Driver oncogene mutations and personalized treatment of lung cancer

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Abstract

Discovery of activating mutation of the EGFR gene in 2004 opened the era of personalized therapy in thoracic oncology. These tumors are highly dependent on the EGFR pathway and inhibition of this pathway results in dramatic induction of apoptosis in vitro, even though cancer cells may have various genetic alterations (oncogene addiction). These observations were soon translated into clinical trials, which reproducibly showed significantly longer progression free survival for those treated with EGFR-tyrosine kinase inhibitors (TKI) than those treated with platinum doublet chemotherapy in patients with lung cancer harboring EGFR mutation. In 2007, it was found that ~5% of adenocarcinoma of the lung harbors EML4-ALK translocation and that these tumors are also addicted to the ALK pathway. We have learned how effective the targeted therapy is, when "addicted oncogene" is pharmacologically inhibited. List of addicted oncogenes is expanding continuously and now it includes HER2, ROS1 or RET in adenocarcinoma of the lung.

Introduction

Recent advances in molecular biology have identified several driver oncogene mutations in human lung cancer that are crucial for development and maintenance of malignant phenotype. Those driver mutations include mutations occurring in the genes for epidermal growth factor receptor (EGFR), anaplastic lymphoma kinase (ALK), ROS1, RET, HER2, etc. When these targets are pharmacologically inhibited, growth of cancer cells is effectively impaired and substantial apoptosis is induced in vitro. In patients with EGFR mutation or ALK translocation, it has been shown that progression free survival is significantly longer when the patients are treated respective selective inhibitor of EGFR or ALK than when they are treated with conventional cytotoxic chemotherapy, leading to overall survival of 24+ months. However, resistance is acquired almost inevitably, mechanisms of which are being elucidated. In this review, current status of driver oncogene-targeted therapies of lung cancer is described and also future perspectives are discussed.
Genomic research revealed temporal and spatial heterogeneity

Inter-tumor heterogeneity

Owing to recent advent of massive parallel sequencing technology, comprehensive analysis of human cancer genome is now possible. It was 2010 when the first genome of human adenocarcinoma of the lung was elucidated. In poorly differentiated adenocarcinoma of a 51-year-old male Caucasian patient with smoking history, as many as 530 somatic single nucleotide variants including one in the KRAS proto-oncogene and 391 others in coding regions, as well as 43 large-scale structural variations were identified. These constitute a large set of new somatic mutations and yield an estimated 17.7 per megabase genome-wide somatic mutation rate. In 2012, exome and genome sequences of 183 lung adenocarcinoma tumor/normal DNA pairs were reported. These analyses revealed a mean exonic somatic mutation rate of 11.9 (range 0.04-117.4)/megabase and identified the majority of genes previously reported as significantly mutated in lung adenocarcinoma. In this study, top 10 mutated genes were TP53, KRAS, EGFR, STK11, KEAP1, ATM, NFI, SMARCA4, ARID1A and BRAF. Korean group also reported similar results that known somatic mutations in EGFR, KRAS, NRAS, BRAF, PIK3CA, MET, and CTNNB1 as well as novel mutations in LMTK2, ARID1A, NOTCH2, and SMARCA4 were present in 200 adenocarcinoma of the lung in Korean patients. The two studies share lot of similarity, however it is of interest to note that, unlike in Imielenski’s report, Korean researchers identified 45 fusion genes, 8 of which were chimeric tyrosine kinases involving ALK, RET, ROS1 (three different fusion partners), FGFR2, AXL, and PDGFRA were also found. Of these, last three are novel. Cancer genomes are actually far more complicated than ever expected and also there are considerable heterogeneities in terms of number of genetic alterations as well as mutated genes among different patients.

Intra-tumor or intra-patient heterogeneity

A comprehensive analysis of tumor heterogeneity in renal cell carcinoma using whole-exome sequencing was recently published. Multiple sites of primary tumors, as well as metastatic sites, before and after treatment with everolimus in a single patient were analyzed. As a result, 128 mutations were detected in this particular patient. Surprisingly, only 40 of them were ubiquitously present at every tumor site, and 31 were present mainly in primary tumor sites, 28 were shared only by metastatic sites, and 29 were unique to specific regions (private mutations). Furthermore, when the tumors from four different renal cancer patients were compared, less than one-third of the mutations were ubiquitously present. This study is the first comprehensive analysis on tumor heterogeneity and revealed that tumors are actually more complicated than ever expected. As those authors concluded, intra-tumor heterogeneity can lead to the underestimation of the tumor genomics landscape as portrayed by single-tumor biopsy specimens. In lung cancer, such detailed studies on tumor heterogeneity have not been published. However, considering the complexity of genomes of lung cancer as described above, heterogeneity at least in a degree that was seen in renal cancer should be present in lung cancer.

