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journal or publication title	Cancer Science
volume	103
number	7
page range	1189-1194
year	2012-07-01
URL	http://hdl.handle.net/2297/45963

doi: 10.1111/j.1349-7006.2012.02279.x

Review Article

Ligand-triggered resistance to molecular targeted drugs in lung cancer: Roles of hepatocyte growth factor and epidermal growth factor receptor ligands

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(Received February 5, 2012/Revised March 7, 2012/Accepted March 13, 2012/Accepted manuscript online March 21, 2012/Article first published online May 9, 2012)

Recent advances in molecular biology have led to the identification of new molecular targets, such as epidermal growth factor receptor (*EGFR*) mutations and echinoderm microtubule-associated protein-like 4 (*EML4*) – anaplastic lymphoma kinase (*ALK*) fusion gene, in lung cancer. Dramatic response has been achieved with *EGFR* inhibitors (gefitinib and erlotinib) and an *ALK* inhibitor (crizotinib) in lung cancer expressing corresponding targets. However, cancer cells acquire resistance to these drugs and cause recurrence. Known major mechanisms for resistance to molecular targeted drugs include gatekeeper mutations in the target gene and activation of bypass survival signal via receptors other than the target receptors. The latter mechanism can involve receptor gene amplification and ligand-triggered receptor activation as well. For example, hepatocyte growth factor (HGF), the ligand of a tyrosine kinase receptor Met, activates Met and the downstream PI3K/Akt pathway and triggers resistance to *EGFR* inhibitors in *EGFR* mutant lung cancer cells. Moreover, *EGFR* ligands activate *EGFR* and downstream pathways and trigger resistance to crizotinib in *EML4-ALK* lung cancer cells. These observations indicate that signals from oncogenic drivers (*EGFR* signaling in *EGFR*-mutant lung cancer and *ALK* signaling in *EML4-ALK* lung cancer) and ligand-triggered bypass signals (HGF-Met and *EGFR* ligands-*EGFR*, respectively) must be simultaneously blocked to avoid the resistance. This review focuses specifically on receptor activation by ligand stimulation and discusses novel therapeutic strategies that are under development for overcoming resistance to molecular targeted drugs in lung cancer. (*Cancer Sci* 2012; 103: 1189–1194)

Lung cancer is the leading cause of cancer death worldwide. Several oncogenic drivers, including epidermal growth factor receptor (*EGFR*) mutations^(1,2) and echinoderm microtubule-associated protein-like 4 (*EML4*) – anaplastic lymphoma kinase (*ALK*) fusion gene,⁽³⁾ have been recently discovered in lung cancer. Dramatic results have been achieved with *EGFR* inhibitors^(4,5) and an *ALK* inhibitor⁽⁶⁾ in lung cancer expressing corresponding targets, and a paradigm shift in treatment is occurring. However, some patients who have responded to those inhibitors acquire resistance to them within a few years and experience the recurrence of cancer.^(7,8) This clinical issue now needs to be resolved.

A gatekeeper mutation occurring at the binding site of a molecular targeted drug is a typical mechanism for development of acquired resistance.⁽⁹⁾ Another crucial mechanism is bypass signals to prevent apoptosis.⁽¹⁰⁾ In addition to receptor gene alterations (e.g. gene mutations and gene amplification)

besides target genes, activation of receptors by ligands plays an important role in bypass signaling.⁽¹¹⁾ In recent years, vigorous attempts have been made to overcome resistance through therapies combining drugs that alleviate resistance due to gatekeeper mutations and drugs that block bypass signals. This paper reviews resistance to molecular targeted drugs caused by receptor activation as a result of ligand stimulation. Using lung cancer as an example, this paper describes the molecular mechanisms for that resistance and therapies to overcome it.

