Amplification of *MET* may identify a subset of cancers with extreme sensitivity to the selective tyrosine kinase inhibitor PHA-665752

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The success of molecular targeted therapy in cancer may depend on the selection of appropriate tumor types whose survival depends on the drug target, so-called "oncogene addiction." Preclinical approaches to defining drug-responsive subsets are needed if initial clinical trials are to be directed at the most susceptible patient population. Here, we show that gastric cancer cells with high-level stable chromosomal amplification of the growth factor receptor MET are extraordinarily susceptible to the selective inhibitor PHA-665752. Although MET activation has primarily been linked with tumor cell migration and invasiveness, the amplified wild-type MET in these cells is constitutively activated, and its continued signaling is required for cell survival. Treatment with PHA-665752 triggers massive apoptosis in 5 of 5 gastric cancer cell lines with *MET* amplification but in 0 of 12 without increased gene copy numbers ($P = 0.00016$). *MET* amplification may thus identify a subset of epithelial cancers that are uniquely sensitive to disruption of this pathway and define a patient group that is appropriate for clinical trials of targeted therapy using MET inhibitors.

gene amplification | molecular marker | oncogene addiction | targeted therapy

Genetic events that arise and are selected during tumor progression may become essential for tumor survival, a phenomenon generally described as "oncogene addiction" (1). The recent success of molecularly targeted agents in the treatment of human cancer appears to depend on the clear identification of drug targets that drive tumorigenesis in subsets of tumors. As such, imatinib (Gleevec) is highly effective in chronic myeloid leukemia cells harboring the *BCR-ABL* translocation, gastrointestinal stromal tumor cells with activating mutations of *C-KIT*, and chronic myelomonocytic leukemia cells with rearrangements of the platelet-derived growth factor receptor, all of which are effectively targeted by the inhibitor (2–4). Similarly, most non-small-cell lung cancers (NSCLC) with activating mutations in the epidermal growth factor receptor (EGFR) appear to be highly sensitive to treatment with the specific EGFR inhibitors gefitinib (Iressa) and erlotinib (Tarceva) (5–7). Collectively, these observations have suggested that genetically defined subsets of cancers may share dependence on a specific signaling pathway and that small-molecule inhibitors targeting these pathways would be most effectively tested in patient populations identified by appropriate molecular markers.

Clues to the identity of genes that are critical to tumor growth and survival and, hence, may serve as therapeutic targets, have emerged from analysis of gross chromosomal rearrangements, including allelic gains and losses. The most striking example is amplification of a locus on chromosome 17q, found in ≈30% of all breast cancers, which targets the HER2 (ERBB2) growth factor receptor (8). Although overexpression of HER2 in breast cancer is associated with an adverse prognosis, response to Herceptin, an antibody targeting the receptor, is specifically correlated with tumors that have high-level *HER2* gene amplification (9). In searching for additional genetic markers that may be correlated with therapeutic response, we analyzed an inhibitor of MET, a growth factor receptor known to be activated in subsets of epithelial cancers and linked to cancer cell migration and tumor invasiveness. A fraction of gastric cancer cell lines appears to be exquisitely sensitive to inhibition of MET signaling using a specific tyrosine kinase inhibitor. In these cells, which are marked by high-level amplification of wild-type *MET*, constitutive activation of the receptor drives proliferative and survival signals. The preclinical identification of patients with such a responsive tumor subset may guide early-phase clinical trials of MET inhibitors, particularly in gastric cancer, where 10–20% of primary tumors are known to have *MET* amplification (10–12).

Results and Discussion

Screening of Cancer Cell Lines for Sensitivity to a MET Tyrosine Kinase Inhibitor. The genetic heterogeneity underlying differential responsiveness of lung cancers to the EGFR tyrosine kinase inhibitors gefitinib and erlotinib is recapitulated in lung-cancer-derived cell lines. Whereas most NSCLC cell lines have an IC_{50} for gefitinib of ≈10 μM, rare cell lines harboring activating mutations in *EGFR* typically demonstrate a 50- to 100-fold enhancement in sensitivity, as measured by cell killing (6, 13–15). To test the predictive value of such an *in vitro* drug-sensitivity screen, we treated 40 cell lines representing diverse tumor types with gefitinib at concentrations ranging from 100 nM to 10 μM. Extreme sensitivity (100 nM) was observed with NCI-H1650, the only NSCLC cell line in our panel with the del E746-A750 activating mutation in *EGFR* (Fig. 1) (14). Variable degrees of sensitivity were evident in other cell lines tested, but none had a degree of cell killing comparable to <1 μM gefitinib. The NCI-H1975 NSCLC cell line harbors both a L858R-sensitizing mutation in *EGFR* and the T790M drug-resistance mutation, and, hence, it scored as relatively resistant in the assay. Consistent with the lower gefitinib sensitivity of the remaining cell lines, they did not harbor activating *EGFR* mutations. To extend this analysis to other tyrosine kinase inhibitors, we screened the same cancer cell line panel for sensitivity to a specific MET tyrosine kinase inhibitor PHA-665752 (16) (Pfizer). Extreme sensitivity (100 nM) to this drug was observed for one gastric cancer cell

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Abbreviations: EGFR, epidermal growth factor receptor; HGF, hepatocyte growth factor; HSR, homogeneously staining region; NSCLC, non-small-cell lung cancer; qPCR, quantitative PCR; siRNA, small interfering RNA.

