Review

Acquired resistance of non-small cell lung cancer to epidermal growth factor receptor tyrosine kinase inhibitors

Fariz Nurwidya*, Fumiyuki Takahashi, Akiko Murakami, Isao Kobayashi, Motoyasu Kato, Takehito Shukuya, Ken Tajima, Naoko Shimada, Kazuhisa Takahashi

Department of Respiratory Medicine, Juntendo University Graduate School of Medicine, 2-1-1 Hongo, Bunkyo, Tokyo 113-8421, Japan

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Activation of epidermal growth factor receptor (EGFR) triggers anti-apoptotic signaling, proliferation, angiogenesis, invasion, metastasis, and drug resistance, which leads to development and progression of human epithelial cancers, including non-small cell lung cancer (NSCLC). Inhibition of EGFR by tyrosine kinase inhibitors such as gefitinib and erlotinib has provided a new hope for the cure of NSCLC patients. However, acquired resistance to gefitinib and erlotinib via EGFR-mutant NSCLC has occurred through various molecular mechanisms such as T790M secondary mutation, MET amplification, hepatocyte growth factor (HGF) overexpression, PTEN downregulation, epithelial-mesenchymal transition (EMT), and other mechanisms. This review will discuss the biology of receptor tyrosine kinase inhibition and focus on the molecular mechanisms of acquired resistance to tyrosine kinase inhibitors of EGFR-mutant NSCLC.

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Contents

1. Introduction ............................................................. 83
2. Factors associated with EGFR-TKI sensitivity ...................... 84

Abbreviations: EGFR, epidermal growth factor receptor; NSCLC, non-small cell lung cancer; TKI, tyrosine kinase inhibitor; WT, wild type; KRAS, Kirsten RNA associated rat sarcoma 2 virus gene; PTEN, phosphatase and tensin homolog; HGF, hepatocyte growth factor; IGF1R, insulin-like growth factor-1 receptor; TGF, transforming growth factor-α; EMT, epithelial-mesenchymal transition; EMP-1, epithelial marker protein-1; PI3K, phosphoinositide 3-kinase; RECIST, response evaluation criteria in solid tumors; BCRP, breast cancer resistance protein; ABCG2, ATP-binding cassette G2; CAF, cancer-associated fibroblast; mTOR, mammalian target of rapamycin; BRAF, v-Raf murine sarcoma viral oncogene homolog B1

*Corresponding author. Tel.: +81 803 690 2529; fax: +81 358 021 617.
E-mail addresses: fariz@juntendo.ac.jp (F. Nurwidya), fumiyuki@dol.hi-ho.ne.jp (F. Takahashi), amuraka@juntendo.ac.jp (A. Murakami), isao-k@juntendo.ac.jp (I. Kobayashi), mtkatou@juntendo.ac.jp (M. Kato), tshukuya@juntendo.ac.jp (T. Shukuya), tajiken@juntendo.ac.jp (K. Tajima), naokoh0421@hotmail.com (N. Shimada), kztakaha@juntendo.ac.jp (K. Takahashi).

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1. Introduction

In the 28 years since the isolation of the cDNA encoding the epidermal growth factor receptor (EGFR) and the deduction of its amino-acid sequence, intensive research efforts have led to important insights into the molecular mechanisms of receptor tyrosine kinase (RTK) function [1]. One major category of RTKs is further classified into the following groups: group 1 containing ERBB family members, group 2 containing insulin growth factor-1 receptor (IGF1R), and group 3 comprising hepatocyte growth factor receptor c-MET [2]. There are 4 members of the ERBB family of RTKs: epidermal growth factor EGFR, also known as ERBB1/HER1, ERBB2/HER2/NEU, ERBB3/HER3, and ERBB4/HER4 [3]. The ERBB1, ERBB3, and ERBB4 receptors are composed of an extracellular ligand-binding region consisting of glycosylated domains; a transmembrane domain containing a single hydrophobic anchor sequence; an intracellular region containing the catalytic tyrosine kinase domain, and a carboxy-terminal region containing several tyrosine residues that become phosphorylated after receptor activation [4]. Although ERBB2 has no known ligand, it is the preferred dimerization partner for other ERBB receptor [5,6]. This family is important for proper regulation of many developmental, metabolic, and physiological processes mediated by epidermal growth factor (EGF), transforming growth factor-α (TGFα), and multiple other ligands [7]. EGFR-dependent pathways also play important roles in the development and progression of human epithelial cancers, including non-small cell lung cancer (NSCLC) [8].

