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Mechanisms of Epidermal Growth Factor Receptor Tyrosine Kinase Inhibitor Resistance and Strategies to Overcome Resistance in Lung Adenocarcinoma

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Somatic mutations that lead to hyperactivation of epidermal growth factor receptor (EGFR) signaling are detected in approximately 50% of lung adenocarcinoma in people from the Far East population and tyrosine kinase inhibitors are now the standard first line treatment for advanced disease. They have led to a doubling of progression-free survival and an increase in overall survival by more than 2 years. However, emergence of resistant clones has become the primary cause for treatment failure, and has created a new challenge in the daily management of patients with *EGFR* mutations. Identification of mechanisms leading to inhibitor resistance has led to new therapeutic modalities, some of which have now been adapted for patients with unsuccessful tyrosine kinase inhibitor treatment. In this review, we describe mechanisms of tyrosine kinase inhibitor resistance and the available strategies to overcoming resistance.

Keywords: Drug Resistance; Receptor, Epidermal Growth Factor; Adenocarcinoma of Lung; Genes, erbB-1

Introduction

Lung cancer remains a major cause of cancer death worldwide. In the year 2012, 1,824,701 new lung cancer cases were diagnosed, and 1,590,000 patients died of this devastating disease worldwide[1]. Non-small cell lung cancer (NSCLC) comprises 80%–85% of lung cancers and more than half of NSCLC cases are discovered at an advanced stage, which requires systemic treatment. Among the changes of the pathologic subtypes of lung cancer, the proportion of adenocarcinoma, which has higher instances of driver mutations, is gradually increasing.

Epidermal growth factor receptor (EGFR, ErbB1, HER1) is a cell surface receptor from the ErbB receptor family and is one of four related receptor tyrosine kinases, along with HER2/c-neu (ErbB2), HER3 (ErbB3), and HER4 (ErbB4)[2]. After ligand binding, EGFR changes from an inactive monomer to an active homodimer. It may also form a heterodimer with another member of the ErbB receptor family. EGFR dimerization induces intrinsic protein-tyrosine kinase activity that results in autophosphorylation of its C-terminal tyrosine residues[3,4]. Autophosphorylation of EGFR activates downstream signaling cascades such as the AKT, mitogen-activated protein kinase (MAPK), and JNK pathways, which ultimately leads to DNA synthesis, cell cycle progression, and proliferation[5].

Mutation, amplification, or dysregulation of the EGFR family is proposed to occur in approximately 30% of epithelial cancers. Somatic mutations that lead to EGFR hyperactivation are associated with a number of cancers, including NSCLC, colorectal cancer, and glioblastoma multiforme[6,7]. These somatic mutations involving the EGFR tyrosine kinase domain

(TKD) lead to the persistent activation of its downstream signaling pathways, which results in uncontrolled cell proliferation[8].

EGFR tyrosine kinase inhibitors (TKI) have been widely described as being useful for the treatment of lung cancer patients with EGFR-TKI–sensitizing mutations, and their use has led to a doubling of progression-free survival[9-13] and a lengthening of overall survival by more than 2 years. However, emergence of resistant cancers has become the ultimate reason for treatment failure, which creates new challenges for the daily management of patients with *EGFR* mutations[14]. In this review, we describe the mechanism behind EGFR-TKI resistance and the strategies available for overcoming resistance to EGFR-TKI.

