Supporting Information

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SI Results

Gefitinib was immobilized to sepharose 6B via an epoxy linkage as previously described (Fig. S2*A*) (1). This affinity reagent was used to bind proteins in extracts of three human lung cancer cell lines. H3255 has a known oncogenic mutation in *EGFR* (L858R), and gefitinib readily induces apoptosis in this cell line (2). In the H1975 cell line, *EGFR* has a T790M mutation in addition to the L858R mutation; as previously shown (3) gefitinib does not induce apoptosis in this cell line. The H2030 cell line has a *KRAS* mutation and no mutation in *EGFR*; this line is also refractory to the apoptotic effect of gefitinib.

Although we expected to find many proteins bound to gefitinib in the affinity experiments, we predicted that a higher proportion of the compound target (EGFR) would be recovered from the gefitinib-affinity column from the sensitive cell line (H3255) than from the insensitive cell lines (H1975 and H2030). After elution with gefitinib (1 mM), proteins in the eluate were identified by liquid chromatography-tandem mass spectrometry (LC-MS/MS). The numbers of tryptic peptides from three independent experiments (for each cell line) were merged and then protein identities were established. A summary of the number of proteins eluted specifically from the gefitinib-affinity column is shown in the Venn diagram in Fig. S2*B*. A total of 246 gefitinib-binding proteins were identified in eluates of at least one cell line (Figure S2*B*), and 62 of these were common to all three. Others were cell-line specific (H3255, 41; H1975, 13; and H2030, 61) or found in two of the three eluates. Fourteen gefitinib-interacting proteins that were highly represented in the eluate from the gefitinib-sensitive line H3255 are listed in Table S2, and these were considered the highest-ranked gefitinib targets. The top candidate on the list is EGFR, which was not observed in the H2030 extracts and was found at a 12-fold lower level in the H1975 extracts.

To complement the affinity-binding approach, transcription profiling of gefitinib-treated cells was performed to help identify pathways that are regulated by the target of the small molecule. Two gefitinib-sensitive cell lines (HCC827 and H3255) and the insensitive cell line H1975 were treated with gefitinib for 3 h and the Illumina Human HT-12 expression array was used to identify genes encoding RNAs present at higher or lower level after compound treatment. The expression of 484 genes was altered by compound treatment of HCC827 and expression of 645 genes was altered by compound treatment in H3255, at a significance threshold of $P < 0.01$ (Fig. S2*C*). To derive a gefitinib-responsive gene signature, we looked for genes that were regulated by gefitinib treatment in both HCC827 and H3255, and we identified a statistically significant overlap of 112 genes in the two treatment groups ($P = 3.27 \times 10^{-12}$, Fisher's exact test). In contrast to HCC827 and H3255, only 149 genes were regulated by gefitinib treatment of H1975 cells at the $P < 0.01$ significance level, and this is approximately the expected number under the null hypothesis ("no regulated genes"). Moreover, only one of these genes overlapped with the gefitinib-expression signature obtained from HCC827 and H3255 cells, leading to the conclusion that any target modulated by gefitinib in H1975 cells likely has no significant influence on gene transcription under the conditions we used. "Heatmaps" of the expression of the 112 gefitinib-responsive genes in each of the three cell lines is shown in Fig. S2*D*, along with the ratio of the transcript change (gefitinib/control). The 112 gefitinib-regulated genes show the same directionality in both HCC827 and H3255 (Fig. S2*D*, *Left* and *Center*). However, as discussed above, most of the 112 genes that constitute the gefitinib-responsive signature in the two sensitive cell lines were not regulated by the compound in H1975 (Fig. S2*D*, *Right*).

As outlined above, our next step was to use a human gene interaction network based on known pathways and protein–protein interaction data (4) to rank preferential gefitinib targets identified by affinity proteomics (Table S2), on the basis of their connection with the gefitinib transcriptomic signature. Fisher's exact test was used to assess whether a protein was linked to more of the transcriptionally altered genes in the input dataset than expected by chance, given its number of neighbors in the network. A full list of the genes whose protein products showed a statistically significant association with the gefitinib-expression signature is shown in Dataset S1. Of all of the proteins in the network, EGFR was the only target that both showed a significant connection to the gefitinib signature ($P = 0.001$, Fisher's exact test) and was also a candidate on the list of likely gefitinib-binding proteins (Table S2). Several genes whose protein products interact directly with EGFR were regulated at the transcriptional level (e.g., *ERBB3* and *ERRFI1*). These results indicate that treatment of cultured cells with a small molecule, followed by computational analysis of transcriptionally altered genes, could be useful for identifying and ranking possible targets of small molecules for validation studies.