When binding to one of several ligands, EGFR forms homodimers or heterodimers with other family members and activates downstream signals such as the phosphoinositide 3-kinase (PI3K)/Akt, Raf/MEK/Erk, and Jak/Stat signaling pathways, initiating a cascade of signaling events that trigger anti-apoptotic signaling, proliferation, angiogenesis, invasion, and metastasis (Fig. 1) [9,10]. Lung adenocarcinoma cells that depend on EGFR for survival constitutively activate the receptor through a combination of genetic mutations and overexpression of EGFR dimeric partners and their ligands [11].

There are 2 major classes of anti-ERBB therapeutics: ectodomain-binding antibodies and small-molecule tyrosine kinase inhibitors (TKIs) gefitinib and erlotinib, which compete with ATP in the tyrosine-kinase domain [12]. The obvious implication of shutting off EGFR with a specific kinase inhibitor, as well as with antibodies or RNA interference, is extinguished proliferative and survival signals on which the tumor cell is dependent, therefore resulting in tumor cell death [13]. Gefitinib was the first oral EGFR-TKI to become available in clinical practice [14], and Japan was the first country that approved the use of gefitinib [15]. EGFR-mutant lung cancer is extremely sensitive to gefitinib and erlotinib [16]. The effectiveness of

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**Fig. 1 – Activation of EGFR and downstream signaling pathways.** Activated EGFR forms homodimers or heterodimers with other family members and activates downstream signaling such as the PI3K/Akt and Raf/MEK/Erk pathways, thereby initiating a cascade of signaling events that trigger proliferation, angiogenesis, anti-apoptosis, migration, and invasion.
gefitinib in lung cancers harboring mutant EGFRs may reflect 2 phenomena: first, EGFR inhibition of critical anti-apoptotic pathways on which these cells have become strictly dependent; second, alteration of biochemical properties such as the affinity of EGFR-TKI to mutant EGFR [17].

2. Factors associated with EGFR-TKI sensitivity

Data have suggested that individuals in whom gefitinib is efficacious are more likely to have adenocarcinomas of the bronchioloalveolar subtype, never smokers, Asian ethnicity, and female [9,18,19]. In the majority of patients with highly responsive tumors, the tumor contains somatic mutations of the EGFR-encoding gene [20,21]. The mutations can be of 3 different types—deletions, insertions, and missense mutations—and they all target key structures around the ATP binding cleft, including the P-loop, αC-helix, and A-loop [22]. Overall, deletions in exon 19 and the point mutation of L858R constitute approximately 90% of all EGFR-activating mutations and are termed “classical activating mutations” [23]. The deletion in exon 19 is a multi-nucleotide in-frame deletion involving the elimination of 4 amino acids (Leu–Arg–Glu–Ala), and the point mutation is a single nucleotide substitution at nucleotide 2573 (T→G) in exon 21 resulting in the substitution of arginine for leucine at position 858 (L858R) [24]. Moreover, the EGFR gene copy number was also confirmed as a predictor of clinical benefit [25]. However, genotype analysis of the INTACT studies (phase III trials of gefitinib) showed that the addition of gefitinib to standard chemotherapy regimens showed a trend toward an increased odd ratio in EGFR-mutant patients but not in patients with EGFR amplification [26]. Clinical trials have shown significant variability in the response to gefitinib, with higher responses found in Japanese patients compared with a predominantly European-derived population (27.5% versus 10.4% in a multi-institutional phase II trial) [27]. The treatment outcome of gefitinib also improved if, prior to treatment, patients were confirmed to lack the KRAS mutation [28]. From in vitro analysis, Okamoto et al. found that downregulation of anti-apoptotic protein survivin and induction of apoptotic protein BIM are independently required for EGFR-TKI-induced apoptosis [29]. Moreover, an increased copy number of the HER2 gene is associated with gefitinib sensitivity in EGFR-positive patients [30]. We hypothesize that the most predictive factor for sensitivity to EGFR-TKIs is a somatic mutation of EGFR. It has been predicted that the relatively favorable prognosis of lung cancer harboring EGFR-mutations means that these patients will gradually represent a larger proportion of the lung cancer population receiving treatment [31].

