Epidermal growth factor receptor (EGFR) is overexpressed in the majority of NSCLC and it is an important target in the treatment of NSCLC. EGFR is a member of the family of EGF-related tyrosine kinase receptors. Upon ligands binding, the receptors homoor hetero-dimerize. Subsequently, it activates receptors’ intrinsic tyrosine kinase activity and broad downstream signaling cascades, mainly including Ras-Raf-MAP-kinase pathway, PI3K-Akt pathway, and STAT pathway. All these have strong stimulatory effect on cell proliferation, differentiation, survival, angiogenesis and migration (9-11).

EGFR has emerged as a critical tumorigenic factor in the development and progression of NSCLC (12-14). Two specific EGFR tyrosine kinase inhibitors (TKIs), gefitinib (ZD1839, Iressa) and erlotinib (OSI-774, Tarceva), have been developed and used clinically in the treatment of advanced NSCLC. These two drugs disrupt EGFR signaling by competing with adenosine triphosphate (ATP) for the binding sites at tyrosine kinase domain, and thus inhibiting the phosphorylation and activation of EGFRs and the downstream signaling network. Both agents can induce dramatic clinical response in patients who fail chemotherapy. Erlotinib and gefitinib have been shown to have survival benefit in Caucasians and Asians respectively when compared to placebo in controlled double-blinded randomized phase III trials (15, 16).

INTRODUCTION

Lung cancer is one of the most common human cancers and the leading cause of cancer death worldwide (1). Lung cancer is generally classified into two histological types, small cell lung cancer (SCLC) and non–small cell lung cancer (NSCLC). NSCLC accounts for approximately 85% of the cases and it is further divided into squamous-cell carcinoma (SCC), adenocarcinoma (AC), large cell carcinoma, and others (2). Adenocarcinoma has become the most prevalent subtype of NSCLC in recent decades (3, 4). The treatment of lung cancer is mainly based on the stage of cancer, patients’ performance status, comorbidity, etc (5). For patients with early stage disease (stage I or II) surgical resection is considered the primary therapeutic choice. It is worth taking notice, however, that majority of NSCLC cases have reached locally advanced (stage III) or metastatic stage (stage IV) at the time of diagnosis (6), and chemotherapy is usually recommended as the first line therapy.

Chemotherapy is often considered too toxic, particularly for elderly patients and patients with poor performance status. The well-established platinum-based regimen can only bring modest survival benefit by increasing the median survival time about three months in average (7, 8). In recent years more effort has been put onto the development of molecular-targeted drugs. Epidermal growth factor receptor (EGFR) is overexpressed in the majority of NSCLC and it is an important target in the treatment of NSCLC. EGFR is a member of the family of EGF-related tyrosine kinase receptors. Upon ligands binding, the receptors homoor hetero-dimerize. Subsequently, it activates receptors’ intrinsic tyrosine kinase activity and broad downstream signaling cascades, mainly including Ras-Raf-MAP-kinase pathway, PI3K-Akt pathway, and STAT pathway. All these have strong stimulatory effect on cell proliferation, differentiation, survival, angiogenesis and migration (9-11).

EGFR has emerged as a critical tumorigenic factor in the development and progression of NSCLC (12-14). Two specific EGFR tyrosine kinase inhibitors (TKIs), gefitinib (ZD1839, Iressa) and erlotinib (OSI-774, Tarceva), have been developed and used clinically in the treatment of advanced NSCLC. These two drugs disrupt EGFR signaling by competing with adenosine triphosphate (ATP) for the binding sites at tyrosine kinase domain, and thus inhibiting the phosphorylation and activation of EGFRs and the downstream signaling network. Both agents can induce dramatic clinical response in patients who fail chemotherapy. Erlotinib and gefitinib have been shown to have survival benefit in Caucasians and Asians respectively when compared to placebo in controlled double-blinded randomized phase III trials (15, 16).
double-blinded randomized phase III trials (15,16). However, among unselected NSCLC patients the objective response rate is only about 10% (17,18). Female patients, nonsmokers, East Asians, and patients with lung adenocarcinoma are noted to have higher response rates (17-19). In addition, many laboratories have found a number of other factors which are associated with EGFR-TKIs sensitivity. In order to better understand and interpret these basic and clinical research knowledge and accelerate the translation of research findings into daily medical practice, we reviewed the literature and carefully evaluated the predictive value of these biomarkers.