Oncogenic Drivers of Lung Cancer

Alteration in genes such as *KRAS*,⁽¹²⁾ *EGFR*,^(1,2) *EML4-ALK*,⁽³⁾ *DDR2*,⁽¹³⁾ and *ROS*⁽¹⁴⁾ are known to be oncogenic drivers for lung cancer. Selective inhibitors of *EGFR* gene mutations and the *EML4-ALK* fusion gene have been developed and the clinical efficacy of these drugs has been demonstrated,^(4–6,15) resulting in their routine clinical use. *EGFR* gene mutations such as exon 19 deletion and a point mutation in L858R in exon 21 cause activation of *EGFR*; the two account for 90% or more of the *EGFR* gene mutations overall.⁽¹⁶⁾ Lung cancers with such *EGFR*-activating mutations respond to a reversible *EGFR*-tyrosine kinase inhibitors (TKIs) such as gefitinib or erlotinib at a rate of 70–80%.⁽⁷⁾ Recent phase III clinical trials revealed that *EGFR*-TKI therapy to treat lung cancer with *EGFR*-activating mutations resulted in a median survival time (MST) of about 30 months.^(4,5) This is clearly a breakthrough in treatment, given that the usual MST for non-small cell lung cancer is 10–14 months.^(17,18) *EML4-ALK* is a fusion gene that was first discovered as a factor for the development of lung cancer, which are considered typical solid tumors.⁽³⁾ *EML4-ALK* is found in about 5% of cases of pulmonary adenocarcinoma. *EML4-ALK* lung cancer is highly prevalent in patients with pulmonary adenocarcinoma who are never or light smokers and aged 50 and under and rarely found in patients with *EGFR*-activating mutations or *KRAS* mutations.^(3,19) Histologically, the cancer often produces an acinar pattern.⁽¹⁹⁾ The cancer responds well (60% and more) to crizotinib,^(6,8) which has *ALK*-inhibiting activity, and has a prognosis on par with *EGFR*-mutant lung cancer treated with *EGFR*-TKI therapy.

Nevertheless, most patients who have lung cancer with *EGFR*-activating mutations and who have undergone *EGFR*-TKI therapy or who have *EML4-ALK* lung cancer and who have undergone *ALK* inhibitor therapy acquire resistance after several years and experience recurrence of the cancer. In

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addition, 20–30% of patients fail to respond to the EGFR-TKI or ALK inhibitor despite having *EGFR* mutations or the *EML4-ALK* fusion gene. In other words, patients with initial resistance pose a clinical problem.

Major Mechanisms for Resistance to Molecular Targeted Drugs in Lung Cancer

Known mechanisms for resistance to targeted drugs include gene mutations produced at the drug's binding site (gatekeeper mutations, such as *EGFR*-T790M and *ALK*-L1196M),^(20–22) activation of bypass signals via receptors other than the target receptors (Met activation in EGFR-TKI resistance),^(11,23) target gene amplification (*ALK* amplification in ALK-TKI resistance),⁽²⁴⁾ and activation downstream of the target.^(25–27) The activation of receptors other than the target receptors can involve amplification of receptor genes or ligand stimulation. This paper focuses specifically on receptor activation by ligand stimulation and reviews recent findings.

Mechanisms of Resistance to EGFR-TKIs

Epidermal growth factor receptor-mutant lung cancer is extremely sensitive to gefitinib and erlotinib. Table 1 shows the major resistance mechanisms to EGFR-TKIs. Hepatocyte growth factor (HGF),^(28,29) a ligand of the tyrosine kinase receptor Met, has been found to bind to Met and activate the phosphatidylinositol 3-kinase (PI3K)/Akt pathway, thereby inducing EGFR-TKI resistance⁽¹¹⁾ (Fig. 1). Hepatocyte growth factor is a factor produced by lung cancer cells^(30,31) as well as by stromal cells (microenvironments) such as fibroblasts.⁽³²⁾ Hepatocyte growth factor is involved in the carcinogenesis,⁽³³⁾

invasion/motility,⁽³⁴⁾ epithelial-to-mesenchymal transition (EMT),⁽³⁵⁾ angiogenesis,⁽³⁶⁾ and metastasis⁽³⁷⁾ in lung cancer, and therefore associates with poor prognosis of the patients^(38,39) (Fig. 2). Moreover, regardless of whether it is produced by cancer cells or fibroblasts, HGF induces EGFR-TKI resistance.⁽⁴⁰⁾ None of the other receptor ligands, including EGF, transforming growth factor (TGF)- α , and insulin-like growth factor (IGF)-1, could induce EGFR-TKI-resistance in *EGFR* mutant lung cancer cells,^(11,41) indicating that EGFR-TKI resistance is induced selectively by HGF.