3. Acquired resistance

Unfortunately, 20–50% of patients with clinical or biology predictors for EGFR sensitivity are resistant to gefitinib, referred to as primary or de novo resistance [32]. Thus, in other words, these patients did not respond to initial treatment with EGFR-TKI. Although the majority of sensitive EGFR-mutant lung cancer patients initially shows a good clinical response, drug resistance invariably occurs and the disease progresses, which leads to acquired resistance [32]. Acquired resistance occurs in patients who (1) have been
treated with a single-agent EGFR-TKI (e.g., gefitinib or erlotinib); (2) have either a tumor with an EGFR mutation associated with drug sensitivity or an objective clinical benefit from treatment with an EGFR-TKI, or both; (3) suffer from systemic progression of disease [response evaluation criteria in solid tumors (RECIST) or WHO] while on continuous treatment with gefitinib or erlotinib; and (4) have no intervening systemic therapy between cessation of gefitinib or erlotinib and initiation of new therapy [33]. Therefore, it is crucial to identify molecular mechanisms of gefitinib-resistance in lung cancers. Mechanisms are usually classified into 3 major categories: (1) issue of ATP-binding site of mutated EGFR; (2) bypass signaling of EGFR pathways; and (3) others mechanisms. All of these mechanisms will be discussed in the following sections (Fig. 2).

3.1. Secondary mutation T790M

One secondary EGFR mutation is a substitution of threonine to methionine at codon 790 (the “gatekeeper” residue; T790M) [34]. In vitro, the T790M mutation activates wild type (WT) EGFR, and the introduction of the T790M mutation increases the ATP affinity of the oncopgenic L858R mutant by more than an order of magnitude, which is the primary mechanism of how the T790M mutation confers drug resistance [35]. However, even within recurrent gefitinib-resistant NSCLCs containing the secondary T790M EGFR mutation, this acquired mutation is only present in a subset of the resistant tumor cells in vitro [36]. A clinicopathologic study also indicated that the T790M mutation is sometimes present in a minor population of tumor cells during the development of NSCLC, suggesting that the detection of a small population of T790M mutant alleles may be useful for predicting gefitinib resistance of NSCLCs with sensitive EGFR mutations [37].

3.2. MET amplification

Although MET amplification is present in untreated NSCLC, EGFR mutation can activate the MET protein in NSCLC in vitro [38]. Moreover, from in vitro and in vivo studies, ligand binding of EGFR also causes MET amplification [39]. Interestingly, in vitro data have demonstrated that the MET inhibitor XL880 is a more efficient inhibitor of lung adenocarcinoma cells with EGFR T790M and MET amplification than either reversible (erlotinib) or irreversible (CL-387 or CL-785) EGFR inhibitors [40]. EGFR T790M and MET amplification accounts for 60–70% of all known causes of acquired resistance to gefitinib; however, other mechanisms of acquired resistance are the subject of further investigation.