We hope this brief review could provide useful information for clinicians, patients, and research professionals, help clinicians to select the right subgroup of NSCLC patients for EGFR-TKI therapy with high frequency of success, and to stimulate future research interest and effort in targeted therapy for NSCLC patients.

1. Somatic mutations in EGFR

Somatic mutation is the mutation that occurs only in somatic cells, which are in contrast to germ cells. A number of somatic mutations have been identified in the EGFR gene in NSCLC. In general these mutations can be classified into three major types: in-frame deletion, insertion, and mis-sense mutation. Most of the mutations are located in the tyrosine kinase coding domain (exons 18-21) of the EGFR gene. The amino acids 746~753 encoded by exon 19 and amino acid 858 encoded by exon 21 are two mutation hotspots, which accounts for over 80% of all the detected mutations.

Gefitinib sensitive mutations

A number of retrospective studies have reported that two activating mutations, small in-frame deletion in exon 19 (746~753) and substitution of leucine for arginine at amino acid 858 in exon 21 (L858R), have striking correlation with EGFR-TKI sensitivity (20-28). This discovery has been claimed as the most significant molecular event in lung cancer (29). Both activating mutations are able to enhance kinase activity of EGFR and the activation of its downstream signaling, and play a pivotal role in supporting NSCLC cell survival (20,30). When specific EGFR-TKIs are applied, the excessive survival signals that cancer cells are “addicted to” are counteracted and dramatic apoptosis occurs (30, 31).

Deletion in exon 19 and L858R are usually more common in women, East Asians, light smokers (less than 15 pack-years), and patients with adenocarcinoma (reviewed in (40)). Some studies have reported that exon 19 deletion is superior to L858R in prediction of response rates and survival (26,39,41). However, conflict results indicate there is no significant difference observed between these two mutations (33,34). More studies are required to clarify this issue.

EGFR-TKIs resistant mutations

T790M, D761Y, L747S, and insertion in exon 20 are associated with resistance to EGFR-TKIs (42-47). T790 is located at the key position in ATP binding cleft of EGFR and is considered the gatekeeper residue. The introduction of T790M mutation increases ATP affinity of receptors, which relatively attenuates the binding of EGFR-TKIs (48). T790M is mainly present in relapsed tumors after an initial response and secondary to EGFR-TKIs therapy (42,43), and it accounts for about half of acquired resistance to gefitinib or erlotinib (44). Therefore, T790M has been considered a specific marker for acquired resistance to EGFR-TKIs. L747S, D761Y and insertions in exon 20 also confer modest resistance to EGFR-TKIs. However, they are not as common as T790M among NSCLC patients with acquired resistance to EGFR-TKIs.

Table 1. Prospective studies of gefitinib/erlotinib in EGFR mutation positive NSCLC patients