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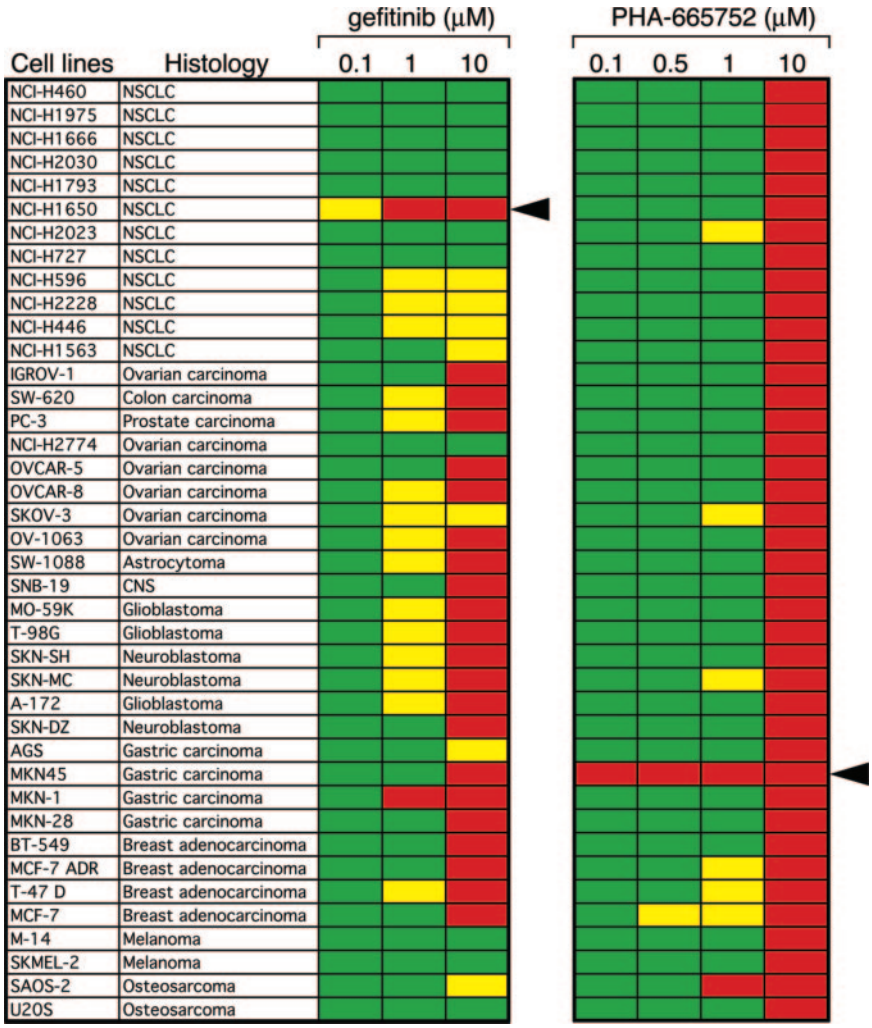

Fig. 1. Drug sensitivity profile of 40 human cancer cell lines treated with gefitinib or PHA-665752. Cells were cultured and analyzed in triplicate within microtiter plates. Cell numbers were quantitated by DNA staining, 3 days after addition of various concentrations of drugs and expressed as a fraction of matched untreated cultures. For each drug concentration, cell lines with relative drug sensitivity (<50% of untreated control growth) are shown in red, intermediate sensitivity (50–75%) in yellow, and drug insensitivity (>75%) in green. Arrowheads denote cell lines with unique drug sensitivity to gefitinib (NCI-H1650) or to PHA-665752 (MKN45).

line MKN45. As with gefitinib, other cell lines demonstrated variable degrees of cell killing, but none had a similar response <1 μM of PHA-665752 (Fig. 1). The MKN45 cell line is known to have amplification of *MET* (17), pointing to a potential genetic mechanism underlying its extraordinary drug sensitivity. None of the other 39 cell lines had *MET* gene amplification, as determined by quantitative PCR (qPCR) analysis (data not shown).

***MET* Amplification and Constitutive Activation in Human Gastric Cancer Cells.** Overexpression of *MET* has been reported in many epithelial cancers, but gene amplification is most common in gastric cancer, where 10–20% of all primary tumors and up to 40% of the scirrhous histological subtype have increased *MET* gene copy numbers (10–12). Analysis of a panel of gastric cancer cell lines by using qPCR identified increased *MET* gene copy number in 5 of 17 (29%) cases (Fig. 2*A*). In all 5 cell lines, FISH analysis showed the amplified gene copies to be integrated within a chromosomal locus, consistent with so-called homogeneously staining regions (HSRs) (Fig. 2*B*). HSR-amplification is characteristically stable in the absence of selection, indicating that the increased *MET* gene copy number represents targeted amplification of this locus rather than reflecting general aneuploidy. FISH and qPCR analyses were consistent in identifying the subset of cell lines with MET amplification, with higher fold amplification apparent by FISH (Fig. 2 *A* and *B*), presumably reflecting the effect of low-level copy-number variability in the control locus used in qPCR analysis, resulting in underestimation of the true extent of *MET* amplification. A cutoff of 8-fold gene amplification, as measured by qPCR, provided a clear distinction between gastric cancer cells with low-level aneuploidy (Amp^-) versus those with high-level specific HSR-amplification of *MET* (Amp^+).

