paradigm of cancer therapy from

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empirical cytotoxic chemotherapy to molecular-targeted cancer therapy (Lee 2017).

At present, there are three generations of EGFR-TKIs proved to be effective in clinical trials. The representative first-generation TKIs are gefitinib and erlotinib, which play an anti-tumor role by reversibly competing with ATP, binding to the tyrosine kinase domain through EGFR intracellular protein tyrosine kinase (Huang and Fu 2015; Seshacharyulu et al. 2012). Different from the first generation, the second-generation TKIs, such as dacomitinib and afatinib, are irreversible EGFR-TKIs, which can irreversibly inhibit three different members of the ERBB family, including EGFR, HER2 and HER4 (Subramaniam et al. 2014). Osimertinib, the third generation TKI, can irreversibly inhibit tyrosine kinase phosphorylation as well as reverse the T790M resistance mutation of patients (Cross et al. 2014). Though these EGFR-TKIs are effective in the treatment of EGFR mutated NSCLC, most patients developed drug resistance after 6 to 13 months, especially with the treatment of first/second-generation TKIs (Mok et al. 2009; Wu et al. 2015, 2017; Yang et al. 2016). Therefore, overcoming drug resistance has become an urgent clinical need to improve the survival of patients harbored EGFR mutations. A comprehensive understanding of the drug resistance mechanism of TKIs targeting NSCLC will be very beneficial to the follow-up drug research and the optimization of clinical strategies. In this review, the resistance mechanisms of EGFR-TKIs are discussed from the perspective of different targets. We also concern about the clinical strategies to overcome TKIs resistance in the current clinical research, and put forward to the possible direction of basic research as well as clinical development.

Gene alterations related to drug resistance

"On-target" and "off-target" have been classified as two main targeting resistance mechanisms proposed by Rotow and Bivona (2017). In detail, the alteration of primary target of the drug signifies on-target resistance, which decreased the inhibition effects of drugs when they interact with targets. Off-target resistance means changes of other related signaling pathways. We mainly elaborated on the molecular mechanisms of resistance of EGFR-TKIs targeted therapy for NSCLC based on these two levels in this review.

On-target resistance mechanism

EGFR mutations

HER family of receptor tyrosine kinases includes four members, respectively are EGFR (HER1 or ErbB1), HER2 (nue/ErbB2), HER3 (ErbB3) and HER4 (ErbB4) (Wheeler et al. 2008). EGFR mutations are limited to 4 exons (exons 18–21), and can be divided into 3 types (deletions, insertions and missense point mutations) (Shigematsu and Gazdar 2006). Most EGFR mutations occur in exon 19 (exon19 deletion, Del19) and exon 21 (exon 21 substitution, L858R) (Yang et al. 2020; Yasuda et al. 2012). These two mutations result in impaired endocytosis of EGFR, increasing the tyrosine phosphorylation. T790M mutation is another important mutation in clinic. It is the most common resistant mechanism to TKIs (Kobayashi et al. 2005). Studies have showed that T790M mutation could cause acquired resistance to first/second generation TKIs in 50–60% patients (Kosaka et al. 2006). At position 790 (T790M) in exon 20, threonine is replaced by methionine in specific kinase domain. Threonine 790 has been designated as a "gatekeeper" residue (Kon et al. 2014), and its mutation leads to the conformational change of EGFR kinase region, which makes TKI drugs unable to approach the active center of tyrosine kinase, weakening the reversible binding with TKI drugs. Table 1 lists the common resistance mechanisms of EGFR-mutant NSCLC. Actually, apart from single gene mutation, the important roles of hybrid mutations and secondary mutations playing in drug resistance have been gradually realized in recent years. The combination type of mutations is related to the prognosis of patients, and also essential to the change of therapeutic strategy. For instance, a study found that NSCLC patients harboring Del19 mutation was more likely to develop T790M secondary mutation than those with L858R mutants (Ke et al. 2017). More evidence revealed that patients of Del19 with T790M had a superiority of overall survival. This result may be caused by

Table 1 Common resistance mechanisms of EGFR-mutant NSCLC

Mutation type	Location	Common mutation	Frequency	References
Short in-frame deletion	Exon 19	Glu 746-Ala 750	45%	Qian et al. (2016)
Single nucleotide substitution	Exon 18 to 21	L858R	40–45%	Qian et al. (2016)
		T790M	1–79% (Dependent on the detection method and tested population)	Yu et al. (2014)
In-frame duplication/insertion	Exon 20	Within the range of codons 762 to 774	4–13%	Hasako et al. (2018)

the different interaction sites with drug between Del19 and L858R (Furuyama et al. 2013). Both of the mutations are located near the active site cleft of kinase. Del19 removes several residues from the loop leading into the αC-helix, while L858R lies in the activation loop of the kinase. This distinction results in different phosphorylation inhibition degree of gefitinib of EGFR, Akt and Erk1/2 in Del19 cells than in L858R cells (Zhu et al. 2008).