First and Second Generation EGFR-TKI

It is well established that 50%–80% of NSCLC overexpress EGFR. Consequently, there have been numerous treatments developed using antibodies and small molecules that inhibits EGFR. The first generation EGFR-specific inhibitors, gefitinib (ZD1839, Iressa) and erlotinib (Tarceva), inhibit EGFR activity by reversibly binding to the adenosine triphosphate (ATP)-binding site in the TKD (Table 1). In the early 2000s, the IDEAL I/II study, which recruited previously treated NSCLC patients, was performed to compare the efficacy and adverse effects between the 250 mg and 500 mg treatments of gefitinib. The researchers found that daily administration of 250 mg of gefitinib had an 18.4% response rate with a 95% confidence interval, 11.5–27.3. The difference in the response rate between 250 mg and 500 mg was not significant. However, the 250 mg treatment group had a lower frequency of side effects, and so the U.S. Food and Drug Administration (U.S. FDA) approved gefitinib 250 mg daily for the treatment of NSCLC[15]. The subsequent phase III study, ISEL, which compared placebo and gefitinib treatment groups in previously treated NSCLC patients, failed to demonstrate any survival benefits in the gefitinib treatment group. This led to the discontinuation of gefitinib use in the United States[16]. However, in 2004, mutations in the EGFR-TKD were identified and some of the patients who had mutations in EGFR-TKD showed an excellent response to the EGFR-TKI[8,17]. The L858R point mutation in exon 21 and deletion mutations in exon 19 that makes up the leucine-arginine-glutamine-alanine (LREA) motif (amino acids 747 to 750) were identified as key sensitizing mutations that predict a positive response to EGFR-TKI. Following these trials, patients with *EGFR* mutations were recruited for further studies, and a response rate of 60%–80%, along with a progression-free survival of approximately 10 months[18,19]. These results helped to establish that specific genetic mutations could predict response rate, progression-free survival, and survival rate following drug treatment, and they highlight

Table 1. EGFR-TKIs currently in use or under development

	Generic/drug name	Trade name	Company	Binding to EGFR-TKD	Wild type GI$_{50}$ (nM)	Mutant type GI$_{50}$ (nM)	L858R/T790M GI$_{50}$ (nM)
1st generation	Gefitinib	Iressa	Astra Zeneca	Reversible	107	NA	NA
	Erlotinib	Tarceva	Roche	Reversible	209–449	3.2	2,253
2nd generation	Afatinib	Giotrif	BI	Irreversible	15	NA	NA
	Dacomitinib (PF-00299804)		Pfizer	Irreversible	NA	NA	NA
3rd generation	Osimertinib	Tagrisso	Astra Zeneca	Irreversible	131–480	17	15
	Rociletinib		Clovis	Irreversible	3,930–4,275	7	33
	Olmutinib	Olita	Hanmi/BI	Irreversible	2,225	9	10
	ASP8273		Astellas	Irreversible	NA	NA	NA
	EGF816		Novartis	Irreversible	333	2–8	5

EGFR-TKI: epidermal growth factor receptor tyrosine kinase inhibitor; TKD: tyrosine kinase domain; NA: not available; BI: Beringer Ingelheim.

the importance of predictive biomarkers for drug response.

Acquired resistance to the first generation EGFR-TKI had accelerated the clinical development of second generation EGFR-TKI, which is characterized by irreversible covalent attachments to the EGFR-TKD and additional activity against other receptors which belong to EGFR family or structurally similar receptors[20]. Several drugs with these characteristics had started clinical trials but most of the second generation EGFR-TKI, except afatinib, had failed because of lack of clinical efficacy or limitation of clinical dosing due to toxicity in NSCLC.

EGFR Mutations: Sensitizing vs. Non-sensitizing *EGFR* Mutations

Activating *EGFR* mutations are frequently identified in lung adenocarcinoma, and advances in genotyping technology has facilitated the discovery of such mutations in small size samples[21,22]. The most frequently detected type of *EGFR* mutation is an in-frame deletion of exon 19 (E19del) near the LREA motif that accounts for approximately 45% of mutations in the TKD, as well as the L858R point mutation of exon 21 that accounts for approximately 40% of TKD mutations[23-25]. In addition to E19del and L858R mutations, less frequent mutations, such as point mutations in exon 18 at position G719 (approximately 3% occurrence rate) and the exon 21 L861Q mutant (approximately 2%), are sensitive to EGFR-TKI[26-28]. On the other hand, an in-frame insertion within exon 20 of *EGFR*, which accounts for 4%–10% of all TKD mutations, and other rare mutations including L747S, D761Y, T790M, and T854A confer resistance to EGFR-TKI[29-31].