As expected, all 5 Amp^+ cells displayed dramatic elevation in MET protein expression, compared with the 12 Amp^- cells (Fig. 3*A*). Remarkably, Amp^+ cells also displayed high levels of baseline MET activation, as measured by phosphorylation of tyrosine residues 1,234/1,235 and 1,349 (Fig. 3*A*). MET phosphorylation was not due to the presence of activating mutations, as determined by nucleotide sequencing of the entire coding sequence in all 17 gastric cancer cell lines (data not shown). MET activation in Amp^+ cells also appeared to be independent of its ligand, hepatocyte growth factor (HGF)/scatter factor, based on three observations. First, whereas Amp^- cells had low levels of MET phosphorylation under standard culture conditions but

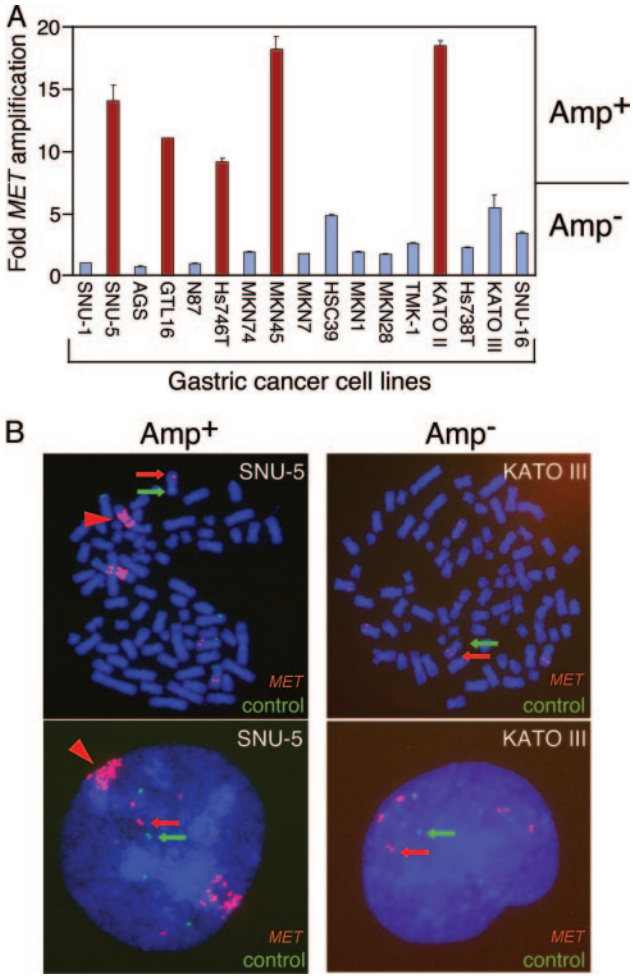

Fig. 2. *MET* genomic amplification in human gastric cancer cell lines. (*A*) Human gastric cancer cell lines screened for the presence of *MET* amplification by using qPCR. The relative *MET* copy number is derived by comparison with an unrelated control locus, *TOP3A*, at chromosome locus 17p11. Cell lines with high-level *MET* amplification (Amp$^+$) are shown in red, whereas the cells with no or low-level copy number increase of *MET* (Amp$^-$) are shown in blue. All Amp$^+$ cells have HSR-amplification of *MET*. (*B*) Representative metaphase (*Upper*) and interphase (*Lower*) FISH analysis of human gastric cancer cell lines, showing amplification of *MET* within characteristic HSRs in Amp$^+$ cells. In SNU-5 cells (Amp$^+$) with high-level amplification, the *MET* signal (red) is present in HSRs (red arrowhead) that are distinct from the endogenous gene locus (chromosome 7q31, red arrow). Control probe on the opposite arm of chromosome 7 (chromosome 7p21) is shown in green (green arrow). In KATO III cells (Amp$^-$), the low-level increased *MET* gene copy number is associated with five individual copies of chromosome 7 (aneuploidy).

