

Drug Delivery and Drug Resistance: EGFR-Tyrosine Kinase Inhibitors in Non-Small Cell Lung Cancer

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Abstract: The epidermal growth factor receptor (EGFR) pathway has emerged as an important target in the treatment of non-small cell lung cancer (NSCLC). Currently available EGFR inhibitors approved for the therapy of NSCLC include the small molecules erlotinib and gefitinib. They are administered orally and interfere with the intracellular tyrosine kinase domain. These agents appear most effective in patients with tumors which are highly dependent on the EGFR signaling pathway because of EGFR activating mutations or amplifications. The use of EGFR tyrosine kinase inhibitors prolongs survival in metastatic NSCLC. However, despite the dramatic initial responses to treatment in some cases, NSCLC eventually becomes resistant to EGFR-tyrosine kinase inhibitors (TKIs). Mechanisms of resistance include *EGFR* secondary mutations that interfere with drug binding. Besides, molecular cross-talk and redundancy between EGFR and other signaling pathways creates alternative pathways for tumor cell proliferation promoting resistance to EGFR inhibition such as the amplification of the receptor tyrosine kinases MET. Polymorphisms in *EGFR* and EGFR pathway-related genes, as well as in genes involved in drug metabolism and intake/efflux might also predict clinical outcome and toxicity.

Therefore, understanding the mechanisms involved in the EGFR signalling and resistance to EGFR inhibition will provide new insights for the development of more effective targeted treatments against NSCLC.

Keywords: EGFR, c-MET, tyrosine kinases, mutations, erlotinib, gefitinib, resistance.

INTRODUCTION

The activity of anti-cancer drugs can be affected by different reasons. As a general rule, individual variation in drug effects might stem from variability in the pharmacokinetics and/or pharmacodynamics of the therapeutic compound and may have both genetic and non genetic causes. Most drugs are given systemically and therefore are subject to changes in absorption, metabolism and delivery to the targeted tissues. Tumors can be located in body parts into which drugs can not easily penetrate or might have increased expression of proteins involved in drug efflux/metabolism. Modification in drug targets might also affect their effects.

One of the most common treatments for cancer is the use of cytotoxic drugs. This strategy is based on the differences in cell division rates between normal cells and cancer cells. However, this treatment includes many side effects. There are three major mechanisms of drug resistance in cancer cells. First, the uptake of water-soluble drugs which requires transporters to enter the cancer cells can be decreased. Second, resistance may occur due to the specific properties or genetic background of the cancer cell itself or the genetic or epigenetic changes that occur after the toxic chemotherapy. In particular, cancers are characterized by several alterations which might affect the ability of cytotoxic drugs to kill the cancer cells such as the changes in cell

cycle, reduced apoptosis, increased repair of DNA damage and altered metabolism [1]. Third, cancer cell might have increased energy-dependent efflux of hydrophobic drugs that can easily enter the cells by diffusion through the plasma membrane.

Therefore, to reduce both the side effects of the cytotoxic drugs and the above-mentioned mechanism of drug resistance, new molecular therapies are being developed which attack the specific signalling pathways that play an important role in the development, growth and progression of the tumors. These new drugs are targeted to specific molecular targets or genes involved in cancer development ("targeted therapy") and should not only reduce the side-effects but also result in higher efficacy and activity against tumors.

One of the most important mechanisms in signalling pathways in cells is the phosphorylation of proteins that is carried out by protein kinases. Protein kinases are involved in the regulation of cell proliferation, migration, differentiation, metabolism and apoptosis. Two important protein kinases are the tyrosine kinases and serine/threonine kinases. Tyrosine kinases catalyze the phosphorylation of tyrosine amino acid residues whereas serine/threonine kinases catalyze the phosphorylation of serine/threonine amino acid residues in proteins. Tyrosine kinases are highly regulated in the cell as they have important regulatory effects in cell homeostasis and signalling pathways. There are two classes of tyrosine kinases: receptor tyrosine kinases and cellular tyrosine kinases. Receptor tyrosine kinases have an intracellular catalytic tyrosine kinase domain, a hydrophobic transmembrane domain and an extracellular ligand binding

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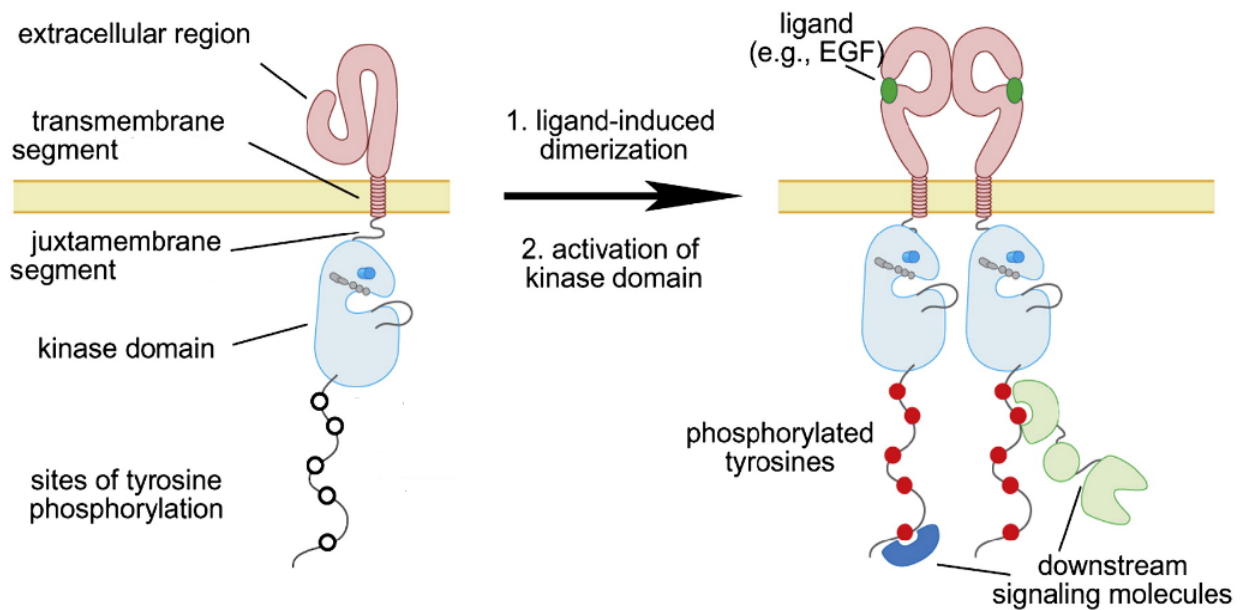