SI Materials and Methods

Cell Lines and Materials. All cells used in this study are of human origin. Normal human bronchiolar epithelial primary cells (NHBE) and bronchiolar epithelial cell growth medium (BGEM) were purchased from Lonza. H3255 and 11–18 lung adenocarcinoma cell lines were gifts from Dr. B. Johnson (Dana-Farber Cancer Center, Boston, MA) and Dr. K. Hagiwar (Saitama Medical School, Saitama, Japan), respectively. Drs. J. Minna and A. Gazdar (University of Texas Southwestern Medical Center, Dallas, TX) kindly provided the following cell lines: HCC4011, H820, HCC1195, HCC4019, H1487, H1648, and H2405. All other human lung cancer cell lines and Eagle's MEM were obtained from American Type Culture Collection. RPMI-1640 media were prepared by the Memorial Sloan-Kettering Cancer Center Media Preparation Lab. FBS (HyClone), DharmaFECT 1 transfection reagent, and all siRNAs were obtained from Thermo Scientific. cDNAs in the pCMV6 entry vector (TruOrfs clones) and the anti-FLAG antibody (4C5-AntiDDK high-affinity antibody) were obtained from Origene. All other antibodies, Opti-MEM serum-reduced media, AlamarBlue proliferation dye, Geneticin and Hoechst 33342 nuclear stain were purchased from Cell Signaling Technologies. SOD1 activity kit was purchased from Cayman Chemicals. Fugene 6 transfection reagent was obtained from Roche Diagnostics. All tissue culture materials were obtained from Fisher Scientific. Ammonium tetramolybate and triethylenetetramine tetrahydrochloride were purchased from Sigma-Aldrich.

Growth of Cell Lines. All lung cancer cell lines with the exception of H820, HCC4011, and HCC4006, were grown in RPMI-1640 media supplemented with 10% (vol/vol) FBS and 1% (vol/vol) streptomycin solution (complete growth media) in a humidified incubator with 5% CO_2 and 95% air. Cells were maintained in 175 cm^2 tissue culture flasks and subcultured when cells reached ~60–75% confluency. H820, HCC4006, and HCC4011 were grown in 10-cm dishes under the conditions and growth media described above with the exception that the FBS concentration was 5%. NHBE cells were grown in BEGM supplemented with 2% (vol/vol) bovine pituitary extract and a mixture of specific

growth factors provided with the media. For affinity chromatography studies, cells were grown in 25-cm diameter tissue culture plates and cell extracts were made from cultures at 60% confluence.

Transfection of Cell Lines with siRNAs and Determination of Growth Rate. For siRNA studies, cells were seeded into 96-well black ViewPlate (Perkin-Elmer) at a density of 5,000 (A549, H460, and H1975) or 10,000 (H358) cells per well. Twenty-four hours after plating, cells were transfected with 100 nM siRNA using 0.2 μL DharmaFECT 1 transfection reagent per well, according to the manufacturer's instructions. Growth was determined 96 h following siRNA by fixing cells with 3% (vol/vol) paraformaldehyde for 10 min, staining with 10 μM Hoechst 33342 DNA marker (20 mins), and then imaging cells using an IN Cell Analyzer 2000 wide-field epifluorescence microscope (GE Health Care). Images were obtained from nine fields per well at 10× magnification and Hoechst-stained nuclei counted using the object recognition function of Developer 1.7 software. Alternatively, the relative number of viable cells were determined by adding AlamarBlue dye (5) at a final concentration of 10% (vol/vol) and measuring the fluorescence 3–5 h later using a Perkin-Elmer Victor^3 V multilabel plate reader (Ex, 530 nm; Em, 590 nm). For inhibitor studies, cells were grown, treated, and IC_{50} values determined as described previously (6, 7).