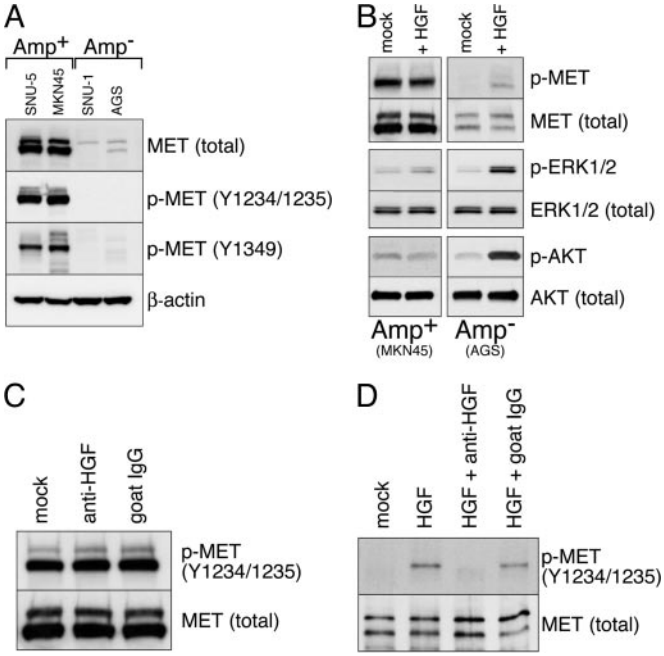

Fig. 3. Constitutive activation of MET in Amp$^+$ cells. (*A*) MET is constitutively activated in the Amp$^+$ cells. Immunoblotting analysis, demonstrating high levels of MET protein expression in two representative Amp$^+$ cell lines, compared with two Amp$^-$ cell lines. Immunoblotting using two phospho-specific MET antibodies (against Y1234/1235 and Y1349) shows strong baseline phosphorylation of the receptor only in Amp$^+$ cells (β-actin loading control). (*B*) Effect of HGF on MET activation in Amp$^+$ and Amp$^-$ cells. Representative immunoblotting analysis of cells serum-starved for 24 h and treated with 40 ng/ml HGF for 10 min. Phosphorylation of MET (Y1234/1235) is induced by HGF in Amp$^-$ cells, but it is unaltered in Amp$^+$ cells treated with HGF (total MET expression in these cells is shown as control). Phosphorylation of the downstream effectors ERK1/2 (T202/Y204) and AKT (S473) is also strongly induced in Amp$^-$ cells treated with HGF but unaltered by HGF treatment in Amp$^+$ cells. Blots probed with phospho-specific antibodies were exposed for a short time to illustrate signaling differences and to avoid potential signal saturation associated with longer exposure times. (*C*) Neutralizing HGF antibody does not affect MET activation in Amp$^+$ cells. Representative Western blot, demonstrating unaltered baseline activation of MET in Amp$^+$ cells (MKN45) treated with neutralizing anti-HGF antibody. Cells were serum starved for 24 h and subsequently treated with 5 μg/ml anti-HGF antibody or goat IgG control in serum-free media for another 24 h, by using standard conditions for neutralization of HGF (30). (*D*) Neutralizing HGF antibody can functionally inactivate HGF-mediated MET activation in Amp$^-$ cells. As control for *C*, Amp$^-$ cells (AGS) were treated with HGF alone, with neutralizing antibody to HGF, or goat IgG (control). Suppression of HGF-induced MET activation in Amp$^-$ cells confirms effective HGF neutralizing activity of this anti-HGF antibody.

demonstrated HGF-induced receptor autophosphorylation accompanied by phosphorylation of downstream effectors ERK1/2 and AKT, no such increase in baseline MET phosphorylation or activation of downstream signaling was evident in Amp$^+$ cells treated with HGF (Fig. 3*B*). Second, no *HGF* mRNA expression was detectable by quantitative RT-PCR in 4 of 5 Amp$^+$ cell lines (see Table 1, which is published as supporting information on the PNAS web site), arguing against an autocrine signaling loop. Finally, treatment of Amp$^+$ cells with neutralizing anti-HGF antibody did not affect the levels of MET phosphorylation (Fig. 3*C*), whereas it effectively suppressed HGF-induced MET activation in Amp$^-$ cells (Fig. 3*D*). Thus, Amp$^+$ cells appear to exhibit constitutive ligand-independent MET activation, which may result from receptor dimerization associated with the very high levels of protein expressed on the cell surface, an effect that has been reported for MET (18) and other receptor tyrosine kinases (19).

***MET* Amplification As Molecular Marker of Susceptibility to a Tyrosine Kinase Inhibitor.** To test the potential therapeutic relevance of these observations, we treated gastric cancer cell lines with the specific MET kinase inhibitor PHA-665752. This small-molecule inhibitor has an IC_{50} against MET of 9 nM, compared with an IC_{50} of 3.8 μM and >10 μM for EGFR and platelet-derived growth factor receptor, respectively (16). In 5 of 5 Amp$^+$ cells, treatment with PHA-665752 for 96 h resulted in a dramatic reduction in cell numbers, whereas treatment had no effect in any of the 12 Amp$^-$ cells ($P = 0.00016$, Fisher's exact test, two-sided) (Fig. 4*A*; and see Table 2, which is published as supporting information on the PNAS web site). Cell viability for these experiments was assessed by vital dye staining and ex-