As more sensitive and precise tumor genotyping systems have been made available for clinical applications, rare *EGFR* mutations of unknown biological and clinical significance are frequently encountered in routine practice[22]. Different responses to EGFR-TKI have been reported for mutations within the same approximate genomic location. For example, in-frame insertions within *EGFR* exon 20 were originally considered to confer EGFR-TKI resistance with a low response rate (5%) and short interval of disease control; however, A763_Y764insFQEA has been reported to be a sensitizing mutation to EGFR-TKI[31]. These findings indicate that more attention and collaborative efforts are required to elucidate the biological and clinical significance of these rare and diverse *EGFR* mutations.

Mechanisms of EGFR-TKI Resistance

Even if EGFR-TKI is administered after confirmation of a mutation in the EGFR-TKD, some patients do not respond to EGFR-TKI at all (*de novo*/intrinsic resistance), and the remaining patients who had a favorable response at initial treatment eventually exhibit disease progression after 10–14 months (acquired resistance). Several mechanisms regulating resistance have been identified, and they may be classified as intrinsic and acquired resistance (Figure 1)[32].

Intrinsic Resistance to EGFR-TKI

1. *BCL2-like 11* intron 2 deletion polymorphism

BCL2-like11 (*BIM*) is a pro-apoptotic molecule of the BCL2 family and is required for TKI-induced apoptotic cell death. The intron 2 deletion polymorphism of BIM causes a splicing switch between exon 4 and exon 3, resulting in a BIM isoform variant lacking the pro-apoptotic BCL2-homology 3 (BH3) domain. The BH3 domain interacts with other members of the BCL2 protein family, including BCL2, BCL2L1/BCL-X(L), and MCL1 and inhibits their anti-apoptotic function. The intron 2 deletion polymorphism confers intrinsic TKI resistance for NSCLC patients with *EGFR* mutations, resulting in significantly inferior responses[33]. The *BIM* intron 2 deletion polymorphism is rare in Western populations but is found in about 12%–15% of Far East Asian populations. This polymorphism does not increase the risk of lung cancer; instead, it limits the response to EGFR-TKI[33,34].