There are also some patients with other mutations, such as L747S (Yamaguchi et al. 2014a), D761Y (Balak et al. 2006) and T854A (Bean et al. 2008), which reduce the sensitivity of the mutant EGFR to bind to EGFR-TKIs as well. With the development of medicinal measurement technique and the awareness of important role that gene mutation plays in TKI drug resistance, there is an increasing number of new mutations have been detected and reported in recent years, including some rare insertion mutations (Woo et al. 2014). However, their specific mechanism is still not clear.

Off-target resistance mechanism

An overview of off-target resistance on type, frequency, molecular mechanism and possible targeted therapy are summarized in Table 2. More details are included in each part.

HER2 amplification

Takezawa et al. (2012) firstly reported that HER2 amplification was a new mechanism of acquired resistance to EGFR-TKIs in EGFR-mutant NSCLC tumors (Takezawa et al. 2012). Interestingly, in human model of NSCLC, EGFR-T790M mutation and HER2 amplification are often mutually exclusive. However, Shtiegman et al. (2007) proved that the amplification of HER2 could help the EGFR-L858R mutation individual cells escape ubiquitination by c-Cbl and enhance ligand-mediated receptor desensitization (Shtiegman et al. 2007). Activated ERK1/2 signaling pathway has been demonstrated as an important cause of drug resistance caused by HER2 amplification (Scrima et al. 2017). In recent years, the promising effect of trastuzumab against HER2 mutation are being widely explored. For instance, the American National Comprehensive Cancer Network (NCCN) has included trastuzumab and afatinib as potential therapy options for NSCLC patients with HER2 mutation (Eberlein et al. 2015). Besides, a phase II trial enrolled 24 patients for treatment with trastuzumab and paclitaxel (de Langen et al. 2018). The results revealed that this combination treatment has a better objective response rate and median duration of response than taxane monotherapy. Another ongoing phase II study of trastuzumab deruxtecan in HER2-overexpressing patients also showed obvious antitumor activity so far (Nakagawa et al. 2021). However, the risk and safety still need further estimation.

HGF/c-MET pathway

C-MET is the receptor for hepatocyte growth factor (HGF). Studies have found that 5% to 10% of patients developed acquired resistance to TKI due to c-MET gene amplification (Lei et al. 2020; Zhang et al. 2019b). C-MET amplification is one of discovered causes of gefitinib resistance, driving ErbB3 dependent activation of PI3K. This pathway is thought to be specific to EGFR/ErbB family receptors and independent of EGFR kinase activity (Engelman et al. 2007). Combinatorial therapy should be considered for these patients with resistant NSCLC carrying c-MET amplification (Wang et al. 2019). For instance, the c-MET inhibitor onartuzumab with erlotinib combination treatment has reported a benefit in a randomized phase II trial for c-MET immunohistochemistry (IHC)-positive patients (Spigel et al. 2013). Activation of HGF/c-MET signaling pathway was also sporadic reported about acquired resistance to TKIs by affecting the c-MET gene. Evidence showed HGF induced gefitinib resistance of lung adenocarcinoma cells with EGFR-activating mutations by restoring the PI3K/Akt signaling pathway via phosphorylation of c-MET in an ErbB3-independent manner (Yano et al. 2008). Therefore, combined therapy with EGFR and HGF-c-MET inhibitors

Table 2 Common mechanisms of off-target resistance

Resistance type	Frequency	Mechanisms	Inhibition drugs
HER2 amplification	2–23% (Swanton et al. 2006)	Activating ERK1/2	Trastuzumab/deruxtecan
MET mutation	2–3% (Sierra and Tsao 2011)	Activating PI3K	Onartuzumab
VEGF overexpression	30–40% (Dong et al. 2010)	Inducing autocrine	Bevacizumab/Apatinib
IGF1R activation	Not available	Activating downstream transcription factors	Figitumumab
EMT	20% (Yochum et al. 2019)	TGF-β-mediated signaling pathway changes	TWIST1 inhibitor harmine
PTEN mutation	4.5% (8of 176) (Jin et al. 2010)	Regulating ubiquitin process	miRNA inhibitors (e.g., miR-221)
K-RAS mutation	15–25% (Kempf et al. 2016)	Stimulating downstream pathways	Sotorasib (K-RAS-G12C)
BRAF mutation	1% (Ohashi et al. 2012)	Activating MEK-ERK	Vemurafenib/Dabrafenib
STAT3 activation	Not available	Operating downstream IL-6	Napabucasin

may overcome the drug resistance and prolong the effective time of EGFR-TKI treatment.