3.3. Hepatocyte growth factor (HGF) overexpression

In vivo, HGF stimulation to MET signaling plays key role in growth, motility, invasion, metastasis, angiogenesis, wound healing, and tissue regeneration [41]. Yano et al. [42] showed in their in vitro study that HGF, a ligand of the MET oncoprotein, induces gefitinib resistance of lung adenocarcinoma cells with EGFR-activating mutations by restoring the phosphatidylinositol

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**Fig. 3 – Potential therapies to reverse resistance.** T790M can be targeted by PF00299804 (irreversible pan-ERBB inhibitor), WZ4002 (covalent pyrimidine EGFR inhibitor), PKC412 (reversible EGFR-T790M inhibitor), E7050 (MET-TKI), afatinib (ERBB inhibitor), and cetuximab (monoclonal antibody against EGFR). Meanwhile, the MET receptor can be inhibited by E7050 and SU11274 (small-molecule MET inhibitor), whereas TAK-701, a monoclonal antibody to HGF, inhibited the phosphorylation of MET. The IGF1R inhibitor AG1024 enhanced gefitinib-induced apoptosis, and AEW541, a selective inhibitor of IGF1R, eliminated the emergence of erlotinib-tolerant cells. IGF1R inhibitor BMS 536924 suppressed the WZ4002-resistant clone and restored EGFR inhibitor sensitivity. YW327.6S2, a monoclonal antibody to Axl, enhanced the effect of erlotinib in reducing tumor growth, while SGI-7079 (Axl inhibitor) reversed erlotinib resistance in Axl-expressing cells.
3-kinase/Akt (PI3K/Akt) signaling pathway via phosphorylation of MET but not EGFR. In tumor samples from lung adenocarcinoma patients with acquired gefitinib-resistance, strong immunoreactivity for HGF was also detected [42]. In the presence of an EGFR-TKI, HGF promotes the selection of cells with MET amplification and encourages the emergence of resistance through MET amplification [16]. Moreover, HGF accelerates the development of MET amplification both in vitro and in vivo; gefitinib resistance due to MET amplification or autocrine HGF production can be cured in vivo by combined EGFR and MET inhibition [43].

3.4. ERBB3 activation

Although ERBB3 is unique among the EGFR family because it lacks tyrosine kinase activity, its 6 tyrosine phosphorylation sites effectively couple the protein to the PI3K/Akt pathway by providing excellent binding sites for PI3K [44]. Compensatory ERBB3 signaling and sustained PI3K/Akt pathway activation have been implicated in the resistance to TKIs in targeted therapies towards other ERBB family members in vitro [45,46]. Downregulation of ERBB3 by an ERBB3-specific short hairpin RNA (shRNA) leads to substantial inhibition of Akt phosphorylation and significantly inhibits cell growth in both resistant and parental HCC827 cells [47]. Application of an ERBB3 antibody, MM-121, is also effective in vivo in reversing the resistance to gefitinib by preventing reactivation of ERBB3 [48].

3.5. Insulin-like growth factor 1 receptor (IGF1R)

IGF1R is a transmembrane RTK that is responsible for mediating IGF bioactivity [49] and has been suggested to play a role in acquired resistance to TKIs [50]. Gefitinib treatment was found to stimulate the IGF1R pathway and downstream signaling by forming an EGFR-IGF1R heterodimer [51]. From in vitro and in vivo studies using gefitinib-resistant (GR) A431 squamous cancer cells, the IGF1R was found hyperphosphorylated, and inhibition of IGF1R signaling restored the ability of gefitinib to downregulate PI3K/Akt signaling and inhibit GR-A431 cell growth [52]. Using gefitinib-resistant PC9 cells, treatment with AG1024 (an IGF1R inhibitor) combined with gefitinib resulted in an in vitro synergistic effect in inducing apoptosis, inhibiting cell proliferation, and decreasing the expression of phosphorylated EGFR (p-EGFR), p-Akt, and p-ERK, which suggests that addition of an anti-IGF1R modulator in combination with gefitinib treatment may be more effective than a single-agent approach [53].