2. Compound *EGFR* mutations

Not only have advances in sequencing technology enabled researchers to acquire more accurate data, but they have also provided a higher probability of identifying a greater number of mutations in the EGFR-TKD from smaller samples. Compound *EGFR* mutations, defined as double or multiple mutations in the EGFR-TKD, are frequently detected with newer sequencing technology. A study analyzing 61 cases of *EGFR* mutation-positive lung adenocarcinoma with next-generation sequencing (NGS)–based repeated deep sequencing found that compound *EGFR* mutations account for 24.6% of *EGFR* mutation-positive lung adenocarcinoma. The majority of compound *EGFR* mutations are a combination of atypical mutations and typical mutations, such as E19del and L858R[22,35]. Kobayashi et al.[36] showed that compound *EGFR* mutations in which an EGFR-TKI–sensitizing mutation—such as G719X, E19del, L858R, or L861Q—coexist with rare mutations of other residues of the TKD that are somewhat sensitive to EGFR-TKI. Peng et al.[37] compared the clinical outcome between NSCLC patients with L858R single mutation with patients harboring both a L858R mutation and a co-mutation in *EGFR* exon 18–21 and found no significant differences in overall survival and progression-free survival. Another study addressed the clinical significance of compound *EGFR* mutations and found that patients with rare atypical mutations and
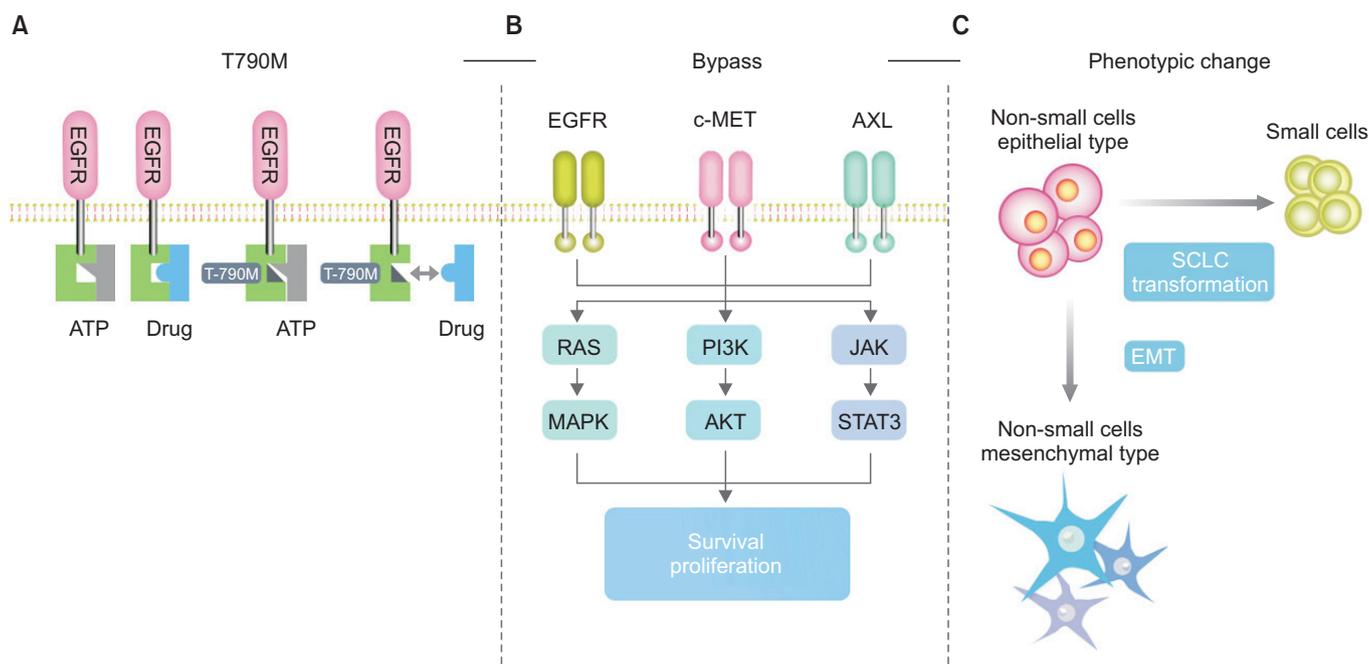
Figure 1. Resistance mechanism against first generation epidermal growth factor receptor tyrosine kinase inhibitor (EGFR-TKI). (A) Mutations in the *EGFR* gene. T790M mutation induces conformational changes on the ATP-binding pocket of EGFR–tyrosine kinase domain, inhibiting interaction with the drug target site. (B) Activation of alternative signaling pathways. *MET* amplification, and overexpression of phosphoinositide 3-kinase (PI3K)/AKT, mitogen-activated protein kinase (MAPK), and AXL bypass the dependency on EGFR activation and can promote survival and proliferation. (C) Phenotypic changes, small cell lung cancer transformation, and epithelial-mesenchymal transition (EMT) confer resistance to EGFR-TKI. SCLC: small cell lung cancer.

either E19del or L858R had a less favorable outcome compared with patients who have a single typical mutation. Compound mutations that contain sensitizing mutations, such as G719X or L858R, seem to have favorable responses to EGFR-TKI. On the other hand, compound mutations consisting of rare atypical mutations have a poor response to EGFR-TKI[35]. Taken together, the patients with compound *EGFR* mutations have a shorter overall survival than those with simple mutations and, therefore, they should be closely monitored during follow-up.

3. Co-alteration of other genes

The concept that any given cancer will have a single driver mutation is now being challenged by data acquired from sequencing multiple genes simultaneously. Although *EGFR* mutations are the only driver mutation in many cases of lung adenocarcinoma, driver mutations in other genes are found intermittently with advances in sequencing technology. When the *EGFR* mutation status was reassessed with target enriched NGS in anaplastic lymphoma kinase (ALK) rearrangement-positive NSCLCs, the co-mutation rate was found to be as high as 15.4%. In this study, authors evaluated the response to TKIs in 14 NSCLC patients with EGFR and ALK co-alteration. Among them, three were treated with EGFR-TKI and had poor

