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MET gene amplification or *EGFR* mutation activate MET in lung cancers untreated with EGFR tyrosine kinase inhibitors

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Abstract

We analyzed *MET* protein and copy number in NSCLC with or without *EGFR* mutations untreated with EGFR tyrosine kinase inhibitors (TKIs). *MET* copy number was examined in 28 NSCLC and 4 human bronchial epithelial cell lines (HBEC) and 100 primary tumors using quantitative real-time PCR. Positive results were confirmed by array comparative genomic hybridization and fluorescence *in-situ* hybridization. Total and phospho-MET protein expression was determined in 24 NSCLC and 2 HBEC cell lines using Western blot. *EGFR* mutations were examined for exon 19 deletions, T790M, and L858R. Knockdown of EGFR with siRNA was performed to examine the relation between EGFR and MET activation. High-level *MET* amplification was observed in 3 of 28 NSCLC cell lines and in 2 of 100 primary lung tumors that had not been treated with EGFR-TKIs. MET protein was highly expressed and phosphorylated in all the 3 cell lines with high *MET* amplification. In contrast, 6 NSCLC cell lines showed phospho-MET among 21 NSCLC cell lines without *MET* amplification ($p = 0.042$). Furthermore, those 6 cell lines harboring phospho-MET expression

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without *MET* amplification were all *EGFR* mutant ($p = 0.0039$). siRNA-mediated knockdown of EGFR abolished phospho-MET expression in examined 3 *EGFR* mutant cell lines of which *MET* gene copy number was not amplified. By contrast, phospho-MET expression in 2 cell lines with amplified *MET* gene was not down-regulated by knockdown of EGFR. Our results indicated that *MET* amplification was present in untreated NSCLC and *EGFR* mutation or *MET* amplification activated MET protein in NSCLC.

Keywords

MET; amplification; *EGFR*; gefitinib; lung cancer

Acquired somatic alterations within the cancer genome such as mutations, gene amplification and deletions cause activation of oncogenes or inactivation of tumor suppressor genes, and form the genetic basis of malignancies.[1] Cancer-specific somatic mutations have been identified in several protein kinases,[2–4] including mutations within the epidermal growth factor receptor (*EGFR*) gene in non-small cell lung cancer (NSCLC).[5–7]

The finding of mutations in the tyrosine kinase domain of *EGFR* in lung adenocarcinoma is of great clinical interest, because many of these tumors are responsive to tyrosine kinase inhibitors (TKIs).[5,6,8] Although most *EGFR* mutant NSCLC initially respond to TKI, the vast majority of these tumors ultimately become resistant to the drug treatment. In approximately half of these cases, resistance is due to the occurrence of a second point mutation in *EGFR* exon 20 (T790M).[9–12] Recently Engelman *et al*.[13] reported that *MET* proto-oncogene (*MET*) amplification led to gefitinib resistance in lung cancers lacking the secondary T790M mutation. Bean *et al*.[14] also reported that *MET* was amplified in lung tumors with acquired resistance more frequently than in untreated lung tumors and accounted for about 20% of cases of acquired resistance to TKIs. *MET* encodes a heterodimeric transmembrane receptor tyrosine kinase for the hepatocyte growth factor.[15–17] Deregulation of MET signaling has been shown to contribute to tumorigenesis in various cancers via activating mutations (*e.g*. papillary renal carcinomas)[18] or via high-level *MET* amplification (*e.g*. gastric cancers).[19] Although *MET* amplification in NSCLC may mainly occur after TKI-induced acquired resistance, its status in previously untreated NSCLC has received scant attention. Besides, MET protein status should also be evaluated to understand the functional effect of *MET* amplification. Furthermore, it is of interest to explore the relation between *EGFR* alteration and MET protein status because recent reports indicated that mutated or amplified EGFR can drive MET activity.[20]

In the current study, we investigated the status of *MET* copy number by quantitative real-time PCR in cell lines and primary lung cancers not previously treated with EGFR-TKIs. We also studied expression of total and phosphorylated MET protein (phospho-MET) in NSCLC cell lines by Western blot and investigated the relation among MET protein expression, *MET* copy number and *EGFR* mutational status. Furthermore, we examined the relation between TKI-sensitivity and MET status in NSCLC cell lines. Finally, we performed siRNA-mediated knockdown of EGFR using *EGFR* mutant or *MET* amplified NSCLC cell lines to see if EGFR influenced MET protein status.

Material and methods

Cell lines

Most of the human lung cancer cell lines examined in this study were established by the authors (A.F.G and J.D.M)[21] at one of 2 locations. The prefix NCI-H- (abbreviated as H-) indicates cell lines established at the National Cancer Institute-Navy Medical Oncology Branch, National Naval Medical Center, Bethesda, MD and the prefix HCC- indicates lines established

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at the Hamon Center for Therapeutic Oncology Research, the University of Texas Southwestern Medical Center at Dallas, Dallas, TX. A549 was obtained from American Type Culture Collection (Manassas, VA). NCI-H3255 was obtained from Dr. Bruce Johnson (Lowe Center for Thoracic Oncology, Dana-Farber Cancer Institute, Boston, MA).[6] PC-9 was obtained from Immuno-Biological Laboratories (Takasaki, Gunma, Japan). All the cancer cell lines except for NCI-H3255 were maintained in RPMI 1640 (Invitrogen, Carlsbad, CA) supplemented with 5 or 10% fetal bovine serum (FBS). NCI-H3255 was maintained in ACL-4.[22,23] *EGFR* mutational status in these cell lines above was available.[3] For control non-malignant cell lines, we utilized 4 human bronchial epithelial cell lines (HBECs, HBEC3KT, HBEC5KT, HBEC17KT and HBEC30KT), which were initiated by the authors (J.D.M and A.F.G).[24,25] The HBEC cell lines were maintained in Keratinocyte-SFM medium (Invitrogen) with bovine pituitary extract (BPE) and human recombinant epidermal growth factor (EGF). All cell lines were incubated at 37°C in a humidified atmosphere with 5% CO_2.