To determine the clinical significance of resistance triggered by HGF, a joint study of Japanese patients with *EGFR*-mutant lung cancer was conducted at 12 facilities.⁽⁴²⁾ The study analyzed the prevalence of HGF, T790M, and *Met* amplification in tissues from patients with EGFR-TKI resistance (Fig. 3). Of 23 tumors with acquired resistance, 14 had high levels of HGF expression (61%), 12 had T790M (52%), and two had *Met* amplification (9%). High levels of HGF expression were detected most often. Of 45 tumors that did not respond to EGFR-TKI despite having *EGFR* mutations, 13 had high levels of HGF expression (29%), 0 had T790M (0%), and two had *Met* amplification (4%). High levels of HGF expression were again detected most often. Results thus suggested that HGF induces acquired and intrinsic resistance to EGFR-TKI and is the most prevalent factor for resistance, at least in Japanese patients with *EGFR*-mutant lung cancer. Therefore, HGF is an important therapeutic target for overcoming EGFR-TKI resistance in *EGFR* mutant lung cancer.

In all of the patients with acquired resistance, HGF originated from cancer cells. Interestingly, two of 13 patients with intrinsic resistance had high levels of HGF (15%) expressed by stromal cells rather than cancer cells. Expression of HGF is

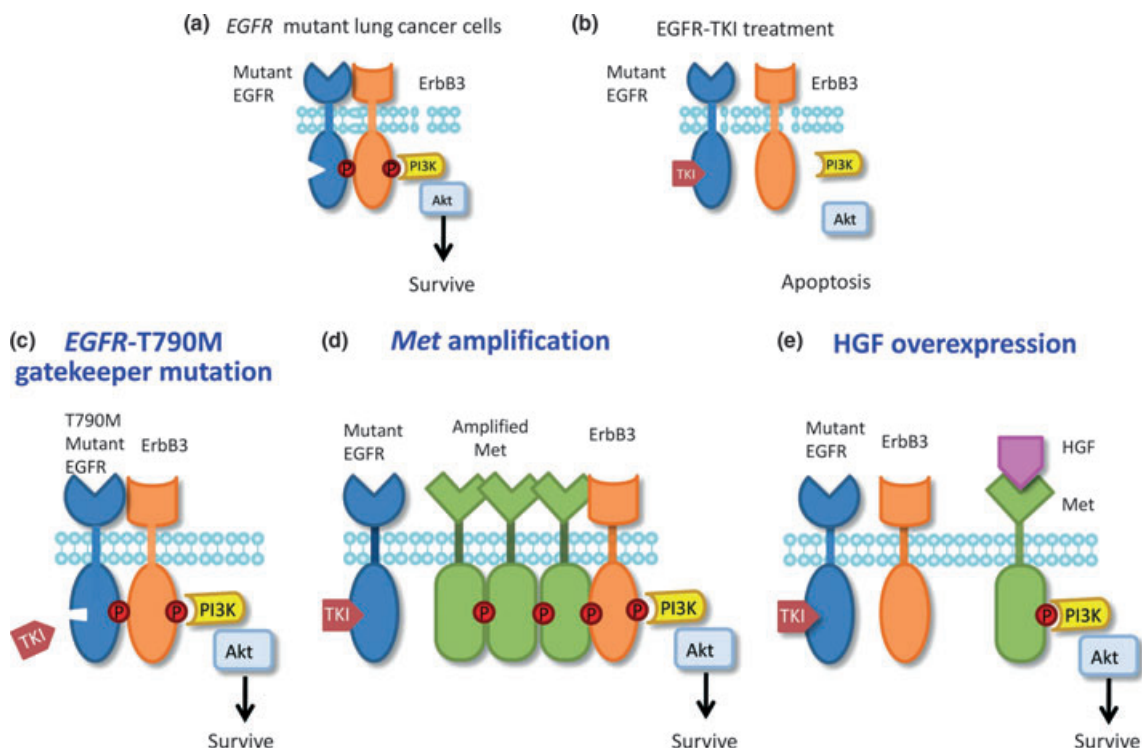


Fig. 1. Resistance signals to epidermal growth factor receptor-tyrosine kinase inhibitors (EGFR-TKIs) in *EGFR* mutant lung cancer cells. (a) Mutant EGFR associates with ErbB3 and transduces survival signal through PI3K/Akt pathway. (b) EGFR-TKIs, such as gefitinib and erlotinib, bind to tyrosine kinase domain of mutant EGFR and shut off the signal and induce apoptosis. (c) *EGFR*-T790M