Fig. (1). General view of the ligand-induced dimerization and activation process of EGFR.

domain [2]. Dimerization of the two receptor tyrosine kinases occurs when the ligand binds to the receptors. This results in the phosphorylation of the tyrosine residues of the intracellular catalytic domains which leads to an active conformation and results in the activation of signalling pathway within the cell (Fig. 1).

The regulation of tyrosine kinases is essential for the control of cell growth and cancer may stem from the unregulated cell proliferation. This can be caused by abnormally constitutively active tyrosine kinases which are independent of ligand binding, because of mutations or over-expression. Therefore, tyrosine kinase inhibitors (TKIs) can be used as anti-cancer agents targeting the specific unregulated tyrosine kinase activities. TKIs can stabilize tumor development, growth and progression in many tumour types, have less side-effects when compared to cytotoxic drugs and interact synergistically with chemotherapy and radiotherapy.

The Epidermal Growth Factor Receptor (EGFR) pathway is part of a complex signal-transduction network central to critical processes in cell proliferation. EGFR is overexpressed in 40-80% of NSCLC and plays a crucial role in cellular proliferation, differentiation, apoptosis and survival [3]. EGFR phosphorylation activates several downstream signaling pathways, including the mitogen-activated protein kinase (MAPK/Erk), phosphatidylinositol-3 kinase/Akt and the signal transducer and activator of transcription (STAT) proteins. These modulate gene transcription and protein translation, thereby stimulating tumour-cell proliferation, migration, invasion, angiogenesis and inhibition of apoptosis. EGFR expression is common in a number of epithelial tissues and in various solid tumour types, where high levels of EGFR expression correlate with more aggressive disease, poorer prognosis and reduced chemosensitivity.

Against this background, EGFR was identified as an attractive target for the development of anticancer drugs, and

the treatment of non-small cell lung cancer (NSCLC) has been improved by the development of the EGFR-TKIs.

TYROSINE KINASE INHIBITORS

Tyrosine kinase receptors have an important role in the development and progression in most of the cancer types. Most TKIs are designed to target the EGFR, VEGFR and PDGFR tyrosine kinase families. The EGFR family includes the tyrosine kinase receptors EGFR (ErbB1, Her1), ErbB2 (Her2), ErbB3 (Her3) and ErbB4 (Her4). When these kinase receptors get abnormally activated, cell growth is deregulated due to decreased apoptosis and angiogenesis in epithelial malignancies. Therefore several TKIs have been developed for inhibiting the abnormal signalling in the cell which are required for cell growth and division. TKIs which target EGFR tyrosine kinases and are used in NSCLC are listed in Table 1.

Gefitinib and erlotinib have been approved by FDA for the treatment of patients with locally advanced or metastatic NSCLC after failure of at least one prior chemotherapy regimen [4] (Table 1). Furthermore, gefitinib has been recently registered in Europe for the first-line treatment of patients with *EGFR* activating mutations. Similarly, gefitinib is already an established therapy for pre-treated NSCLC in the Asia-Pacific region, where consultations with regulatory authorities are ongoing to discuss its potential use in first-line therapy.

They are orally bioavailable and they selectively and reversibly bind to the ATP-binding site of the EGFR intracellular tyrosine kinase domain. They have a common chemical backbone structure and show similar disposition characteristics in humans after administration [5] with a similar bioavailability, of approximately 60% [6].

SELECTIVITY AND EFFICACY

Protein tyrosine kinases have a tyrosine kinase domain which consists of a C-terminal and an N-terminal lobe. The binding site for the downstream proteins is the C-terminal

Table 1. Tyrosine Kinase Inhibitors (TKIs) of the EGFR Tyrosine Kinase Family Used in the Treatment of NSCLC

Inhibitor	Tyrosine Kinase Target	Cancer Target	Clinical Status
Gefitinib (Iressa, ZD1839)	EGFR	Lung, Breast	Approved
Erlotinib (Tarceva, OS1774)	EGFR	Lung	Approved
Lapatinib (Tykerb, GW572016)	ErbB1, ErbB2	NSCLC, breast, gastric	Approved/Phase III
Canertinib (CI10333)	EGFR, ErbB2, ErbB3, ErbB4	NSCLC, breast	Phase I/II
EKB-569	EGFR, ErbB2	NSCLC, colorectal	Phase II
BIBW2992	EGFR, ErbB2	NSCLC, breast	Phase II

lobe. Tyrosine kinase inhibitor selectivity is mostly obtained by targeting the highly conserved ATP binding site of the tyrosine kinases which is in the cleft between the two lobes [7]. In order to obtain the maximum selectivity, tyrosine kinase inhibitors are designed to specifically attack the ATP binding site of the tyrosine kinases. Selectivity is also obtained by using the proximal regions of the ATP binding site. The adenine region is used by the inhibitors to increase their potency and the sugar region is used for selectivity in EGFR inhibitors as they have different amino acids when compared to the other receptors [8]. Selectivity pocket and channel are not used by ATP-binding and play an important role in increasing the inhibitor selectivity and binding affinity, while the phosphate binding region can also be used to improve selectivity [9]. Hubbard *et al.* suggested that juxtamembrane regions can also be a good target to obtain specificity and increase affinity [10]. This region is located between the transmembrane segment and kinase domain and it autoinhibits the catalytic activity of the receptor tyrosine kinases. This region can be targeted for the drugs as the sequences are not similar in different juxtamembrane segments in different receptor tyrosine kinase families.