Western blot analysis

Preparation of total cell lysates and Western blot were done as described previously.[25] Primary antibodies used were mouse monoclonal anti-Met (25H2, Cell Signaling, Beverly, MA), rabbit monoclonal anti-phospho-Met (3D7, $Tyr^{1234/1235}$; Cell Signaling), rabbit polyclonal anti-EGFR (Cell Signaling) and mouse monoclonal anti-actin (Sigma-Aldrich, St. Louis, MO) antibodies. Actin levels were used as a control for protein loading. Peroxidase-labeled anti-rabbit or anti-mouse antibodies (Amersham Pharmacia, Piscataway, NJ) were used as the second antibody.

Tumor samples

We analyzed 100 serially collected primary Japanese lung cancers from patients who underwent surgery in Okayama University Hospital (Okayama, Japan) from 2005 to 2007. Resected tumors were frozen at −80°C until DNA was extracted. Corresponding non-malignant peripheral lung tissue was also available. Genomic DNA was obtained from frozen primary lung tumors, corresponding non-malignant peripheral lung tissue and cell lines by standard phenol-chloroform (1:1) extraction followed by ethanol precipitation or by using DNeasy Tissue Kit (Qiagen, Valencia, CA). Institutional Review Board permission and informed consent were obtained at Okayama University Hospital. Clinico-pathological characteristics including age, sex, smoking status (pack-years of exposure), histologic subtype and clinical stage were available.

Quantitative real-time PCR for MET copy number evaluation

Quantitative real-time PCR (qPCR) was performed using Applied Biosystems 7300 Real-Time PCR System (Applied Bio-systems, Foster City, CA). We evaluated *MET* copy number in each sample by comparing the *MET* locus to the reference Line-1, a repetitive element for which copy numbers per haploid genome are similar among all of the human normal and neoplastic cells.[26,27] We also chose Type III collagen gene (*COL8A1*, 3q12-q13.1) as another reference gene so as to confirm reliability of this qPCR assay. Primer sequences for *COL8A1* were reported previously.[28] *MET* gene copy number in Human Genomic DNA (EMD Biosciences, Darmstadt, Germany) was set as 2 and copy numbers greater than 5 were considered as high-level amplification. Further information including primer sequences for *MET* and the formula calculating *MET* copy number is shown in Supporting Data.

Tiling path array comparative genomic hybridization (CGH)

Array hybridization was performed as previously described.[29] Detailed information is shown in Supporting Data.

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Fluorescence *in-situ* hybridization (FISH)

To confirm the results of *MET* copy number obtained by qPCR, FISH was performed using cell lines NCI-H820 and NCI-H3255. Detailed method of FISH is shown in Supporting Data.

EGFR mutational analyses

EGFR mutations in exons 19 (deletions) or 21 (L858R point mutation) were detected by mutant-enriched PCR as described previously.[30] The common deletions of exon 19 were distinguished from wild type based on PCR product length polymorphisms using 12% polyacrylamide gel electrophoresis (PAGE). For the point mutation in exon 21 (L858R), *Sau*96I digestion, which specifically digests the mutant type, was done prior to PAGE analysis.

Secondary *EGFR* T790M point mutations in exon 20 were detected by direct sequencing as described previously.[31] Briefly, PCR products were incubated using ExoSAP-IT (Amersham Biosciences, Piscataway, NJ) and sequenced directly using Applied Biosystems PRISM dye terminator cycle sequencing method (Applied Biosystems). All sequence variants were confirmed by independent PCR amplifications and bidirectional sequencing.

Determining TKI-sensitivity in lung cancer cell lines by MTS assay

The MTS colorimetric assay (Promega, Madison, WI) was performed as per the manufacturer's instructions. This assay is based on the conversion of MTS into soluble formazan by endogenous dehydrogenase enzymes found in metabolically active cells. Cells were plated at $1 \times 10^3 – 4 \times 10^3$ cells/well in tissue culture-treated 96-well plates. The following day, TKI (gefitinib) was added to each plate in a dilution series across the plate such that 8 different concentrations of the drug were tested. On day 5, 20 μl of MTS was added, followed by 1-hr incubation at 37°C and then the absorbance was measured at 490 nm on a plate reader.