gatekeeper mutation results in preventing EGFR-TKIs to bind EGFR, and thereby induces resistance. (d) Amplified *Met* associates with ErbB3, transactivates the downstream signaling pathway, PI3K-Akt, and thereby induces resistance. (e) Hepatocyte growth factor (HGF) phosphorylates Met and activates PI3K-Akt pathway, independent of EGFR or ErbB3, and thereby induces resistance. "P" indicates phosphorylation.

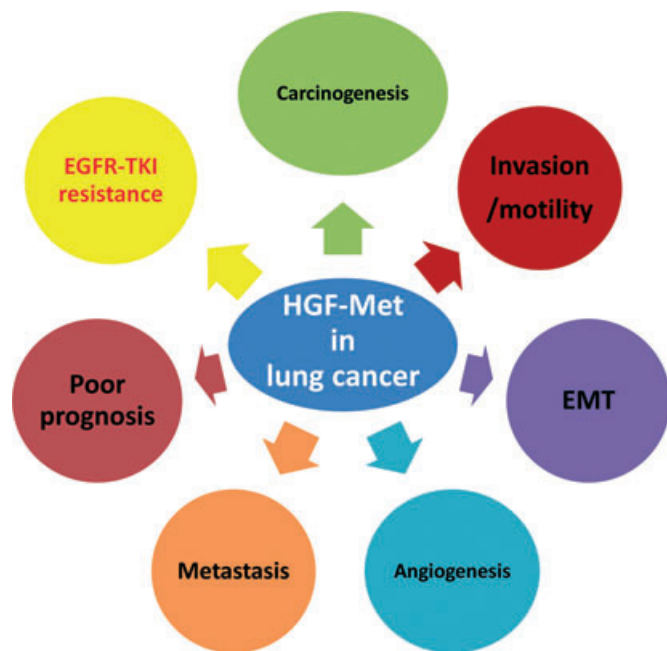


Fig. 2. Role of hepatocyte growth factor (HGF)-Met in lung cancer. Hepatocyte growth factor is involved in the carcinogenesis, invasion/motility, epithelial-to-mesenchymal transition (EMT), angiogenesis, and metastasis, and therefore associates with poor prognosis of lung cancer. Moreover, HGF triggers resistance of *EGFR* mutant lung cancer cells to reversible epidermal growth factor receptor-tyrosine kinase inhibitors (EGFR-TKIs), irreversible EGFR-TKIs, and mutant EGFR selective TKIs.

Table 1. Major mechanisms of epidermal growth factor receptor-tyrosine kinase inhibitor (EGFR-TKI) resistance in *EGFR* mutant lung cancer

	Acquired resistance	Intrinsic resistance	Reversible resistance	Reference
Alteration of target gene				
Gatekeeper mutation (secondary mutation:T790M)	○		?	20, 21
Activation of bypass signal				
Receptor gene amplification (Met)	○			23
Activation of ligand (HGF)	○	○	?	11
Receptor activation by epigenetic mechanism			○	50
Alteration of downstream				
PIK3CA mutation	○			26
PTEN loss	○			25
BIM suppression		○		27
Others				
SCLC transformation	○			26
EMT	○			26

○, involved. EMT, epithelial-to-mesenchymal transition; HGF, hepatocyte growth factor; PTEN, phosphatase and tensin homolog; SCLC, small cell lung cancer.