Generation of Stable Cell Lines. H358 cells were seeded at a density of 200,000 cells per well of a six-well tissue culture plate in RPMI-1640 growth media supplemented with only 10% FBS (vol/vol). Twenty-four hours later, cells were transfected with either 1 μg pCMV6 empty vector or pCMV6-SOD1 (with FLAG and MYC tags at the C terminus) using Fugene 6 transfection reagent (Roche Diagnostics) according to the manufacturer's instructions. A geneticin-resistant population of cells was selected by growing the transfected cells in 400 μg/mL geneticin (added 48 h after transfection) for an additional 21 d. After this time, cells were grown in complete growth media without any selection antibiotic. Cells were used for experiments after a minimum of 7 d out of selection medium.

Preparation of Cell Extracts and Affinity Chromatography. Cells (thirty 20-cm dishes of cells at 60% confluency) were lysed in a buffer containing 10 mM Hepes, pH 7.5, 150 mM NaCl, 0.5% Triton X-100, 10% glycerol, 1 mM EDTA, 10 mM $Na_4O_7P_2$, and 10 mM NaF. At the time of the experiment, the following was added to the lysis buffer: 1 mM Na_3VO_4, 10 μg/mL aprotinin, 10 μg/mL leupeptin, 1 mM phenylmethylsulphonyl fluoride, and 0.2 mM dithiothrietol. Lysates were precleared by centrifugation (10 min, 10,000 *g*, 4 °C) and then the NaCl concentration adjusted to 1 M. The cleared lysates were then loaded onto a chromatography column containing 500 μL affinity matrix and allowed to pass through at a flow rate of ∼100 μL/min at 4 °C. The affinity matrices were then washed with 10 column volumes of ice-cold lysis buffer (with 150 mM NaCl).

Mass Spectrometry and Protein Identification. Protein mixtures were denatured, and concentrated into a single, 1-mm wide stack by electrophoresing through an SDS stacking gel until entering the separation gel. Gels were then stained with Coomasie dye, protein stack excised, and proteins digested with trypsin, and then samples processed as described (8). Analysis of the resulting peptides was performed with a QSTAR-Elite hybrid quadrupole time-of-flight mass spectrometer (AB/MDS Sciex) equipped with a NanoSpray ion source (AB/MDS Sciex). Initial protein identification from LC-MS/MS data was done using the Mascot search engine (Matrix Science, version 2.2.04), National Center for Biotechnology Information and International Protein Index databases. One missed tryptic cleavage site was allowed, precursor ion mass tolerance = 0.4 Da, fragment ion mass tolerance = 0.4 Da, protein modifications were allowed for Met-oxide, Cys-acrylamide, and N-terminal acetylation. MudPit scoring was typically applied with "require bold red" activated, and using significance threshold score of $P < 0.01$.

SOD1 Enzymatic Assay. The SOD1 assay uses a tetrazolium salt for detection of superoxide radicals produced in a coupled-enzyme assay. In brief, SOD1 purified from bovine erythrocytes (0.2 U/mL) was incubated with 0.1 mM hypoxanthine, radical detector (tetrazolium salt), and inhibitors for 20 min at room temperature in 96-well plates. Reaction was initiated by the addition of xanthine oxidase to all wells and allowed to proceed for 20 min at room temperature on a shaker. The absorbance of each sample was measured at 450 nm using a plate reader. A standard curve of SOD1 activity was generated using purified SOD1 (0–0.25 U/mL) and then SOD1 enzymatic activity in the samples was calculated by linear regression.

Gene Expression Profiling of Cells Exposed to Small Molecules. Data files were exported using GenomeStudio software, $\log_2$ transformed, quantile normalized, and analyzed using Partek Genomics Suite (v6.5). Expression levels were compared between treatment and control groups for each cell line using a Welch's *T* test with $P < 0.01$ considered significant. Genes significantly deregulated in the sensitive cell lines upon treatment (and not in the resistant line in the case of gefitinib treatments) were then identified using the Venn function in Partek. Values for each of these overlapping genes were normalized and scaled from 0 to 100 across the sample set and heatmaps were generated using Genesis software (PMID: 11836235). Fisher's exact test was used to determine whether there was a significant overlap in the regulated genes between cell lines that were treated with the same compound.

