Hepatocyte Growth Factor Expression in *EGFR* Mutant Lung Cancer with Intrinsic and Acquired Resistance to Tyrosine Kinase Inhibitors in a Japanese Cohort

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Introduction: This study was performed to determine the incidence rates of resistance factors, i.e., high-level hepatocyte growth factor (HGF) expression, epidermal growth factor receptor (EGFR) T790M secondary mutation, and *MET* amplification, in tumors with intrinsic and acquired EGFR tyrosine kinase inhibitor (TKI) resistance in *EGFR* mutant lung cancer.

Methods: Ninety-seven specimens from 93 *EGFR* mutant lung cancer patients (23 tumors with acquired resistance from 20 patients, 45 tumors with intrinsic resistance from 44 patients [nonresponders], 29 sensitive tumors from 29 patients) from 11 institutes in Japan were analyzed. HGF expression, *EGFR* T790M secondary mutation, and *MET* amplification were determined by immunohistochemistry, cycleave real-time polymerase chain reaction, and fluorescence in situ hybridization, respectively.

Results: High-level HGF expression, *EGFR* T790M secondary mutation, and *MET* amplification were detected in 61, 52, and 9% of tumors with acquired resistance, respectively. High-level HGF expression was detected in 29% of tumors with intrinsic resistance (nonresponders), whereas *EGFR* T790M secondary mutation and *MET* amplification were detected in 0 and 4%, respectively. HGF expression was significantly higher in tumors with acquired resistance than in sensitive tumors ($p < 0.001$, Student's t test). Fifty percent of tumors with acquired resistance showed simultaneous HGF expression with *EGFR* T790M secondary mutation and *MET* amplification.

Conclusions: High-level HGF expression was detected more frequently than *EGFR* T790M secondary mutation or *MET* amplification in tumors with intrinsic and acquired EGFR-TKI resistance in *EGFR* mutant lung cancer in Japanese patients. These observations provide a rationale for targeting HGF in EGFR-TKI resistance in *EGFR* mutant lung cancer.

Key Words: EGFR-TKI, EGFR mutation, HGF, Acquired resistance, Intrinsic resistance.

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Epidermal growth factor receptor (EGFR)-activating mutations, in-frame deletion in exon 19, and L858 point mutation in exon 21 are selectively expressed in a population with lung cancer.[1,2] *EGFR*-activating mutations are detected considerably more frequently in nonsmokers, females, adenocarcinomas, and patients from East Asia, including Japan.[3–5] The reversible EGFR tyrosine kinase inhibitors (EGFR-TKIs) gefitinib and erlotinib show dramatic therapeutic efficacy, response rates of 70 to 80%, and significant prolongation of progression-free survival (PFS) compared

with standard first-line cytotoxic chemotherapy in patients with *EGFR* mutant lung cancer.[6–9] However, patients almost always develop acquired resistance to EGFR-TKIs after varying periods.[6,9,10] In addition, 20 to 30% of patients with *EGFR*-activating mutations show intrinsic resistance to EGFR-TKIs.[4] Therefore, intrinsic and acquired resistance to EGFR-TKIs are major problems in management of *EGFR* mutant lung cancer.

Two genetically conferred mechanisms—*EGFR* T790M secondary mutation (T790M secondary mutation)[11,12] and *MET* gene amplification[13]—induce acquired resistance to EGFR-TKIs in *EGFR* mutant lung cancer. In addition, we recently demonstrated the occurrence of hepatocyte growth factor (HGF)-induced resistance.[14] HGF, a ligand of MET,[15] induces EGFR-TKI resistance by activating MET, which restores phosphorylation of downstream MAPK-ERK1/2 and PI3K-Akt pathways,[14] using Gab1 as an adaptor.[16] HGF may be involved in both intrinsic and acquired resistance to EGFR-TKIs in *EGFR* mutant lung cancer.[14]

T790M secondary mutation, *MET* amplification, and high-level HGF expression were detected in clinical specimens from *EGFR* mutant lung cancer patients who acquired resistance to EGFR-TKIs,[11–14,16–18] indicating the clinical relevance of all three resistance mechanisms in lung cancer. Although the number of cases in each study was limited (<30 cases/study), probably because of low availability of biopsy specimens from resistant tumors, *EGFR* T790M secondary mutation and *MET* amplification were estimated to have occurrence rates of 50%[11,12,17,19] and up to 20%,[13,16,17] respectively, in patients showing acquired resistance to EGFR-TKIs. Nevertheless, the incidence of HGF-induced resistance has not been determined. In addition, the incidence rates of these three resistance factors in intrinsic resistance (nonresponders) are unknown.