responses to gefitinib, whereas the eight treated with ALK inhibitors had favorable responses, suggesting that signaling from *ALK* rearrangement may override EGFR[38]. Another study found that mutations in exon 9 and 20 of the *PIK3CA* gene occurred in 3.9% of squamous cell cancers and 2.7% of adenocarcinomas. Among 34 NSCLC cases harboring *PIK3CA* mutations, 17 cases had a co-mutation in *EGFR* exon 18–21 and 4 cases had a co-mutation in *KRAS* exon 2–3, indicating that *PIK3CA* mutations are frequently accompanied by *EGFR/ KRAS* mutations. These studies revealed the importance of identifying *PIK3CA* mutations by demonstrating that patients with *PIK3CA* single mutation have a poorer prognosis than those with co-mutation of *PIK3CA* and *EGFR/KRAS*[39].

Acquired Mechanism of Resistance

1. Alteration of EGFR

The T790M gatekeeper mutation, localized in exon 20 of EGFR, has been found for the first time to confer resistance to EGFR-TKI[40] and can be identified in about half of patients who experience resistance after treatment with first generation EGFR-TKI[41]. This threonine-to-methionine substitution leads to an increased affinity of EGFR to ATP compared with

its affinity to first generation of EGFR-TKI. The bulkier methionine residue at position 790 sterically inhibits the interaction with the inhibitor, effectively preventing its binding to the TKD while preserving catalytic activity. The T790M mutation has been rarely identified in the EGFR-TKI naïve patients in Western countries[42]. A few EGFR-TKI–resistant mutations have also been identified, such as T854A in exon 21, D761Y, and L747S, but their clinical relevance remains unclear[43-45].

Three drugs have been developed for patients with the T790M mutation and are ready for clinical application: CO-1686 (Clovis, Rociletinib), which was first developed specifically for T790M; AZD9291 (AstraZeneca, Tagrisso, Osimertinib); and BI1482694/HM61713 (Hanmi, Olita, Olmutinib). Third generation *EGFR* mutant selective inhibitors (EMSIs) are characterized by sparing wild type *EGFR*, selective inhibition of T790M, and irreversible covalent binding to target. A brief comparison is available in Table 1[16]. CO-1686 demonstrated its efficacy in treating T790M-positive NSCLC during TIGER-X (Phase 2), TIGER-1 (Phase 2/3), TIGER-2 (Phase 2), and TIGER-3 (Phase 3) studies; however, hyperlipidemia, nausea, vomiting, and corrected QT interval prolongation became primary concerns. AZD9291 also demonstrated its efficacy in treating T790M-positive patients through AURA1, AURA2, and FLAURA trails. Although it had less severe adverse effects than gefitinib, there were reports of skin rashes, diarrhea, and, in some cases, interstitial pneumonitis. AZD9291 was first approved by the U.S. FDA for the treatment of *EGFR* mutation–positive NSCLC among the third generation EMSI and is now available in our medical fields. ASP8273 (Astellas) and EGF816 (Novartis) were recently developed; however, resistance against these third generation EGFR-TKI eventually develop, possibly through the C797S mutation.