Gene-Expression Microarray Analysis of Lung Cancer Cell Lines and Clinical Lung Cancer Specimens. Affymetrix U133A and U133B array data for lung cancer cell lines were downloaded from the Gene Expression Omnibus (accession no. GSE4824) and normalized using robust multichip average (RMA) algorithm (PMID: 12925520). For cell lines in which IC_{50} values for inhibition of growth were determined, expression levels for genes of interest were assessed for correlations with IC_{50} values using a Spearman test and implemented in GraphPad Prism. Affymetrix U133A microarray data for 58 lung adenocarcinoma tumors and 49 normal lung tissue samples (31 pairs) and a set of 26 lung adenocarcinoma/adjacent normal pairs were downloaded from the Gene Expression Omnibus (accession nos. GSE10072 and GSE7670, respectively) and normalized using RMA (PMID: 12925520). The resulting normalized intensity values were compared between tumor and normal samples using Welch's *T* test (for unpaired analysis) or a paired *t* test (for paired analysis) with a two-tailed *P* value ≤ 0.05 considered significant.

Proliferation, DNA Synthesis, and Apoptosis Measurements. To determine rates of proliferation, cells were seeded at a density of 25,000 cells per well of a six-well plate. The number of cells was counted starting 48 h later by detaching cells with trypsin and counting cells using a hemocytometer. Cells were plated in duplicates and two experiments were performed 80 d apart. To measure rates of DNA synthesis, cells were seeded at a density of 20,000 cells per well in 24-well tissue culture plates. Twenty-four hours later, cells were fed fresh growth media containing 0.5 μCi/mL of H^3-methyl thymidine for 1 h. Cells were then washed three times with ice-cold 10% trichloroacetic acid (vol/vol) and lysed in 0.5 M NaOH. Radiation was detected by liquid scintillation counting. To determine the apoptosis index, cells were plated at a density of 30,000 cells per well

in 96-well tissue culture plates. Caspase-3/7 activity was determined 24 h later in cell homogenates using a commercially available fluorescence-based assay (Promega; ApoONE caspase-activity kit).

Western Blotting. Cells grown in 25-cm diameter dishes were lysed at 60% confluency with 1 mL lysis buffer per plate [25 mM Tris-HCl, pH 7.6, 150 mM NaCl, 1% Nonidet P-40 (vol/vol), 1% sodium deoxycholate (wt/vol) and 0.1% SDS (wt/vol)]. At the time of the experiment, the following was added to the lysis buffer: 1 mM Na_3VO_4, 10 μg/mL aprotinin, 10 μg/mL leupeptin, 1 mM phenylmethylsulphonyl fluoride, and 0.2 mM dithiothrietol. For Western blotting, 50 μg whole-cell extracts were resolved on 4–20% gradient gels, transferred onto nitrocellulose membranes, and then immunoblotted for the desired protein.

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8. Erdjument-Bromage H, et al. (1998) Examination of micro-tip reversed-phase liquid chromatographic extraction of peptide pools for mass spectrometric analysis. *J Chromatogr A* 826:167–181.

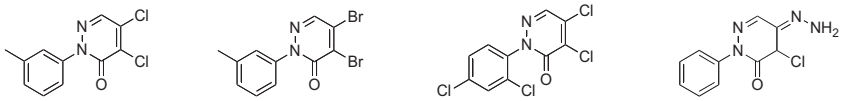

Fig. S1. Structures of active and inactive LCS-1 analogs. LCS-1: 4,5-dichloro-2-*m*-tolylpridazin-3(2*H*)-one; LCS-1.28: 4,5-dibromo-2-*m*-tolypridazin-3(2*H*)-one; LCS-1.34: 4,5-dichloro-2(2,4-dichlorophenyl)pyridazin-3(2*H*)-one; LCS-1.11: (Z)-4-chloro-5-hydrazono-2-phenyl-4,5-dihydropyridazin-3(2*H*)-one. LCS-1, LCS1.28 and LCS-1.34 have similar activity profiles against lung cancer cell lines; LCS-1.11 does not affect the growth of these cells (7).