Oncogene addition

Driver and passenger mutation

Although there are a lot of genetic changes in a single human tumor, it is now known that not all of them are equally important for development and maintenance of cancer. Indeed, it is likely that most of them have made no contribution at all. These mutations, called “passenger mutations” are caused by exposure to mutagens, or genetic instability or many mitoses but they are neutral genetic changes that are unrelated to cancer development. In contrast, one to a few genetic changes are essential for development, growth or survival of cancer cells. In other words, cancer cells are dependent on (addicted to) one or a few oncogenes for the malignant phenotype, even though they may have multiple genetic changes (oncogene addiction). When this addicted gene, “Achilles heel”, is targeted, cancer cells are profoundly inhibited for their growth or survival. This forms theoretical basis of targeted therapy of cancer such as imatinib for chronic myeloid leukemia, or gefitinib or erlotinib for lung cancer, etc.

Heterogeneity of driver oncogene

However, if there is a heterogeneity of mutation of addicted oncogenes, therapeutic effect of
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Targeted drugs would be very limited. Fortunately, not all mutations are that heterogeneous. In the patient with renal cancer exemplified above, mutation of the VHL tumor suppressor gene was present throughout the tumor sites examined. Then what about lung cancer? As discussed later, lung cancer with mutation in the gene encoding epidermal growth factor receptor (EGFR) is addicted to the EGFR signaling and very sensitive to tyrosine kinase inhibitors (TKI) of the EGFR. As in the case of VHL gene mutation, EGFR mutation is not heterogeneous according to detailed analysis by Yatabe et al.7 In this analysis, no discordant EGFR mutation patterns were detected among 77 paired primary and metastatic site samples or among 54 primary and recurrent tumor pairs.7 Furthermore, 3 parts each from 50 lung cancers and 100 parts each from 5 tumors also showed identical EGFR mutation status.7 Thus, the heterogeneity of driver gene mutation, such as that of VHL in renal cell cancer or EGFR in lung cancer, is rare, although there are some conflicting data.

Search for driver oncogenes

Driver gene mutation is a good target for the cancer therapy by its definition. Several empirical approaches can be used to help identify the driver mutations in specific types of human cancer. Since KRAS and EGFR mutations are known to be mutually exclusive, it is postulated, that if the new gene mutations occur in tumors without know driver gene mutations, that mutation can be a new driver gene mutation. Mutations of the ALK, ROS1, HER2 are the cases.8 More directly, screening of libraries of inhibitors9 or short hairpin RNAs10 that specifically inhibit many gene expression may be able to identify proteins on which survival of cancer cells are dependent.

EGFR (Epidermal growth factor receptor)

EGFR biology (Fig.1)

EGFR was originally identified as a 170kDa protein that showed increased phosphorylation when bound to EGF in the A431 squamous cell carcinoma cell line in 1978.11 There is a family of proteins closely related to EGFR. This family consists of EGFR (also known as ERBB1/HER1), ERBB2/HER2/NEU, ERBB3/HER3 and ERBB4/HER4.12 Binding of a family of specific ligands to the extra-cellular domain of ERBB (except for ERBB2) leads to the formation of homodimers and heterodimers.12 ERBB2 does not have corresponding ligands but is a preferred dimerization partner.12 Dimerization consequently stimulates intrinsic tyrosine kinase activity of the receptors and triggers autophosphorylation of specific tyrosine residues within the cytoplasmic regulatory domain. These phosphorylated tyrosines serve as specific binding sites for several adaptor proteins. Several signal transducers then bind to these adaptors to initiate multiple signaling pathways, including mitogen-activated protein kinase, phosphatidylinositol 3-kinase/AKT and the signal transducer and activator of transcription (STAT)3 and STAT5 pathways.12 These eventually result in cell proliferation, migration and metastasis, evasion from apoptosis, or in angiogenesis, all of which are associated with cancer phenotypes.

EGFR as therapeutic target and early clinical trials in lung cancer

Various cancers including non-small cell lung cancer (NSCLC) often overexpress EGFR, and this high expression is usually associated with poor prognosis. Therefore, EGFR is regarded as one promising target for cancer therapy. EGFR-targeted therapy can be classified into two types: small-molecule EGFR tyrosine kinase inhibitors (TKIs) and antibodies against the extracellular domain of the EGFR. EGFR-TKIs, such as gefitinib (Iressa) and erlotinib (Tarceva), were the first targeted drugs to become clinically available for the treatment of NSCLC. They are both in the aniline-quinazoline class of reversible EGFR-TKIs that compete with ATP. During its early clinical development, female patients, those who had never smoked, and those of Asian descent with adenocarcinomas were often found to be remarkably sensitive to EGFR-TKIs.13