96-well plate data were imported into an in-house *D*atabase of *I*n *VI*tro drug *S*ensitivity Assays (DIVISA by Luc Girard, manuscript in preparation) where IC_{50}s are calculated as well as various statistical analyses. To calculate IC_{50} values, the data was background-subtracted (columns 1 and 12 typically contained media with no cells or drugs and served as background values), and fitted to the DRC model (R package 'drc' by Christian Ritz and Jens Streibig, http://www.bioassay.dk) to generate a sigmoidal curve from which the concentration that inhibits 50% of the cells (IC_{50}) was determined.

siRNA-mediated knockdown of EGFR

siRNAs targeting *EGFR* were designed through the RNAi Co. Ltd website (http://www.rnai.co.jp/e_index.html) based on siDirect online software system,[32] which can efficiently select siRNA sequences avoiding off-target effects and were chemically synthesized by RNAi Co., Ltd. (Tokyo, Japan). siRNA sequences targeting 2 different regions of *EGFR* are as follows (sense and anti-sense, respectively): siEGFR-1 (5′-GCU ACG AAU AUU AAA CAC UUC-3′ and 5′-AGU GUU UAA UAU UCG UAG CAU-3′) and siEGFR-2 (5′-CCG CAA AUU CCG AGA CGA AGC-3′ and 5′-UUC GUC UCG GAA UUU GCG GCA-3′). Cells were transfected with 10 nM of siRNA using Lipofectamine™ RNAiMAX (Invitrogen) according to the manufacturer's protocol. Control cells were treated with RNAiMAX alone or with scrambled negative control siRNA sequence (RNAi). Cells were grown and harvested 72 hours after the transfection for Western blot analysis.

Statistical analyses

The differences of significance among categorized groups were compared using Fisher's exact tests. All statistical analyses in this study were performed using Prism 4 software (GraphPad Software, San Diego, CA).

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Results

MET gene status in NSCLC cell lines

We investigated 28 NSCLC and 4 HBEC cell lines for *MET* gene copy number. Ten NSCLC cell lines with *EGFR* activating mutations were available (all adenocarcinomas from patients untreated with TKIs). Two of these also had the T790M mutation in exon 20 of *EGFR* (Table I). NCI-H1650 is known to have a homozygous deletion of the *PTEN* gene and absence of its protein.[20,28] *MET* copy numbers of the 4 HBEC cell lines were near diploid (Fig. 1). Cell lines NCI-H1993 and A549 were used as positive and negative controls for *MET* copy number evaluation, respectively.[13] We also obtained similar results of *MET* copy numbers in cell lines and tumors using Type III collagen gene (*COL8A1*, 3q12-q13.1) as another reference gene (representative results are shown in Supporting Table I).

High-level *MET* amplification was observed in 1 cancer cell line with *EGFR* mutation and 2 cell lines without *EGFR* mutation (Fig. 1 and Table I). There was no difference in *MET* amplification status between *EGFR* mutant and wild type cell lines. Copy number status was confirmed using CGH in cell lines NCI-H1993 and A549, and one primary tumor (Fig. 2) and using FISH for NCI-H3255 and NCI-H820 (Fig. 3). In addition, we confirmed that samples with copy numbers ranging from 3 to 4 obtained by qPCR were not amplified by CGH (results are shown in Supporting Table II). Furthermore, mean copy number of corresponding normal lung tissue in 100 samples obtained by qPCR was 2.3 and maximal and minimum values were 3.7 and 1.7, respectively. Therefore, we chose copy number more than 5 as high-level amplification. The FISH analyses for NCI-H3255 revealed polysomy and NCI-H820 revealed clustered amplification in addition to polysomy. Summary of *MET* FISH results on 3 additional cell lines (HCC827, NCI-H1993, NCI-H2073) studied as well as NCI-H3255 and NCI-H820 are shown in Supporting Table III.

Protein expression status of MET and its association with MET copy number and EGFR mutation

We investigated MET protein expression status in *EGFR* mutant ($n = 10$) or wild type ($n = 14$) cell lines as well as HBEC cell lines ($n = 2$) and correlated the results with *MET* copy number and *EGFR* mutational status.

Total MET protein expression was present in all *EGFR* mutant cell lines and in 9 of 14 wild type NSCLC cell lines. MET phosphorylation sites at tyrosine residues ($Tyr^{1234/1235}$) are located in the activation loop of the kinase domain and are indicative of kinase activation. As shown in Fig. 4, 7 (NCI-H1975, HCC4011, NCI-H3255, NCI-H820, PC-9, HCC4006, and HCC827) of 10 *EGFR* mutant and 2 (NCI-H1993 and NCI-H1648) of 14 wild type cell lines expressed phospho-MET. All the cell lines expressing phospho-MET also expressed total MET protein. All 3 cell lines (NCI-H820, NCI-H1993 and NCI-H1648) with increased *MET* copy number (1 mutant and 2 wild types), expressed phospho-MET. By contrast, of the 21 cell lines lacking *MET* increased copy number, 6 cell lines (NCI-H1975, HCC4011, NCI-H3255, PC-9, HCC4006 and HCC827) expressed phospho-MET, indicating that phospho-MET expression was significantly correlated with increased copy number ($p = 0.042$). Furthermore, those 6 cell lines (NCI-H1975, HCC4011, NCI-H3255, PC-9, HCC4006 and HCC827) expressing phospho-MET without *MET* amplification were all *EGFR* mutant, suggesting that *EGFR* mutation was also associated with phospho-MET expression ($p = 0.0039$). The HBEC cell lines had total MET but lacked phospho-MET expression.