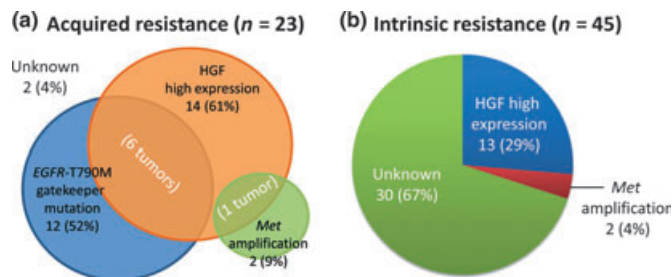


Fig. 3. Incidence of resistance factors in *EGFR* mutant lung cancer resistant to epidermal growth factor receptor-tyrosine kinase inhibitors (EGFR-TKIs). Results of a joint study of Japanese patients with *EGFR*-mutant lung cancer conducted at 12 facilities to determine the clinical significance of resistance triggered by hepatocyte growth factor (HGF). (a) Of 23 tumors with acquired resistance, 14 had high levels of HGF expression (61%), 12 had T790M (52%), and two had *Met* amplification (9%). High levels of HGF expression were detected most often. T790M and HGF are often both present in tumors that have developed acquired resistance to gefitinib and erlotinib. (b) Of 45 tumors that did not respond to EGFR-TKI despite having *EGFR* mutations, 13 had high levels of HGF expression (29%), 0 had T790M (0%), and two had *Met* amplification (4%). High levels of HGF expression were again detected most often. These results suggest that HGF induces acquired and intrinsic resistance to EGFR-TKI and is the most prevalent factor for resistance.

enhanced by inflammatory cytokines (IL-1, TNF, and IFN) and suppressed by TGF- β .⁽²⁹⁾ However, mechanisms for the enhanced expression of HGF by cancer-associated fibroblasts (CAF) or lung cancer cells as leads to EGFR-TKI resistance have yet to be clarified.

Relationships between resistance factors themselves. The aforementioned analysis found that no tumors expressed both T790M and *Met* amplification at the same time. This corroborates previous results indicating that T790M and *Met* amplification are almost mutually exclusive.^(26,41,43) An interesting finding is that six of 12 tumors expressing T790M were found to also have high levels of HGF expression while one of two tumors with *Met* amplification also had high levels of HGF expression.⁽⁴²⁾ Thus, HGF is often joined by other factors for resistance, such as T790M and *Met* amplification. The biological significance of this coexistence is described below.

HGF and *Met* amplification. Although Turke *et al.*⁽⁴¹⁾ only noted the phenomenon in the HCC827 cell line, they reported that small numbers of cancer cells with *Met* amplification (0.14%) may already be present. In the presence of EGFR-TKI, HGF promotes the selection of cells with *Met* amplification and encourages the emergence of resistance through *Met* amplification. Similarly, examination of clinical specimens from specific patients yielded results indicating the phenomenon whereby HGF accelerated *Met* amplification by expanding pre-existing *Met*-amplified clones.

Hepatocyte growth factor and T790M. Onizuka *et al.*⁽⁴⁴⁾ analyzed tissues from 10 patients with *EGFR*-mutant lung cancer who developed acquired resistance to gefitinib. They detected T790M in seven patients, and they reported that HGF was overly expressed in five of six patients that were available for analysis. In 27 patients who developed acquired resistance to gefitinib or erlotinib, Turke *et al.*⁽⁴¹⁾ detected T790M in 15. Of the 15, 11 (73%) had increased levels of expression, compared with levels before treatment, or high levels of HGF expression. Thus, HGF often co-exists with T790M.

Irreversible EGFR-TKIs that form a covalent bond with EGFR were expected to alleviate resistance due to a secondary *EGFR* mutation, the T790M mutation, and its clinical development is underway. However, irreversible EGFR-TKIs have not performed as well as expected in numerous clinical trials conducted here in Japan and abroad.⁽⁴⁵⁾ The current authors found

that HGF induced resistance to reversible EGFR-TKIs as well as to irreversible EGFR-TKIs.^(46,47) T790M and HGF are often both present in tumors that have developed acquired resistance to gefitinib and erlotinib, suggesting that high levels of HGF expression in a tumor may be a reason why irreversible EGFR-TKIs have not been effective in clinical trials. Presumably, a combination of an HGF inhibitor and irreversible EGFR-TKI would be of clinical benefit in treating tumors with high levels of HGF expression.