Here, we performed a large-scale study in 23 tumors with acquired resistance from 20 patients, 45 tumors with intrinsic resistance from 44 patients (nonresponders), and 29 sensitive tumors from 29 patients to determine the incidences of the three resistance factors not only in acquired resistance but also in intrinsic resistance (nonresponders) to EGFR-TKIs in Japanese patients with *EGFR* mutant lung cancer.

MATERIALS AND METHODS

Patient details are described in the Supplementary information (http://links.lww.com/JTO/A197).

Definition of Sensitivity to EGFR TKI

Here, tumors with *EGFR* mutation known to be associated with drug sensitivity (i.e., G719X, exon 19 deletion, and L858R) were obtained from patients before or after treatment with a single EGFR-TKI.[9]

Sensitive tumors were defined as those obtained from patients whose tumors showed a decrease in diameter of at least 30% (either documented partial response or complete response) associated with EGFR-TKI treatment in imaging studies (Response Evaluation Criteria in Solid Tumors [RECIST] version 1.0). Tumor specimens were obtained before EGFR-TKI treatment.

Tumors with acquired resistance were defined as described previously.[9] Briefly, cases showing objective clinical benefit from treatment with an EGFR TKI as defined by either documented partial or complete response (RECIST) or significant and durable (>6 months) clinical benefit (stable disease as defined by RECIST) and systemic progression of disease (RECIST), while on continuous treatment with gefitinib or erlotinib within the last 30 days were defined as showing acquired resistance. Tumor specimens were obtained after systemic progression of disease.

As intrinsic resistance (nonresponders) has not been clearly defined, tumors without response to treatment with an EGFR TKI, i.e., either documented stable disease or progressive disease (RECIST), were defined as showing intrinsic resistance (nonresponders). Tumor specimens were obtained either before or after EGFR-TKI treatment.

Patients

Ninety-seven tumor specimens with EGFR mutations were obtained from 93 lung cancer patients, all of whom provided written informed consent, at 11 institutes in Japan. This study was approved by the Institutional Review Boards of each institute.

Patients' characteristics are shown in Table 1. Eighty-seven patients had adenocarcinomas, one had large cell carcinoma, two had squamous cell carcinoma, two had adenosquamous carcinoma, and one had undifferentiated non-small cell carcinoma. As the first EGFR-TKI, gefitinib and erlotinib were given to 82 and 10 patients, respectively, and the dual inhibitor of EGFR and VEGFR2, vandetanib,[20] was given to 1 patient.

Exon 19 deletion and L858R point mutation in exon 21 of *EGFR* were detected in 40 and 57 of the 97 tumors, respectively (Table 1). Two of these tumors had both exon 19 deletion and L858R point mutation. Two tumors without exon 19 deletion or L858R had G719X. Twenty-three tumors with acquired resistance were obtained from 20 patients after EGFR-TKI treatment. Forty-five tumors with intrinsic resistance (nonresponders) were obtained from 44 patients either before (41 tumors from 41 patients) or after (four tumors from three patients) EGFR-TKI treatment. Twenty-nine sensitive tumors were obtained from 29 patients before EGFR-TKI treatment.

Immunohistochemistry for HGF

Immunohistochemical staining was conducted on formalin-fixed, paraffin-embedded tissue sections (4 μm thick) of tumor specimens with microwave antigen retrieval in 0.01 M citrate buffer (pH 6.0). We used rabbit polyclonal antibody against HGF-α (IBL, Gunma, Japan) at 1:20 dilution as a primary antibody and EnVision/HRP Polymer Reagent (Dako, Glostrup, Denmark) and DAB (3,3′-diaminobenzidine tetrahydrochloride) Liquid (Dako) for detection.