The effect of EGFR protein for MET phosphorylation

We performed siRNA-mediated knockdown of EGFR to see if activated EGFR affected MET activity. As shown in Figure 5, MET phosphorylation was decreased or abolished after EGFR

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was knocked down in 3 *EGFR* mutant cell lines (HCC827, HCC4011 and HCC4006), which had relatively strong phospho-MET expression. By contrast, phospho-MET was retained after knockdown of EGFR in 2 *MET* amplified cell lines (NCI-H820 and NCI-H1993). NCI-H820 also had exon 19 deletions in *EGFR* gene.

The relation between MET status and TKI sensitivity

We compared the MET status to TKI sensitivity in NSCLC cell lines (Table I). We analyzed the relation between phospho-MET expression and IC_{50} value of gefitinib in *EGFR* mutant cell lines without T790M or PTEN loss and in *EGFR* wild type cell lines. It seems that there is no relation between phospho-MET expression and IC_{50} value of gefitinib in both groups.

MET amplification in previously untreated lung cancers

Information about 100 primary lung cancers is presented in Table II. *EGFR* activating mutations were found out in 28 of 100 primary tumors and there were no T790M mutations in this sample set. *MET* amplification was found in 2 of 100 untreated lung tumors. Of interest, one of them also had a L858R mutation, and was refractory to gefitinib. Detailed information of 2 patients (patient nos. 1 and 2) with *MET* amplification is provided in Supporting Data.

Discussion

We found that many NSCLC cell lines and bronchial epithelial cells expressed total MET protein. However, a more restricted subset of MET-expressing NSCLC cell lines also expressed the phosphorylated forms. Phospho-MET expression was significantly related to increased *MET* copy number and was significantly more frequent in *EGFR* mutant cell lines. As additional evidence for the relation between *EGFR* mutation and MET activation, recent reports suggested that other tyrosine kinases were also activated in cells with EGFR activation by *EGFR* mutations,[20] supporting our results. Indeed, the results of siRNA experiments confirmed effect of activated EGFR to MET status. Three *EGFR* mutant cell lines harboring phospho-MET expression without *MET* amplification lost or decreased phospho-MET expression whereas *MET* amplified cell lines with or without *EGFR* mutations retained phospho-MET expression after knockdown of EGFR, indicating that *EGFR* mutation or *MET* amplification activate MET protein.

Regarding clinical samples, we found increased *MET* copy number in 2 of 100 previously untreated primary resected lung cancers. For clinical samples, contamination with non-malignant cells is invariably present (estimated average per tumor = 50% tumor cells, 50% non-malignant cells). Thus cut-off value of amplification may be reduced. One of these 2 *MET* amplified tumors also had an activating *EGFR* mutation (L858R), but lacked the resistance associated with T790M mutation. This patient had an advanced tumor and died within 20 days after the start of gefitinib treatment. While he would appear to have been refractory to gefitinib therapy, the short survival duration renders a firm conclusion difficult. We did not examine phospho-MET protein status in primary tumors because formalin fixed materials were unsuitable for such studies.[33] We neither had enough frozen clinical samples for Western blot analysis. It is mandatory to confirm the relation among phospho-MET expression, *MET* copy number and *EGFR* mutations in primary tumors.

We studied Japanese patients and found the expected high frequency of *EGFR* mutations in NSCLC arising in patients of East Asian ethnicity.[7] The frequency of *MET* amplification in untreated lung cancers was relatively low. These results were similar with the previous report.[14] On the other hand, Beau-Faller *et al*. recently reported relatively higher frequency (21%) of *MET* amplification in 106 non-Asian NSCLC.[34] Their definition of “amplification” is normalized ratio over mean plus 2 standard deviations of 30 normal lung DNA samples. Even

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if we use this definition for our sample set, that is, normalized ratio over mean plus 2 standard deviations of 100 normal lung DNA samples, we only have 8 samples with *MET* amplification out of 100 tumors. The discrepancy of frequency of *MET* amplification between them may be explained by ethnic difference.

We also examined the IC_{50} of gefitinib to investigate the impact of phospho-MET on NSCLC cell lines, but the effect of gefitinib did not depend on MET status but depended on *EGFR* mutation or PTEN status. Our results suggested that although biological significance of MET activation as a result of *MET* amplification by gefitinib exposure caused acquired resistance to gefitnib,[13] MET activation was not solo factor for inherent resistance to gefitinib.

The clinical course of our 2 cases with *MET* amplification suggests that tumors with *MET* amplification are very aggressive in nature, that is, *MET* amplification may have the potential to be involved with development, progression or metastasis of lung cancer, consistent with the known relation of MET expression and tumor invasion and metastasis.[35] Lutterbach *et al*. reported that lung cancer cell lines with *MET* amplification were dependent on MET for growth and survival.[36] Although phospho-MET expression was not analyzed in our clinical samples because of technical issues, further investigation for phospho-MET expression status in primary tumors is important to understand biological significance of MET expression in lung cancer.