VEGF pathway

Vascular endothelia growth factor (VEGF) is essential in the progress of both physiological and pathological angiogenesis. VEGF receptor 2 (VEGFR2) is a kind of receptor tyrosine kinase which primarily mediates the VEGF signals, and it is also related to VEGF-induced cell proliferation in NSCLC (Devery et al. 2015). It has been well documented that in NSCLC, high expression of VEGFR2 often related to poor prognosis, since VEGF/VEGFR2 has potential to drive tumor cell proliferation through an autocrine signaling pathway (Pau et al. 2009; Seto et al. 2006). Besides, the enhanced VEGF level is often associated with EGFR-TKIs resistance (Larsen et al. 2011). A preclinical model has demonstrated simultaneous use of EGFR and VEGFR2 inhibitors were effective in antitumor treatment, supporting that combination strategy has a significant potential in future EGFR-TKIs resistance therapy (Tonra et al. 2006). A study included 311 Chinese NSCLC patients compared the combination of erlotinib and VEGF inhibitor bevacizumab to erlotinib (Le et al. 2021). Patients in combined therapy group had progression-free survival (PFS) improvement of 6.7 months at the primary endpoint. Other evidence revealed that patients with acquired resistance to EGFR-TKIs may also get profit of apatinib by its inducing effect on cell cycle arrest and VEGFR signaling pathway inhibition (Song et al. 2019).

IGF1 pathway

Insulin-like growth factor 1(IGF1) is an intermediate of many growth hormone responses, and has potential to stimulate the growth of some type of cancers. IGF1 receptor (IGF1R) is a tyrosine kinase-containing heterotetramer on cell surface, which mediated the biological activities of the IGFs (Werner et al. 2019). Activation of IGF1R signaling pathway is responsible for afatinib resistance in NSCLC patients harboring the T790M mutation (Lee et al. 2016). In addition, NSCLC cells with gefitinib-resistance are also related to the activity of IGF1R, since cell lines that were already resistant to gefitinib were found to have increased total-IGF1R and phosphorylated-IGF1R expression (Denduluri et al. 2015). Guerard et al. (2018) discovered that amphiregulin induced the nuclear accumulation of IGF1R. As a result, cell cycle was arrested through p21 (WAF1/CIP1) upregulation, and the induction of apoptosis was prevented in response to gefitinib (Guerard et al. 2018). Another study showed that hypoxia activated IGF1R, stimulating the growth of NSCLC stem cells which were already resistant to gefitinib (Murakami et al. 2014). This result suggests that IGF1R pathway might be a promising target for overcoming gefitinib resistance in NSCLC.

EMT

The epithelial-mesenchymal transition (EMT) phenotype is a malignant cancer phenotype characterized by augmented invasion, metastasis and chemoresistance (Kurimoto et al. 2016). Studies have demonstrated that many cytokines induced acquired resistance to EGFR-TKIs by promoting the occurrence of EMT (Xue et al. 2017; Yamaguchi et al. 2014b). Suda et al. (2011) added EMT-inducing ligand transforming growth factor β (TGF-β) to HCC4006 lung cancer cells in vitro, both morphological changes of cells and resistance to erlotinib were observed after 2 weeks treatment (Suda et al. 2011). It is reported that Forkhead box protein M1 (FOXM1), pyruvate dehydrogenase kinase 4 (PDK4) and Connexin 26 (Cx26) may be the essential regulators of EMT and are associated with TKIs resistance (Kong et al. 2014; Yang et al. 2015). Induction of EMT via the activation of the TGF-β/Smad canonical signaling pathway has been reported to be involved in the EGFR-TKIs induced resistance (Zhang et al. 2019a). TGF-β2 signaling links with EGFR-TKI drug-escape, cancer cells undergoing early adaptive drug escape are susceptible to TGF-β2 inhibition (Thiagarajan et al. 2016). In addition, activation of TGF-β-mediated signaling was sufficient to induce EMT occurrence and an increased secretion level of TGF-β-dependent interleukin 6 (IL-6) unleashed previously addicted lung tumor cells from their EGFR dependency (Yao et al. 2010). Thus, precise inhibition of both EGFR and TGF-β is a possible way to overcome drug resistance. Yochum et al. (2019) demonstrated that genetic silencing of TWIST1, the transcription factors for EMT, can inhibit the growth of EGFR-mutant NSCLC cells (Yochum et al. 2019). Overexpression of TWIST1 leads to resistance to erlotinib and AZD9291 in NSCLC patients. Inhibition of TWIST1 may be helpful to slow down the occurrence of EGFR-TKI resistance.