Evaluation of HGF Expression

The percentages of cancer cells with positive cytoplasmic and/or membrane HGF immunoreactivity were evaluated (0 to 100%), and the modal intensity of the positively staining cells on a scale ranged from 0 to 3+ (0, complete

TABLE 1. Patient Characteristics

Number of Patients	Acquired Resistance ($n = 20$)	Intrinsic Resistance ($n = 44$)	Sensitive ($n = 29$)	Total ($n = 93$)
Age				
Median	59.5	65.5	65	64
Range	32–85	34–76	42–86	32–86
Gender				
Male	6	26	10	42
Female	14	18	19	51
Smoking history				
Former/current Smoker	3	21	11	35
Never smoker	17	23	18	58
Histological type				
Adeno	19	39	29	87
Large cell	0	1	0	1
Squamous cell	0	2	0	2
Undifferentiated non-small cell carcinoma, or adenosquamous	1	2	0	3
EGFR-TKI treatment				
Gefitinib	19	36	27	82
Erlotinib	1	7	2	10
Vandetanib	0	1	0	1
Number of Tumors	***n* = 23**	***n* = 45**	***n* = 29**	***n* = 97**
EGFR mutation status				
Exon 19 deletion	12	14[a]	14[a]	40
L858R	11	30	16	57
G719X	0	2	0	2

[a] One patient's tumor had both exon 19 deletion and L858R point mutation.

absence of staining; 1+, weaker staining than normal bronchial epithelium; 2+, similar staining to normal bronchial epithelium; and 3+, clearly more intense staining than normal bronchial epithelium) (Supplementary Figure 1, http://links.lww.com/JTO/A197). The percentage and intensity were multiplied to give a scoring index (*H* score) ranging from 0 to 300, according to a previously reported method with minor modifications.[16] Turke et al.[16] reported that HGF expression was significantly higher in specimens with acquired resistance (mean ± SD: 205 ± 106) compared with pretreatment (126 ± 112). On additional evaluation with specimens showing acquired resistance from patients whose tumors were obtained only after acquiring EGFR-TKI resistance, HGF expression was similar (176 ± 126) to that of specimens with acquired resistance in patients with paired tumor specimens; they concluded that these findings with clinical specimens supported the suggestion that HGF mediated resistance to EGFR-TKIs. Therefore, we defined high-level HGF expression as *H* score ≥200 in this study. Evaluation was performed independently by two investigators (KT and MN) blinded to individual clinical information.

Cycleave Real-Time Polymerase Chain Reaction Assay for T790M Mutation

Details of the cycleave real-time polymerase chain reaction (PCR) assay have been described previously.[21] Briefly, tumor cell-rich areas in hematoxylin and eosin-stained sections were marked under a microscope, and tissues were scratched from the area of another deparaffinized unstained section. Pieces of the scratched tissue were incubated with 1× PCR buffer containing 100 μg/mL proteinase K for 1 hour at 54°C. After heat inactivation at 95°C for 3 minutes, the solution was used directly as the template DNA for the assay. Then, exon 20 of the *EGFR* gene was amplified by real-time quantitative PCR assay on a SmartCycler (Cepheid, Sunnyvale, CA) using Cycleave PCR Core kits (TaKaRa Co. Ltd., Ohtsu, Japan) with a T790M-specific cycling probe and a wild-type cycling probe. This assay detected as few as 5% cancer cells with T790M mutation in a background of cells with wild-type T790M in *EGFR*.

MET Amplification

Formalin-fixed, paraffin-embedded tissue sections (4 μm thick) were subjected to dual-color fluorescence in situ hybridization using a MET/CEP7 probe cocktail (Kreatech Diagnostics, Amsterdam, The Netherlands) according to the manufacturer's instructions. Staining was evaluated as reported previously.[22,23]

Statistical Analysis

Statistical significance was determined by Student's *t* test. All statistical analyses were performed using GraphPad

TABLE 2. Expression of HGF, T790M Secondary Mutation, and *MET* Amplification in EGFR-TKI-Resistant Tumors Obtained from *EGFR* Mutant Lung Cancer Patients

	Acquired Resistance ($n = 23$)	Intrinsic Resistance ($n = 45$)	Sensitive ($n = 29$)
High-level HGF expression	14 (61%)	13[a] (29%)	3[b] (10%)
EGFR T790M secondary mutation	12 (52%)	0	0
MET amplification	2 (9%)	2 (4%)	0

[a] High-level HGF expression was detected in the stroma in two patients.
[b] High-level HGF expression was detected in the stroma in one patient.