In conclusion, *MET* amplification is present in a subset of untreated lung cancers and cell lines with or without *EGFR* mutations, and *EGFR* mutation or *MET* amplification lead to activation of MET. Further studies clarifying biological significance of MET activation may shed light on the pathogenesis of lung cancer.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Abbreviations

BAC
: bacterial artificial chromosome

CGH
: comparative genomic hybridization

EGFR
: epidermal growth factor receptor

MET
: *MET* proto-oncogene

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NSCLC
non-small cell lung cancer

PCR
polymerase chain reaction

qPCR
quantitative real-time PCR

SCLC
small cell lung cancer

siRNA
small interfering RNA

TKI
tyrosine kinase inhibitor

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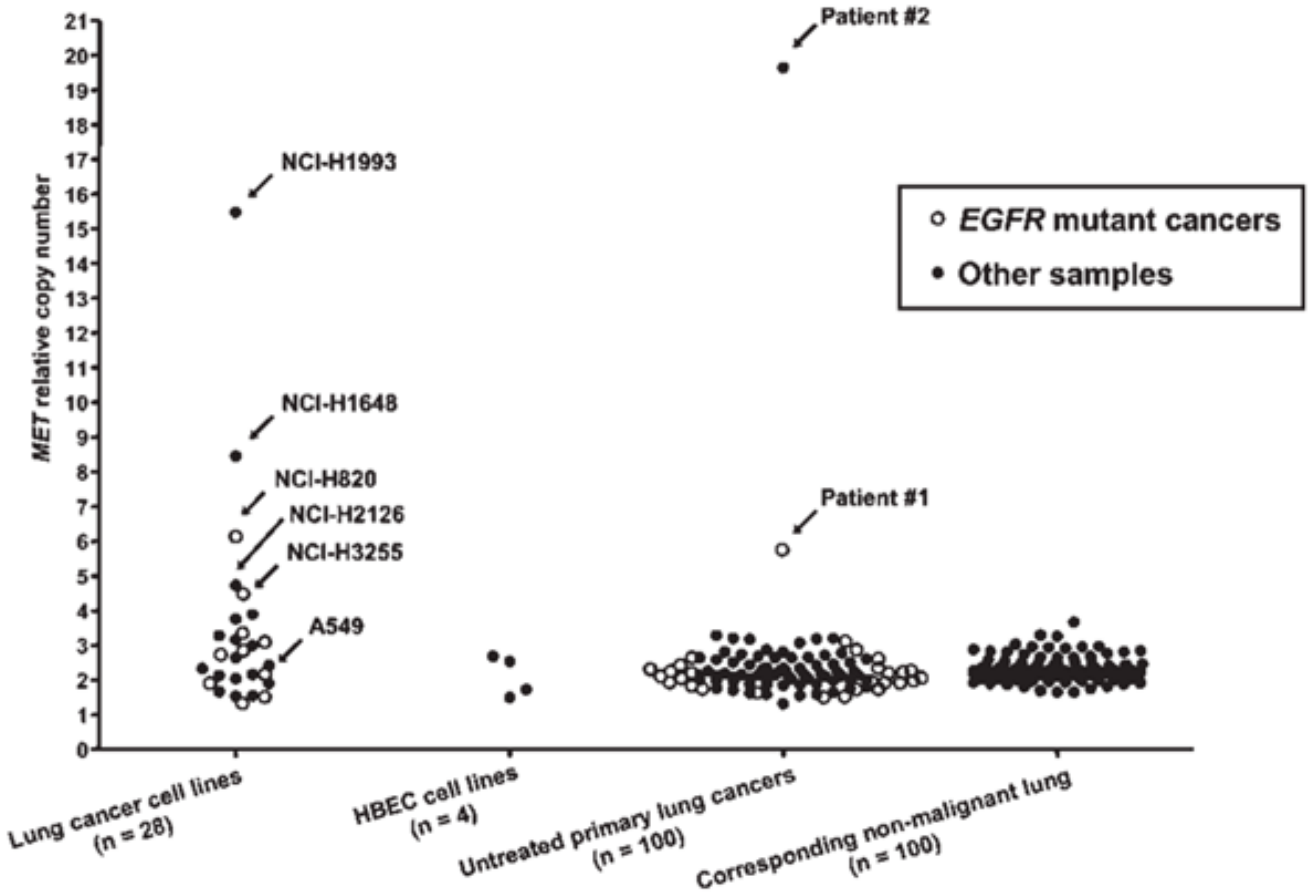

Figure 1.

MET gene copy number by quantitative real-time PCR in lung cancer cell lines and untreated primary tumors. *MET* copy numbers were expressed as relative values compared to pooled human genomic DNA from multiple healthy donors. Twenty-eight lung cancer cell lines (10 *EGFR* mutant and 18 *EGFR* wild type cell lines) were analyzed for *MET* copy number. NCI-H1993 and A549 were used as positive and negative controls, respectively. For primary lung tumors, corresponding adjacent non-malignant tissues (*n* = 100) were also available. Copy numbers greater than 5 were considered as high-level amplification.