The selectivity of a tyrosine kinase inhibitor is very important in the sense of focusing on tumor cells instead of normal cells to minimize the side effects. Furthermore, the tyrosine kinase inhibitors, gefitinib and erlotinib, are more effective in tumor cells harbouring specific mutations, such as the deletion mutations in exon 19 or different point mutations (including the most common, L858R) in EGFR-TK domain [11]. Similarly an inhibitor which is used for attacking the overexpression, such as MET inhibitor for MET overexpression, may result in inhibition of MET in tumor cells, but also in some normal cells with high expression of MET. Therefore, selectivity is very important for attacking only the desired tumor cells instead of normal cells, minimizing the side effects.

Selectivity and specificity are increased when an inhibitor is able to bind to different conformational states of the enzyme. Erlotinib can be an example for this as it can recognize and bind to different conformations of EGFR with high affinity. This ability seems to be more important than the sequence differences of the kinase domain or interaction differences with binding site residues [12]. Thus, the crystal structure of tyrosine kinases is very important in designing tyrosine kinase inhibitors with higher specificity and selectivity. As a summary, to increase the selectivity and to minimize the side-effects, it is important to design tyrosine kinase inhibitors which specifically block certain tyrosine

kinase or kinases that are found in the abnormal signalling. For increasing the selectivity of tyrosine kinase inhibitors different mutational status, different sequences and conformation states of kinases are also being investigated.

In general, different tumor types express different number and types of tyrosine kinases while the same type of tumors have similar receptor tyrosine kinases both in expression and numbers. Administration of tyrosine kinase inhibitors that specifically target one of these kinases can be effective in tumor types depending on mutations in specific oncogenes for their development, such as BCR-ABL in leukaemia, specifically targeted by imatinib, according to the “oncogene addiction” theory [13]. However, most human cancers display multiple genetic abnormalities, which might differ also between subsets of the same type of cancer. Therefore, a tyrosine kinase inhibitor which inhibits many kinases will be more effective in cancer types which occur due to overexpression of several kinases. However, although this administration seems to be more advantageous as multikinase inhibitor can inhibit several types of receptors that are overexpressed, there is a still a high variability in the number and the types of kinases which might differ also between subsets of the same type of cancer, also in NSCLC.

Furthermore, EGFR mutations and more importantly overexpression of EGFR have a crucial role in the formation of NSCLC, but also in the metastatic behaviour. Therefore, the variability in EGFR overexpression in NSCLC can reduce the efficacy of the tyrosine kinase inhibitors. Besides, some tyrosine kinase inhibitors might also increase the risk of metastasis whereas some tyrosine kinase receptors reduce this risk. Since EGFR family plays a very important role in metastasis, it is very important to inhibit the specific receptor tyrosine kinases that increase the risk of metastasis, while not affecting the suppressive receptor tyrosine kinases.

Finally, some receptor tyrosine kinases might be tumor suppressor genes and the role of one tyrosine kinase receptor in one type of cancer can be different in another cancer type. Consequently, although mostly receptor tyrosine kinases play a role in the formation of tumors, they might also have a tumor suppressive properties in different types of cancer. Therefore, there is a possibility of a tyrosine kinase inhibitor to promote tumor growth such as the tyrosine kinase inhibitor canertinib which can stimulate tumor growth as it non-selectively targets the EGFR family members. This implies that inhibiting a tyrosine kinase can have different effects in different cancer types, and this should be carefully investigated.

PHARMACOKINETICS

The pharmacokinetics of a drug is determined by its absorption, distribution, metabolism and excretion. The bioavailability of a drug is dependent on these four properties which are associated with the molecular weight, hydrophobicity, hydrophilicity, hydrogen bonding and active transport of the drug. Gefitinib and erlotinib have wide pharmacokinetic variability in cancer patients [14]. Furthermore, it has been shown that the pharmacokinetic parameters of gefitinib and erlotinib are different. Administration of erlotinib at approved daily dose (150mg) achieved approximately 3.5 fold higher steady-state plasma concentration than gefitinib with the recommended dose (250mg) [15]. Food intake and administration of the drugs with food might also increase erlotinib bioavailability [5]. Therefore, gefitinib has lower bioavailability and higher systemic clearance than erlotinib [14]. *In vitro* studies also showed that erlotinib is less susceptible than gefitinib to metabolism by major liver enzymes and higher plasma erlotinib exposure is achieved despite administration of a lower erlotinib daily dose when compared with gefitinib [14]. Finally, the approved erlotinib dose is administered at its maximum tolerated dose while gefitinib dose is one third of its maximum tolerated dose [16]. These data might explain why the use of erlotinib could result in a clinical advantage over gefitinib as its systemic exposure is higher than gefitinib.

It has been found that smoking also has an effect on gefitinib and erlotinib pharmacokinetics. In particular, smoking resulted in a higher erlotinib clearance [17]. The effect of smoking status on gefitinib is not clear yet although it has been reported to be more effective in patients with NSCLC who had no smoking history than for the smokers. However, the better survival in such subpopulation might be due to their favorable natural history rather than the gefitinib treatment effects [18]. Indeed, no smoking status can be accepted as an important favorable clinical response to both gefitinib and erlotinib.