Prism Ver. 4.01 (GraphPad Software, Inc., San Diego, CA). All tests were two sided, and $p < 0.05$ was taken to indicate statistical significance.

RESULTS

HGF Expression, T790M Secondary Mutation, and MET Amplification in Tumors with Acquired Resistance

Among 23 tumors with acquired resistance from 20 patients, *EGFR* T790M secondary mutation was detected in 12 tumors (52%) from 11 patients (60%) (Table 2). *MET* amplification was detected in two tumors (9%) from two patients (10%). As HGF is a soluble cytokine, evaluation of HGF is not as simple as that for genetically conferred T790M secondary mutation and *MET* amplification, which can be designated as plus or minus. As described in the Materials and Methods section, we defined high-level HGF expression as *H* score ≥200 in this study. High-level HGF expression was detected in 14 tumors (61%) from 13 patients (60%). In these 14 tumors, HGF was predominantly expressed in cancer cells.

The high HGF expression was simultaneously detected in 6 of 12 tumors positive for T790M secondary mutation (50%) (Table 3, Figure 1). High-level HGF expression was also detected simultaneously in one of two tumors positive for *MET* amplification (50%). These results suggested possible interactions among these three resistance factors, consistent with previous reports.[16,17]

Expression of HGF, T790M Secondary Mutation, and MET Amplification in Tumors with Intrinsic Resistance (Nonresponders)

T790M secondary mutation was not detected in 45 tumors with intrinsic resistance from 44 patients (nonresponders), but *MET* amplification was detected in two tumors (4%) (Table 2). *EGFR* D761Y secondary mutation was detected in two tumors (4%) from one patient[24](Supplementary Table 1, http://links.lww.com/JTO/A197). In contrast, high-level HGF expression in cancer cells was detected in 11 tumors (24%) from 11 patients. In addition, HGF was detected at high levels in stromal cells in two tumors (4%) from two patients (data not shown). In total, high-level HGF expression was detected in 13 tumors with intrinsic resistance (29%). Notably, high-level HGF expression was simultaneously detected in one of two *MET* amplification-positive tumors (50%) (Table 2). These results suggested the involvement of HGF in intrinsic resistance to EGFR-TKIs in *EGFR* mutant lung cancer in Japanese patients.

Expression of HGF, T790M Secondary Mutation, and MET Amplification in Sensitive Tumors

Neither *EGFR* T790M secondary mutation nor *MET* amplification was detected in 29 sensitive tumors from 29 patients. High-level HGF expression was detected in two tumors (7%) (Supplementary Table 2, http://links.lww.com/JTO/A197). High levels of HGF were detected in stromal cells in one tumor (3%). In total, a high level of HGF expression was detected in three sensitive tumors (10%). Thus, although high HGF expression level was detected even in sensitive tumors, the incidence of high HGF expression was much lower in sensitive tumors than in those with acquired or intrinsic resistance. In addition, mean *H* score of HGF in tumors with acquired resistance was significantly higher than that in sensitive tumors ($p < 0.001$, Student's *t* test) (Figure 2). There was no significant difference in mean *H* score of HGF between tumors with intrinsic resistance (nonresponders) and sensitive tumors.

DISCUSSION

Our previous studies[14,25,26] documented HGF-mediated resistance to EGFR-TKIs in *EGFR* mutant lung cancer, which was also confirmed by other groups.[16,27] Here, we demonstrated that a high level of HGF expression was detected most frequently in tumors with intrinsic and acquired resistance to EGFR-TKIs in *EGFR* mutant lung cancer in Japanese patients. Our data indicated that although T790M secondary mutation and *MET* amplification are predominantly responsible for acquired resistance, HGF may be responsible not only for acquired resistance but also for intrinsic resistance to EGFR-TKIs.