However, all four phase III trials that compared chemotherapy with EGFR-TKI to chemotherapy alone were negative.14–17 It was not until the BR.21 was reported at the American Society of Clinical Oncology (ASCO) meeting in 2004 when erlotinib exhibited significant survival advantage in a phase III trial.18 In this trial, patients with NSCLC randomized to erlotinib arm had a median overall survival (OS) of 6.7 months, which was significantly longer than that of placebo (4.7 months).18 By contrast, a similar
Fig. 1 EGFR signal transduction pathway.
Binding of a family of specific ligands to the extra- cellular domain of ERBB (except for ERBB2, see below) leads to the formation of homodimers and heterodimers. This process is mediated by rotation of domains I and II, leading to promotion from a tethered configuration to an extended configuration. This exposes the dimerization domain. Upon ligand binding, kinase domain dimerizes asymmetrically in a tail-to-head orientation. Dimerization consequently stimulates intrinsic tyrosine kinase activity of the receptors and triggers autophosphorylation of specific tyrosine residues within the cytoplasmic regulatory domain. These phosphorylated tyrosines serve as specific binding sites for several adaptor proteins, such as phospholipase Cγ, CBL, GRB2, SHC and p85. For example, tyrosine-X-X-methionine (where X is any amino acid) is a motif for the p85 binding site. Several signal transducers then bind to these adaptors to initiate multiple signalling pathways, including mitogen-activated protein kinase, phosphatidylinositol 3-kinase/AKT and the signal transducer and activator of transcription (STAT) 3 and 5 pathways. These eventually result in cell proliferation, migration and metastasis, evasion from apoptosis, or in angiogenesis, all of which are associated with cancer phenotypes. ERBB3 lacks tyrosine kinase activity because of substitutions in crucial residues in the tyrosine kinase domain. However, it has many binding sites for p85, a regulatory subunit of phosphatidylinositol 3-kinase, and thus is a preferred dimerization partner.

Discovery of EGFR mutation
In 2004, it was found that a subset of lung cancer patients harbors an activating mutation of the EGFR gene. EGFR mutations are found almost exclusively in adenocarcinoma and are more frequent in females, patients who have never smoked, and those of Asian ethnicity. In general, about 40% of East-Asian NSCLC patients and 15% of Caucasian NSCLC patients have EGFR gene mutation. About 90% of these EGFR mutations are either short in-frame deletions in exon 19 (usually deletion of five amino acids) or point mutations that result in a substitution of leucine for arginine at amino acid 858 (L858R).

The most striking discovery was that lung cancers with EGFR mutation are very sensitive to EGFR-TKIs. Cells with EGFR mutation become highly dependent on the EGFR pathway, and cancer cells are vulnerable to inhibition of this pathway despite the presence of other genetic alterations. The mutated version of the EGFR can have ~30 times higher affinity for EGFR-TKIs than does wild-type EGFR. These two facts are considered to form the basis of the sensitivity of EGFR-mutated tumors. Consequently, IC₅₀ values of lung cancer cell lines with EGFR mutation are in the range of several tens of nM, which is several hundredths to thousandths that of wild-type EGFR. Subsequent retrospective and prospective studies showed that the response rate to EGFR-TKIs of patients with EGFR mutation is 70%-80% and...
that, when treated with EGFR-TKIs, patients with EGFR mutation have a significantly longer survival than those with wild-type EGFR.26

Clinical trials with patient selection according to clinical background (Table 1)

The observation that EGFR-TKI trials in unselected patients did not produce satisfactory outcomes led to the clinical trials with patient selection. The IPASS study was the first phase III trial to compare gefitinib with standard chemotherapy as a first-line treatment in which patients were selected by clinical background (i.e., only Asian patients with adenocarcinoma who had never smoked or who smoked very lightly were enrolled).27 Although gefitinib was superior in terms of progression-free survival (PFS), the survival curves crossed at 6 months; chemotherapy was better initially but gefitinib therapy was better later.27 Molecular subset analysis that included one-third of the patients showed that the benefit was limited to patients with EGFR mutation and that gefitinib treatment was detrimental for those without a mutation. This observation resulted in the general consensus that the presence of EGFR mutation is a predictive marker for the outcome of EGFR-TKI treatment.27 Similarly First-SIGNAL trial for patients with no smoking history showed the similar results.28

Clinical trials with patient selection according to EGFR mutation (Table 1)