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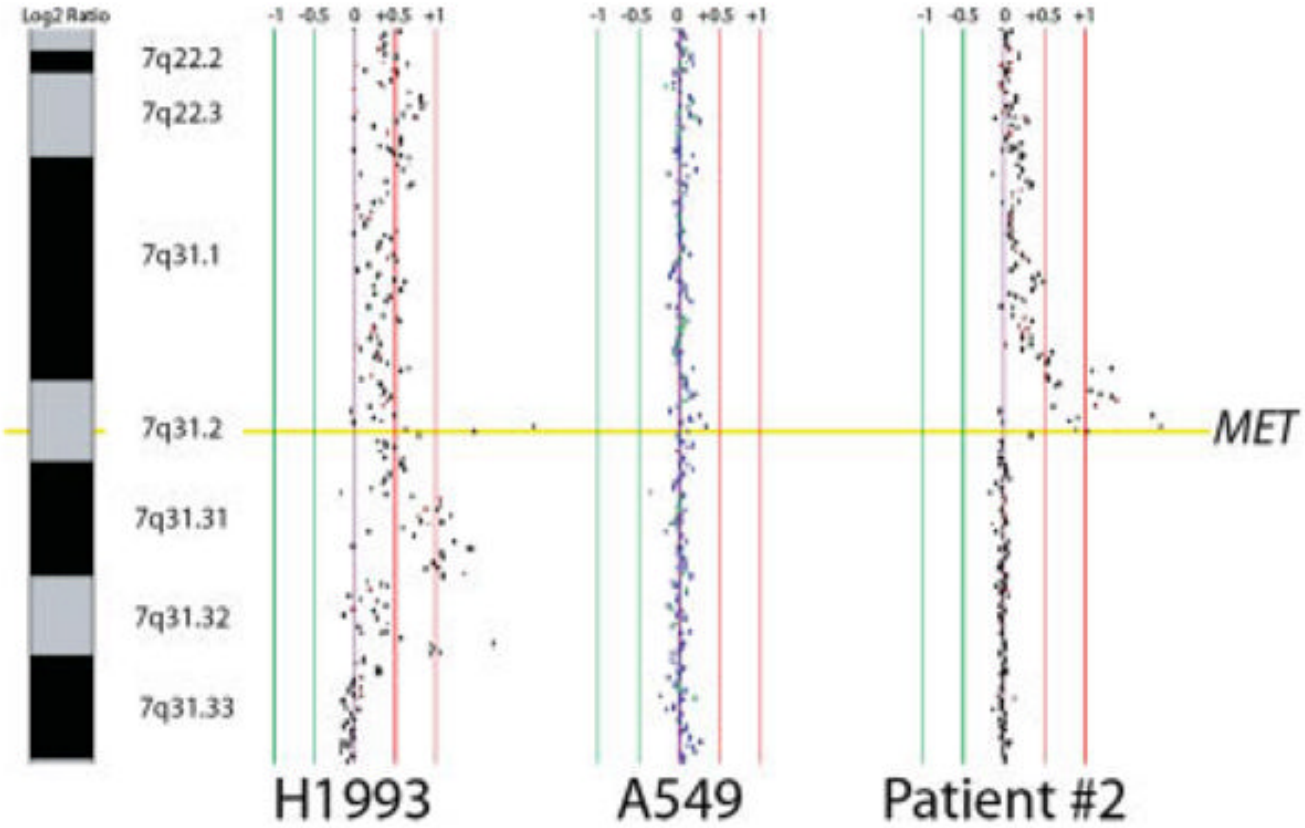

Figure 2.

Array CGH. Alignment of representative array CGH profiles at the *MET* gene locus for lung cancer cell lines and a primary lung tumor. Normalized log 2 signal intensity ratios were plotted using *SeeGH* software. Vertical lines denote log 2 signal ratios from −1 to 1 with copy number increases to the right (red lines) and decreases to the left (green lines) of 0 (purple line). Each black dot represents a single BAC clone. The region containing *MET* is shaded yellow. One cell line (NCI-H1993) and one clinical tumor (Patient no. 2) displaying *MET* gain/amplification are depicted along with cell line A549, which has near diploid copy number for *MET*. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

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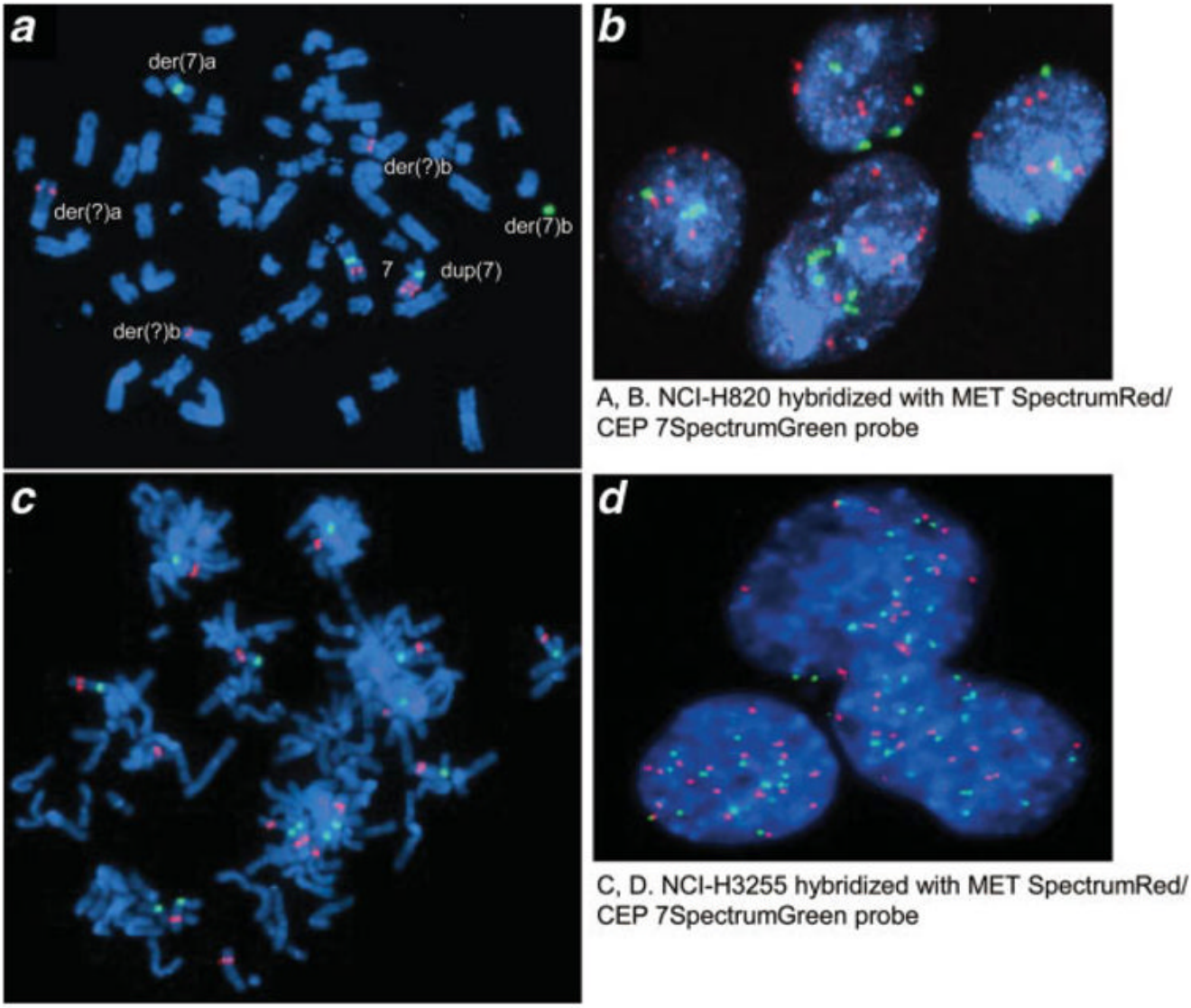