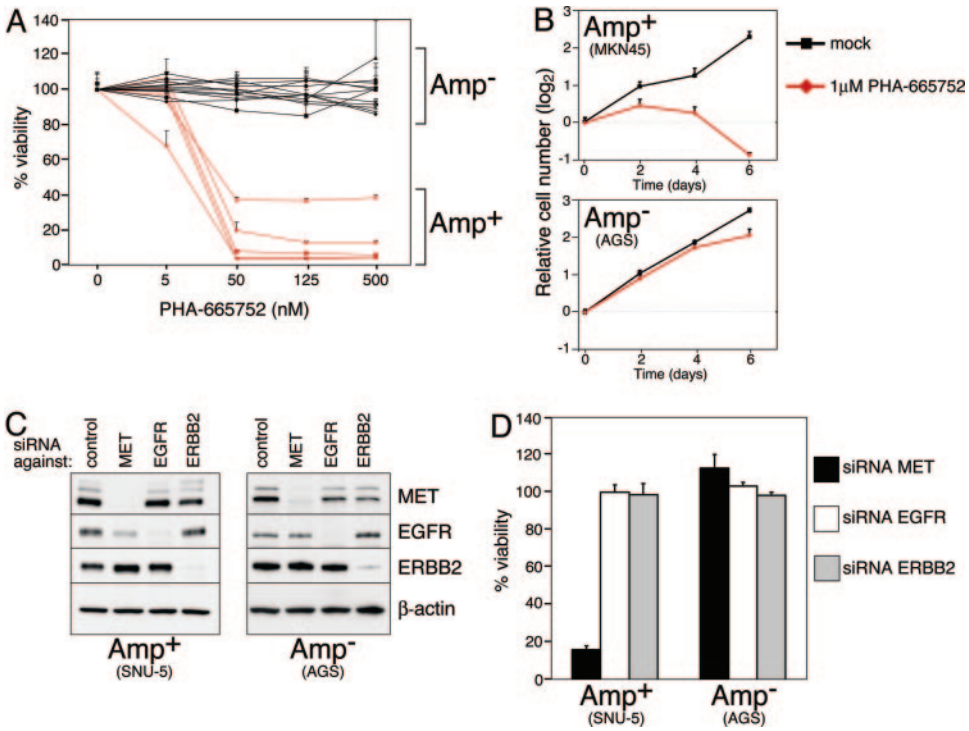

Fig. 4. Selective killing of gastric cancer cell lines with *MET* amplification after MET inhibition. (*A*) Sensitivity of Amp$^+$ cells (red) and Amp$^-$ cells (black) to increasing concentrations of PHA-665752. Cells were grown for 96 h at various concentrations of PHA-665752, and their viability was assessed by using MTT assays. Results are plotted as percent viability of treated cells compared with untreated matched controls. Experiments were performed in triplicate, with standard deviations shown. (*B*) Growth curve of representative Amp$^+$ and Amp$^-$ cells treated with PHA-665752. Cells were grown for up to 6 days in the presence or absence of PHA-665752 (1 μM), and relative cell numbers were measured by using the fluorescent nucleic acid dye SYTO60 and expressed as a fraction of the number of cells plated. Experiments were performed in triplicate, with standard deviations shown. (*C*) Effective knockdown of targeted receptor tyrosine kinases by using siRNAs. Immunoblotting analysis of MET, EGFR, and ERBB2 protein levels after treatment of Amp$^+$ and Amp$^-$ cells with specific siRNAs for 48 h. The relative exposure time of MET signal in Amp$^-$ immunoblots was increased to demonstrate effectiveness of siRNA knockdown (β-actin loading control). (*D*) Selective killing of Amp$^+$ cells after siRNA-mediated knockdown of MET. Viability in Amp$^+$ and Amp$^-$ cells, measured by using the MTT assay, was compared 96 h after knockdown of MET or unrelated receptors (EGFR and ERBB2). Cell viability is plotted as a percentage of cells treated with a nonspecific (control) siRNA duplex. Experiments were performed in triplicate, with standard deviations shown.

pressed as a fraction of viable cells in matched untreated cultures. To determine whether this effect represented cell death or growth arrest, we compared the effect of PHA-665752 on the proliferation of Amp$^+$ and Amp$^-$ cells as a function of time. Amp$^+$ cells underwent an initial arrest in proliferation, followed by cell death (Fig. 4*B*). In contrast, the proliferation rate of Amp$^-$ cells was unaffected by the presence or absence of PHA-665752 (Fig. 4*B*).

To confirm that the differential effects of PHA-665752 are truly attributable to its effect on MET, we transfected cells with small interfering (si)RNA targeting the *MET* receptor transcript. Effective and specific knockdown of MET protein expression was demonstrated by immunoblotting analysis (Fig. 4*C*). As control for nonspecific effects on growth factor signaling, we also tested siRNA targeting EGFR and ERBB2. Consistent with the effect of PHA-665752, a marked reduction in cell viability was evident in Amp$^+$ cells after MET knockdown, whereas no such effect was observed in Amp$^-$ cells (Fig. 4*D*). Amp$^+$ cells were not affected by knockdown of other receptors, such as EGFR or ERBB2.

To address the mechanism by which PHA-665752 triggers cell death in Amp$^+$ cells, we first tested the effect of drug treatment on MET-dependent signaling. PHA-665752 (50 nM) effectively suppressed the constitutive MET autophosphorylation in Amp$^+$ cells (Fig. 5*A*). Most significantly, treatment with this concentration of PHA-665752 also effectively abrogated the baseline phosphorylation of downstream effectors of growth factor receptors, such as ERK1/2, AKT, STAT3, and FAK. Thus, constitutive activation of these proliferative and survival pathways in Amp$^+$ cells appears to depend specifically on baseline MET signaling. In contrast, in Amp$^-$ cells, where MET is not constitutively autophosphorylated, PHA-665752 had no effect on baseline phosphorylation of ERK1/2, AKT, STAT3, or FAK, indicating that these effectors are likely to be activated through alternative growth factor receptors (Fig. 5*A*).

Suppression of essential growth-factor-mediated survival pathways has been linked to the induction of apoptosis. Consistent with this model, both cleaved caspase-3-staining and PARP-cleavage assays demonstrated apoptosis in Amp$^+$ cells treated with PHA-665752 but not in Amp$^-$ cells under identical conditions (Fig. 5 *B* and *C*). The early induction of apoptosis by PHA-665752 in SNU-5 cells is accompanied by a prominent PARP-cleavage signal at 72 h, compared with a more delayed but prolonged cell death in MKN45 cells (Fig. 3*B*). Thus, a subset of gastric cancer cell lines defined by targeted *MET* amplification appears to depend on constitutive activation of this growth factor receptor for their survival and show exquisite sensitivity to cell killing by the tyrosine kinase inhibitor PHA-665752.