Subsequently, two Japanese trials (NEJ00229 and WJTOG340530) selected patients according to the presence of EGFR mutation. The PFS of patients with EGFR mutation treated with gefitinib was around 10 months, whereas the PFS for those treated with platinum doublet chemotherapy was around 6 months. These trials clearly confirmed that the determinant of clinical efficacy is the presence of EGFR mutation and not the clinical background of the patient. Subsequently, results of the OPTIMAL study,31 EURTAC study32 that used erlotinib and Lux-Lung 3 study that used afatinib33, an irreversible EGFR-TKI, for patients with lung cancer harboring EGFR mutation were published. However, to our disappointment, in any of these trial, the large difference in PFS was not translated into a significant difference in OS. This was explained by the fact that a significant proportion of the patients in the chemotherapy arm received EGFR-TKI upon progression. However, considering that median survival time (MST) of ECOG 1594 published in 2002 comparing four different platinum double chemotherapy ranged from 7.4 to 8.1 months34, MST > 24 months as shown in the table is really amazing.

<table>
<thead>
<tr>
<th>Study</th>
<th>Patient group</th>
<th>EGFR-TKI</th>
<th>PFS(months)</th>
<th>HR for OS(months)</th>
<th>OS(months)</th>
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<tr>
<td></td>
<td></td>
<td>N TKI Chemotx</td>
<td>TKI Chemotx</td>
<td>PFS(95%CI)</td>
<td>TKI Chemotx</td>
</tr>
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<td>Subset analyses of patients selected by clinical background</td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>IPASS</td>
<td>Asian, light-non-smoker, adenocarcinoma</td>
<td>gefitinib</td>
<td>261 9.5 6.3</td>
<td>0.48(0.36-0.64)</td>
<td>21.6 21.9</td>
</tr>
<tr>
<td>First SIGNAL</td>
<td>Korean, non-smoker, adenocarcinoma</td>
<td>gefitinib</td>
<td>42 8.4 6.7</td>
<td>0.61(0.31, 1.22)</td>
<td>30.6 26.5</td>
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<tr>
<td>Phase III trials for patients selected by EGFR mutation</td>
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</tr>
<tr>
<td>NEJ002</td>
<td>Japanese,</td>
<td>gefitinib</td>
<td>228 10.8 5.4</td>
<td>0.32(0.24-0.44)</td>
<td>27.7 26.6</td>
</tr>
<tr>
<td>WJTOG3405</td>
<td>Japanese,</td>
<td>gefitinib</td>
<td>172 9.6 6.6</td>
<td>0.52(0.38-0.72)</td>
<td>35.5 38.8</td>
</tr>
<tr>
<td>OPTIMAL</td>
<td>Chinese</td>
<td>erlotinib</td>
<td>154 13.7 4.6</td>
<td>0.16(0.10-0.26)</td>
<td>22.7 28.9</td>
</tr>
<tr>
<td>EURTAC</td>
<td>Caucasian</td>
<td>erlotinib</td>
<td>173 9.7 5.2</td>
<td>0.37(0.25-0.54)</td>
<td>19.3 19.5</td>
</tr>
<tr>
<td>Lux Lung 3</td>
<td>Caucasian 26% Asian 72%</td>
<td>afatinib</td>
<td>345 11.1 6.9</td>
<td>0.58(0.43-0.78)</td>
<td>N/A N/A</td>
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</tbody>
</table>
ALK (Anaplastic lymphoma kinase)

Discovery of ALK translocation

In 2007, Soda et al. identified the gene resulting from the fusion of that for EML4 (echinoderm microtubule-associated protein-like 4) and the gene for ALK (anaplastic lymphoma kinase) as a transforming activity in mouse 3T3 fibroblasts from DNA of lung cancer in a Japanese man with a smoking history. In the same year, Rikova et al., also reported presence of EML4-ALK in lung cancer by mass spectrometric analysis of phosphotyrosine immunoprecipitated from lung cancer specimens. Like EGFR, ALK is also receptor tyrosine kinase (RTK) whose ligand is not very well known. This EML4-ALK fusion gene results from a small inversion within chromosome 2p (Fig.2). By fusing the coiled-coil domain of EML4 with the kinase domain of ALK, the ALK protein dimerizes without ligand binding, leading to oncogenic activation. Subsequently, various vari-

Fig. 2  Mechanism of activation of ALK in lung cancer

Fig. 3  Variants of ALK fusion partners (modified from Sasaki et al. and Horn et al.)
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Other driver oncogenes in lung cancer

**HER2**

Activating mutation in the kinase domain of the human HER2/EGFR/NEU gene is present in about 3% of adenocarcinoma of the lung. As in the case of EGFR mutation, HER2 mutations are more frequent in never-smoking females with adenocarcinoma and have mutual exclusive relationship with other driver gene mutations such as EGFR, ALK, or KRAS. HER2 mutations mainly occur as a small insertion in exon 20, especially four amino acid insertion at codon 776 (YVMA-776779ins). Lung cancer with HER2 mutations shows high sensitivity to HER2-TKI. There are two case reports that described clinical activity of trastuzumab (anti Her2 ntibody) for patients with HER2+ lung cancer. De Greve et al. reported that objective response was obtained in three of three patients with lung cancer harboring HER2 mutation treated with afatinib, an irreversible ERBB family blocker. However, recent phase II study of dacomitinib, another irreversible pan HER inhibitor, is disappointing with a response in 3 of 18 patients with HER2 mutation.