Gefitinib and erlotinib may also have different drug-drug interaction properties. It has been shown that administration of a single dose of rifampicin (a potent CYP3A4 – isoenzyme involved in the metabolism of gefitinib - inducer) significantly reduces gefitinib systemic exposure by 83%, while administration of itraconazole (a potent CYP3A4 inhibitor) significantly increases it by 78% [19]. It has been found that romidepsin increased the sensitivity of erlotinib synergistically in NSCLC cell lines including EGFR wild type cell lines and TKI resistant EGFR mutant cell lines due to the enhanced apoptosis. These observations support a role for the combination of a histone deacetylase inhibitor and erlotinib in the treatment of NSCLC [20].

However, overall response rates to these drugs remain modest and it is difficult to evaluate their pharmacokinetics in order to determine the better therapy for each patient [21]. First of all, pharmacokinetics of a drug may be different in every patient due to the genetic differences in metabolisms. Second, the pharmacokinetics of a drug can change over time in the same patient. Third, environmental factors, such as diet, smoking and body condition can also affect drug metabolism. Finally there are several similarities in physical and chemical properties in tyrosine kinase inhibitors.

Therefore, further comparative pharmacokinetic/pharmacogenetic studies should be done in order to determine the most effective and safe tyrosine kinase inhibitor and to find out the mechanisms that have an important role in the development of the variability in drug absorption, distribution, metabolism, and excretion, as well as drug interaction and clinical effects for the EGFR-TKIs.

RESISTANCE

A major hurdle in cancer treatment is that tumor cells have or acquire drug resistance. This happens also for the use of TKIs. The resistance to EGFR-TKIs in NSCLC can be caused by different mechanisms such as the occurrence of resistant mutations in tyrosine kinase receptors, such as the *EGFR* T790M, or the activation of other signalling pathways, such as MET overexpression, or the altered metabolism or activity of the influx/efflux proteins, resulting in lower concentrations of the drugs.

Genetic Alterations Affecting EGFR-TKIs Activity

Higher response rates to EGFR-TKIs have been associated with the female gender, adenocarcinoma histology, Asian ethnicity, non-smoker status [21].

Specific activating mutations within the EGFR-TK domain are associated with these clinical characteristics and are predictive of EGFR-TKIs activity [22]. Prospective studies with gefitinib or erlotinib in patients harboring EGFR activating mutations reported response rates ranging from 60 to 82%, suggesting that these drugs might be a valuable alternative to chemotherapy in selected NSCLC patients [23]. In contrast, recent studies also identified mutations in EGFR associated with resistance.

In response to ligand binding EGFR is activated *via* dimerization and the phosphorylation of the tyrosine kinase domain which results in the activation of different downstream signalling pathways such as MAPK, STAT, SRC/FAK and PI3K/AKT which forms an intersecting network and stimulates cell growth and proliferation [24]. When EGFR is mutated, cell growth is triggered without restrictions. Deregulation of protein synthesis, cell cycle, cell growth, cell division, apoptosis and angiogenesis results in carcinogenesis. EGFR alterations and mutations, involving either the receptor itself or the downstream effectors, are frequent in NSCLC [25]. The identification of these mutations and alterations which result in different sensitivity to tyrosine kinase inhibitors in EGFR-directed therapies is crucial for the selection of the right tyrosine inhibitors and decrease resistance.

EGFR Mutations in NSCLC

Initial *in vitro* studies showed that mutated EGFR significantly increased and prolonged activation when compared to wild type [26], favouring EGFR-TKIs activity, but there are different subtypes of EGFR mutations.

Mutation frequency in NSCLC is recognized to vary across ethnic groups, with a notably higher prevalence observed in East-Asian trials (30% to 60%), than in North American studies (10% to 20%) (Fig. 2). Approximately 90% of the EGFR mutations affect regions within exon 18 to 21, which code for tyrosine kinase domain [27]. In-frame deletion in exon 19 accounts for approximately 50% of the

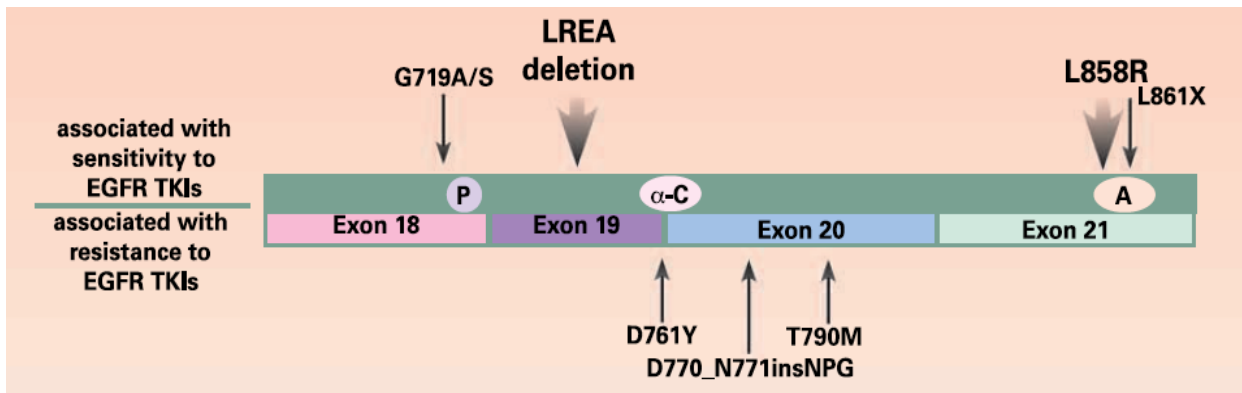


Fig. (2). Mutations associated with sensitivity to gefitinib or erlotinib in EGFR gene in NSCLC (top). Mutations associated with resistance to gefitinib or erlotinib (bottom).