3.6. HER2 amplification

Lung tumor samples and adjacent normal lung cells from EGFR mutant (L858R) mice resulted in erlotinib-resistant tumors after long-term erlotinib treatment [54]. Seven of 19 (37%) erlotinib-resistant tumors showed more than 2-fold increase in HER2 expression compared with normal lung cells. The study also revealed that HER2 amplification, based on fluorescence in situ hybridization (FISH) analysis, was observed in 3 of 26 tumor samples from patients with acquired resistance to gefitinib or erlotinib.

3.7. BRAF gene mutations

Among 697 patients with lung adenocarcinoma, BRAF mutations were present in 18 patients (3%; 95% CI: 2–4%) [55]. In one in vitro study, ectopic expression of BRAF V600E or BRAF G469A conferred resistance to erlotinib in PC9 cells that harbored drug-sensitive EGFR mutations [56]. Ectopic expression of these mutants, but not their WT counterpart, led to constitutive pERK activation, even in the presence of erlotinib or afatinib. Moreover, examination of 195 clinical EGFR-TKI-resistant tumor samples revealed that 2 patients had a BRAF mutation [56].

3.8. PTEN downregulation

The PTEN tumor suppressor is a central negative regulator of the PI3K/Akt signaling cascade and influences multiple cellular functions, including cell growth, survival, proliferation, and migration in a context-dependent manner [57]. From in vitro and in vivo studies, gefitinib-resistant PC-9/GEFs cell lines showed a marked downregulation of PTEN expression and increased Akt phosphorylation, and knockdown of PTEN expression using small interfering RNAs (siRNAs) specific for PTEN in PC-9 cells resulted in drug resistance to gefitinib [58]. Based on an in vivo study, restoration of PTEN expression alters the sensitivity of prostate cancer cells to EGFR inhibition [59]. Meanwhile, among lung cancer patients with EGFR mutations, survival was longer in those with high PTEN expression than in those with low PTEN expression [60].

3.9. EMT

Emerging data show that resistance to TKIs is related to EMT. An in vitro study confirmed that increased protein expression of vimentin, combined with the loss of E-cadherin, claudin 4, and claudin 7 by immunoblotting, is associated with gefitinib resistance in NSCLC cell lines [61]. In contrast, restoration of gene expression associated with the epithelial phenotype can sensitize NSCLC cells to targeted therapies [62]. Once a cancer cell has transitioned to a mesenchymal-like phenotype, cellular dependence on EGFR signaling is reduced and alternate growth factor pathways are activated [63]. Further clinical trials confirmed a benefit in patients with NSCLC with high expression of E-cadherin and who received erlotinib, contrary to the E-cadherin-negative patients who had an overall deteriorating condition after erlotinib treatment [64].

3.10. ATP-binding cassette G2 transporter

From in vitro studies, the human ABCG2 (BCRP, breast cancer resistance protein) transporter causes cancer drug resistance by actively extruding a variety of cytotoxic drugs and displays a high-affinity interaction with several tyrosine kinase receptor inhibitors, including gefitinib [65,66]. However, an in vitro study also indicated that gefitinib reverses ABCG2-mediated drug resistance by direct inhibition other than competitive inhibition, as a ABCG2 ligand in 3 cell lines overexpressing ABCG2 [67]. The apparent discrepancy between these studies is most likely due to the selected concentrations of gefitinib [68]. For gefitinib concentrations of 0.1–1 μM, gefitinib is a
ABC2 ligand and is transported by ABCG2; in contrast, gefitinib inhibits ABCG2 at higher concentration, e.g., 10 μM [66]. The first evidence in which expression of ABCG2 was found associated with acquired resistance to gefitinib was reported in a case-study of a Japanese patient [69]. Recent clinical evidence showed that of the 10 lung adenocarcinoma patients defined as long-term responders, who experienced more than 3 years of response to gefitinib, 6 patients were stained positive for ABCG2 by immunohistochemistry [70).