Concluding Remarks and Clinical Implications. Analysis of the role of MET in malignancy has largely focused on its effect in promoting cell motility, invasion, and metastasis rather than its primary transforming potential. However, the ability of MET itself to drive tumorigenesis is evident from its central role in initiating human papillary renal carcinoma (20) and in a number of mouse models with ectopic expression of the activated receptor (21–23).

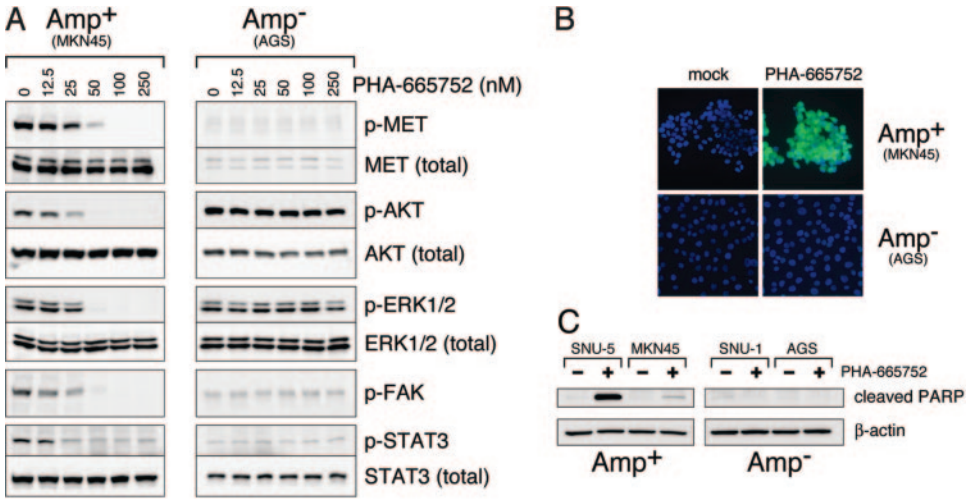

Fig. 5. Suppression of MET-dependent signals by PHA-665752 in Amp$^+$ cells and induction of apoptosis. (*A*) Immunoblotting analysis, demonstrating inhibition of MET autophosphorylation (Y1234/1235) by PHA-665752. Abrogation of baseline phosphorylation of downstream effectors [ERK1/2 (T202/Y204), AKT (S473), STAT3 (Y727), and FAK (Y576/Y577)] is evident after drug treatment in Amp$^+$ cells but not in Amp$^-$ cells. PHA-665752 was added for 3 h before protein extraction (representative blots shown). (*B*) Induction of apoptosis in Amp$^+$ cells, but not in Amp$^-$ cells, 72 h after treatment with PHA-665752 (1 μM), measured by staining for cleaved caspase-3 (green). Cells are costained with DAPI (blue) to show nuclei. (*C*) Immunoblotting analysis for PARP cleavage to demonstrate induction of apoptosis in Amp$^+$ cells, but not Amp$^-$ cells, after treatment with PHA-665752 (500 nM for 72 h) (β-actin loading control).

Our observations raise the possibility that, even in human cancers, where MET deregulation may or may not be the initiating genetic event, amplification of the gene encoding this receptor may lead to dependence on its transduced signals (i.e., oncogene addiction), thereby identifying a potential target for therapeutic intervention.

The use of panels of tumor-derived cell lines to identify subtypes with extraordinary sensitivity to molecularly targeted drugs may provide an effective functional screening approach in the preclinical development of targeted agents. Although the studies described here were limited to gastric cancer cell lines cultured *in vitro*, the dramatic difference between Amp$^+$ and Amp$^-$ cells is likely to also be evident in mouse xenograft models. Interestingly, in its initial testing, PHA-665752 showed effective killing in only one cell line in culture, GTL16, and it induced marked tumor regression in mouse xenografts derived from these cells (16). Of note, GTL16 is a gastric cancer cell line with >10-fold *MET* amplification (24), consistent with our proposed link between *MET* amplification and susceptibility to MET inhibition. Clinical trials of MET inhibitors, once available, are now required to test this molecular correlation.

Our observations suggest that *MET* amplification in gastric cancers may constitute an important molecular marker for targeted therapy. As MET inhibitors enter the clinical arena in the near future, our results would predict that patients with gastric cancer positive for HSR-amplification of *MET* may constitute a subset at high likelihood for drug response. Selection of tumors in early-phase clinical trials that are readily genotyped for markers such as HSR-amplification of *MET* may allow demonstration of drug effectiveness while limiting the size of patient cohorts required for such clinical studies. In contrast to the large clinical trials of gefinitib involving unselected cases of NSCLC that were required to demonstrate a small responsive subset (25–28), this strategy relies on preclinical identification of cancer types most likely to show a clinical response. If successful, this approach to identifying critical molecular markers of drug susceptibility may facilitate the clinical testing of targeted agents in cancer therapy.