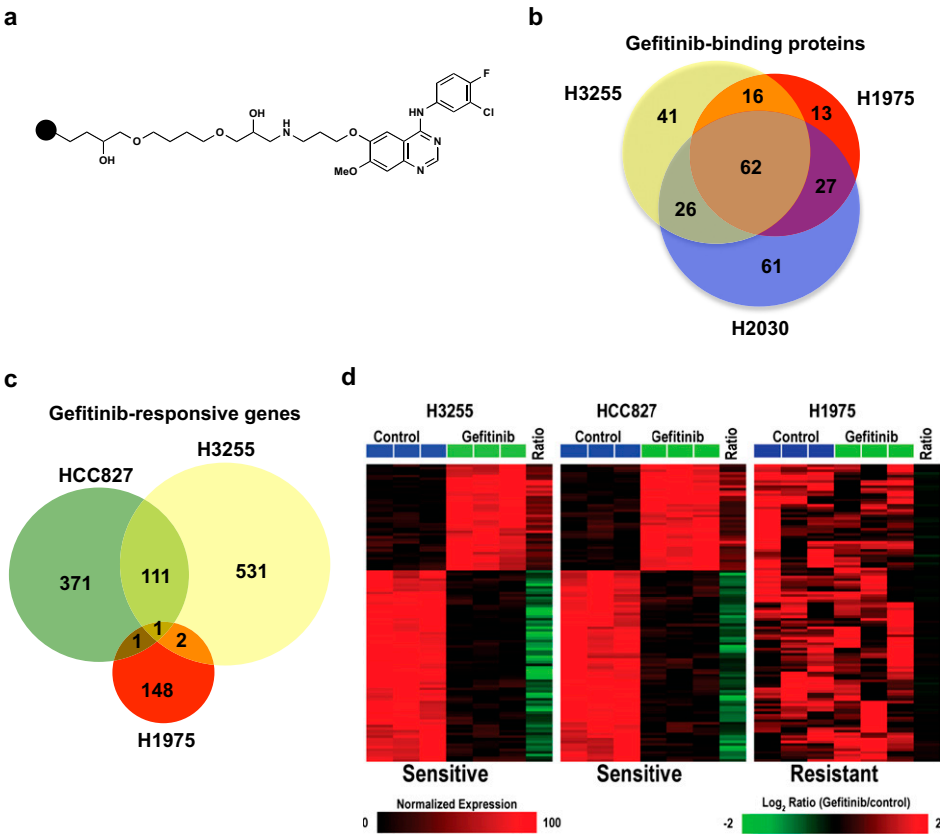

Fig. S2. Confirmation of EGFR as a major gefitinib target using affinity proteomic and genomic approaches. (*A*) Gefitinib was immobilized to sepharose 6B beads (closed circles) to create an affinity reagent to isolate binding partners for gefitinib in extracts of H1975, H2030, and H3255 cell lines. (*B*) The numbers of proteins eluted with unbound gefitinib (1 mM) from the gefitinib-affinity column are shown in the Venn diagram. Affinity chromatography and MS identification were performed at least three times for each cell line. (*C*) HCC827, H3255, and H1975 cells were treated for 3 h with 100 nM gefitinib and then gene expression profiles were determined using the Illumina Human HT-12 expression array. The numbers of gefitinib-responsive genes ($P < 0.01$) in each cell line are shown in the Venn diagram. The 112 genes that were regulated in both HCC827 and H3255 comprise the gefitinib gene signature. (*D*) "Heatmaps" of the altered expression of the 112 gefitinib-responsive genes in HCC827 (*Left*) and H3255 (*Center*) cells. Most of these genes (111) were not regulated in H1975 cells (*Right*). The ratio (gefitinib/control) of the average expression of each gene in three replicates is shown in the last column of the heatmaps.

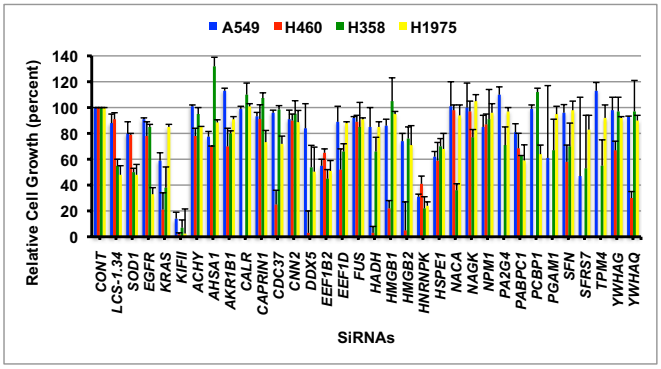

Fig. S3. Only siRNAs to SOD1 mimic the effect of LCS-1 on growth of lung cancer cell lines. Cells were transfected with siRNAs that target genes whose products were candidate LCS-1–binding proteins in 96-well plates. Ninety-six hours following transfection, cells were fixed and labeled with Hoechst nuclear stain; images were acquired using an IN Cell Analyzer 2000 wide-field epifluorescence microscope as described in *SI Materials and Methods*.