Activation of Alternative Pathways

1. *MET* amplification

MET amplification was first identified in EGFR-TKI–sensitive lung cancer cells that develop resistance after prolonged exposure to gefitinib as a result of focal amplification of the *MET* gene. *MET* amplification was identified in four of 18 lung cancers that had developed resistance to EGFR-TKI through ErbB3-dependent activation of phosphoinositide 3-kinase (PI3K)[47]. Although the frequency of *MET* amplification in treating naïve NSCLC is rare, *MET* amplification is the second most commonly acquired resistance mechanism, occurring up to 20% during EGFR-TKI treatment. Cell lines with *MET* gene amplification are extremely sensitive to MET inhibition, and the majority of preclinical and clinical data demonstrate that co-inhibition of MET and EGFR may be an effective strategy to overcoming resistance. A trial involving use of the MET inhibitor onartuzumab in combination with erlotinib in heavily treated patients had promising results: MET-positive patients assessed by immunohistochemistry had increased progression-free survival and overall survival compared with MET-negative patients[48]. Following the METLung phase III trial, which compared the combination of onartuzumab and erlotinib with erlotinib alone in MET-positive and *EGFR* mutation-positive patients did not show any differences between the groups at interim analysis, and so the trial was terminated early. Ficlatuzumab is an anti-MET monoclonal antibody that targets the hepatocyte growth factor. In a phase II trial recruiting *EGFR* sensitizing mutation-positive Asian patients, the combination of ficlatuzumab and gefitinib did not improve response rate or progression-free survival. However, a subset of patients with *EGFR* sensitizing mutation positive and MET-low expression patients benefited from this combination, suggesting ficlatuzumab may inhibit emergence of resistant clones[49]. The MARQUEE trial (MET inhibitor ARQ 197 plus erlotinib versus erlotinib plus placebo in NSCLC) and ATTENTION trial (Asian trial of tivantinib plus erlotinib versus erlotinib for NSCLC without *EGFR* mutation) were both phase III trials that evaluated tivantinib, a selective MET inhibitor used previously to treat unselected patients. Both studies did not reveal a difference in overall survival. Cabozantinib (XL184/BMS-907351; Exelixis/Bristol Myer Squibb), foretinib (XL880, EXEL-2880, GSK1363089; Exelixis/GSK), MetMAb (Genentech-Roche), and crizotinib (PF-2341066; Pfizer) have been developed as MET inhibitors and are undergoing clinical trials[50]. Although MET poses as an ideal biological target whose amplification is frequently observed in EGFR-TKI treated patients, its clinical benefit is still modest.

2. *HER2* amplification

HER2 amplification or overexpression is associated with more aggressive types of cancer. *HER2* amplification is a rare event that occurs in 1%–2% of untreated lung adenocarcinoma as measured by fluorescence *in situ* hybridization (FISH), a microarray-based genomic copy number analysis. A study that adapted FISH to measure *HER2* amplification found that 12% of tumors had acquired resistance to EGFR-TKI, and that this event is mutually exclusive to those with the T790M mutation[51]. Because afatinib is a second generation irreversible pan-HER inhibitor, it was expected to be effective in the patients previously treated with first generation EGFR-TKI. LUX-Lung 4 trial, a phase II single-arm study, enrolled 62 patients that were previously treated with gefitinib and/or erlotinib and also with afatinib. Although not all the patients in this study harbored *HER2* amplification, the effect of afatinib was modest and had an overall response rate of 8.2%, and progression-free survival was found to be 4.4 months[52]. Based on these findings, Takezawa et al.[51] suggested that the combination of afatinib and cetuximab would be beneficial for patients without the T790M mutation who experienced acquired

resistance after EGFR-TKI treatment. The combination of afatinib and cetuximab induces tumor regression by decreasing the phosphorylation of EGFR and total EGFR expression, resulting in inhibition of its signaling pathways[53]. In a phase Ib trial that combined cetuximab and afatinib for 126 NSCLC patients whose disease progressed during EGFR-TKI treatment, the overall response rate was 29% and progression-free survival was 4.7 months. Interestingly, this combination was equally effective in T790M-positive and negative patients[54], implying that the efficacy of the combination is attribute to the intrinsic effect of afatinib or associated with any EGFR-TKI combined with cetuximab needs to be clarified.

3. *PIK3CA* mutation

PIK3CA, which encodes the catalytic subunit of PI3K, is occasionally mutated in lung cancer. Mutations usually occur in its helical domain (exon 9) or kinase domain (exon 20), and the E542K, E545K, E545Q, H1047L, and H1047R mutations have been identified as activating mutations of *PIK3CA*[32]. In addition, *PIK3CA* mutations have been detected in a small percentage (approximately 5%) of *EGFR*-mutated lung cancers with acquired resistance to EGFR-TKI therapy. Downstream mutations in *PIK3CA* are rare, but have been identified as a mechanism of resistance[55]. Recently, a study demonstrated that *PIK3CA* mutations frequently coexist with *EGFR* or *KRAS* mutations[56]. NSCLC patients with *PIK3CA* mutation have a poor prognosis. The role of mutant *PIK3CA* in oncogenic signaling requires further investigation, including development of novel targets for therapeutic intervention in cancers harboring *PIK3CA* mutations.