PTEN mutation

Phosphatase and tension homolog (PTEN) inactivation can specifically raise EGFR activity. By disintegrating newly formed ubiquitin ligase Cbl complexes, PTEN impairing the ligand-induced ubiquitylation and degradation of the activated receptor (Jin et al. 2018). A recent study demonstrated that EGFR-mutant patients harboring PTEN deletion had a shorter PFS and overall survival (OS) than those with intact PTEN. Moreover, PTEN deletion was identified as a cause of resistance to EGFR-TKIs in advanced NSCLC patients with EGFR sensitive mutations (Gkountakos et al. 2019). PI3K/Akt signaling pathway is regulated by PTEN deletion

negatively, and it was found that CpG hyper methylation of the PTEN gene contributed to the decreased expression of PTEN during acquired resistance to gefitinib or erlotinib (Maeda et al. 2015). Up-regulation of PTEN provides a new idea for improving drug resistance of EGFR-TKIs. Hopkins et al. (2013) found a translational variant leading to a longer PTEN protein (Hopkins et al. 2013). This kind of special protein is a membrane-permeable lipid phosphatase which is able to enter other cells and antagonize PI3K signaling. There are also some other possible methods to increase PTEN expression level. For instance, statins was suggested to promote the activity of transcription factor peroxisome proliferator-activated receptor-γ, consequently upregulating PTEN mRNA levels (Teresi et al. 2006). Regulating microRNA is another way which deserves trying to control PTEN expression. For example, highly expressed microRNA-21 (Zhang and Peng 2017), microRNA-25-3p (Sun et al. 2021) and microRNA-103a-3p (Li et al. 2021) are responsible for the proliferation and invasion of NSCLC cells via targeting PTEN. Controlling these microRNAs expression may resume the function of PTEN and re-sensitize the drug-resistant cancer cells to EGFR-TKIs.

RAS mutation

K-RAS is a member of the RAS family. Researchers used a mass spectrometry to detect genetic mutations related to EGFR-TKIs resistant lung cancer, and found that K-RAS was one of the drug resistance mutations (Tian et al. 2017). 5.9% K-RAS mutation could be found in EGFR mutated lung adenocarcinoma patients who developed acquired resistance to erlotinib (Cardona et al. 2017). K-RAS inhibitors have been under clinical or pre-clinical investigation for cancers with K-RAS mutation. Sotorasib, a small molecule inhibitor of K-RAS, was administered orally once daily to 129 patients (59 with NSCLC) harboring K-RAS p.G12C mutation. The results showed 32.2% of the NSCLC patients had a considerable control, leading to a median PFS of 4 months (Hong et al. 2020). Other compound such as KYA1797K (Park et al. 2019) also showed significant efficacy in animal models, more details are under further study.

BRAF mutation

According to statistics, approximately 1% patients of lung cancers with resistance to EGFR-TKIs were found to have mutations in BRAF (Ohashi et al. 2012). The BRAF mutations consists of three types, BRAF-V600E, BRAF-G469A/V, and BRAF-D594G, in which BRAF-V600E accounts for 50% in total (Tissot et al. 2016). Ho et al. (2017) has reported that activation of downstream MEK-ERK signaling pathway was the reason why BRAF-V600E mutation caused osimertinib resistance (Ho et al. 2017). Evidence suggests that BRAF inhibition demonstrated antitumor activity in NSCLC, and strategy combined BRAF and MEK inhibitors has a great potential to achieve a superior efficacy (Trojaniello et al. 2019; Tu et al. 2015). For example, in a clinical cohort, researchers found that trametinib ± dabrafenib, LXH254 and lifirafenib had a potent inhibition effect on both BRAF V600E and non-V600 mutant NSCLC cell lines (Negrao et al. 2020).

STAT3 gene expression signal activation

High expression and sustained activation of signal transducer and activator 3 of transcription (STAT3) can promote cancer cell proliferation and tumor growth (Hu et al. 2019). Lee et al. (2014) found that a tremendous number of drug-treated ''oncogene-addicted'' cancer cells engaged a positive feedback loop leading to STAT3 activation, promoting cell survival as a result. They also proved the role of STAT3 following erlotinib treatment in limiting the overall drug response (Lee et al. 2014). Specifically, inhibition of an oncogenic kinase in "addicted" cancer cells by transient STAT3 activation through IL-6R and EGFR, significantly contributes to resistance of the cell population to drug treatment and consequently limits the efficacy of these agents. Another study also demonstrated that EGFR inhibition resulted in the adaptive activation of both STAT3 and Src-YAP1 signaling, potentially operating downstream of IL-6, to promote cell survival and limit the initial response to EGFR-TKIs treatment in EGFR-mutant lung cancer (Chaib et al. 2017). Moreover, STAT3 polymorphism rs4796793 is possible to be a potential biomarker for the prediction of prognosis and susceptibility in lung cancer patients (Gong et al. 2019). MicroRNA-218 was proved to be a tumor suppressor in lung cancer by regulating IL-6/STAT3 signaling pathway (Yang et al. 2017). These results revealed that in the future, we may have more ways associated with STAT3 to predict tumor progression, which is of great significance for the evaluation and adjustment of patient's treatment strategy.