**ROS1**

In 2007, Rikova et al., described ROS1 fusion in adenocarcinoma of the lung for the first time. ROS1 is a sevenless superfamily of RTK. They collected proteins with phosphotyrosine by immunoprecipitation in 41 lung cancer cell lines and 150 tumors and then these proteins were analyzed by mass spectrometry. In addition to known activation of EGFR or MET gene product, they identified 2 ROS1 fusions (with SLC34A2 and CD74 each) as well as ALK fusions. In 2012, using a ROS1 FISH (fluorescent in-situ hybridization) assay, Berghenthon et al., screened 1,073 patients with NSCLC and found that 18 (1.7%) harbored rearranged ROS1. Patients with ROS1 rearrangements were significantly younger and more likely to be never-smokers. The HCC78, ROS1-rearranged NSCLC cell line, and 293 cells transfected with CD74-ROS1 showed evidence of sensitivity to crizotinib that also has ROS1-TKI activity. The patient treated with crizotinib showed tumor shrinkage, with a near complete response. Takeuchi et al., subsequently identified 13 ROS1 fusion positive adenocarcinoma. In addition to previous known fusion partners (i.e. CD74 and...
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SLC34A2), they identified TPM3, SDC4, EXR and LRIG3 as fusion partners of ROS1. In a phase II study of crizotinib which also possesses ROS1 inhibitory effect for 13 patients with ROS1 rearrangement, response rate was 54% (7/13) with a median duration of treatment of 20 weeks.54

RET
RET gene also encodes for a RTK and is known to be frequently mutated in medullary thyroid cancer and have translocation resulting in fusion proteins in papillary thyroid cancer. Korean researchers55 followed by two Japanese and one US groups42'56'57 reported that RET translocations similar to those of ALK or ROS1 are present in ~1% of adenocarcinoma of the lung. The fusion partner is mostly KIF5B also known as an occasional fusion partner for ALK and is rarely CCDC6 which is a common fusion partner in thyroid cancer, NCOA4,58 or TRIM33.59 As expected, lung cancer cells with RET fusion are sensitive to RET inhibitor such as vandetanib, sorafenib or sunitinib at least in vitro.56'57 Recently, preliminary data for the first three patients with RET fusion-positive lung cancer treated with the RET inhibitor cabozantinib (XL-184) was reported. Confirmed partial responses in two patients and prolonged stable disease approaching of 31 weeks were observed.59

MET
MET is RTK for hepatocyte growth factor (HGF). Activation of MET signaling leads to epithelial-mesenchymal transition, cell scattering, angiogenesis, proliferation, enhanced cell motility, invasion, and metastasis.60 MET gene can be activated either by amplification or mutation. Amplification of the MET gene in untreated lung cancer is reported to occur in ~5% of adenocarcinoma,61 while mutation of the MET is found about 3%.62–63 However, in contrast to sporadic papillary-type renal cell carcinomas, childhood hepatocellular carcinomas, and head and neck squamous cell carcinomas where MET mutations occur in the tyrosine kinase domain, MET mutations in lung cancer are mostly splice mutations that result in deletion of exon 14 encoding the juxtamembrane domain.61–63 This juxtamembrane domain is required for the binding with c-Cbl E3-ligase that leads to ubiquitination and receptor degradation. Therefore, deleting this domain is expected to cause overexpression of MET. However, when sensitivity to MET-TKI was compared among several cell lines with different MET status, presence of the MET mutation was not associated with sensitivity.64,65 Rather, presence of MET amplification but not MET overexpression is correlated with sensitivity.64,65 Ou et al., reported a patient with MET amplification who showed a dramatic response upon treatment of crizotinib, an ALK-MET-ROS1 inhibitor.66