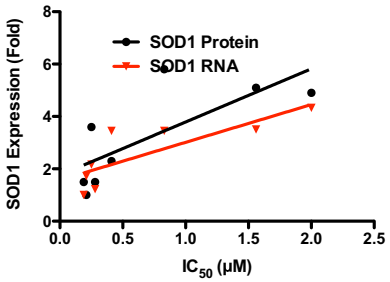

Fig. S4. Correlation between SOD1 levels and sensitivity to LCS-1. Cell lines for which data were available for sensitivity, *SOD1* RNA and SOD1 protein were used. There is a significant, inverse correlation between sensitivity to LCS-1 and the level of *SOD1* RNA (Spearman's $r = 0.8167$, $P = 0.0108$) or SOD1 protein (Spearman's $r = 0.8264$, $P = 0.0154$).

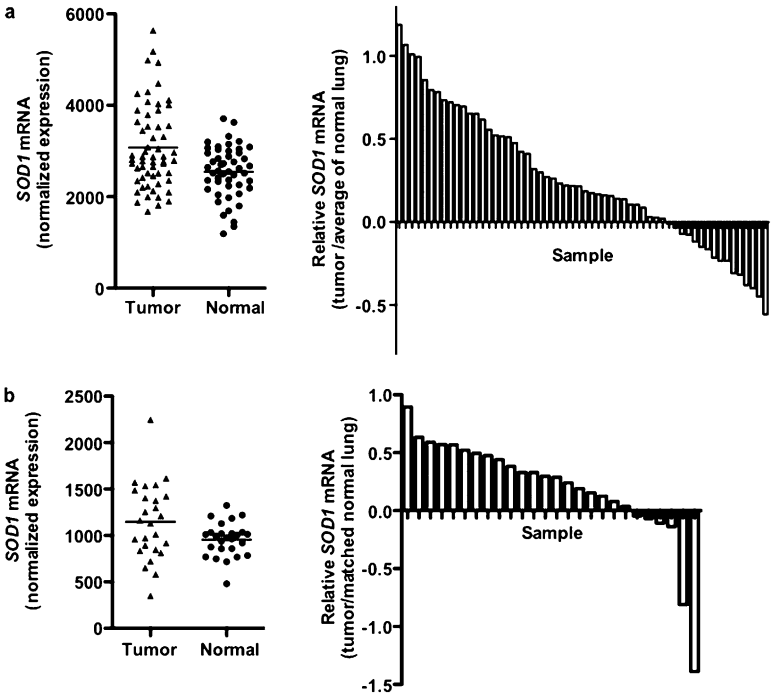

Fig. S5. *SOD1* is expressed at higher levels in some human lung cancers than in normal lung tissue. (*A*) *SOD1* expression in human lung adenocarcinomas and normal lung tissue is shown at *Right* for (*A*) 58 lung adenocarcinoma samples and 49 normal lung tissue (including 31 pairs) or (*B*) 26 lung adenocarcinomas paired with adjacent normal lung. The ratio of SOD1 mRNA (tumor/normal) is shown for each tumor/normal pair at *Right*. Data were obtained from the Gene Expression Omnibus (accession nos. GSE10072 and GSE7670 for *A* and *B*, respectively) and analyzed as described under *SI Materials and Methods*.

Table S1. Cell lines, known genetic alterations, IC_{50} for inhibition of cell growth by LCS-1, and relative *SOD*1 expression