By-pass pathway

A study demonstrated that TNF1-driven adaptive response is detected in NSCLCs with EGFR activating mutations. Activation of EGFR signaling leads to downregulation of TNF mRNA rapidly, and the inhibition of EGFR signaling resulted in ascending TNF mRNA stability by decreasing miR-21 levels. Meanwhile, NF-κB is activated by TNF, then increases the transcription of TNF mRNA in a feed-forward loop in return, which may be a major mechanism of resistance to EGFR inhibition (Gong et al. 2018). Aberrant activation of NF-κB upregulates the transcription of numerous genes involved in cancer cell proliferation, survival, metastasis and therapeutic resistance (Li and Sethi

2010). For example, NF-κB takes part in initial EGFR inhibitor treatment, promoting tumor cell survival because EGFR oncogene inhibition induces the production of EGFR-TRAF2-RIP1-IKK complex (Blakely et al. 2015), which is related to an NF-κB-mediated transcriptional survival program. Bivona et al. (2011) had proved that the inhibition of NF-κB pathway can enhance or restore erlotinib sensitivity (Bivona et al. 2011). This result indicates a possible way to overcome EGFR-TKIs resistance.

Tumor microenvironment

In recent years, immunosuppressive effects of EGFR mutations have been reported in tumor microenvironment, such as regulatory cells (Tregs) (Mascia et al. 2016), immunoregulatory cytokines (Jia et al. 2019), and exosomes (Poggio et al. 2019). Actually, the immunosuppressive environment caused by TGF-β, IL-10 and IL-35 secretion from Tregs in tumors actively attenuates and subverts the antitumor immune responses of $CD4^+$ T cells, $CD8^+$ T cells and natural killer cells (Chin and Wang 2016; Frydrychowicz et al. 2017). Moreover, studies have shown that the level of amphiregulin in plasma in patient with NSCLC counts for poor prognosis (Chang et al. 2011). Another research found that amphiregulin could mediate suppressive function of Tregs via the EGFR/glycogen synthase kinase (GSK)-3/forkhead box P3 (Foxp3) axis (Wang et al. 2016).

Apicella et al. (2018) found that under long-term treatment with TKIs, glycolysis and lactic acid production increased in EGFR- or MET-addicted cancer cells (Apicella et al. 2018). Interestingly, the secreted lactate is the key molecule to instruct cancer-associated fibroblasts to produce HGF activating MET-dependent signaling in cancer cells, leading to sustained resistance to TKIs. EGFR-TKIs have been shown to down-regulate the expression of programmed death ligand (PD-L1), a prognostic indicator related to immunotherapy, in selected NSCLC with sensitive EGFR mutations that expressed high level of PD-L1(Tang et al. 2015). Actually, erlotinib up-regulated major histocompatibility complex (MHC)-I and MHC-II proteins in interferon (IFN)-γ treated keratinocytes but abrogated IFN-γ-induced expression of PD-L1 (Im et al. 2016). CD47 and calreticulin, those kinds of proteins associated with immune recognition, could affect the clearance of tumor cells by phagocytosis, contributing to cancer cell immune escape and adaptive drug resistance. In addition, Liu et al. (2019) observed that hypofractionated (high dose with a low frequency treatment) EGFR TKI treatment is more potent than standard strategy (Liu et al. 2019). The hypofractionated EGFR TKI triggers greater innate sensing for type I IFN and CXCL10 production through the Myd88 signaling pathway to enhance tumor-specific T cell infiltration and reactivation. This result provides evidence of proper combination of EGFR TKIs and immunotherapy in future treatment exploration.

Clinical strategy to overcome EGFR-TKIs drug resistance

Though the third generation of TKIs as monotherapy can make an improvement sometimes when the resistance of the first and second TKIs appears (Tan et al. 2018), it still lacks long-term efficacy due to drug resistance. Therefore, combination of EGFR-TKIs with other treatments are considered an effective strategy to overcome drug resistance. A series of recent studies have shown that EGFR-TKIs combination therapy has shown survival benefits. Relevant ongoing clinical trials about EGFR-TKIs combined with other treatments are listed in Table 3.