cases and it eliminates four amino acids (LREA) that lie downstream of lysine residue at position 745 [28]. The missense mutation at codon 858 that leads to the substitution of leucine by an arginine (L858R) accounts at least 35% of EGFR mutations in NSCLC. The remaining 5% mutations are the insertions in exon 20. Finally, there are also rare substitutions which span exon 18 to 21 [25]. The mutations in EGFR affect sensitivity to EGFR inhibitors. In lung cancer, activating EGFR tyrosine kinase domain mutations are associated with increased clinical efficacy of erlotinib and gefitinib [26].

The mechanisms of how EGFR mutations are involved in NSCLC development and the tumors become sensitive to treatment are not fully understood. Previous studies showed that the EGFR exon 19 deletion and L858R mutation triggers the ligand-independent activation and extends the receptor kinase activity after it is stimulated by the ligand binding [22, 29]. *In vitro* studies suggested that the mutations in EGFR kinase domain are sufficient for oncogenic transformation as exon 19 deletion, exon 20 insertion and exon 21 L858R can transform fibroblasts [28].

However, patients with EGFR mutations can hardly achieve a complete response. The continued treatment of the tumor by gefitinib or erlotinib cures the drug-sensitive cells whereas a population of tumor cells which gain resistance to gefitinib or erlotinib are also developed. To understand the mechanisms of acquired resistance, tumors from NSCLC patients who showed initial sensitivity to gefitinib or erlotinib and then developed an acquired resistance were analyzed. These studies showed that additional EGFR mutations are also found in the tumors with acquired resistance [30]. In patients with tumors bearing gefitinib- or erlotinib-sensitive EGFR mutations, resistant subclones containing an additional EGFR mutation emerge in the presence of drug [31]. Therefore, lung adenocarcinomas from patients who respond to the tyrosine kinase inhibitors gefitinib or erlotinib usually harbor somatic gain-of-function mutations in exons encoding the kinase domain of the EGFR [31]. Despite the initial responses, patients eventually progress by mechanisms of acquired resistance.

Acquired resistance can develop by several mechanisms. About 50% of the resistance to gefitinib and erlotinib are caused by secondary mutations such as the exon 20 point mutation (T790M) or the insertion in exon 20

(D770V/N771insNPG) in the EGFR gene [32]. The insertion involving exon 20 has been related with a poor tumor response and can also be related to primary resistance [33]. The tyrosine kinase domain mutation which is a point mutation in exon 19 (D761Y) is also associated with drug resistance [34]. However the most important mutation associated with drug resistance and tumor relapse [35] is the exon 20 point mutation (T790M), which changes threonine into methionine at position 790 in the catalytic cleft of the EGFR tyrosine kinase domain [24] and is associated with resistance. This resistance might be due to the blocking of the binding of erlotinib or gefitinib to the kinase ATP-binding pocket [34]. Other *in vivo* studies suggested that the acquired resistance might be due to the alterations in receptor turnover [36].

Therefore, characterization of the genetic alterations of the relapsed tumor could help to decrease the resistance and optimize the therapy.

Mutations outside the exons encoding the kinase domain are rare in lung cancers [22], however the EGFRvIII mutations have also been found in some NSCLC, especially in squamous cell carcinomas [37]. In particular a recent study genotyped the EGFRvIII mutation status in 252 surgically treated lung cancer cases, and this mutation was detected in 8 of 252 patients [38]. All patients were male, smokers, and 7 had squamous cell carcinoma. Of note, EGFR gene copy number was significantly higher in EGFRvIII mutants, suggesting that EGFRvIII gene mutation might be one of the mechanisms of increased EGFR copy number. However, further studies are needed to confirm the mechanisms of EGFRvIII mutations for possible anti-EGFR therapy for lung cancer.

EGFR activating mutations are also associated with amplification, and increased EGFR copy number has also been reported to be associated with response and survival to EGFR tyrosine kinase inhibitors [24]. It has been shown in a previous study that the patients with amplification of EGFR had significantly improved response of gefitinib when compared with the patients who have normal, low or no EGFR copy number [39]. Furthermore, MET amplification has also been identified as a mechanism of acquired resistance to gefitinib and erlotinib through secondary mutations in different studies [40, 41].

Finally, Sos *et al.* showed that in EGFR-dependent cells, PTEN loss partially uncouples mutant EGFR from downstream signaling and activates EGFR, thereby contributing to erlotinib resistance [42]. The clinical relevance of these findings is supported by the observation of PTEN loss in 1 out of 24 primary EGFR-mutant non-small cell lung cancer (NSCLC) tumors. These results suggested a novel resistance mechanism in EGFR-mutant NSCLC involving PTEN loss [42]. These findings and observations should help to guide the search for more effective therapy with tyrosine kinase inhibitors against a specific subset of lung cancers.

MET Overexpression in NSCLC with EGFR Mutations

In many cancer types, drug resistance is caused by the overexpression and amplification of the target kinase and in NSCLC, an important mechanism involved in resistance to gefitinib and erlotinib is the amplification of MET. The amplification of MET is observed in approximately 20% of the NSCLC tumors resulting in the activation of PI3K/Akt signalling by ErbB3 phosphorylation without the involvement of EGFR and ErbB2 [39]. It has been found that MET physically interacts with both EGFR and Her2 in a NSCLC cell line with overexpression and overactivation of MET. Therefore, combined use of a dual EGFR/Her2 inhibitor with a MET inhibitor yields maximal growth inhibition [43].