Involvement of other receptor ligands. In squamous cell carcinoma (carcinomas of the head and neck and cervical carcinoma) models with cancer harboring wild-type EGFR, EGFR-TKI resistance due to activation of IGF-1R have been reported in preclinical models.⁽⁴⁸⁾ Acquired resistance due to activation of IGF-1R has not yet been demonstrated in *EGFR*-mutant lung cancer.

Some lung cancer patients who respond well to treatment with EGFR-TKIs, and who later experience therapy failure, demonstrate a second response to EGFR-TKI re-treatment after a “drug holiday”.⁽⁴⁹⁾ Recent study showed that activation of IGF-1R may be involved in a reversible “drug-tolerant” state,⁽⁵⁰⁾ though this has yet been proven in studies with clinical specimens.

Mechanisms of Resistance to Anti-EGFR Antibody

Cetuximab, a chimeric anti-EGFR antibody, had a statistically significant survival benefit when used together with anticancer agents to treat advanced non-small cell lung carcinoma (FLEX trial).⁽⁵¹⁾ Subsequent biomarker analysis revealed that cetuximab was more efficacious in patients with high levels of EGFR expression than in those with low levels of EGFR expression.⁽⁵²⁾ Elucidation of the molecular mechanisms of cetuximab resistance in patients with high levels of EGFR expression should thus help to sustain the benefits of treatment.

Yamada *et al.*⁽⁵³⁾ examined mechanisms for cetuximab resistance using lung cancer cell lines expressing high levels of wild-type *EGFR* or mutant *EGFR*. Both H292 (wild-type *EGFR*) and Ma-1 (*EGFR*-mutant; exon 19 deletion) cell lines were sensitive to cetuximab, but HGF induced cetuximab resistance by activating PI3K/Akt pathway (Fig. 4). In a xenograft

model in SCID mice, H292 tumors inoculated with fibroblasts producing large amounts of HGF were resistant to cetuximab. Cancer-associated fibroblasts may express high levels of HGF depending on the patient.⁽⁴⁰⁾ These observations suggest that in clinical settings there are some patients in whom CAFs express high levels of HGF and induce cetuximab resistance. This finding needs to be verified using clinical specimens.

Mechanisms of Resistance to ALK-TKIs

Crizotinib, an ALK-TKI, responds well to *EML4-ALK* lung cancer (60% or more) and has therapeutic efficacy comparable to that of EGFR-TKI in treating *EGFR*-mutant lung cancer. However, almost every patient who responds well to crizotinib will, without exception, experience recurrence due to acquired resistance.⁽¹²⁾ The mechanisms for acquired resistance include gatekeeper L1196M mutations,⁽²²⁾ *ALK* gene amplification,⁽²⁴⁾ and other *ALK* gene mutations (L1152R, C1156Y, F1174L).^(54–57)

With regard to resistance due to ligands, EGFR ligands (EGF, amphiregulin, HB-EGF, and TGF- α) are reported to induce crizotinib resistance. Sasaki *et al.*⁽⁵⁵⁾ reported that cells derived from pleural effusions of patients with *EML4-ALK* lung cancer who developed acquired resistance to crizotinib displayed the gatekeeper L1196M mutation and high levels of expression of EGFR ligands (EGF and amphiregulin). This suggests the existence of a mechanism of resistance due to activation of an EGFR pathway in an autocrine manner. We reported that exogenously added EGFR ligands activated EGFR pathway and induced resistance to crizotinib in a paracrine manner (Fig. 4).⁽⁵⁸⁾ Crizotinib has Met-inhibiting action, so HGF did not induce crizotinib resistance. Other receptor ligands, such as IGF-1 and platelet-derived growth factors (PDGFs), also failed to induce resistance. These observations indicate that when the signal of *EML4-ALK*, an oncogenic driver, is blocked in *EML4-ALK* lung cancer cells, a bypass signal from another receptor will be activated, leading to resistance.