Many clinical studies were carried out to evaluate the effect of first-line combination EGFR-TKIs plus chemotherapy 10 years ago. However, the results of these studies were negative (Rebuzzi et al. 2020). The main problem is before experiments, the type and status of EGFR mutation of patients were not detected and selected. Therefore, patients who are not likely to respond to EGFR-TKIs were also included in these studies, leading to unsatisfied results. Recent clinical studies showed that the combined treatment of EGFR-TKIs and chemotherapy exhibited significant clinical benefits. Han et al. (2017) found that the PFS and OS of patients with lung adenocarcinoma harboring sensitive EGFR mutations in the combination group received gefitinib and pemetrexed/carboplatin were longer than that of patients in the chemotherapy or gefitinib alone (Han et al. 2017). Similarly, Noronha et al. (2019) demonstrated that patients accepted gefitinib with additional pemetrexed-carboplatin chemotherapy got a significantly prolonged PFS and OS (Noronha et al. 2019). Hosomi et al. (2020) conducted a phase III study to evaluate the efficacy of EGFR-TKI combined with platinum doublet chemotherapy in untreated advanced NSCLC patients with EGFR mutations (Hosomi et al. 2019). Compared with gefitinib alone, carboplatin combined with gefitinib improved PFS in patients with EGFR mutations. However, the OS of this kind of treatment requires further validation.

EGFR-TKIs combined with antiangiogenic therapy are also extensively investigated in recent years. Seto et al. (2014) demonstrated that a prolonged median PFS in the treatment of erlotinib plus bevacizumab (16.0 months) compared with erlotinib alone (9.7 months) (Seto et al. 2014). Another study also confirmed that bevacizumab plus erlotinib combination therapy improved PFS compared with erlotinib alone in patients with EGFR-positive NSCLC (Saito et al. 2019). Besides, Nakagawa et al. (2019)

Table 3 The ongoing clinical trials of EGFR-TKIs combination therapy

Identifier	Interventions	Phase	Locations
NCT03381066	Gefitinib/Pemetrexed/Cisplatin/Vinorelbine	III	Korea
NCT02759614	Erlotinib/Bevacizumab	III	China
NCT02454933	AZD9291/MEDI4736	III	Canada
NCT03428022	Apatinib/EGFR-TKI	III	China
NCT04001777	Osimertinib/APG-1252	I	China
NCT00977470	Erlotinib/Hydroxychloroquine	II	United States
NCT03940703	Osimertinib/Tepotinib	II	United States
NCT03758287	Gefitinib/CT053PTSA	I/II	China
NCT03126799	Erlotinib/Bevacizumab	II	Korea
NCT01859026	Erlotinib/MEK162	I	United States
NCT02438722	Afatinib/Cetuximab	II/III	United States
NCT02411448	Erlotinib/Gefitinib/Osimertinib/Ramucirumab	III	United States
NCT02954523	Osimertinib/Dasatinib	I/II	United States
NCT02971501	Osimertinib/Bevacizumab	II	United States
NCT04035486	Osimertinib/Pemetrexed/Carboplatin/Cisplatin	III	United States
NCT02496663	Osimertinib/Necitumumab	I	United States
NCT03784599	Osimertinib/Trastuzumab emtansine	II	Netherlands
NCT03909334	Osimertinib/Ramucirumab	II	United States
NCT03392246	Osimertinib/Selumetinib	II	United States
NCT02824458	Gefitinib/Apatinib	III	China
NCT03831932	Osimertinib/CB-839	I/II	United States
NCT04028778	Gefitinib/Anlotinib	III	China
NCT02520778	Osimertinib/Navitoclax	I	United States
NCT03133546	Osimertinib/Bevacizumab	II	Ireland
NCT03778229	Osimertinib/Savolitinib	II	United States
NCT02917993	Osimertinib/Itacitinib	I/II	United States
NCT04185883	Afatinib/Sotorasib	I/II	Japan/United States
NCT04820023	BBT-176/Cetuximab	I/II	Korea
NCT04575415	Bevacizumab/Erlotinib/Gefitinib/Icotinib/Afatinib/Dacomitnib/Osimertinib	Unknown	China
NCT04116918	Anlotinib/JS001	Unknown	China
NCT02157883	AZD9291/Itraconazole	I	United States/Belgium/Korea/Netherlands/Taiwan/United Kingdom
NCT04552613	EGFR-TKI/pemetrexed plus carboplatin	Unknown	China
NCT03727867	EGFR-TKI/Stereotactic body radiation therapy	III	China
NCT03543683	Osimertinib/Aspirin	Unknown	China
NCT04970693	Furmonertinib/Radiotherapy	II	China
NCT04413201	Afatinib/Osimertinib	IV	Germany
NCT01982955	Gefitinib/Tepotinib	I/II	44 study locations worldwide
NCT04401059	Elemene/First-generation EGFR-TKI	IV	China
NCT01861223	Afatinib/Nimotuzumab	I/II	Korea
NCT03532698	Osimertinib/Aspirin	Unknown	China
NCT02143466	AZD9291/AZD6094/Selumetinib/MEDI4736	I	United States/Canada/Japan/Korea/Poland/Taiwan/Russian Federation/Ukraine
NCT03054038	Afatinib/Necitumumab	I	United States
NCT03706287	Anlotinib/Pemetrexed/Cisplatin/Carboplatin	I/II	China
NCT04184921	Osimertinib/Aspirin	Unknown	China
NCT03766490	Anlotinib Hydrochloride/Gefitinib/Icotinib	Unknown	China
NCT04206072	D-0316/ Icotinib	II/ III	China
NCT02856893	Osemertinib/Gefitinib	II	France/Jordan/Poland/Slovenia/Spain