KRAS
The KRAS gene encodes for a protein with GTPase activity and oncogenic mutation results in impairment of this enzymatic activity, conferring for KRAS to remain its GTP bound active state. KRAS is the oldest known oncogene and is frequently mutated especially in Caucasian patients compared to Asian patients with adenocarcinoma of the lung (~30% vs. ~10%). However, targeted therapy against mutated KRAS has been unsatisfactory. One reason for this may be the fact that KRAS selective inhibitor is difficult to develop because there are many G-proteins which are important to normal physiologic function and the other is that these tumors are not very dependent on the mutated KRAS. However, not all cancers with KRAS mutations may not be refractory to KRAS inhibition; shRNA targeting KRAS revealed that half of the lung cancer cell lines are resistant to KRAS knockdown, while the other half are not. Further analysis revealed that epithelial mesenchymal transition (EMT) is a characteristic phenotype of KRAS independency.67 Early clinical trials for the development of farnesyl transferase inhibitors, which were expected to inhibit posttranslational processing and membrane localization of KRAS proteins has been unsuccessful.68 Screening of 12 inhibitors in 84 genomically validated cell lines identified that KRAS mutant cells conferred enhanced heat shock protein (HSP) 90 dependency that was also confirmed in vivo.69 In 2012, the results of randomized phase II trial comparing selumetinib (SEL) (AZD6244, ARRY-142866) that inhibits MEK1/2 signaling plus docetaxel (DOC) with docetaxel plus placebo for patients with lung cancer harbouring KRAS mutation who had received prior chemotherapy, was presented. Overall survival was longer for SEL/DOC vs DOC (9.4 mo vs 5.2 mo)
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but did not reach statistical significance. However, response rate (DOC 0%, SEL/DOC 37%; p < 0.0001) and progression free survival (DOC 2.1 mo, SEL/DOC 5.3 mo, 1-sided p = 0.0138), were significantly improved for SEL/DOC. It is noteworthy that this is the first prospective study to demonstrate a clinical benefit of a targeted therapy for patients with KRAS mutant cancer.

**BRAF**

BRAF lies downstream of KRAS, and directly phosphorylates MEK, which in turns phosphorylates ERK. In lung cancer, BRAF mutation is present in 1% to 4% of adenocarcinoma. In contrast to melanoma where BRAF mutation is frequent and ~90% of BRAF mutation is V600E, this is detected in only half of NSCLC patients with BRAF mutation. BRAF mutations occur most often in former or current smokers compared with patients never smoked. In melanoma with BRAF mutation, selective inhibitors of BRAF (vemurafenib) or MEK (trametinib) have been shown to improve clinical outcome. One patient with lung cancer harboring BRAF V600E is reported to obtain partial response to another BRAF-TKI, debrafenib. However, her tumor recurred after 8 months. Interestingly, the recurrent tumor had three acquired mutations in *KRAS*, *CDKNA2*, and *TP53* genes in addition to original BRAF V600E.

**Driver oncogenes in Squamous cell carcinoma of the lung**

So far, all of successes of targeted therapy aimed at driver oncogene have been derived from adenocarcinoma of the lung. However, recent efforts have revealed that lung squamous cell carcinomas also have potentially “druggable” mutations that are crucial for maintenance of malignant phenotype. These genetic alterations include *FGFR1* (fibroblast growth factor receptor 1) amplification, *PIK3CA* (phosphatidylinositol 3 kinase) mutation, *PDGFRα* (platelet derived growth factor receptor alpha) amplification, or *DDR2* (discoidin domain RTK 2) mutation. (Table 2) Above all, DDR2 mutations are present in 4% of lung squamous cell lung carcinoma, and DDR2 mutations are associated with sensitivity to dasatinib both in vivo and in a patient.

| Table 2 Driver oncogenes identified human squamous cell carcinoma of the lung. |
|----------------------|----------------------|-------------|
| Gene                | Genetic change       | Frequency |
| FGFR1               | Amplification        | 20-25%     |
| FGFR2               | Mutation             | 5%         |
| PIK3CA              | Mutation             | 9%         |
| PTEN                | Mutation/Deletion    | 18%        |
| CCND1               | Amplification        | 8%         |
| CDKN2A              | Deletion/Mutation    | 45%        |
| PDGFRA              | Amplification/Mutation | 9%      |
| EGFR                | Amplification        | 10%        |
| EGFRvIII            | Large deletion       | 5%         |
| BRAF                | Mutation             | 3%         |
| DDR2                | Mutation             | 4%         |
| ERBB2               | Amplification        | 2%         |

**Acquired resistance**

**Clinical definition**

Although targeted therapy to addicted oncogene initially shows a dramatic response, acquired resistance inevitably develops after a median of about 10 months. Clinical definition of this acquired resistance is proposed by Jackman et al.; Le; 1) previous treatment with a single-agent EGFR TKI (eg, gefitinib or erlotinib), 2) either or both of the following: a tumor that harbors an EGFR mutation known to be associated with drug sensitivity or objective clinical benefit from treatment with an EGFR TKI, 3) systemic progression of disease (Response Evaluation Criteria in Solid Tumors (RECIST) or WHO) while on continuous treatment with gefitinib or erlotinib within the last 30 days, 4) and no intervening systemic therapy between cessation of gefitinib or erlotinib and initiation of new therapy. This relatively simple definition is expected to lead to a more uniform approach to investigating this problem. **Mechanisms responsible for acquired resistance**