Many NSCLC cell lines express total MET protein and a subset of MET-expressing NSCLC cell lines also expressed the phosphorylated forms. In particular, phospho-MET expression was significantly related to increased MET copy number and was significantly more frequent in EGFR mutant cell lines [44]. As an additional evidence for the relation between EGFR mutation and MET activation, it has been suggested that other tyrosine kinases were also activated in cells with EGFR activation by EGFR mutations [44]. Guo *et al.* also showed that three EGFR mutant cell lines harboring phospho-MET expression without MET amplification lost or decreased phospho-MET expression whereas MET amplified cell lines with or without EGFR mutations retained phospho-MET expression after knockdown of EGFR, indicating that EGFR mutation or MET amplification activate MET protein [45]. Therefore, MET amplification is present in a subset of untreated lung cancers and cell lines with or without EGFR mutations, and EGFR mutation or MET amplification lead to activation of MET. The combination of MET inhibitors and EGFR inhibitors can offer a more effective treatment for NSCLC patients with gefitinib and erlotinib resistance. For example a second tyrosine kinase inhibitor which is against MET should be administered with gefitinib or erlotinib.

EGFR Polymorphisms

Several studies have investigated the polymorphisms in EGFR and EGFR related pathways and their relation with EGFR TKIs in NSCLC. Many of those studies are focused on the intron 1 CA repeat polymorphism. The CA simple sequence repeat 1 (CA-SSR1) is a highly polymorphic locus in intron 1 of EGFR and it contains 14-21 CA dinucleotide repeats [46, 47]. Length of CA repeat is inversely correlated with EGFR gene transcription therefore shorter alleles have higher transcription [48]. In tumors, short CA repeats are associated with higher EGFR expression when compared

with long CA repeats. Another functional EGFR polymorphism is the -216G/T polymorphism which is located at the promoter region of EGFR and changes guanine to thymine. It is located in an important binding site for transcription factors and the T allele is associated with a higher EGFR expression [24].

Most of the studies suggest that shorter CA repeat length is associated with better response and survival in patients with EGFR TKIs [47, 49]. It has been shown that the classical EGFR mutations predicted sensitivity to gefitinib and there was a trend toward shorter CA repeats being associated with longer survival among the patients with drug-sensitive EGFR mutation [50]. Nie *et al.*, also showed the association of improved survival with shorter CA repeats in Chinese patients who were treated with gefitinib for the CA repeat polymorphism [51]. Similarly, it has been found that classical EGFR mutations are associated with response and survival in patients treated with gefitinib for EGFR mutation and CA repeat polymorphism with a trend towards higher response rates among patients with low CA repeats [52]. However, Gregorc *et al.*, evaluated patients treated with gefitinib for EGFR intron 1 and -216G/T polymorphisms. They showed that the outcome of treatment with gefitinib in patients with NSCLC is dependent on the genetic variability in the promoter region of EGFR gene. They did not find any relation with the intron 1 CA repeat polymorphism while the ones with 216G/T polymorphism showed lower response rates [53]. Furthermore, Nomura *et al.*, showed that the two EGFR promoter polymorphisms are unrelated to survival in NSCLC patients who were not treated with EGFR TKIs after adjusting for sex, ethnicity, age and smoking. These authors suggest that EGFR intron 1 does not affect the growth of NSCLC independent of the treatment with gefitinib [54].

In conclusion, several common germline polymorphisms in the promoter region of the EGFR gene can be associated with clinical benefit, progression time and survival of NSCLC patients with gefitinib treatment and further studies in this setting are warranted.

CONCLUSION

Targeted therapy focuses on the differences in the genetic background between the tumor cells and the normal cells and it aims to attack specifically the tumor cells. Currently, EGFR inhibition is an important strategy used for the treatment of NSCLC. EGFR inhibitors, such as the tyrosine kinase inhibitors erlotinib and gefitinib are one of the most commonly used treatment strategies. Alterations in EGFR have a major importance in EGFR tyrosine kinase inhibition response, whereas modifications that occur on the downstream effectors could also lead to resistance. Secondary mutations result in resistance by interfering with the binding of tyrosine kinase inhibitors, thus resulting in a decreased sensitivity. The altered pathways are also different in individuals because of the genetic diversity in metabolism.

Therefore, a better and more detailed characterization of the mechanisms of resistance causing events would help to decrease the resistance and optimize the therapy. In the future, finding better predictors of response, resistance and survival could be critical to determine the patients who might get the best benefit from specific treatments.

