Supplementary Table 1. Comparison of *MET* copy numbers by qPCR using Line-1 and *COL8A1* as references

Samples	*MET* copy number by qPCR*	
	Line-1	*COL8A1*
NCI-H1993	15.5	21.8
NCI-H1648	8.5	6.2
PC-9	2.8	1.5
A549	2.3	1.4
Patient #1	5.7	9.8
Patient #2	19.6	17.1

*Copy numbers greater than 5 were considered as high-level amplification.

Only representative samples were analyzed.

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Article¶

***MET* Gene Amplification or *EGFR* Mutation Activate MET in Lung Cancers Untreated With EGFR Tyrosine Kinase Inhibitors ¶**

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Supplementary Materials and Methods¶

Quantitative real-time PCR for MET copy number evaluation¶

PCR primer sequences for *MET* were used to generate a 145-bp amplicon in exon 2 by using Primer3 (http://frodo.wi.mit.edu/cgi-bin/primer3/primer3_www.cgi) and modifying the sequences. Primer sequences for *MET* are as follows (forward and reverse, respectively): 5'-ATC AAC ATG GCT CTA GTT GTC-3' and ... [1]

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Supplementary Table 2. CGH results in lung cancer cell lines with *MET* copy number around 3 to 4 by qPCR

Cell lines	*MET* copy number by qPCR	CGH status (Fold change relative to ploidy)
NCI-H1819	3	1.03
NCI-H2073	3.8	1.19
NCI-H2126	4.7	1.13
NCI-H2170	3.3	1.14
NCI-H3255	4.5	1.37
HCC1195	3.9	1.26
HCC2935	3.3	1.34
HCC4011	3.1	1.32

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Supplementary Table 3. The results of FISH for *MET* in lung cancer cell lines

Cell Lines	Ploidy	CEP7		*MET*					*MET*/CEP7	Comments
		Mean/cell	SD	Mean/cell	SD	% cells with ≤2 signals	% cells with 3 signals	% cells with ≥ 4 signals		
HCC827	hypo 3n	5.90	1.70	4.23	1.71	12.00	16.00	72.00	0.72	High Polysomy
NCI-H820	hyper 3n	3.83	0.78	5.94	1.20	0.00	0.00	100.00	1.55	High Polysomy + Gene duplication
NCI-H1993	hyper 2n-hypo 4n	8.60	2.21	8.30	2.52	0.00	2.00	97.00	0.97	Gene Amplification
NCI-H2073	hyper 2n	5.29	2.17	2.63	1.18	65.00	14.00	21.00	0.50	Low Polysomy
NCI-H3255	hypo 3n-hyper 6n	13.89	1.73	13.80	1.68	0.00	0.00	100.00	0.99	High Polysomy


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Quantitative real-time PCR for MET copy number evaluation

PCR primer sequences for *MET* were used to generate a 145-bp amplicon in exon 2 by using Primer3 (http://frodo.wi.mit.edu/cgi-bin/primer3/primer3_www.cgi) and modifying the sequences. Primer sequences for *MET* are as follows (forward and reverse, respectively): 5'-ATC AAC ATG GCT CTA GTT GTC-3' and 5'-GGG AGA ATA TGC AGT GAA CC-

3'. Subsequently, this *MET* amplicon sequence was compared with the human genome using BLAST (http://www.ncbi.nlm.nih.gov/blast/Blast.cgi) to determine its uniqueness. Primer sets for both *MET* and Line-1 were additionally confirmed to generate a single anticipated size amplicon (as evaluated by gel electrophoresis). PCR was carried out in 20 μl volume containing 10 ng of genomic DNA, 200 nM each primer (for both *MET* and Line-1, in independent reactions) and 1X Power SYBR Green PCR Master Mix (Applied Biosystems). Conditions for qPCR reaction were as follows: one cycle of 95°C for 10 min, 40 cycles of 94°C for 30 s, 57°C for 30 s, and 70°C for 30 s. Fluorescent signal was detected and recorded during the extension step (70°C) of each cycle. Each amplification reaction was checked for the absence of nonspecific PCR products by dissociation-curve analysis. PCRs for each primer set were performed in triplicate, and mean values were calculated. Quantification was based on the standard curve method. Human Genomic DNA (EMD Biosciences, Darmstadt, Germany), consisting of a mixture of human whole blood pooled from 6-8 male and female donors, was run in every assay as a calibrator sample. Copy number change of *MET* gene relative to the Line-1 and the calibrator were determined by using the formula $(T_{MET}/T_{\text{Line-1}})/(C_{MET}/C_{\text{Line-1}})$, where T_{MET} and $T_{\text{Line-1}}$ were determined from sample DNA by

using *MET* and Line-1, and C_{MET} and $C_{Line\text{-}1}$ were determined from the calibrator sample by using *MET* and Line-1. We confirmed the results of samples for increased *MET* copy number by repeating the experiments three or more times, by using *COL8A1* gene as another reference gene, and by array comparative genomic hybridiazation (CGH) (see below).