The mechanisms responsible for this resistance can be classified in 4: 1) the emergence of a single recurrent missense mutation within the kinase domain of the target gene (the so-called gatekeeper mutation) that interferes with the drug’s ability to interact with a target, 2) activation of additional kinases that bypass inhibitory
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1) Gatekeeper mutation: When secondary mutation that changes threonine to methionine at codon 790 of the EGFR gene (T790M) or leucine to methionine at codon 1196 of the ALK gene (L1196M) occurs, affinity between kinase and ATP exceeds that between kinase and TKI. Therefore, although the tumor is still dependent on the original kinase, downstream signal is no more inhibited and resistance emerges. 2) Bypass mechanism: sometimes other kinase is activated and it bypasses the original inhibition. In the case of EGFR, activation of MET, AXL, HER2 by gene amplification or other mechanism or upregulation of IGFR via IGF binding protein have been reported. In addition, role of HGF, a ligand to MET, is proposed. In the case of ALK, activation of EGFR or KIT by mutation, overexpression or amplification have been reported. 3) Inactivation of downstream negative regulator: Inactivation of PTEN, a phosphatase that inactivate PI3K, has been reported both in vitro and in a patient as a resistance mechanism of EGFR-TKI. 4) Activation of direct downstream molecule: Point mutation of PI3K or MAPK in the case of EGFR and KRAS mutation in the case of ALK are classified in this category.

Fig. 5 Incidence of mechanisms of acquired resistance to 1) EGFR-TKI and 2) ALK-TKI.

Effect of TKI on the original kinase and re-activate downstream signaling in the presence of TKI, 3) inactivation of negative effector of downstream signaling, and 4) activation of a molecule downstream to the original target. It has been shown that about half of the mechanisms for acquired resistance to EGFR-TKI is due to T790M gatekeeper mutation and MET.
amplification only accounts for ~5% of acquired resistance (Fig. 5).82,83 Small cell transformation with or without PI3K mutation82, epithelial mesenchymal transition84 are also proposed as a mechanism of resistance.

Similar but not identical mechanisms have been found in the case of treatment of crizotinib for ALK+ lung cancer (Fig. 5). L1196M secondary gate-keeper mutation was the first that causes acquired resistance to crizotinib. Since leucine at codon 1196 of ALK corresponds to threonine at codon 790 of EGFR by sequence homology, L1196M is expected to confer resistance through mechanisms similar to T790M of the EGFR gene. In contrast to the fact that the secondary mutations other than T790M is extremely rare in the case of EGFR gene, it does not appear that L1196M is a single dominant form since several other mutations have been reported such as 1151Tins, L1152R, C1156Y, F1174C/L, G1202R, D1203N, S12036Y, and G1269A.85 In addition, ALK amplification has been proposed as one of resistant mechanisms. Activation of EGFR, KRAS, c-KIT have been also reported as ALK-independent resistance mechanisms.86,87

**Overcoming acquired resistance (Fig. 6)**

Several strategies have been proposed and tested to overcome acquired resistance caused by EGFR-TKI. In clinical practice, EGFR-TKI is usually converted to cytotoxic chemotherapy. In the NEJ002 trial that compared gefitinib with carboplatin/paclitaxel in the first line setting, as high as 65% of the patient received any chemotherapy and 62% of them was platinum-based as the second line treatment in patients originally allocated to the gefitinib group.88 However, it is shown that in patients who develop acquired resistance, stopping erlotinib or gefitinib results in symptomatic progression, increase in SUV (max), and increase in tumor size, suggesting that some tumor cells remain sensitive to epidermal growth factor receptor blockade.89 This phenomenon often termed as “disease flare” is reported to occur in 14 of 61 patients (23%), when disease flare is defined as hospitalization or death attributable to disease progression.90 Disease flare is also experimentally mimicked and the data suggested that patients may benefit from continued treatment with an EGFR TKI, even after developing T790M-mediated resistance.91 and this concept is being tested in several clinical trials, including “IMPRESS” in which patient with acquired resistance to gefitinib are randomized between cisplatin/pemetrexed and cisplatin/pemetrexed plus gefitinib (ClinicalTrials.gov, number NCT01544179).

**Mechanism dependent overcoming**

It would be nicer, however, if we could cope with acquired resistance according to the underlying molecular mechanism. To overcome T790M gatekeeper mutations, “irreversible” EGFR inhibitors were developed. These irreversible EGFR inhibitors such as afatinib or dacomitinib form a covalent bond to cysteine 797 of EGFR and show in vitro activity in cancer cells with T790M resistant mutations at a clinically achievable concentration.92,93 They are also active in cancer harboring exon 20 insertion mutations that are resistant to EGFR-TKIs.