Figure 3.

Fluorescence *in-situ* hybridization (FISH) with *MET* SpectrumRed/CEP7 Spectrum-Green probe set in *EGFR* mutant lung cancer cell lines NCI-H820 (*a*, *b*) and NCI-H3255 (*c*, *d*)., Both cell lines are shown to have high level of polysomy for chromosome 7, in which *MET* gene is located. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

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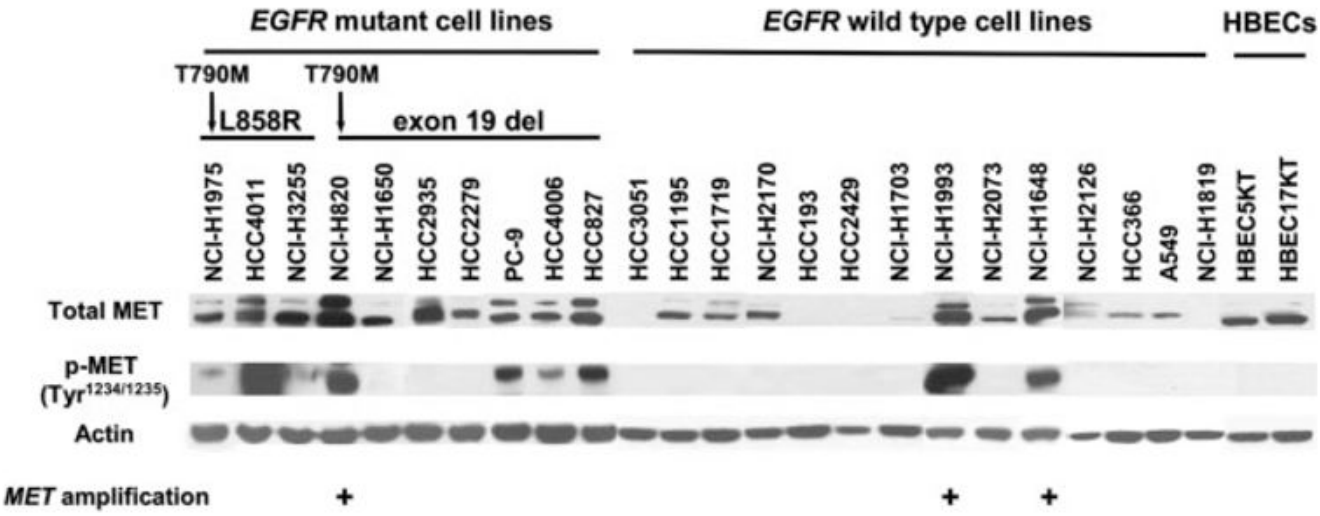

Figure 4.

MET protein expression in *EGFR* mutant or wild type cell lines. Whole cell lysates were subjected to Western blot using the antibodies indicated in this column. MET phosphorylation sites at tyrosine residues ($Tyr^{1234/1235}$) are located in the activation loop of the kinase domain and are indicative of kinase activation. All the 3 cell lines (NCI-H820, NCI-H1993 and NCI-H1648) with *MET* amplification had strong expression of phospho-MET (p-MET). Of interest, in 16 NSCLC cell lines with total-MET expression but no *MET* amplification, while 6 (NCI-H1975, HCC4011, NCI-H3255, PC-9, HCC4006 and HCC827) of 9 *EGFR* mutant cell lines showed phospho-MET, phosphorylation was not observed among 7 *EGFR* wild type cell lines.

