NRF2 regulates serine biosynthesis in non–small cell lung cancer

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Tumors have high energetic and anabolic needs for rapid cell growth and proliferation1, and the serine biosynthetic pathway was recently identified as an important source of metabolic intermediates for these processes2,3. We integrated metabolic tracing and transcriptional profiling of a large panel of non–small cell lung cancer (NSCLC) cell lines to characterize the activity and regulation of the serine/glycine biosynthetic pathway in NSCLC. Here we show that the activity of this pathway is highly heterogeneous and is regulated by NRF2, a transcription factor frequently deregulated in NSCLC. We found that NRF2 controls the expression of the key serine/glycine biosynthesis enzyme genes PHGDH, PSAT1 and SHMT2 via ATF4 to support glutathione and nucleotide production. Moreover, we show that expression of these genes confers poor prognosis in human NSCLC. Thus, a substantial fraction of human NSCLCs activates an NRF2-dependent transcriptional program that regulates serine and glycine metabolism and is linked to clinical aggressiveness.

Uniformly labeled [13C]glucose ([U-13C]glucose) is metabolized via the glycolytic intermediate 3-phosphoglycerate (3-PG) to serine M3 (with three 13C-labeled atoms) and, subsequently, to glycine M2 via the glycolytic intermediate 3-phosphoglycerate (3-PG) to serine (Fig. 1a). To profile the activity of the serine/glycine biosynthesis pathway in NSCLC, we labeled a panel of 79 human NSCLC cell lines with [13C]glucose and quantified serine and glycine labeling via gas chromatography combined with mass spectrometry (GC/MS). We determined 6 and 24 h to be the optimal time points to detect labeling of serine and glycine (Supplementary Fig. 1). At 24 h, the fractional abundance of serine M3 and glycine M2 ranged from 0–40% (Fig. 1b,c), with significant correlation between the time points (Supplementary Fig. 2). Neither serine nor glycine labeling correlated with cellular doubling times (Supplementary Fig. 3).

There was a significant correlation between serine M3 and glycine M2 labeling at both time points, indicating that the glycine produced from glucose was derived from serine (Fig. 1d,e). As has been reported previously4,5, de novo serine synthesis conferred the ability to grow in the absence of extracellular serine (Fig. 1f,g). Thus, the serine biosynthesis pathway is not uniformly operant in NSCLC, and regulatory mechanisms exist for controlling the activation of this pathway in a subset of cell lines.

To identify a mechanism for increased serine biosynthesis in NSCLC, we correlated serine and glycine biosynthesis with gene expression using data from the Broad-Novartis Cancer Cell Line Encyclopedia (CCLE)5. We observed a significant correlation between serine and glycine labeling and the expression of the PHGDH gene encoding phosphoglycerate dehydrogenase (Fig. 1h and Supplementary Table 1), which catalyzes the first and rate-limiting step in serine biosynthesis5. Serine-high cell lines (with a serine M3 z score >0.5 at 24 h) were sensitive to PHGDH silencing (Fig. 1i), and ectopic PHGDH rescued the proliferation of ‘serine-low’ cell lines (serine M3 z score <0.5 at 24 h) in serine-deficient medium (Fig. 1j and Supplementary Fig. 4).

Previous studies have shown a role for PHGDH copy number gain in increasing serine biosynthetic activity5,6. However, we did not find evidence for this mechanism in NSCLC cell lines (Supplementary Fig. 5 and Supplementary Table 2). To investigate alternative mechanisms of PHGDH regulation, we performed gene set enrichment analysis (GSEA)6,7 on the genes whose expression positively correlated with serine and glycine biosynthesis. Interestingly, the gene set with targets of the transcription factor nuclear factor erythroid-2–related factor 2 (NRF2; encoded by NFE2L2) was the top hit (Supplementary Fig. 6), suggesting that NRF2 might be a regulator of PHGDH and the serine biosynthetic pathway.

Next, we examined NRF2 localization (Supplementary Fig. 7) and found a significant correlation of nuclear NRF2 with serine biosynthesis (Fig. 2a). Additionally, we ranked the cell lines according to expression of NRF2 target genes (Supplementary Fig. 8 and

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Supplementary Table 3), which significantly correlated with NRF2 abundance (Supplementary Fig. 7). ‘NRF2-high’ cell lines had significantly higher serine M3 labeling at 6 and 24 h and glycine M2 labeling at 24 h (Fig. 2b,c). Next, we silenced NRF2 (NFE2L2) and found that it regulates the expression of serine/glycine biosynthetic pathway genes (PHGDH, PSAT1, PSPH, SHMT1 and SHMT2) (Fig. 2d).
ATF4 transcriptionally activates serine biosynthetic genes in response to serine starvation in NSCLC cells. Interestingly, nuclear ATF4 expression correlated with both serine labeling and NRF2 protein expression (Supplementary Fig. 11). ATF4 has been reported as both a direct transcriptional target and heterodimerization partner of NRF2. In agreement with transcriptional regulation of ATF4 by NRF2, we observed marked reduction in ATF4 mRNA expression, binding of RNA polymerase II to the ATF4 promoter and the levels of newly synthesized ATF4 mRNA upon NRF2 knockdown (Fig. 3a and Supplementary Figs. 12a and 13a,b), with no effect on ATF4 mRNA stability (Supplementary Fig. 13c,d). Furthermore, ATF4 protein levels were decreased upon NRF2 knockdown (Fig. 3b), but NRF2 did not regulate ATF4 translation (Supplementary Fig. 14). Notably, ATF4