The mechanism of intrinsic resistance to EGFR-TKIs is not well understood. To our knowledge, this is the first study with more than 40 clinical specimens indicating the incidence of resistance factors in intrinsic resistance to EGFR-TKIs in *EGFR* mutant lung cancer. Here, we found that a high level of HGF expression was most frequently (29%) detected in tumors with intrinsic resistance, compared with T790M secondary mutation (0%) and *MET* amplification (4%). It is noteworthy that although the high HGF expression level was detected in cancer cells in tumors with acquired resistance, HGF expression was detected in both cancer cells (10/12 tumors) and host stroma cells (2/12 tumors) in tumors with intrinsic resistance (nonresponders). HGF was reported to be produced by not only cancer cells but also stromal cells.[15] Our data clearly indicated that both cancer cells and stromal cells are sources of HGF, which induces intrinsic EGFR-TKI resistance in *EGFR* mutant lung cancer. As HGF-induced resistance could be reversed by anti-HGF antibody and the natural HGF inhibitor NK4,[25,27] highly produced HGF in

TABLE 3. Summary of Tumors with Acquired Resistance

ID	Gender	Histological Type	*EGFR* Mutation Status	Treatment	BOR	PFS	HGF	T790M	*MET* Amplification
KZ-1	M	Ad	Exon 19 del	Erlotinib	PR	254	60	−	+
KZ-2	F	Ad	L858R	Gefitinib	CR	1041	40	−	−
KZ-3	F	Ad	L858R	Gefitinib	PR	366	200	−	−
OK1—1	M	Ad	Exon 19 del	Gefitinib	PR	351	290	−	−
OK1—2							300	−	−
OK4—2	F	Ad	Exon 19 del	Gefitinib	PR	57	210	+	−
TS-1—3	F	Ad	L858R	Gefitinib	PR	180	90	−	−
TS-1—4							280	+	−
SG2	M	Ad	Exon 19 del	Gefitinib	PR	174	150	+	−
SG3	F	Ad	L858R	Gefitinib	SD	368	110	+	−
SG4	F	Ad	L858R	Gefitinib	PR	60	220	−	+
SG6	M	Ad	Exon 19 del	Gefitinib	PR	352	140	+	−
SG8	F	Ad	L858R	Gefitinib	SD	210	90	+	−
SG9	F	Ad	Exon 19 del	Gefitinib	SD	221	200	+	−
SG10	F	Ad	L858R	Gefitinib	CR	210	210	−	−
TB1—2	M	Ad	Exon 19 del	Gefitinib	PR	1770	230	+	−
TB2—2	F	AdSq	Exon 19 del	Gefitinib	PR	300	300	−	−
AC29—1	M	Ad	L858R	Gefitinib	PR	533	250	−	−
AC29—2							270	+	−
AC24	F	Ad	Exon 19 del	Gefitinib	PR	98	170	+	−
AC26	F	Ad	Exon 19 del	Gefitinib	SD	448	180	+	−
AC28	F	Ad	Exon 19 del	Gefitinib	PR	357	200	+	−
AC31	F	Ad	L858R	Gefitinib	PR	894	200	−	−

Ad, adeno; AdSq, adenosquamous; BOR, best overall response.

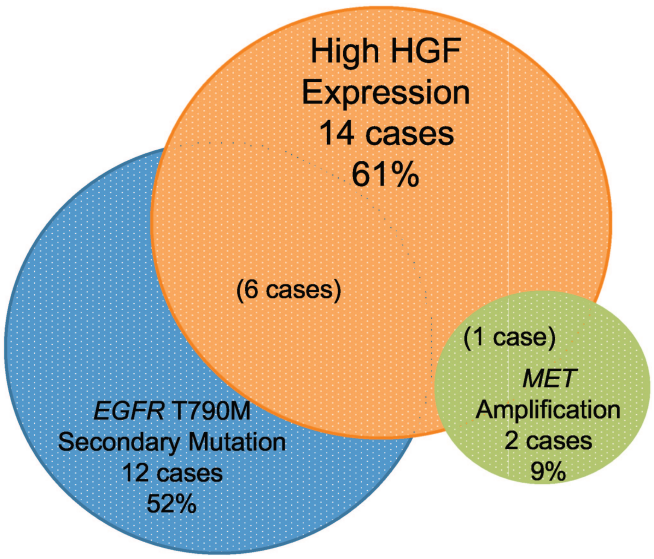

FIGURE 1. Incidences of high-level HGF expression, T790M secondary mutation, and *MET* amplification in 23 tumors with acquired resistance. Values in parentheses are the numbers of cases in which the tumors expressed two resistance factors simultaneously.