<table>
<thead>
<tr>
<th>Author</th>
<th>No. of participating patients with EGFR mutations</th>
<th>Ethnicity</th>
<th>EGFR mutation screening method</th>
<th>Overall response and disease control rate (%)</th>
<th>Complete response (%)</th>
<th>Partial response (%)</th>
<th>Stable disease (%)</th>
<th>Median progression-free survival (Months)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yoshikawa K et al. (35)</td>
<td>21</td>
<td>Japanese</td>
<td>Gene scan &amp; cycleave real-time quantitative PCR technology</td>
<td>91</td>
<td>3 (14)</td>
<td>16 (76%)</td>
<td>0</td>
<td>7.7</td>
</tr>
<tr>
<td>Sunaga N, et al. (32)</td>
<td>21</td>
<td>Japanese</td>
<td>Sequencing</td>
<td>91</td>
<td>3 (14)</td>
<td>13 (62%)</td>
<td>3 (14%)</td>
<td>12.9</td>
</tr>
<tr>
<td>Inoue A, et al. (34)</td>
<td>16</td>
<td>Japanese</td>
<td>Sequencing</td>
<td>88</td>
<td>0</td>
<td>12 (75%)</td>
<td>2 (13%)</td>
<td>9.7</td>
</tr>
<tr>
<td>Ashina H, et al. (33)</td>
<td>16</td>
<td>Japanese</td>
<td>Sequencing</td>
<td>81</td>
<td>2 (13%)</td>
<td>10 (62%)</td>
<td>1 (6%)</td>
<td>8.9</td>
</tr>
<tr>
<td>Paz-Ares L, et al. (36)</td>
<td>21</td>
<td>Caucasian</td>
<td>Gene scan &amp; TaqMan assay</td>
<td>91</td>
<td>6 (29%)</td>
<td>13 (62%)</td>
<td>0</td>
<td>&gt;8</td>
</tr>
<tr>
<td>van Zandwijk N, et al. (37)</td>
<td>13</td>
<td>Caucasian</td>
<td>Sequencing and gene scan</td>
<td>92</td>
<td>1 (8%)</td>
<td>10 (77%)</td>
<td>1 (8%)</td>
<td>14</td>
</tr>
<tr>
<td>Sequist LV, et al. (38)</td>
<td>31</td>
<td>Asian &amp; others</td>
<td>Sequencing</td>
<td>94</td>
<td>1 (3%)</td>
<td>16 (52%)</td>
<td>12 (39%)</td>
<td>9.2</td>
</tr>
</tbody>
</table>
MET amplification

MET is a high affinity tyrosine kinase receptor for hepatocyte growth factor (HGF)/scatter factor. The binding of HGF results in autophosphorylation of MET at multiple tyrosine residues and activation of many downstream signaling components, which produce profound effect on cellular motility, growth, survival, invasion, and metastasis. Alteration of MET pathway contributes to the development and progression of a number of human tumors. Amplification of the MET gene has been detected in gastric cancers (10–20%) and esophageal cancers (50,51).

In addition, activating mutations of MET are observed in papillary renal carcinoma (52). MET amplification has been observed in NSCLC and it is associated with EGFR-TKI resistance (53,54). Its incidence is about 21% (9 out of 43) among patients with acquired resistance. Among untreated patients it occurs much less frequently (about 3%) (53). MET amplification is able to activate ERBB3 (HER3)-dependent PI3K/Akt pathway, and ultimately lead to gefitinib resistance (54). Its occurrence is independent of T790M (53).

3. K-ras mutation

Ras is one of the important molecules in the downstream of EGFR signaling pathway. Ras is able to activate serine/threonine kinase Raf, the mitogen-activated protein kinases ERK1 and ERK2, and a number of nuclear proteins to promote cell proliferation. Ras genes, especially K-ras, have been implicated in the pathogenesis and prognosis of lung cancer (55). Mutated K-ras can be observed among 20–30% NSCLC patients. Majority of the mutations (approximately 80–90%) are guanine to thymine transversion in codon 12, which results in constitutive activation of K-ras protein (56,57). NSCLC patients with K-ras mutations are associated with unfavorable prognosis (58–60).

The correlation of K-ras mutations with EGFR mutations and gefitinib response has been investigated by several groups (61–63). In general, the mutations of EGFR and K-ras are mutually exclusive. NSCLC patients with K-ras mutations have poor sensitivity to EGFR-TKIs (25,64). Screening K-ras mutation among NSCLC patients who are negative for EGFR mutations could provide additional information to avoid EGFR-TKIs.

Type III deletion mutation (EGFRvIII) is the deletion of exons 2–7, a 801bp fragment of EGFR cDNA, which produces a truncated receptor lacking a portion of extracellular ligand binding domain (65). The truncated receptor, however, is oncogenic. It has constitutive kinase activity, which is strong enough to activate downstream signaling cascades and gives cells growth advantage (66,67). EGFRvIII has been identified in a number of human solid tumors, including glioblastoma, breast cancer, ovarian cancer, prostate cancer, and lung cancer (66–69).