Figure 3 NRF2 regulates the expression of serine/glycine biosynthesis genes through ATF4. (a) ATF4 mRNA expression in A549 cells expressing scrambled shRNA or shNRF2. (b) Immunoblot analysis of NRF2, ATF4 and actin expression in the cells from a. (c) Immunoblot analysis of NRF2, ATF4 and serine pathway enzyme expression in lysates from A549 cells expressing scrambled shRNA, shNRF2 1 or ATF4 shRNA (shATF4) 1 or 2. (d) mRNA expression in the cells from c. (e) ATF4 knockdown impairs serine biosynthesis. Cell lines were grown in the presence of [U-13C]glucose for the indicated times, metabolites were extracted and fractional 13C labeling of serine was analyzed by LC/MS. (f) ATF4 rescues serine biosynthesis enzyme expression following NRF2 knockdown. A549 cells were infected with lentivirus encoding mouse ATF4 (mATF4) before infection with lentivirus encoding scrambled shRNA or shRNA targeting NRF2. (g) Immunoblot analysis of NRF2, ATF4 and actin expression in the cells from f. (h) ATF4 rescues the serine biosynthesis defect in A549 cells with shRNA-mediated knockdown of NRF2. Cells were assayed as in e. (i) ATF4 rescues the growth of H1975 cells in serine-deficient medium. Cells expressing luciferase, PHGDH or ATF4 were grown in the indicated media for 3 d, and cell number was normalized to that for cells grown in full medium. (j) Chromatin immunoprecipitation with antibody to ATF4 of the PHGDH, PSAT1 and SHMT2 promoters. Binding was normalized to that in immunoprecipitation with IgG control. P2 is a binding site (Supplementary Fig. 16). Results are the average of three technical (a,d,f,j) or biological (e,h,i) replicates. All error bars, s.e.m.
knockdown reduced the expression and activity of the serine pathway components to an extent similar to that seen with NRF2 knockdown, whereas it did not affect NRF2 expression (Fig. 3c–e). Ectopic expression of NRF2 in NRF2-depleted cells partially rescued NQO1, ATF4 and serine biosynthesis enzyme expression (Supplementary Fig. 12c) and induced expression of these genes in the serine-low cell line H1975 (Supplementary Fig. 12d), confirming the regulation of these genes by NRF2. Similarly, ectopic ATF4 expression rescued the effects of NRF2 silencing on serine biosynthesis enzyme expression (Fig. 3f,g and Supplementary Fig. 12b) and serine labeling from glucose at early time points (Fig. 3h), although defects in serine production were observed at later time points (Supplementary Fig. 15). Furthermore, ectopic ATF4 rescued the growth of H1975 cells in serine-deficient medium (Fig. 3i). We identified the ATF4 binding sites in the PHGDH, PSAT1 and SHMT2 promoters (Supplementary Fig. 16) and found that, although NRF2 itself did not bind to these sites (Supplementary Fig. 16), NRF2 silencing significantly decreased binding of ATF4 (Fig. 3j). These results demonstrate that NRF2 regulates serine biosynthesis gene expression through ATF4.

We examined how PHGDH-derived serine contributes to downstream metabolism (Fig. 4a). PHGDH, NRF2 or ATF4 silencing decreased the incorporation of glucose-derived serine into cystathionine (Supplementary Fig. 17a,b) and the incorporation of glucose-derived glycine into glutathione (Fig. 4b, Supplementary Fig. 17c), without loss of cell viability (Supplementary Fig. 18). Metabolism of serine to glycine results in the production of one-carbon donors (Fig. 4c, Supplementary Fig. 18), without loss of cell viability (Supplementary Fig. 18). We observed a decrease in the PHGDH-derived labeling (M+7) of purines, including inosine monophosphate (IMP), adenosine monophosphate (AMP), adenosine diphosphate (ADP) and inosine, following PHGDH or ATF4 silencing, whereas NRF2 silencing decreased both the ribose (M+5) and PHGDH-derived (M+7) labeling of these purines (Fig. 4c and Supplementary Fig. 17d–f). Labeling in serine-high cell lines of glutathione and purines was significantly higher at 48 h than at 24 h (Fig. 4d,e and Supplementary Fig. 19a,b). Furthermore, the majority of the 13C-labeled serine and glycine had escaped the cell by 24 h of labeling with [13C]glucose owing to equilibration with unlabeled amino acids in the medium, suggesting
that the fractional labeling at 24 h was underestimating the total contribution of PHGDH to these metabolite pools (Supplementary Fig. 20 and Supplementary Note). In support of this notion, PHGDH, NRF2 or ATF4 silencing resulted in significant decreases in the total levels of purines and thymidine nucleotides (Fig. 4f) as well as glutathione, cystathionine and homocysteine (Fig. 4g). In contrast, we did not observe differences in the S-adenosyl methionine (SAM)/S-adenosyl homocysteine (SAH) ratio, which is also modulated by the folate cycle, or in histone or DNA methylation (Supplementary Fig. 21). Furthermore, we observed a significant decrease in the nicotinamide adenine dinucleotide phosphate reduced/oxidized (NADPH/NADP+) ratio in serine-high cells following PHGDH silencing that was not observed in serine-low cells (Fig. 4h). These results demonstrate that the serine biosynthesis pathway supports glutathione and nucleotide production in NSCLC.