3.11. Integrin β1

Integrin signaling critically contributes to the progression, growth, and therapy resistance of malignant tumors [71]. The sensitivity of NSCLC cells to gefitinib has also been reported negatively correlated with integrin β1 expression levels. From an in vitro study using an established human NSCLC cell line PC9/AB2, 576-fold decrease in gefitinib sensitivity was observed compared with the parental PC9 cell line; the gefitinib-resistant PC9 cell line overexpressed integrin β1 [72]. Integrin β1-silenced cells show a defective activation of the EGFR signaling cascade, leading to decreased in vitro proliferation, enhanced sensitivity to cisplatin and gefitinib, impaired migration, and invasive behavior [73].

3.12. Epithelial marker protein-1 (EMP-1)

Jain et al. [74] generated in vivo gefitinib-resistance models in an adenocarcinoma xenograft by serially passing tumors in nude mice in the presence of gefitinib until resistance was acquired. By comparing the gene expression profiles of the gefitinib-sensitive and gefitinib-resistant tumors using whole genome cDNA microarrays, Jain et al. found that EMP-1 was significantly upregulated (up to 34-fold) in resistant tumors. Moreover, EMP-1 expression was correlated with the lack of response to gefitinib in lung cancer patient samples [74]. EMP-1 was also overexpressed ~ 45-fold in gefitinib-resistant cancer-associated fibroblasts (GR-CAFs) compared with the parental CAF [75].

3.13. Activation of Axl

Axl, belonging to the subfamily of RTK, has been implicated in cancer cell viability and migration. Knockdown of Axl resulted reduced tumor growth in a xenograft model [76]. Inhibition of Axl has been implicated to increase chemosensitivity of A549 cells to doxorubicin, carboplatin, and etoposide [77]. Therefore, Axl may be involved in EGFR-TKI resistance. Zhang et al. [78] reported the increased activation of Axl in vitro and in vivo in EGFR-mutant lung cancer models of acquired resistance to erlotinib in the absence of the EGFR T790M secondary mutation or MET activation. Moreover, increased expression of Axl was found in EGFR-mutant lung cancers obtained from individuals with acquired resistance to TKI. Combination of SGI-7079 (an Axl inhibitor) with erlotinib reversed erlotinib resistance in mesenchyme-type cells expressing Axl and in a xenograft model of mesenchymal NSCLC [79].

3.14. Small-cell transformation

Histological analyses of tumor biopsies from 37 EGFR-mutant NSCLC patients with acquired EGFR-TKI-resistant revealed that 5 tumors (14%) transformed from NSCLC to SCLC and were sensitive to standard SCLC treatments [80]. In another study, 4 cases of small-cell transformation were found in 155 patients with acquired resistance to gefitinib or erlotinib (3%, 95% CI: 0–6%) [81]. In 2012, a 46-year-old female was reported with relapsed NSCLC (exon 19 deletion) after 12 months of erlotinib treatment [82]. Although the tumor retained the somatic EGFR exon 19 deletion, with no evidence of the T790M mutation, the biopsy of a liver metastasis confirmed features typical of SCLC. Small-cell histologic transformation may be a phenomenon unique to TKI therapy because the transformation has not been reported in patients who have been treated or are in the process of being treated using cytotoxic chemotherapy.

4. Potential therapies to reverse resistance

Continuous exposure of EGFR-mutant NSCLC cell lines to gefitinib or erlotinib has derived clinically relevant mechanisms of acquired resistance, validating this approach as an in vitro tool to anticipate resistance mechanisms [83]. The characterization of acquired resistance mechanisms to EGFR inhibitors has led to the design of several clinical trials assessing the efficacy of EGFR T790M-inhibiting agents, combination therapy with EGFR TKIs, inhibitors of MET or PI3K/Akt, HSP-90 inhibitors, and RAS-pathway inhibitors [84].