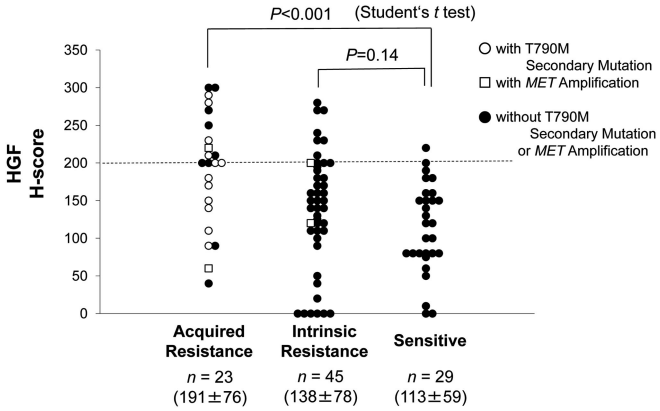

FIGURE 2. HGF expression score (*H* score) in EGFR-TKI-resistant tumors obtained from *EGFR* mutant lung cancer patients. Values in parentheses are mean ± SD of *H* score.

resistant tumors would be an ideal therapeutic target regardless of its origin.

It was of interest that a high level of HGF expression was detected in a small population of sensitive tumors. This was consistent with a previous report[16] indicating high-level HGF expression (*H* score ≥200) in several specimens from responders. Although the reason for the high level of HGF expression in tumors from responders is unclear at present, there are several possible explanations as follows. First, although HGF was expressed at high levels, natural inhibitors such as cleaved HGF and truncated MET, both of which inhibit binding of HGF to MET, may be generated in the tumors.[28,29] Second, negative regulators of MET tyrosine kinase activity such as protein kinase C may be activated and negate the effect of HGF on induction of EGFR-TKI resis-

tance in these tumors.[30] As the amounts of each clinical specimen were limited, we would like to perform further analyses in future studies should sufficient amounts of specimens become available.

Recent studies indicated that multiple resistance factors can be induced simultaneously in a single cancer. For example, Qi et al.[31] reported the simultaneous occurrence of *Met* mutation and activation of the EGFR pathway by ligand overexpression, similar to T790M mutation and HGF overexpression in EGFR mutant lung cancer, which caused resistance to Met-TKIs in gastric cancer. Katayama et al.[32] also reported that *ALK* gene amplification and gatekeeper mutation in *ALK* occurred simultaneously and conferred resistance to ALK inhibitors in EML4-ALK lung cancer. In this study, T790M secondary mutation and the high HGF expression level were simultaneously detected at high incidence (50%) in tumors with acquired resistance. Irreversible EGFR-TKIs were thought to have potential to control acquired resistance caused by T790M secondary mutation, but clinical responses were rarely observed in clinical trials.[33,34] We recently found that HGF induces resistance to not only reversible EGFR-TKIs but also irreversible EGFR-TKIs by activating the MET/PI3K/Akt pathway in *EGFR* mutant lung cancer cells with or without T790M secondary mutation.[26] Taken together, these observations suggest that HGF would be simultaneously expressed with T790M secondary mutation in tumors with acquired resistance and reduce the sensitivity to irreversible EGFR-TKIs in *EGFR* mutant lung cancer patients.