Cell line	Known altered gene	Known mutation/copy number change	IC_{50} for growth inhibition by LCS-1, μM	*SOD1* mRNA, normalized expression
H358	*KRAS*	G13C	0.19 ± 0.04*	852
H23	*KRAS*	G12C	0.20 ± 0.01*	1,417
HCC1195	*TITF1*	Amplification	0.20 ± 0.01*	1,796
H2347	*NRAS*	Q61R	0.20 ± 0.01*	2,428
HCC4011	*EGFR*	L858R	0.20 ± 0.06*	ND
HCC827	*EGFR*	DelE746-A750	0.22 ± 0.03*	1,044
11–18	*EGFR*	L858R	0.24 ± 0.07*	ND
H2444	*KRAS*	G12V	0.24 ± 0.01*	ND
H1975	*EGFR*	L858R/T790M	0.25 ± 0.02*	1,841
H820	*EGFR*	DelE746-749/T790M	0.28 ± 0.07*	3,470
H1648	*MET*	Amplification	0.28 ± 0.02*	2,358
HCC4006	*EGFR*	Del746-748	0.41 ± 0.03	2,953
H2030	*KRAS*	G12C	0.54 ± 0.08	ND
H2405	*MAP2K4*	c.814–1200del387; p?	0.55 ± 0.09	ND
HCC2935	*EGFR*	DelE746-S752, I ins	0.64 ± 0.06	1,468
PC9	*EGFR*	DelE746-A759	0.65 ± 0.02	ND
H2009	*KRAS*	G12A	0.76 ± 0.01	2,150
H2122	*KRAS*	G12C	0.83 ± 0.32	2,973
HCC2279	*EGFR*	DelE746-A750	0.85 ± 0.07	4,347
H3255	*EGFR*	L858R	0.88 ± 0.15	1,828
HCC4019	*KRAS*	G12C	0.92 ± 0.03	ND
H1437	*MET*	R988C	0.98 ± 0.05	2,814
A549	*KRAS*	G12S	1.52 ± 0.07	2,891
H460	*KRAS*	Q61H	1.56 ± 0.06	2,565
H1734	*KRAS*	G13C	1.94 ± 0.24	ND
H1650	*EGFR*	DelE746-A750	2.00 ± 0.35	3,694
NHBE	None	None	2.66 ± 0.22	1,863
WI-38	None	None	5.52 ± 1.2	ND

The IC_{50} for inhibition of growth by LCS-1 (4,5-dichloro-2-*m*-tolylpyridazin-3(2*H*)-one) was determined 96 h after compound treatment using AlamarBlue viability dye. The known mutations in the cell lines and the growth inhibitory activities of LCS-1 in descending order of sensitivity are shown here. Results are the mean ± SE of 2–10 IC_{50} determinations. *IC_{50} for inhibition of growth of tumor cell lines that were 10-fold more sensitive to LCS-1 was compared with that of NHBE and found to be significantly lower ($P < 0.05$). Affymetrix U133A and U133B array data for lung cancer cell lines were downloaded from the Gene Expression Omnibus (accession no. GSE4824), normalized using robust multichip average (RMA) algorithm (PMID: 12925520) and analyzed as described in *Materials and Methods*. *SOD1* expression as a function of IC_{50} is displayed in Fig. 3*A*. ND, not determined.

Table S2. Detection of putative gefitinib targets by affinity proteomics

Identified proteins	Gene ID	Accession no.	MW (kDa)	Number of assigned peptides		
				H3255	H1975	H2030
Epidermal growth factor receptor	EGFR	IPI00018274	134	24	2	ND
Myosin-14	MYH14	IPI00337335	229	14	1	ND
Protein disulfide-isomerase	P4HB	IPI00010796	57	12	ND	2
Galectin-3	LGALS3	IPI00465431	26	12	ND	ND
Protein disulphide-isomerase A3	PDIA3	IPI00025252	57	11	ND	1
Protein disulphide-isomerase A6	PDIA6	IPI00299571	54	10	ND	4
Flavin reductase	BLVRB	IPI00219910	22	9	ND	2
Adipocyte plasma membrane-associated protein	ADMAP	IPI00031131	46	8	ND	ND
Carcinoembryonic antigen-related cell adhesion molecule 1	CEACAM1	IPI00027412	37	8	ND	ND
5′-nucleotidase	NT5E	IPI00009456	63	7	ND	ND
Farnesyl pyrophosphate synthetase	FDPS	IPI00902799	13	6	ND	1
Napsin-A	NAPSA	IPI00005800	48	6	ND	ND
Nucleoside diphosphate kinase, mitochondrial	NME4	IPI00012972	21	6	ND	ND
Alpha-actinin-4	ACTN4	IPI00013808	105	6	ND	ND

Gefitinib-binding proteins were isolated using a gefitinib affinity matrix from extracts of gefitinib-sensitive (H3255) and gefitinib-insensitive cell lines (H1975 and H2030), eluted with 1 mM gefitinib and analyzed by LC-MS/MS. Proteins for which the total number of assigned tryptic peptides were present at higher levels in the eluates of H3255 than in H1975 and H2030 are listed. Results represent the number of merged tryptic peptides (assigned spectra) obtained from three independent experiments for each cell line. Identified proteins were mapped to the International Protein Index (IPI) database (accession nos. given above). ND, not detected.