Tiling path array CGH

Equal amounts (200-400 ng) of sample and reference individual male genomic DNA were differentially labelled with cyanine-3 or cyanine-5 (Perkin Elmer, Foster City, CA) respectively by random priming. Labelled DNA probes were purified using Microcon YM-30 columns (Millipore, Billerica, MA) and precipitated with 100 mg Cot DNA (Invitrogen) before resuspension in 45 ml of hybridization solution containing 80% DIG Easy hybridization buffer (Roche, Laval, QC, Canada), 100 mg sheared herring sperm DNA (Sigma-Aldrich, St. Louis, MO) and 50 mg yeast tRNA (Calbiochem, San Diego, CA). The probe mixture was then denatured at 85°C for 10 minutes before blocking repetitive sequences by incubation at 45°C for 1 hour prior to hybridization to array slides. The array contains 26,363 duplicate spotted bacterial artificial chromosome (BAC) clones

selected to give optimal coverage of the human genome [1, 2]. After hybridization for ~40 hours at 45°C, arrays were washed in 0.1X SSC, 0.1% SDS at room temperature, rinsed in 0.1X SSC and dried by centrifugation.

Array imaging, normalization and data analysis

Immediately after washing, arrays were scanned using a charge-coupled device (CCD) camera system (Applied Precision, Issaquah, WA) and the resulting images were then analyzed using SoftWoRx Tracker Spot Analysis software (Applied Precision). Systematic array biases were removed from all array data files as previously described [3] and *SeeGH* software was used to combine replicates and visualize array data as log2 ratio plots in SeeGH karyograms [4]. Replicate data points that exceeded a standard deviation of 0.1 were excluded from subsequent analysis. Genomic imbalances were identified using aCGH-Smooth [5] as previously described [6]. Log2 ratio data for *MET* was extracted using a moving-average of BAC clones at 10 kb intervals and averaged for the length of the gene and samples with a Log2 ratio >0.5 were considered to contain high-level *MET* amplification.

Fluorescence in-situ hybridization (FISH)

Slides were prepared from fixed cell pellets available in the laboratory from the specimens of 2 *EGFR*-mutant cell lines, NCI-H820 and NCI-H3255. The slides were submitted to a dual-color FISH assay using a mixture of the CEP7 SG probe (Abbott Molecular, Des Plaines, IL) and a homebrew *MET* probe prepared with the human DNA insert from the BAC clone RP11-95I20. The *MET* probe was labeled with SpectrumRed-conjugated dUTPs using the Vysis Nick Translation kit (Abbott Molecular). The slide pre-treatment included wash in 70% glacial acetic acid for 30 sec, incubation in 0.008% pepsin/0.01 M HCl at 37^{o} C for 5 min and in 1% formaldehyde for 10 min, and dehydration in a graded ethanol series. Probe set was applied to the selected hybridization areas, which were covered with glass cover slips and sealed with rubber cement. DNA co-denaturation was performed for 8 min at 85^{o}C and hybridization was allowed to occur at 37^{o}C for 40 hours. Post-hybridization washes were performed with 2x SSC/0.3%NP-40 at 72°C and 2xSSC for 2 min at room temperature followed by dehydration

in a graded ethanol series. Chromatin was counterstained with DAPI (0.3 μg/ml in VECTASHTELD Mounting Medium, Vector Laboratories, Burlingame, CA).

Analysis was performed on epifluorescence microscope using single interference filter sets for green (FITC), red (Texas red), and blue (DAPI) as well as dual (red/green) and triple (blue, red, green) band pass filters. Approximately 20 metaphase spreads and 100 interphase nuclei were analyzed in each cell line and in this setting we roughly estimated the ploidy and identified the chromosomes harboring homologous sequences to the MET/CEP7 probe set. For documentation, images were captured using a CCD camera and merged using dedicated software (CytoVision, Applied Imaging, San Jose, CA).

Supplementary Results

Clinical course of 2 patients with *MET* amplification

Patient #1 was an 82-year-old ever smoker male (20 pack-year exposure) who was diagnosed with NSCLC. Left lower

lobectomy and systemic lymph node dissection revealed a stage IB poorly differentiated adenocarcinoma. He developed recurrent disease with multiple lung and mediastinal lymph node metastases 6 months after surgery. Because his primary tumor harbored an *EGFR* point mutation (L858R) in exon 21 (supplementary Fig. 1), gefitinib was administered. CT scan was taken 10 days after gefitinib treatment because of progressive fatigue but showed no change in tumor size and increased pleural effusion. No evidence of interstitial pneumonitis was revealed. He died 20 days after the beginning of gefitinib administration. As shown in supplementary Fig. 1, he had no T790M mutation in exon 20 of *EGFR*.

Patient #2 was a 70-year-old ever smoker (51 pack-year exposure) who was diagnosed as NSCLC (c-stage IIB). A chest X-ray taken 1 year prior to resection during annual check-up did not show an abnormal shadow, but a lung tumor 9 cm in size was detected at the time of diagnosis. Left upper lobectomy and systemic lymph node dissection combined with chest wall resection were performed for an adenocarcinoma (T3N0M0 stage IIB). His tumor lacked any activating mutation or T790M mutation in *EGFR* (supplementary Fig. 1). He received adjuvant chemotherapy consisting of carboplatin and paclitaxel after surgery. PET/CT scan revealed bilateral metastases of adrenal glands 7 months after surgery.

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Supplementary References

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3. Khojasteh M, Lam WL, Ward RK, MacAulay C. A stepwise framework for the normalization of array CGH data. BMC Bioinformatics 2005;6:274.

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