The incidence of EGFRvIII in NSCLC varies among studies. Okamoto et al and Garcia et al have identified 16% (5 of 32) and 39% (30 of 76) of EGFRvIII using immunochemistry staining (66,70). In contrast, low detected rates have been reported using RT-PCR (2.8%–3.2% or undetectable) (71–73). The study performed in transgenic mouse has revealed that EGFRvIII mutant cancer cells are relatively resistant to EGFR-TKIs, but sensitive to irreversible EGFR inhibitor (71) and anti-EGFR antibody 806 (74).

5. EGFR gene dosage

Gene dosage is the number of copies of a gene present in a cell or nucleus. An increase in gene dosage means the gene is amplified. Gene amplification is a molecular mechanism responsible for oncogene overexpression. By production of multiple copies of a particular gene or genes, the phenotype that the gene confers is amplified in the cell.

High copies of EGFR (amplification or high polysomy) have been detected in approximately 30% of NSCLC patients using fluorescence in situ hybridization (FISH), and it is usually associated with poor clinical prognosis (75). High copies of EGFR probably is an effective predictor for better treatment response to EGFR-TKIs (Table 2) (22,23,76,77). Patients who have increased copies of EGFR gene show significant survival benefit from EGFR-TKIs treatment in both Phase II (53,78) and Phase III clinical trials (Iressa Survival Evaluation in Lung cancer and BR.21) (79,80) (Table 2).

High EGFR copy number is frequently correlated with EGFR somatic mutations (22,27,31,81). This casts doubt about the independent predictive value. Additional preclinical and clinical studies with large sample size are paramount to resolving this issue. Since the mutation rate of EGFR is much lower among Caucasians (~10%) compared with Asians (30–50%) and a substantial portion of patients without EGFR mutations still benefit from EGFR-TKIs treatment, increased EGFR gene copy number could play its unique role in predicting EGFR-TKIs susceptibility. Japanese patients with EGFR gene amplification, however, do not benefit from gefitinib treatment (72).

6. EGFR protein expression

Overexpression of EGFR protein is very common in NSCLC patients (40–80%) (13,14), and it is associated with aggressive clinical behaviors and poor prognosis (82–87). The relationship between EGFR protein level and EGFR-TKIs sensitivity has been studied intensively. Both positive (23,77,79,80) and negative correlation (88,89) have been reported (Table 3).

The conflict observations partially could be attributed to the methodology (immunohistochemistry staining, IHC) applied for EGFR protein quantification because different laboratories use different antibodies, different scoring systems, and different protocols. EGFR protein is often associated with EGFR gene copy number (23,75,90,91). Hirsch et al have recently suggested that patients with FISH and IHC double positive (approximately 23%) probably can benefit more from EGFR-TKIs (77).

7. HER2 expression and gene dosage

HER2 is another member of erbB transmembrane receptor family. It has intrinsic kinase activity. HER2 is known to be a preferred coreceptor for EGFR in the process of EGFR heterodimerization. Increased expression of HER2 is associated with inferior survival in NSCLC patients, and high EGFR and HER2 coexpression has additive impact on unfavorable prognosis (92).

Overexpression of HER2 protein is not associated with gefitinib response and survival. (76,93). Neither is HER2 copy number (78). However, HER2 amplification could predict gefitinib sensitivity and survival among NSCLC patients with increased EGFR copy number. (76,94)
### Table 2. Detected EGFR copy number using FISH and EGFR-TKI treatment response in NSCLC