4. *BRAF*

BRAF mutations are commonly detected in malignant melanoma: 30%–40% of cases have an activating mutation, whereas it is only detected in approximately 1% of NSCLC. The G469A, V600E, and V599E mutations in the *BRAF* gene are found in a number of tumors, including malignant melanoma, colorectal, and lung cancer. V599E is one of the common activating mutations and causes constitutive activation of the RAF/MEKL/ERK signaling cascade, resulting in pro-mitogenic activity and decreased drug response[57]. Vemurafenib and dabrafenib was found to be effective in treating malignant melanoma and are now under clinical evaluation for treating lung adenocarcinoma.

5. AXL

AXL belongs to the Tyro/AXL/Mer (TAM) receptor tyrosine kinase family and is involved in cell survival, growth, and metastasis[58,59]. Recently, several reports have indicated that the aberrantly activated AXL could contribute to the development of acquired resistance to targeted agents such as imatinib, lapatinib, and erlotinib[55,56,60]. According to the combined work of three independent research groups[60], *in vivo* xenograft and *in vitro* cell line cancer models with AXL-mediated resistance were established by the continued treatment with erlotinib. The genetic and chemical inhibition of AXL was able to restore sensitivity to erlotinib in these models. Furthermore, re-biopsy samples acquired from patients with EGFR-TKI resistance were found to have increased expression of AXL as assessed by an immunohistochemistry analysis. Although this evidence is quite compelling, there remain several concerns, such as how accurately an immunohistochemistry analysis of AXL expression can reflect the bypassing of AXL signal activation, which is associated with resistance. In addition, further study is needed to determine the exact proportion of patients that could benefit from the suppression of AXL signaling. Although some multi-kinase inhibitors with the ability to inhibit AXL and several specific AXL inhibitors are under development[61], there are currently no potent selective AXL inhibitors available that can be used in clinical trials, which makes it difficult to confirm the exact role of AXL in acquired resistance to EGFR-TKI.

Phenotype Transformation

1. Small cell transformation

Transformation of small cell lung cancer has been seldom observed after the development of drug resistance. Tumor cells with the small cell lung cancer phenotype that retain their original *EGFR* mutations are effectively treated with standard chemotherapy; however, after a period of conventional cytotoxic chemotherapy, these tumors become sensitized to EGFR-TKI. The mechanism underlying this phenomenon is still unknown.

2. Epithelial-mesenchymal transition

Epithelial-mesenchymal transition (EMT) is a regular physiological process that occurs during embryogenesis, wound healing, and tissue regeneration; however, it is also observed in a few pathological conditions. EMT is characterized by the loss of E-cadherin and the gain of mesenchymal markers, such as vimentin and N-cadherin[32]. Through EMT, cancer cells gain mesenchymal properties and become more motile and invasive. Although the specific mechanism of EMT remains unclear, there may be several mechanisms at play during EMT. Activation of AXL by the PI3K/AKT pathway and loss of E-cadherin by activation of EGFR-MEK-ZEB1-MMP2 axis may induce EMT, thereby promoting cancer cell invasion and progression. Other EMT-inducing signals include those initiated by transforming growth factor β, fibroblast growth factor,

epidermal growth factor, and hepatocyte growth factor, and pathways or factors associated with EMT include Notch, interleukin 6, SOX9, FoxO4, and SRC.

Conclusion

Through the identification of resistance mechanisms during EGFR-TKI treatment, new therapeutic modalities have been adapted for patients with unsuccessful treatments. These adaptations have had favorable results in some cases. Among them, the third generation EMSIs had outstanding therapeutic effects, and some of these drugs are now available for use in the medical field. On the other hand, inhibition of alternative pathways—such as MET amplification, *HER2* amplification, PI3K/AKT/mammalian target of rapamycin signaling, and MAPK signaling—is theoretically sound, and many TKIs have been developed. However, their clinical benefit remains modest, and further clinical evaluation is required. Further evaluation of their biologic impact and careful selection of patients that would benefit from these TKIs may result in effective treatment of patients who were not successfully treated with first generation EGFR-TKI.

Conflicts of Interest

No potential conflict of interest relevant to this article was reported.

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