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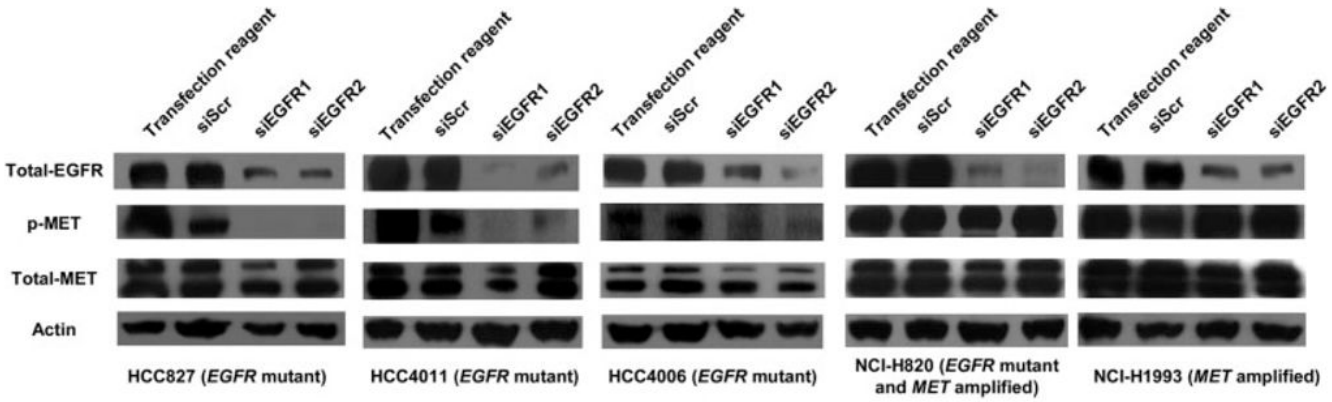

Figure 5.

siRNA-mediated knockdown of EGFR. Knockdown of EGFR was performed using 2 different siRNAs (siEGFR1 and siEGFR2). Control cells were treated with transfection reagent alone or scrambled siRNA sequence (siScr). Knockdown of EGFR decreased or abolished phospho-MET expression in *EGFR* mutant cell lines (HCC827, HCC4011 and HCC4006) whereas phospho-MET expression was retained after knockdown of EGFR in *MET* amplified cell lines (NCI-H820 and NCI-H1993).

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TABLE I

MET COPY NUMBER STATUS IN *EGFR* MUTANT OR WILD TYPE NSCLC CELL LINES

Cell line	Histology	*EGFR* activating mutation	T790M mutation	*MET* copy number by qPCR[1]	p-MET expression by Western blot[2]	IC_{50}s of gefitinib (μM)
NCI-H820	AD	E746-E749 del	Yes	6.1	Yes	3.0
NCI-H1650	AD	E746-A750 del	No	1.9	No	11.7
NCI-H1975	AD	L858R	Yes	2.7	Yes	25
NCI-H3255	AD	L858R	No	4.5	Yes	0.089
HCC827	AD	E746-A750 del	No	2.2	Yes	0.04
HCC2279	AD	E746-A750 del	No	1.5	No	0.048
HCC2935	AD	E746-S752 del, I ins	No	3.3	No	0.11
HCC4006	AD	L747-A750 del, P ins	No	1.3	Yes	0.23
HCC4011	AD	L858R	No	3.1	Yes	0.6
PC-9	AD	E746-A750 del	No	2.8	Yes	0.031
A549	AD	WT	No	2.3	No	Not done
NCI-H226	SQ	WT	No	1.5	Not done	Not done
NCI-H522	AD	WT	No	3.2	Not done	Not done
NCI-H1299	LC	WT	No	2.6	Not done	26.4
NCI-H1648	AD	WT	No	8.5	Yes	36.7
NCI-H1703	SQ	WT	No	1.5	No	Not done
NCI-H1819	AD	WT	No	3	No	19
NCI-H1993	AD	WT	No	15.5	Yes	17.9
NCI-H2073	AD	WT	No	3.8	No	0.031
NCI-H2126	LC	WT	No	4.7	No	21.4
NCI-H2170	SQ	WT	No	3.3	No	3.2
NCI-H2347	AD	WT	No	2.2	Not done	60
HCC193	AD	WT	No	2.1	No	21.1
HCC366	ADSQ	WT	No	1.7	No	30
HCC1195	AD	WT	No	3.9	No	27.6
HCC1719	AD	WT	No	2.4	No	Not done
HCC2429	NSCLC	WT	No	1.9	No	Not done
HCC3051	LC	WT	No	2	No	Not done

AD, adenocarcinoma; SQ, squamous cell carcinoma; LC, large cell carcinoma; ADSQ, adenosquamous cell carcinoma; NSCLC, non-small cell lung carcinoma; WT, wild type.

[1] Copy numbers greater than 5 were considered as high-level amplification.

[2] See also Figure 4.

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TABLE II

CHARACTERISTICS OF PRIMARY LUNG CANCERS (n = 100)

Characteristics	Number
Age	
<66	48
>=66	52
Sex	
Male	69
Female	31
Smoking History	
Never	32
Ever	68
Histology	
Adenocarcinoma	75
Squamous cell carcinoma	16
Adenosquamous cell carcinoma	1
Large cell carcinoma	6
Small cell carcinoma	2
Clinical stage	
IA, IB	74
IIA, IIB	12
IIIA	12
IIIB	2
IV	0
EGFR activating mutations	
Exon 19 deletions	15
Exon 21 point mutation	13
Wild type	72
T790M mutation	0