<table>
<thead>
<tr>
<th>Study subjects</th>
<th>Scoring criteria</th>
<th>Result</th>
<th>Conclusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>81 (Southwest Oncology Group study 0126)</td>
<td>FISH negative</td>
<td>with no or low genomic gain (≤4 copies in 40% cells)</td>
<td>68%</td>
</tr>
<tr>
<td></td>
<td>FISH Positive</td>
<td>high level of polyomysy (≥4 copies in 40% cells) Gene amplification (EGFR/chr7≥2, or ≥15 copies per cell in ≥10% cells)</td>
<td>32%</td>
</tr>
<tr>
<td>102</td>
<td>Disomy</td>
<td>≤2 copies in ≥90% of cells</td>
<td>35%</td>
</tr>
<tr>
<td></td>
<td>Low trisomy</td>
<td>≤2 copies in ≥40% of cells, 3 copies in 10%–40% of the cells, ≥4 copies in &lt;10% of cells</td>
<td>17%</td>
</tr>
<tr>
<td></td>
<td>High trisomy</td>
<td>≤2 copies in ≥40% of cells, 3 copies in ≥40% of cells, ≥4 copies in &lt;10% of cells</td>
<td>2%</td>
</tr>
<tr>
<td></td>
<td>Low polynomy</td>
<td>≥4 copies in 10%–40% of cells</td>
<td>14%</td>
</tr>
<tr>
<td></td>
<td>High polynomy</td>
<td>≥4 copies in ≥40% of cells</td>
<td>20.0%</td>
</tr>
<tr>
<td></td>
<td>Gene amplification</td>
<td>EGFR/chr7≥2, or ≥15 copies per cell in ≥10% cells</td>
<td>13%</td>
</tr>
<tr>
<td>370</td>
<td>Disomy</td>
<td>≤2 copies in ≥90% of cells</td>
<td>69%</td>
</tr>
<tr>
<td></td>
<td>Low trisomy</td>
<td>≤2 copies in ≥40% of cells, 3 copies in 10%–40% of the cells, ≥4 copies in &lt;10% of cells</td>
<td>16%</td>
</tr>
<tr>
<td></td>
<td>High trisomy</td>
<td>≤2 copies in ≥40% of cells, 3 copies in ≥40% of cells, ≥4 copies in &lt;10% of cells</td>
<td>24%</td>
</tr>
<tr>
<td></td>
<td>Low polynomy</td>
<td>≥4 copies in 10%–40% of cells</td>
<td>27%</td>
</tr>
<tr>
<td></td>
<td>High polynomy</td>
<td>≥4 copies in ≥40% of cells</td>
<td>17%</td>
</tr>
<tr>
<td></td>
<td>Gene amplification</td>
<td>EGFR/chr7≥2, or ≥15 copies per cell in ≥10% cells</td>
<td>14%</td>
</tr>
<tr>
<td>25</td>
<td>Disomy</td>
<td>≤2 copies in ≥90% of cells</td>
<td>10%</td>
</tr>
<tr>
<td></td>
<td>Low trisomy</td>
<td>≤2 copies in ≥40% of cells, 3 copies in 10%–40% of the cells, ≥4 copies in &lt;10% of cells</td>
<td>18%</td>
</tr>
<tr>
<td></td>
<td>High trisomy</td>
<td>≤2 copies in ≥40% of cells, 3 copies in ≥40% of cells, ≥4 copies in &lt;10% of cells</td>
<td>2%</td>
</tr>
<tr>
<td></td>
<td>Low polynomy</td>
<td>≥4 copies in 10%–39% of cells</td>
<td>24%</td>
</tr>
<tr>
<td></td>
<td>High polynomy</td>
<td>≥4 copies in ≥40% of cells</td>
<td>34%</td>
</tr>
<tr>
<td></td>
<td>Gene amplification</td>
<td>EGFR/chr7≥2, or ≥15 copies per cell in ≥10% cells</td>
<td>11%</td>
</tr>
<tr>
<td>183</td>
<td>Pooled study subjects from Italy and SWOG study 0126</td>
<td>FISH negative</td>
<td>no or low genomic gain (≤4 copies in 40% cells)</td>
</tr>
<tr>
<td></td>
<td>FISH Positive</td>
<td>Gene amplification (EGFR/chr7≥2, or ≥15 copies per cell in ≥10% cells)</td>
<td>32%</td>
</tr>
</tbody>
</table>