Materials and Methods

Cellular Proliferation and Viability Assays. Cells were plated in 96-well plates in medium containing 4% FBS at ≈4,000 cells per well and, after 24 h, treated with various concentrations of either gefitinib or PHA-665752. For quantitation of cellular proliferation, cells were fixed at appropriate time points in 4% paraformaldehyde, and all plates were stained simultaneously by using the fluorescent nucleic acid stain SYTO60 (Molecular Probes) at 1:8,000 dilution in PBS. Quantitation was done by measuring the absorption at 700 nm by using the Odyssey Imaging System (LI-COR, Lincoln, NE). The relative cell number was obtained by normalizing treated samples to matched untreated specimens. For quantitation of cell viability, cultures were stained after 4 days by using the MTT assay. Briefly, 10 μl of 5 mg/ml MTT (Thiazolyl blue) solution was added to each well and incubated for 2 h at 37°C. For adherent cell lines, the media was removed from each well, and the resultant MTT formazan was solubilized in 100 μl of DMSO. For nonadherent cell lines, the MTT formazan was solubilized by direct addition of 100 μl of acidic isopropanol (0.1 N HCl) to each well. The results were quantitated spectrophotometrically by using a test wavelength of 570 nm and a reference wavelength of 630 nm.

FISH and Mutational Analysis. Bacterial artificial chromosome clone CTD-1013N12, containing the full-length *MET* gene, was used for FISH. PAC RP4–620P6, mapping to 7p21, was used as a control probe. FISH was performed as described in ref. 29. For mutational analysis, genomic DNA was amplified by PCR and sequenced bidirectionally by using BigDye Terminator v1.1 chemistry (Applied Biosystems). Primer sequences and annealing temperatures are provided in Table 3, which is published as supporting information on the PNAS web site.

siRNA-Mediated "Knockdown" of MET Expression and Immunoblotting Analysis. The duplexes targeting *MET*, *EGFR*, and *ERBB2* transcripts were custom SMARTpool mixtures from Dharmacon (Lafayette, CO). siRNA duplexes were transfected by using Lipofectamine 2000 from Invitrogen following the manufacturer's instructions. Briefly, cells were plated in 4% serum and transfected the next day with siRNAs at a final concentration of 40 nM for 5 h, followed by change of culture medium. The transfection was repeated on day 2 under the same conditions. Cell viability was assayed 4 days from the time of the first transfection, by using the MTT assay.

Antibodies. The phospho-MET (Y1234/Y1235), phospho-AKT (S473), phopho-ERK1/2(T202/Y204), phospho-FAK (Y576/Y577), phospho-STAT3 (Y727), AKT, ERK1/2, STAT3, ERBB2, EGFR, cleaved PARP, and cleaved caspase-3 antibodies were from Cell Signaling Technology (Beverly, MA). The phospho-MET (Y1349) antibody was from BioSource International (Camarillo, CA). The total MET antibody (C-12) was from Santa Cruz Biotechnology. The β-actin antibody was from Abcam (Cambridge, MA). The neutralizing HGF goat antibody was from R & D Systems, and the matched goat IgG control antibody was from Sigma. All immunoblots were done with 1:1,000 antibody dilution, except for the β-actin antibody, which was used at 1:10,000 dilution.

Apoptosis Induction Assay. Cells were plated on coverslips in 12-well dishes and grown to ≈75% confluency in 10% serum, followed by incubation in 4% serum and PHA-665752. After 72 h, cells were fixed with 4% paraformaldehyde for 20 min, permeabilized by using 1% Nonidet P-40 for 5 min, and blocked with 3% BSA for 30 min. The coverslips were then incubated overnight at 4°C with cleaved caspase-3 antibody at 1:200 dilution. The next day, the coverslips were washed three times with PBS and incubated with a secondary antibody (goat anti-rabbit FITC-conjugated) for 1 h at 1:250 dilution. After five washes with PBS, coverslips were mounted in Vectashield mounting medium containing DAPI (Vector Laboratories), and staining was visualized by fluorescent microscopy.

Supporting Information. Further description of experimental procedures can be found in *Supporting Materials and Methods*, which is published as supporting information on the PNAS web site.