Preclinical studies have indicated that selective ALK inhibitors (TAE684 and CH5424802) are effective in alleviating gatekeeper mutations and *ALK* gene amplification that induce acquired resistance to crizotinib.^(56,59) This should lead to

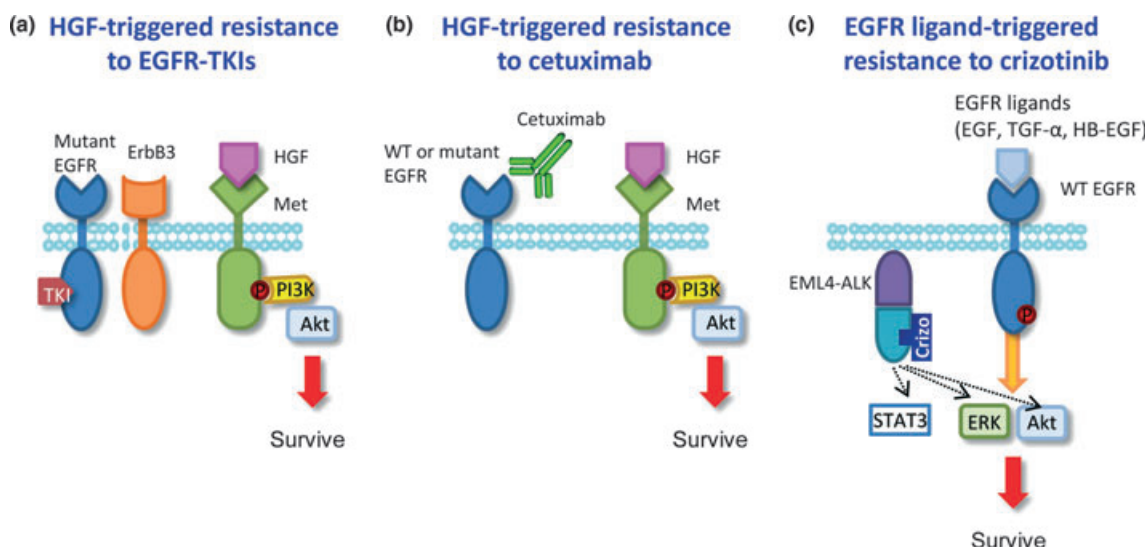
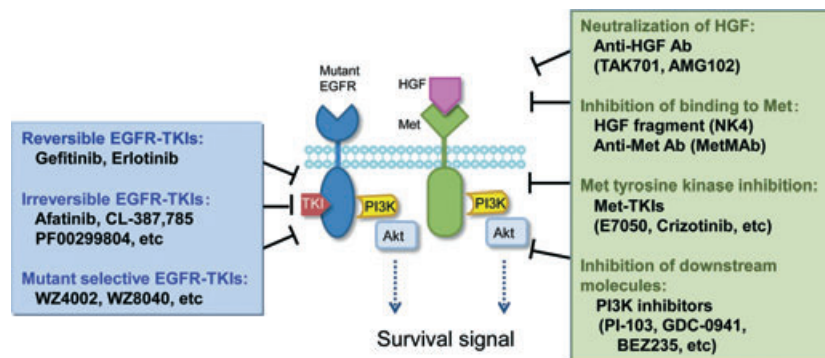


Fig. 4. Ligand-triggered resistance to epidermal growth factor receptor (EGFR) inhibitors and anaplastic lymphoma kinase (ALK) inhibitors. (a) In *EGFR* mutant lung cancer cells, hepatocyte growth factor (HGF) activates Met and downstream PI3K/Akt pathway and triggers resistance to EGFR-tyrosine kinase inhibitors (TKIs). (b) In *EGFR* wild type lung cancer cells, HGF activates Met and downstream PI3K/Akt pathway and triggers resistance to anti-EGFR antibody, cetuximab. (c) In *EML4-ALK* lung cancer cells, EGFR ligands activate EGFR and downstream PI3K/Akt and ERK1/2 pathways and triggers resistance to crizotinib.