Table 3 (continued)

Identifier	Interventions	Phase	Locations
NCT04905550	Almonertinib/Radiotherapy	II	China
NCT02789345	Osimertinib/Ramucirumab/Necitumumab	I	United States/France/Korea/Spain/Taiwan
NCT01156545	BIBW 2992/Simvastatin	II	Korea
NCT04816214	Osimertinib/Capmatinib	III	Unknown
NCT02321293	Gefitinib/Erlotinib/CurcuVIVA™	I	Canada
NCT04755738	Almonertinib/Microwave ablation	II	China
NCT05011487	Osimertinib/Cisplatin/Pemetrexed	II	China
NCT00973310	EGFR-TKI/Radiation therapy	II	China
NCT02296125	AZD9291/Erlotinib/Gefitinib	III	170 study locations worldwide
NCT02151721	Vorinostat/Gefitinib	I	Japan
NCT04077463	Lazertinib/Amivantamab/Carboplatin/Pemetrexed	I	83 study locations worldwide
NCT03516214	EGF816/Trametinib	I	Germany/Spain
NCT02716311	Afatinib/Cetuximab	II	France
NCT01570296	Gefitinib/BKM120	I	Singapore
NCT03455829	Osimertinib/G1T38	I/II	United States
NCT03810807	Osimertinib/Dacomitinib	I	United States
NCT03623750	EGFR-TKI/EGF-PTI/Cyclophosphamide	I/II	Spain
NCT04545710	Osimertinib/Abemaciclib	II	United States
NCT02726568	Icotinib/Radiation	II	China
NCT03151161	Icotinib/Pemetrexed/Carboplatin	II	China
NCT04397432	EGFR-TKI/Elemene	Unknown	China
NCT02037997	Erlotinib/Pemetrexed/Docetaxel	II	China
NCT02098954	Erlotinib/Gemcitabine platinum	Unknown	China
NCT02716116	TAK-788/Pemetrexed/Carboplatin	I/II	69 study locations worldwide
NCT04592666	Almonertinib/Pemetrexed/Carboplatin	II	China
NCT03292133	EGF816/Gefitinib	II	United States
NCT02031601	Erlotinib/Gefitinib/Icotinib/Docetaxel/Pemetrexed/Platinum	IV	China
NCT03497767	Osimertinib/Stereotactic radiosurgery	II	Australia/Singapore
NCT04028778	Gefitinib/Anlotinib hydrochloride	III	China
NCT04541407	Osimertinib/Lorlatinib/Temozolomide	I	United States

*Source: www.ClinicalTrials.gov

demonstrated in the ramucirumab plus erlotinib treated group, patients have a longer PFS (19.4 months) than in the placebo plus erlotinib treated group (12.4 months) (Nakagawa et al. 2019). Ramucirumab is an antagonist of VEGFR, which can specifically bind VEGFR2 and block the coordination of VEGF ligand, VEGF-A, VEGF-C and VEGF-D (Clarke and Hurwitz 2013). Because of the interconnections between VEGF and EGFR, apatinib (a highly selective VEGFR2 inhibitor) can significantly enhance the anti-tumor effect of gefitinib in T790M positive mutant cells (Li et al. 2017).

Other specific mutation inhibitors combined with EGFR-TKIs are reported as well besides of those mentioned above. For example, the combination of EGFR-TKIs gefitinib with the demethylating agent 5-aza-2'deoxycytidine (5-AZA) and the histone deacetyl-transferase (HDAC) inhibitor Trichostatin A (TSA) may be an effective strategy aimed at PTEN inactivation lung cancer (Noro et al. 2007). MEK inhibitors combined with EGFR-TKIs are also explored (Martinelli et al. 2017). Eberlein et al. (2015) reported combination therapy of osimertinib and the MEK inhibitor selumetinib could prevent the emergence of drug resistance in PC9 cells and delayed drug resistance in H1975 cells (Eberlein et al. 2015). Day et al. (2019) demonstrated that the knockdown of TNFAIP8 inhibited EGF and IGF-1-stimulated migration in NSCLC cells. This discovery indicated TNFAIP8 and its effectors may be exploited to realize EGFR and IGF1R regulation, improving the efficacy of molecular-targeted therapies as a result (Day et al. 2019). Jia et al. (2016) tested the activity of a new type of allosteric inhibitor EAI045, which can inhibit L858R/T790M-mutant EGFR with nanomolar potency (Jia et al. 2016). Okura et al.