REFERENCES

- [1] Szakacs G, Paterson JK, Ludwig JA, Booth-Gente C, Gottesman MM. Targeting multidrug resistance in cancer. *Nat Rev* 2006; 5: 219-34.
- [2] Jorissen RN, Walker F, Pouliot N, Garrett TPJ, Ward CW, Burgess AW. Epidermal growth factor receptor: mechanisms of activation and signalling. *Exp Cell Res* 2003; 284: 31-53.
- [3] Woodburn JR. The epidermal growth factor receptor and its inhibition in cancer therapy. *Pharmacol Ther* 1999; 82: 241-50.
- [4] Kim ES, Hirsh V, Mok T, *et al.* Gefitinib versus docetaxel in previously treated non-small-cell lung cancer (INTEREST): a randomised phase III trial. *Lancet* 2008; 372: 1809-18.
- [5] Siegel-Lakshai WS, Beijnen JH, Schellens JH. Current knowledge and future directions of the selective epidermal growth factor receptor inhibitors erlotinib (Tarceva) and gefitinib (Iressa). *Oncologist* 2005; 10: 579-89.
- [6] Frohna P, Lu J, Eppler S, *et al.* Evaluation of the absolute oral bioavailability and bioequivalence of erlotinib, an inhibitor of the epidermal growth factor receptor tyrosine kinase, in a randomized, crossover study in healthy subjects. *J Clin Pharmacol* 2006; 46: 282-90.
- [7] Hubbard SR. Protein tyrosine kinases: autoregulation and small molecule inhibition. *Curr Opin Struct Biol* 2002; 12: 735-41.
- [8] Traxler PM, Furet P, Buchdunger E, Meyer T, Lydon N. 4-(Phenylamino) pyrrolopyrimidines: potent and selective, ATP-site directed inhibitors of the EGF-receptor protein tyrosine kinase. *J Med Chem* 1996; 39: 2285-92.
- [9] Fabbro D, Ruetz S, Buchdunger E, *et al.* Protein kinases as targets for anti-cancer agents: from inhibitors to useful drugs. *Pharmacol Ther* 2002; 93: 79-98.
- [10] Hubbard SR. Juxtamembrane autoinhibition in receptor tyrosine kinases. *Nat Rev Mol Cell Biol* 2004; 5: 464-70.
- [11] Riely GJ, Pao W, Li A R, *et al.* Clinical course of patients with non-small cell lung cancer and epidermal growth factor receptor exon 19 and exon 21 mutations treated with gefitinib or erlotinib. *Clin Cancer Res* 2006; 12: 839-44.
- [12] Verkhivker GM. Exploring sequence-structure relationships in the tyrosine kinase space: functional classification of the binding specificity mechanisms for cancer therapeutics. *Bioinformatics* 2007; 15: 1919-26.
- [13] Luo J, Solimini NL, Elledge SJ. Principles of cancer therapy: oncogene and non-oncogene addiction. *Cell* 2009; 136(5): 823-37.
- [14] Li J, Zhao M, He P, Hidalgo M, Baker SD. Differential metabolism of gefitinib and erlotinib by human cytochrome P450 enzymes. *Clin Cancer Res* 2007; 13(12): 3731-7.
- [15] Lu JF, Eppler SM, Wolf J, *et al.* Clinical pharmacokinetics of erlotinib in patients with solid tumors and exposure-safety relationship in patients with non-small cell lung cancer. *Clin Pharmacol Ther* 2006; 80: 136-45.
- [16] Blackhall F, Ranson M, Thatcher N. Where next for gefitinib in patients with lung cancer? *Lancet Oncol* 2006; 7: 499-507.
- [17] Hamilton M, Wolf JL, Rusk J, *et al.* Effects of smoking on the pharmacokinetics of erlotinib. *Clin Cancer Res* 2006; 12: 2166-71.
- [18] Hotta K, Kiura K, Takigawa N, *et al.* Association of the benefit from gefitinib monotherapy with smoking status in Japanese patients with non-small-cell lung cancer. *Lung Cancer* 2008; 62: 236-41.
- [19] Swaisland HC, Ranson M, Smith RP, *et al.* Pharmacokinetic drug interactions of gefitinib with rifampicin, itraconazole and metoprolol. *Clin Pharmacokinet* 2005; 44: 1067-81.
- [20] Zhang W, Peyton M, Xie Y, *et al.* Histone deacetylase inhibitor romidepsin enhances anti-tumor effect of erlotinib in non-small cell lung cancer (NSCLC) cell lines. *J Thorac Oncol* 2009; 4: 161-6.
- [21] Baselga J, Arteaga CL. Critical update and emerging trends in epidermal growth factor receptor targeting in cancer. *J Clin Oncol* 2005; 23: 2445-59.
- [22] Paez JG, Janne PA, Lee JC, *et al.* EGFR mutations in lung cancer: correlation with clinical response to gefitinib therapy. *Science* 2004; 304: 1497-500.
- [23] Saijo N, Takeuchi M, Kunitoh H. Reasons for response differences seen in the V15-32, INTEREST and IPASS trials. *Nat Rev Clin Oncol* 2009; 6: 287-94.
- [24] Heist RS, Christiani D. EGFR-targeted therapies in lung cancer: predictors of response and toxicity. *Pharmacogenomics* 2009; 10: 59-68.
- [25] Laurent-Puig P, Lievre A, Blons H. Mutations and response to epidermal growth factor receptor inhibitors. *Clin Cancer Res* 2009; 15: 1133-9.
- [26] Lynch TJ, Bell DW, Sordella R, *et al.* Activating mutations in the epidermal growth factor receptor underlying responsiveness of non-small cell lung cancer to gefitinib. *N Engl J Med* 2004; 350: 2129-39.
- [27] Shigematsu H, Gazdar AF. Somatic mutations of epidermal growth factor receptor signalling pathway in lung cancers. *Int J Cancer* 2006; 118: 257-62.
- [28] Greulich H, Chen TH, Feng W, *et al.* Oncogenic transformation by inhibitor-sensitive and -resistant EGFR mutants. *PLoS Med* 2005; 2: e313.
- [29] Amann J, Kalyankrishna S, Massion PP, *et al.* Aberrant epidermal growth factor receptor signalling and enhanced sensitivity to EGFR inhibitors in lung cancer. *Cancer Res* 2005; 65: 226-35.
- [30] Kobayashi S, Boggon TJ, Dayaram T, *et al.* EGFR mutation and resistance of non-small cell lung cancer to gefitinib. *N Engl J Med* 2005; 352: 786-92.
- [31] Pao W, Miller VA, Politi KA, *et al.* Acquired resistance of lung adenocarcinomas to gefitinib or erlotinib is associated with a second mutation in the EGFR kinase domain. *PLoS Med* 2005; 2: 1-11.
- [32] Kosaka T, Yatabe Y, Endoh H, *et al.* Analysis of epidermal growth factor receptor gene mutation in patients with non-small cell lung cancer and acquired resistance to gefitinib. *Clin Cancer Res* 2006; 19: 5764-9.
- [33] Wu JY, Wu SG, Yang CH, *et al.* Lung cancer with epidermal growth factor receptor exon 20 mutations is associated with poor gefitinib treatment response. *Clin Cancer Res* 2008; 14: 4877-82.
- [34] Riely GJ, Politi KA, Miller VA, Pao W. Update on epidermal growth factor receptor mutations in non-small cell lung cancer. *Clin Cancer Res* 2006; 12(24): 7232-41.
- [35] Balak MN, Gong Y, Riely GJ, *et al.* Novel D761Y and common secondary T790M mutations in epidermal growth factor receptor-mutant lung adenocarcinomas with acquired resistance to kinase inhibitors. *Clin Cancer Res* 2006; 12: 6494-501.
- [36] Kwak EL, Sordella R, Bell DW, *et al.* Irreversible inhibitors of the EGF receptor may circumvent acquired resistance to gefitinib. *Proc Natl Acad Sci USA* 2005; 102: 7665-70.
- [37] Ji H, Zhao X, Yuza Y, *et al.* epidermal growth factor receptor variant III mutations in lung tumorigenesis and sensitivity to tyrosine kinase inhibitors. *Proc Natl Acad Sci USA* 2006; 103: 7817-22.
- [38] Sasaki H, Kawano O, Endo K, Yukiue H, Yano M, Fujii Y. EGFRvIII mutation in lung cancer correlates with increased EGFR copy number. *Oncol Rep* 2007; 17: 319-23.
- [39] Cappuzzo F, Hirsch FR, Rossi E, *et al.* Epidermal growth factor receptor gene and protein and gefitinib sensitivity in non-small cell lung cancer. *J Natl Cancer Inst* 2005; 97: 643-55.
- [40] Engelman JA, Zejnullahu K, Mitsudomi T, *et al.* MET amplification leads to gefitinib resistance in lung cancer by activating ErbB3 signaling. *Science* 2007; 316: 1039-43.
- [41] Bean J, Brennan C, Shih JY, *et al.* MET amplification occurs with or without T790M mutations in EGFR mutant lung tumors with acquired resistance to gefitinib or erlotinib. *Proc Natl Acad Sci USA* 2007; 104: 20932-7.
- [42] Sos ML, Koker M, Weir BA, *et al.* PTEN loss contributes to erlotinib resistance in EGFR-mutant lung cancer by activation of Akt and EGFR. *Cancer Res* 2009; 69: 3256-61.
- [43] Agarwal S, Zerillo C, Kolmakova J, *et al.* Association of constitutively activated hepatocyte growth factor receptor (Met) with resistance to a dual EGFR/Her2 inhibitor in non-small-cell lung cancer cells. *Br J Cancer* 2009; 100: 941-9.
- [44] Kubo T, Yamamoto H, Lockwood WW, *et al.* MET gene amplification or EGFR mutation activate MET in lung cancers untreated with EGFR tyrosine kinase inhibitors. *Int J Cancer* 2009; 124: 1778-84.
- [45] Guo A, Villen J, Kornhauser J, *et al.* Signaling networks assembled by oncogenic EGFR and c-Met. *Proc Natl Acad Sci USA* 2008; 105: 692-700.
- [46] Araujo A, Ribeiro R, Azevedo I, *et al.* Genetic polymorphisms of the epidermal growth factor and related receptor in non-small cell lung cancer – a review of the literature. *Oncologist* 2007; 12: 201-10.