Fig. 5. Strategies to treat hepatocyte growth factor (HGF)-triggered resistance. Hepatocyte growth factor-triggered resistance can be overcome by blocking both epidermal growth factor receptor (EGFR) pathway and HGF-Met pathway. Epidermal growth factor receptor signal can be blocked by reversible EGFR-tyrosine kinase inhibitors (TKIs), irreversible EGFR-TKIs, and mutant EGFR selective TKIs. Hepatocyte growth factor-Met signal can be blocked by neutralization of HGF, inhibition of HGF-Met binding, inhibition of Met kinase, and inhibition of downstream molecules such as phosphatidylinositol 3-kinase (PI3K) and mammalian target of rapamycin (mTOR).



better clinical performance by selective ALK inhibitors, and clinical trials of selective ALK inhibitors are underway. However, resistance is anticipated to occur with selective ALK inhibitors as well, and elucidation of the mechanisms by which it occurs is crucial. Yamada *et al.* found that both HGF and EGFR ligands induce resistance to the selective ALK inhibitor TAE684.⁽⁵⁸⁾ This finding indicates that a selective ALK inhibitor produces greater effect against resistance caused by gate-keeper mutation and amplification in *ALK* than does crizotinib, but it also suggests that resistance due to bypass signaling may occur more readily than would occur with crizotinib. Results of clinical trials should indicate whether a selective ALK inhibitor or multikinase inhibitor like crizotinib is more effective at treating *EML4-ALK* lung cancer.

Treatments for Resistance Due to Ligand Stimulation

Signals from oncogenic drivers (EGFR signaling in *EGFR*-mutant lung cancer and ALK signaling in *EML4-ALK* lung cancer) and bypass signals that trigger resistance (HGF-Met and EGFR ligands-EGFR) must be simultaneously blocked to avoid resistance caused by bypass signaling as a result of ligand stimulation.

Hepatocyte growth factor-Met can be inhibited by Met-TKI, HGF-neutralizing antibody,^(11,40,60) or inhibitors of HGF-Met binding such as anti-Met monoclonal antibody MetMAB⁽⁶¹⁾ and natural antagonist NK4.⁽⁴⁰⁾ Inhibitors of downstream molecules, such as PI3K and mammalian target of rapamycin (mTOR), can be also used.⁽⁶²⁾ The current authors have indicated in *in vitro* and *in vivo* models that resistance due to HGF can be alleviated by combined use of EGFR-TKI (gefitinib or erlotinib) and such an inhibitor (Fig. 5).^(40,41,46,47,59,61) Epidermal growth factor receptor-TKIs and anti-EGFR antibody can be used to inhibit EGFR ligands and EGFR (both are used clinically), and resistance to ALK inhibitors can be alleviated through combined use of those EGFR inhibitors.

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Conclusion

Mechanisms for resistance to molecularly targeted drugs have gradually been clarified, and strategies to treat that resistance have been indicated. In lung cancer, Met and EGFR pathways are important for transduction of a bypassing signal to survive, but their receptors also play an important role in hemostasis *in vivo*.⁽²⁹⁾ Thus, the efficacy and safety of therapy by blocking these pathways for long periods must be carefully verified. In many instances, an individual may have several resistant tumors and multiple mechanisms of resistance may be at work. Thus, optimal treatment for each patient requires accurate diagnosis of the mechanism by which the individual patient developed resistance. In such instances, a second biopsy is recommended,⁽²⁶⁾ but in the event of multiple lesions all of their origins would have to be biopsied, a feat that would be rather difficult. A highly reliable, minimally invasive diagnostic technique needs to be developed to achieve that goal.

Acknowledgments

This work was supported by Grants-in-Aid for Cancer Research (Yano, 21390256), Scientific Research on Innovative Areas “Integrative Research on Cancer Microenvironment Network” (S. Yano, 22112010A01) from the Ministry of Education, Culture, Sports, Science, and Technology of Japan, and Project for Development of Innovative Research on Cancer Therapeutics (P-Direct).

Disclosure Statement

Seiji Yano received honoraria from Chugai Pharmaceutical Co., Ltd. and AstraZeneca. Seiji Yano received research funding from Chugai Pharmaceutical Co., Ltd., Kyowa Hakko Kirin Co., Ltd. and Eisai Co., Ltd. Takayuki Nakagawa is an employ of Eisai Co., Ltd.

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