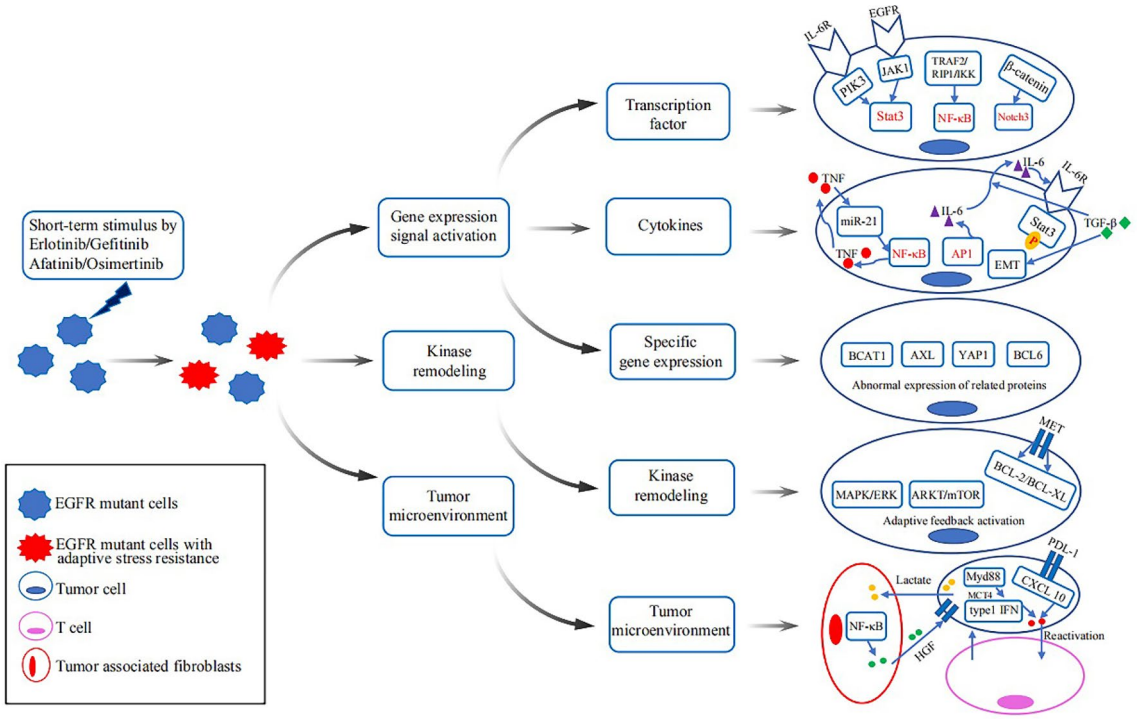

Fig. 1 Targeted resistance mechanisms of epidermal growth factor receptor-inhibitors (EGFR-TKIs)

(2020) conducted a small group of clinical trials to verify the efficacy of a novel AXL inhibitor ONO-7475, which showed that ONO-7475 suppressed the emergence and maintenance of tolerant cells to the initial EGFR-TKIs (Okura et al. 2020). These findings suggest that combined strategy of EGFR-TKI and other signaling pathway inhibitors is a promising therapeutic approach in the future.

Future perspectives

Target therapy has been a revolution in the management of advanced cancers, particularly NSCLC using EGFR-TKIs. However, drug resistance shortly occurs as expected. During the treatment of EGFR-TKIs, cancer cells develop drug resistance owing to EGFR on-target and off-target mechanisms (Fig. 1). Tumor microenvironment also plays imperative roles in EGFR-TKI drug resistance. Although EGFR-TKIs combination therapy has delayed the occurrence and development of drug resistance to some extent, there is large space to be improved and developed. For instance, combinatory treatment of EGFR-TKIs combined with inhibitors of MET, EMT and PI3K/Akt pathways, and antibodies against IGF1R, IL-6 and VEGF should be extensively explored. Owing to the cutting-edge technologies, such as whole-genome sequencing and RNA sequencing, in accurate detection and diagnosis of cancers, precision medicine and individualized therapy are conceived in clinical application. The disease types, genotypes, mutations in specific genes and immune status of patients should be comprehensively analyzed before individualized treatment of lung cancers. Nevertheless, the researches for more in-depth and detailed molecular mechanisms involved in the disease and drug resistance of targeted therapy of NSCLC are still highly demanded to transform NSCLC into a chronic disease.

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Declarations

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