Table S3. LCS-1–binding proteins

Protein name	Gene ID	Accession no.	MW (kDa)	Number of assigned peptides			
				LCS-1.28		LCS-1	
				H1975	H358	H1975	H358
Polyadenylate-binding protein 1	PABPC1	IPI00008524	71	30	3	26	1
Proliferation-associated protein 2G4	PA2G4	IPI00299000	44	6	5	4	4
N-acetylglucosamine kinase	NAGK	IPI00296526	42	6	1	3	1
Nucleophosmin	NPM1	IPI00220740	29	3	11	2	ND
DEAD box polypeptide 5	DDX5	IPI00017617	69	3	5	ND	4
High mobility group protein B1	HMGB1	IPI00419258	25	2	11	3	9
Splicing factor, arg/ser rich 7	SFRS7	IPI00003377	27	2	3	2	2
Cell cycle-associated protein 1	CAPRIN1	IPI00783872	78	2	3	ND	4
Heterogeneous nuclear ribonucleoprotein K	HNRNPK	IPI00216049	51	1	21	2	18
Eukaryotic translation elongation factor 1-delta	EEF1D	IPI00023048	31	1	14	6	8
Fused in liposcarcoma	FUS	IPI00221354	53	1	9	ND	11
Tropomyosin alpha-4 chain	TPM4	IPI00010779	29	1	9	ND	7
14–3-3 protein gamma	YWHAG	IPI00220642	28	1	6	1	5
3-hydroxyacyl-CoA dehydrogenase type-2	HADH	IPI00017726	27	1	6	ND	ND
Adenosylhomocysteinase	AHCY	IPI00012007	48	1	4	2	6
GTP-binding nuclear protein Ran	RAN	IPI00643041	24	1	3	1	9
Heterogeneous nuclear ribonucleoprotein H3	HNRNPH3	IPI00013877	37	1	3	ND	3
Ewing sarcoma breakpoint region 1	EWSR1	IPI00009841	69	1	3	ND	4
Eukaryotic translation initiation factor 2 subunit 3	EIF2S3	IPI00297982	51	1	2	ND	2
Aldo-keto reductase family 1, member B1	AKR1B1	IPI00413641	36	1	1	1	3
Calreticulin	CALR	IPI00020599	48	ND	13	3	10
Stratifin	SFN	IPI00013890	28	ND	12	2	10
Tyrosine 3-monooxygenase/tryptophan monooxygenase activation proteins, beta polypeptide	YWHAQ	IPI00018146	28	ND	10	2	10
High mobility group protein B2	HMGB2	IPI00219097	24	ND	10	1	7
Nascent polypeptide-associated complex subunit alpha	NACA	IPI00023748	23	ND	10	1	6
Hypoxanthine-guanine phosphoribosyltransferase 1	HPRT1	IPI00218493	25	ND	9	1	9
Eukaryotic translation elongation factor 1 beta 2	EEF1B2	IPI00178440	25	ND	7	2	5
10 kDa heat shock protein 1, mitochondrial	HSPE1	IPI00220362	11	ND	6	3	6
Fructose-bisphosphate aldolase	ALDOA	IPI00418262	48	ND	5	1	5
Poly(rC)-binding protein 1	PCBP1	IPI00016610	37	ND	3	1	4
Calponin-2	CNN2	IPI00015262	34	ND	3	1	2
Activator of 90-kDa heat shock protein ATPase homolog 1	AHSA1	IPI00030706	38	ND	2	3	5
Superoxide dismutase [Cu-Zn]	SOD1	IPI00218733	16	ND	2	1	2
Cell division cycle 37 homolog	CDC37	IPI00013122	44	ND	1	1	2

Proteins from whole cell extracts of H358 and H1975 cells were eluted from the LCS-1 and LCS-1.28 affinity columns using 0.5 mM LCS-1 or LCS-1.28, respectively. The identities of the proteins were elucidated by tandem LC-MS/MS and mapped to the IPI database (accession nos. given above). Results represent the number of merged tryptic peptides (assigned spectra) obtained from three independent experiments for each cell line. The 34 proteins listed here were not identified in experiments with the gefitinib affinity column. ND, not detected.

Other Supporting Information Files

Dataset S1 (XLSX)