By covalent binding to EGFR, the local concentration of irreversible EGFR inhibitors may increase substantially (compared with gefitinib and erlotinib, which are reversible inhibitors), thus providing a means for inhibiting EGFR phosphorylation despite the presence of the T790M mutation in vitro [85]. However, acquired resistance against EGFR inhibitors has also occurred [86], and the inhibition of EGFR by currently available second-generation (irreversible) EGFR-TKIs is therefore not sufficient to physiologically prevent the emergence of cells that still depend on EGFR signaling [87]. Combination of afatinib, an irreversible ErbB-family blocker, and cetuximab, a monoclonal antibody against EGFR, induced dramatic shrinkage of erlotinib-resistant T790-mutant tumors in mice; in combination these agents efficiently depleted both phosphorylated and total EGFR levels [88] (Fig. 3). In a double-blind, randomized, phase IIb/III trial (LUX-Lung 1 study) of patients with lung adenocarcinoma (stage IIIb/IV), who had progressed after chemotherapy (1–2 lines) and at least 12 weeks of erlotinib or gefitinib treatment, afatinib-treated patients had longer median progression-free survival [89] and improved quality of life compared with the best supportive care [90]. It has also been reported that of 4 patients who had the gefitinib-resistant EGFR T790M mutation, 2 responded to cetuximab-containing chemotherapy [91].

Lee et al. [92] recently identified indolocarbazole compounds, including a clinically well-tolerated FL13 inhibitor PKC412, as potent reversible inhibitors of EGFR T790M that do not inhibit wild-type EGFR in vitro. EGFR T790M mutants were inhibited at concentrations of PKC412 as low as 3–30 nM, whereas wild-type EGFR was not detectably affected by PKC412 concentrations as
high as 10 μM. Therefore, the toxicity in skin of these inhibitors, reflecting activity against wild-type EGFR, may be reduced. In previous study, Engelma et al. [93] showed that PF00299804, an irreversible pan-ERBB inhibitor, is an effective agent in vitro and in vivo in lung tumors with the EGFR T790M acquired resistance mutation. Using NSCLC cell lines harboring endogenous T790M mutations, and NSCLC cell lines and Ba/F3 cells engineered to express EGFR T790M in cis to an activating mutation, PF00299804 inhibited EGFR phosphorylation in all EGFR T790M proteins, whereas gefitinib was ineffective even at 10 μM. Oral administration of PF00299804 causes significant antitumor activity, including marked tumor regressions in a variety of human tumor xenograft models that contain the double mutation L858R-T790M [94]. Nevertheless, it has also been reported that PF00299804-resistant cells emerged through focal amplification of EGFR that preferentially involved the T790M-containing allele [95]. Resistance to PF00299804 arises at least in part through selection of a pre-existing EGFR T790M-amplified clone both in vitro and in vivo using a xenograft model.

Much recent attention has been focused on new covalent pyrimidine EGFR inhibitors. Using functional pharmacological screens, Zhou et al. [96] identified a covalent pyrimidine EGFR inhibitor, from an irreversible kinase inhibitor library, which specifically inhibited EGFR T790M, known as WZ4002. This agent was 100-fold more potent against EGFR T790M, and up to 100-fold less potent against wild-type EGFR, than quinazoline-based EGFR inhibitors in vitro. WZ4002 was much more effective against T790M because the anilinopyrimidine scaffold of WZ4002 was an intrinsically better fit for the T790M-positive NSCLC [96]. Although WZ4002 inhibited the growth of H1975 cells with a gatekeeper T790M mutation, WZ4002 did not inhibit the growth of HCC827ER cells with MET amplification [97].

The combinational targeting of both MET and ERBB receptors pathways could enhance anti-tumor activity by circumventing resistance to EGFR-targeting agents [98]. Obstruction of MET signaling in resistant cells restores their sensitivity to EGFR inhibitors, and only the simultaneous blockage of both MET and EGFR impairs tumor growth [99]. Using erlotinib-resistant lung cancer cell line H1975, which expresses L858R/T790M-EGFR in cis, Tang et al. [100] tested the effect of MET inhibition using the small-molecule inhibitor SU11274. SU11274 plus erlotinib potentiated MET inhibition of downstream cell proliferative survival signaling. This study suggested that MET-based targeted inhibition using small-molecule MET inhibitor is a potential treatment strategy for T790M-EGFR-mediated erlotinib-resistant NSCLC.