- [47] Zhang W, Weissfeld JL, Romkes M, *et al.* Association of the *EGFR* intron 1 *CA* repeat length with lung cancer risk. *Mol Carcinog* 2007; 46: 372-80.
- [48] Gebhart F, Zanker KS, Brandt B, *et al.* Modulation of epidermal growth factor receptor gene transcription by a polymorphic dinucleotide repeat in intron 1. *J Biol Chem* 1999; 274: 13176-80.
- [49] Buerger H, Gebhardt F, Schmidt H, *et al.* Length and loss of heterozygosity of an intron 1 polymorphic sequence of *EGFR* is related to cytogenetic alterations and epithelial growth factor receptor expression. *Cancer Res* 2000; 60: 854-7.
- [50] Ichihara S, Toyooka S, Fujiwara Y, *et al.* The impact of epidermal growth factor receptor gene status on gefitinib-treated Japanese patients with non-small cell lung cancer. *Int J Cancer* 2007; 120: 1239-47.
- [51] Nie Q, Wang Z, Zhang GC, *et al.* The epidermal growth factor receptor intron 1 (*CA*)_n microsatellite polymorphism is a potential predictor of treatment outcome in patients with advanced lung cancer treated with gefitinib. *Eur J Pharmacol* 2007; 570: 175-81.
- [52] Han SW, Jeon JK, Lee KH, *et al.* Intron 1 *CA* polymorphism and mutations of epidermal growth factor receptor and gefitinib responsiveness in non small cell lung cancer. *Pharmacogenet Genomics* 2007; 17: 313-9.
- [53] Gregorc V, Hidalgo M, Spreafico A, *et al.* Germline polymorphisms in *EGFR* and survival in patients with lung cancer receiving gefitinib. *Clin Pharmacol Ther* 2008; 83(3): 477-84.
- [54] Nomura M, Shigematsu H, Li L, *et al.* Polymorphisms, mutations and amplification of the *EGFR* gene in non-small cell lung cancers. *PLoS Med* 2007; 4: e125.

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