MET amplification has been detected in ~20% of tumors with acquired resistance to EGFR-TKIs in *EGFR* mutant lung cancer,[13,16,17] while the incidence reported in Japanese patients is rare.[14,18] Here, we detected *MET* amplification in two tumors (9%) with acquired resistance, suggesting that *MET* amplification can be detected in a significant proportion of tumors with acquired resistance even in Japanese patients. One case with high-level HGF expression and *MET* amplification (KZ-1) was treated with gefitinib and PFS was 254 days. The other case with low HGF and *MET* amplification (SG4) was treated with erlotinib and PFS was 60 days (Table 3). Although it is not possible to make definitive conclusions based on the data from only these two cases, the shorter PFS in the former case tentatively supports the observation that HGF accelerates expansion of preexisting clones with MET amplification.[16] Notably, simultaneous expression of these two factors was also detected in one tumor with intrinsic resistance (nonresponder). However, the mechanism by which HGF is induced in *EGFR* mutant lung cancer is still not well defined. Further examinations are warranted to elucidate the interaction between HGF expression and *MET* amplification in *EGFR* mutant lung cancer.

Among 68 resistant tumors, high-level HGF expression, T790M secondary mutation, and *MET* amplification were not detected in one tumor with acquired resistance and 31 tumors with intrinsic resistance, indicating the involvement of other mechanisms of resistance in these tumors. *EGFR* D761Y secondary mutation in exon 20 was detected in two tumors from the same patient.[24] *EGFR* D761Y mutation was originally identified in recurrent brain metastasis and was shown to induce intermediate-grade resistance to EGFR-TKIs.[35] In addition, rare secondary mutations (other than T790M and D761Y) or a preexisting resistance mutation in a minority of clones may also be involved in intrinsic resistance. Moreover, it was recently reported that a subpopulation of cancer cells that transiently exhibit a distinct phenotype characterized by engagement of IGF-1R activity, hypersensitivity to HDAC inhibition, and altered chromatin showed an intrinsic ability to tolerate exposure to EGFR-TKI.[36] Minor secondary mutations, a preexisting resistance mutation in a minority of clones, or chromatin-mediated drug resistance mechanisms may be involved in resistant tumors without high HGF expression, T790M secondary mutation, and *MET* amplification.

To overcome the HGF-induced resistance to EGFR-TKI in *EGFR* mutant lung cancer, double blockade of the EGFR pathway and HGF-MET pathway is therefore theoretically necessary.[14,16,27] To inhibit mutant EGFR with or without T790M secondary mutation, EGFR mutant-specific inhibitors were developed in addition to irreversible EGFR-TKIs.[37] To inhibit HGF-MET signaling, several inhibitors, including anti-HGF antibody, NK4 (natural antagonist of MET), and MET-TKIs, were developed.[16,25–27] Further studies are essential to determine optimal combined therapy with best efficacy and safety. In addition, a prospective study is required to determine whether immunohistochemical detection of HGF would be sufficiently reliable to identify patients with HGF-induced resistance to EGFR-TKIs. As levels of HGF in peripheral blood are correlated with clinical outcome to EGFR-TKIs in patients with non-small cell lung cancer,[38,39] such noninvasive methods may facilitate individual therapy for overcoming HGF-induced resistance to EGFR-TKIs in *EGFR* mutant lung cancer patients.

Recent studies indicated at least three important roles of HGF in EGFR-TKI resistance in *EGFR* mutant lung cancer. First, HGF induces resistance to reversible EGFR-TKIs, gefitinib, and erlotinib, by restoring MET/Gab1/PI3K/Akt pathways.[14,16] Second, HGF accelerates expansion of preexisting *MET*-amplified cancer cells and facilitates MET amplification-mediated resistance during EGFR-TKI treatment.[16] Third, after acquiring resistance to reversible EGFR-TKIs, HGF induces resistance of lung cancer cells with T790M secondary mutation to irreversible EGFR-TKIs.[24] Here, we detected high-level HGF expression frequently in tumors with intrinsic and acquired resistance to EGFR-TKIs in *EGFR* mutant lung cancer in Japanese patients. These findings indicate the value of HGF as a therapeutic target for EGFR-TKI-resistant *EGFR* mutant lung cancer. Therefore, combined therapy with EGFR-TKIs and HGF-MET inhibitors in patients with HGF-induced resistance may improve the clinical outcome of *EGFR* mutant lung cancer.

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