Another MET inhibitor under intensive investigation is E7050. This agent could reverse the HGF-induced EGFR resistance in PC9 cells [101,102] and sensitize HCC827ER (MET amplification), PC-9/HGF (HGF-transfectant), and HGF-treated H1975 cells (T790M mutation) to WZ4002, inhibiting EGFR and MET phosphorylation and their downstream molecules [97]. In summary, these findings suggest that the combination of a mutant-selective EGFR-TKI (WZ4002) and a MET-TKI (E7050) is effective for suppressing the growth of erlotinib-resistant tumors caused by the gatekeeper T790M mutation, MET amplification, and HGF overexpression [97].

Combined treatment with IGF1R inhibitor AGI024 enhanced gefitinib-induced growth inhibition and apoptosis as well as downregulated phosphorylation of Akt, EGFR, and IGFR [103–105]. Because in vitro cross-talk between the EGFR and IGF1R signaling pathways exist in gefitinib-resistant H358 mucinous NSCLC cells, anti-IGF1R treatment has been shown to sensitize H358 cells to gefitinib-induced apoptosis [106]. Using a ‘drug-tolerant’ PC9 cell model with >100-fold reduced gefitinib sensitivity, Sharma et al. [107] demonstrated an altered chromatin state that requires the histone demethylase KDM5A. Four different HDAC inhibitors (TSA, SAHA, MS-275, and Scriptaid), as well as AEW541, a selective inhibitor of IGF1R, virtually eliminated the emergence of drug-tolerant cells. Interestingly, activation of IGF1R has also been observed in PF29804-resistant or WZ4002-resistant clones (with no T790M mutation) of PC9, and the IGF1R inhibitor BMS 536924 restored EGFR inhibitor sensitivity [108].

PI3K/mammalian target of rapamycin inhibition is a potentially effective therapeutic strategy against NSCLC with acquired resistance to EGFR inhibition because robust PI3K and mTOR inhibition are likely to provoke autophagy [109]. TAK-701, a humanized monoclonal antibody to HGF, in combination with gefitinib, inhibited the phosphorylation of MET, EGFR, extracellular signal-regulated kinase, and AKT in HCC827-HGF cells, resulting in the suppression of cell growth, indicating that autocrine HGF-MET signaling contributed to gefitinib resistance in these cells [110]. Combination therapy with TAK-701 and gefitinib also markedly inhibited the growth of HCC827-HGF tumors in vivo [110].

Ye et al. [111] developed a phage-derived monoclonal antibody (YW327.6S2) that recognizes both human and murine Axl, and this antibody binds to both human and murine Axl with high affinity. In an A549 xenograft model, YW327.6S2 enhanced the effect of erlotinib, reducing tumor growth. However, whether this agent can reverse EGFR-TKI resistance may need further investigation.

Interestingly, one study demonstrated increased sensitivity to gefitinib in KRAS-mutant NSCLC cell lines. Compared with gefitinib alone, a combination of gefitinib with lovastatin showed significantly enhanced cell growth inhibition and cytotoxicity in gefitinib-resistant A549 and NCI-H460 human NSCLC cells [112].

5. Conclusions

Acquired resistance is a complex biological process and generates an urgent need for appropriate biomarkers both for treatment selection as well as monitoring [113]. Investigation of accurate biomarkers that may be involved in NSCLC is a research area that needs to be further explored. One challenge is that once a particular agent reverses TKI resistance, the NSCLC cells are capable to adapt to anti-TKI resistance agents. Nevertheless, the expression of drug-resistant genes that render NSCLC resistant to TKIs make drug-resistance proteins highly attractive targets for new therapies in combination with TKI.

Conflicts of interest

The authors have no conflicts of interest.
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