### Table 3. EGFR protein expression and EGFR-TKI treatment response

<table>
<thead>
<tr>
<th>Sample size</th>
<th>Scoring criteria</th>
<th>Results</th>
<th>Conclusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>325 (Phase III clinical trial BR.21 study)</td>
<td>Negative</td>
<td>&lt;10% cells positive for membranous staining</td>
<td>43%</td>
</tr>
<tr>
<td></td>
<td>Positive</td>
<td>≥10% of tumor cells positive for membranous staining</td>
<td>57%</td>
</tr>
<tr>
<td>100</td>
<td>Negative</td>
<td>0–99</td>
<td>40%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>100–199</td>
<td>58%</td>
</tr>
<tr>
<td></td>
<td>Positive</td>
<td>200–299</td>
<td>58%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>300–400</td>
<td>58%</td>
</tr>
<tr>
<td>200 (Pooled study subjects from Italy and SWOG study 0126)</td>
<td>Negative</td>
<td>0–99</td>
<td>39%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>100–199</td>
<td>58%</td>
</tr>
<tr>
<td></td>
<td>Positive</td>
<td>200–299</td>
<td>61%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>300–400</td>
<td>61%</td>
</tr>
<tr>
<td>379 (Phase III Iressa Survival Evaluation in Lung Cancer)</td>
<td>Negative</td>
<td>0–99</td>
<td>30%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>100–199</td>
<td>70%</td>
</tr>
<tr>
<td></td>
<td>Positive</td>
<td>200–299</td>
<td>70%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>300–400</td>
<td>70%</td>
</tr>
<tr>
<td>50</td>
<td>0/1+</td>
<td>Negative to faint immunoreactive cells</td>
<td>54%</td>
</tr>
<tr>
<td></td>
<td>2+/3+</td>
<td>Medium to strong immunoreactive cells</td>
<td>46%</td>
</tr>
</tbody>
</table>

*Percentage of positive tumor cells per slides x dominant intensity pattern of staining"
8. Akt phosphorylation

The phosphatidylinositol 3’-kinases (PI3K)/Akt pathway is one of the important downstream signal transduction pathways of EGFR. It plays critical role in regulating cell survival and apoptosis. Akt activation is able to protects cells from apoptosis by inactivating pro-apoptotic proteins. (95,96) Increased PI3K/Akt activity has been observed in NSCLC. Positive p-Akt expression is associated with better gefitinib responsiveness and prognosis. (77,97,98) Conflicting result have also indicated that p-Akt is not associated with EGFR-TKI efficacy (99).

Gene expression signature and mass spectrometry

Gene expression signature and mass spectrometry are fast growing area in cancer research. Although both biotechnologies are costly, they are robust for new biomarkers discovery. For patients who are negative for EGFR mutations and/or other markers, gene expression and mass spectrometry analysis probably could introduce new insight into clinical practice to assure better clinical outcomes. By comparing the gene expression patterns of gefitinib sensitive and gefitinib resistant lung cancer, Balko and Coldren et al have found several novel markers associated with gefitinib sensitivity. (100,101) In addition, they have generated a multivariate model, which is supposed to provide more accurate prediction for EGFR-TKI sensitivity than single biomarkers or clinical characteristics (100).

Mass spectrometry is currently the most powerful analytic proteomic tool. Using mass spectrometry Taguchi et al have performed a multicohort cross-institutional study to investigate serum predictive biomarkers for clinical outcome after EGFR-TKIs treatment. They have identified eight distinct peaks and developed an algorithm, which could be used for patients selection and to predict prognosis after EGFR-TKI treatment (102). However, there are some concerns regarding the predictive value because the identities of the eight discriminatory peaks remain unknown and there are no other validation tests performed beyond their laboratory.

DISCUSSION

Identifying a panel of predictive markers is important for selection of advanced NSCLC patients for EGFR-TKI therapy. Although several important demographic and clinical factors are associated with treatment response, EGFR somatic mutations are still the most effective predictor for EGFR-TKI sensitivity. EGFR mutation screening could be number one test to provide the most direct and valuable information to help clinicians to make treatment decision. Among EGFR mutation negative patients, other predictive markers, such as EGFR copy number detected by FISH or K-ras mutation could provide important information in deciding the use of EGFR-TKIs for NSCLC patients.

REFERENCES

Molecular Predictors of EGFR-TKI Sensitivity in Advanced Non-Small Cell Lung Cancer


MOLECULAR PREDICTORS OF EGFR-TKI SENSITIVITY IN ADVANCED NON–SMALL CELL LUNG CANCER