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1. Weinstein, I. B. (2002) *Science* **297,** 63–64.
2. Apperley, J. F., Gardembas, M., Melo, J. V., Russell-Jones, R., Bain, B. J., Baxter, E. J., Chase, A., Chessells, J. M., Colombat, M., Dearden, C. E., *et al.* (2002) *N. Engl. J. Med.* **347,** 481–487.
3. Druker, B. J., Talpaz, M., Resta, D. J., Peng, B., Buchdunger, E., Ford, J. M., Lydon, N. B., Kantarjian, H., Capdeville, R., Ohno-Jones, *et al.* (2001) *N. Engl. J. Med.* **344,** 1031–1037.
4. Heinrich, M. C., Corless, C. L., Demetri, G. D., Blanke, C. D., von Mehren, M., Joensuu, H., McGreevey, L. S., Chen, C. J., Van den Abbeele, A. D., Druker, B. J., *et al.* (2003) *J. Clin. Oncol.* **21,** 4342–4349.
5. Lynch, T. J., Bell, D. W., Sordella, R., Gurubhagavatula, S., Okimoto, R. A., Brannigan, B. W., Harris, P. L., Haserlat, S. M., Supko, J. G., Haluska, F. G., *et al.* (2004) *N. Engl. J. Med.* **350,** 2129–2139.
6. Paez, J. G., Janne, P. A., Lee, J. C., Tracy, S., Greulich, H., Gabriel, S., Herman, P., Kaye, F. J., Lindeman, N., Boggon, T. J., *et al.* (2004) *Science* **304,** 1497–1500.
7. Pao, W., Miller, V., Zakowski, M., Doherty, J., Politi, K., Sarkaria, I., Singh, B., Heelan, R., Rusch, V., Fulton, L., *et al.* (2004) *Proc. Natl. Acad. Sci. USA* **101,** 13306–13311.
8. Slamon, D. J., Clark, G. M., Wong, S. G., Levin, W. J., Ullrich, A. & McGuire, W. L. (1987) *Science* **235,** 177–182.
9. Pauletti, G., Dandekar, S., Rong, H., Ramos, L., Peng, H., Seshadri, R. & Slamon, D. J. (2000) *J. Clin. Oncol.* **18,** 3651–3664.
10. Kuniyasu, H., Yasui, W., Kitadai, Y., Yokozaki, H., Ito, H. & Tahara, E. (1992) *Biochem. Biophys. Res. Commun.* **189,** 227–232.
11. Nessling, M., Solinas-Toldo, S., Wilgenbus, K. K., Borchard, F. & Lichter, P. (1998) *Genes Chromosomes Cancer* **23,** 307–316.
12. Sakakura, C., Mori, T., Sakabe, T., Ariyama, Y., Shinomiya, T., Date, K., Hagiwara, A., Yamaguchi, T., Takahashi, T., Nakamura, Y., *et al.* (1999) *Genes Chromosomes Cancer* **24,** 299–305.
13. Koizumi, F., Shimoyama, T., Taguchi, F., Saijo, N. & Nishio, K. (2005) *Int. J. Cancer* **116,** 36–44.
14. Sordella, R., Bell, D. W., Haber, D. A. & Settleman, J. (2004) *Science* **305,** 1163–1167.
15. Tracy, S., Mukohara, T., Hansen, M., Meyerson, M., Johnson, B. E. & Janne, P. A. (2004) *Cancer Res.* **64,** 7241–7244.
16. Christensen, J. G., Schreck, R., Burrows, J., Kuruganti, P., Chan, E., Le, P., Chen, J., Wang, X., Ruslim, L., Blake, R., *et al.* (2003) *Cancer Res.* **63,** 7345–7355.
17. Rege-Cambrin, G., Scaravaglio, P., Carozzi, F., Giordano, S., Ponzetto, C., Comoglio, P. M. & Saglio, G. (1992) *Cancer Genet. Cytogenet.* **64,** 170–173.
18. Bergstrom, J. D., Hermansson, A., Diaz de Stahl, T. & Heldin, N. E. (1999) *Br. J. Cancer* **80,** 650–656.
19. Brennan, P. J., Kumagai, T., Berezov, A., Murali, R. & Greene, M. I. (2000) *Oncogene* **19,** 6093–6101.
20. Schmidt, L., Duh, F. M., Chen, F., Kishida, T., Glenn, G., Choyke, P., Scherer, S. W., Zhuang, Z., Lubensky, I., Dean, M., *et al.* (1997) *Nat. Genet.* **16,** 68–73.
21. Gallego, M. I., Bierie, B. & Hennighausen, L. (2003) *Oncogene* **22,** 8498–8508.
22. Jeffers, M., Fiscella, M., Webb, C. P., Anver, M., Koochekpour, S. & Vande Woude, G. F. (1998) *Proc. Natl. Acad. Sci. USA* **95,** 14417–14422.
23. Takayama, H., LaRochelle, W. J., Sharp, R., Otsuka, T., Kriebel, P., Anver, M., Aaronson, S. A. & Merlino, G. (1997) *Proc. Natl. Acad. Sci. USA* **94,** 701–706.
24. Ponzetto, C., Giordano, S., Peverali, F., Della Valle, G., Abate, M. L., Vaula, G. & Comoglio, P. M. (1991) *Oncogene* **6,** 553–559.
25. Fukuoka, M., Yano, S., Giaccone, G., Tamura, T., Nakagawa, K., Douillard, J. Y., Nishiwaki, Y., Vansteenkiste, J., Kudoh, S., Rischin, D., *et al.* (2003) *J. Clin. Oncol.* **21,** 2237–2246.
26. Giaccone, G., Herbst, R. S., Manegold, C., Scagliotti, G., Rosell, R., Miller, V., Natale, R. B., Schiller, J. H., Von Pawel, J., Pluzanska, A., *et al.* (2004) *J. Clin. Oncol.* **22,** 777–784.
27. Herbst, R. S., Giaccone, G., Schiller, J. H., Natale, R. B., Miller, V., Manegold, C., Scagliotti, G., Rosell, R., Oliff, I., Reeves, J. A., *et al.* (2004) *J. Clin. Oncol.* **22,** 785–794.
28. Kris, M. G., Natale, R. B., Herbst, R. S., Lynch, T. J., Jr., Prager, D., Belani, C. P., Schiller, J. H., Kelly, K., Spiridonidis, H., Sandler, A., *et al.* (2003) *J. Am. Med. Assoc.* **290,** 2149–2158.
29. Mohapatra, G., Moore, D. H., Kim, D. H., Grewal, L., Hyun, W. C., Waldman, F. M., Pinkel, D. & Feuerstein, B. G. (1997) *Genes. Chromosomes Cancer* **20,** 311–319.
30. Wong, A. S., Pelech, S. L., Woo, M. M., Yim, G., Rosen, B., Ehlen, T., Leung, P. C. & Auersperg, N. (2001) *Oncogene* **20,** 1318–1328.