![Fig. 6 Strategies to overcome acquired resistance to EGFR-TKI.](image-url)
gefitinib, and erlotinib. However, these compounds are more active for wild-type EGFR than EGFR with T790M, because the IC50 of these compounds for wild-type EGFR is lower than that for EGFR T790M. Therefore it is anticipated that dose limitation due to inhibition of wild-type EGFR would result in inadequate clinical activity against cancer cells harboring T790M. Indeed, in Lux-Lung I study for patients clinically enriched for EGFR mutation who failed gefitinib or erlotinib after initial successful treatment, there was no overall survival advantage for patients treated with afatinib over those treated with placebo.

3rd generation EGFR-TKI

To overcome this drawback, chemical libraries were screened to find one that selectively inhibits EGFR-T790M while sparing the wild-type T790M. A covalent pyrimidine EGFR inhibitor, WZ4002, showed 30- to 100-fold more potency against EGFR T790M and up to 100-fold less potent against wild-type EGFR than are quinazoline-based EGFR inhibitors. Although no results of clinical trials using this compound have been reported, CO-1686, similarly identified so-called 3rd generation EGFR-TKIs, is currently being assessed in clinical trials. (http://www.clovisoncology.com/products-companion-diagnostics/co-1686/)

Afatinib in combination with cetuximab

Anti-EGFR antibodies bind to EGFR and induce endocytosis and depletion of phosphorylated and total EGFR from the cell surface. Indeed, the combination of an irreversible EGFR-TKI, afatinib with cetuximab was assessed in vivo and achieved marked shrinkage of erlotinib-resistant tumors with T790M. Importantly, this result suggests that most of the tumors are still addicted to the EGFR pathway even after acquiring resistance. Following this result, a phase I/II study of afatinib in combination with cetuximab was conducted enrolling 100 patients with clinically defined acquired resistance to EGFR therapy. Disease control rate and response rate were 94% and 40%, respectively. Interestingly, the response was similar in both T790M+ (38%) and T790M- (47%) tumors. However, median progression-free survival was 4.7 months.

Other approaches to acquired resistance

Tumor resistance caused by MET amplification may be treated with combined treatment of EGFR-TKI plus MET-TKI or MET antibodies. Heat shock protein 90 (Hsp90) is a 90 kDa molecular chaperone that is required for folding, stabilization and functioning of proteins including several oncogene products such as EGFR, MET and EML4-ALK. Several studies suggested

Fig. 7 Phenotypic changes in HCC827 lung cancer cells from “oncogene addiction” to “acquired resistance” and further to “drug addiction” during adaptation to intensive EGFR and MET kinase inhibition.
that HSP90 inhibition may be beneficial for patients with acquired resistance to EGFR-TKI\textsuperscript{99–101}

Local therapy as countermeasure for acquired resistance

It is also predicted that even when patients with acquired resistance are treated with inhibitors that effectively target respective resistant mechanisms, cancer cells are smart enough to escape it using another mechanism (Fig. 7). Therefore, genetic heterogeneity that involves driver gene mutations becomes more evident when specific therapeutic pressure is present,\textsuperscript{102} and this should be kept in mind when making treatment strategy. It is suggested that this tumor heterogeneity may account for the benefit of local therapy including surgery in selected patients by eliminating an evolutionary reservoir of phenotypic tumor cell diversity. Yu et al. recently studied 18 patients with EGFR mutation who received elective local therapy for tumors with acquired resistance.\textsuperscript{103} Among these 18 patients, 13 underwent surgical resection (11 lung and two adrenal resections).\textsuperscript{103} The median overall survival from local therapy was as amazingly long as 41 months. Of course, these patients constitute a highly selected group of patients with tumors of a more indolent nature. However, that report clearly indicates that local therapies can be a useful treatment option for these patients. Roles of surgery in the era of personalized medicine is reviewed in our recent article.\textsuperscript{104}

Conclusion

I have described biology of driver oncogenes in lung cancer and current situation of clinical development of new treatment strategies. In 2000, lung cancer was either small cell or non-small cell lung cancer. However, it is now clear that NSCLC is not a one-size-fits-all disease but that there are many NSCLC subsets with specific genetic alterations that are critical to the growth and survival of these cancers with profound therapeutic implication (Fig. 8) Lung cancer still remains the leading cause of cancer deaths worldwide. However, through these efforts, we will be able to considerably prolong patient survival making lung cancer more chronic disorder, and in someday we may be able to cure it.

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