We next asked whether this pathway promotes tumorigenesis. PHGDH silencing significantly impaired the soft agar growth of serine-high but not serine-low cell lines (Fig. 5a). Interestingly,

Figure 5 Activation of the serine biosynthesis pathway promotes tumorigenesis in NSCLC. (a) PHGDH knockdown impairs the soft agar growth of serine-high but not serine-low cell lines. (b,c) Soft agar growth correlates with serine (b) and glycine (c) labeling at 24 h. Each cell line was plated at 5,000 cells/well, and the number of colonies was determined after 14 d. P values were calculated by Student’s t distribution with n = 2 degrees of freedom. (d) PHGDH knockdown impairs the xenograft growth of a serine-high cell line (PC9; left) but not a serine-low cell line (H1373; right). Results are the averages for five tumors. (e) Immunoblot analysis of PHGDH expression of the cell lines and xenografts from d upon injection and at the end point. (f) Patients with high NRF2 protein expression (Z score >0.5) demonstrate elevated serine pathway gene expression in samples from The Cancer Genome Atlas (TCGA) lung adenocarcinoma cohort. Boxes represent mean values, and error bars represent s.e.m. (g) Gene expression of PHGDH, PSAT1 and SHMT2 in the Director’s Consortium Lung adenocarcinoma data set clusters patients into cohorts with high and low expression. (h) Kaplan-Meier survival analysis of patients with high (n = 29; red) or low (n = 414; blue) expression of PHGDH, PSAT1 and SHMT2 based on the patient clustering from g. Median survival was 36 months (high expression) versus 73.2 months (low expression). The P value was calculated using the Mantel-Cox test. All error bars, s.e.m.; *P < 0.05, **P < 0.01, ***P < 0.001.

Figure 6 Model of the regulation of serine/glycine biosynthesis by NRF2. An ATF4 transcriptional program, indirectly activated by NRF2 or KEAP1 gene mutations, regulates the expression of serine/glycine biosynthesis enzymes. These enzymes produce serine and glycine from the glycolytic intermediate 3-PG and funnel the carbon into glutathione and nucleotides via the folate and transsulfuration cycles. NRF2- and ATF4-regulated enzymes are shown in red. 3-PG, 3-phosphoglycerate; 3-PP, 3-phosphohydroxy pyruvate; 3-PS, 3-phosphoserine; GSH, glutathione.
we observed a significant correlation between serine and glycine labeling and colony number in soft agar (Fig. 5b,c). Furthermore, PHGDH silencing impaired the xenograft growth of the serine-high cell line PC9 (Fig. 5d, left) but not the serine-low cell line H1373 (Fig. 5d, right). We observed that all tumors re-expressed PHGDH at the end point (Fig. 5e), suggesting that PHGDH was required for tumor formation. Next, we examined whether serine pathway gene expression correlated with the overall survival of human patients. Human tumors with high NRF2 protein expression displayed significantly poorer prognosis (cancergenome.nih.gov/). Furthermore, we found that high expression of PHGDH, PSAT1 and SHMT2 conferred a significantly poorer prognosis (Fig. 5g,h) and was associated with higher tumor grade (Supplementary Fig. 22b–d). These results demonstrate that, in human NSCLC, NRF2 regulates the expression of serine biosynthetic enzymes, which correlates with poor prognosis.

We have demonstrated a striking heterogeneity in the activity of the serine biosynthetic pathway in NSCLC. Notably, intracellular amino acid labeling from [13C]glucose is underestimated because of rapid exchange with 13C-containing amino acids from the medium, which is likely mediated by amino acid antiporters (Supplementary Note). Caution should be used when interpreting data from 13C-labeling experiments because of these exchange mechanisms.

Heterogeneity in the activity of metabolic or signaling pathways is a common phenomenon across tumors, cell lines and even between cells from the same tumor. Here, by systematically analyzing the serine/glycine biosynthesis pathway in a large, highly annotated panel of NSCLC cell lines, we identified NRF2 as the molecular driver of this pathway. NRF2 is frequently deregulated in NSCLC through somatic amplification and altered glucose metabolism in human melanoma. Regulation of the pentose phosphate pathway to supply ribose for nucleotides24,25. NRF2 indirectly regulates ATF4 transcriptional activity via PSAT1 and PHGDH to supply the substrates for glutathione and nucleotide biosynthesis24,25. NRF2 regulates the expression of genes involved in anabolic metabolism24,25. Here we demonstrate that NRF2 regulates a serine biosynthesis metabolic program via ATF4 and PHGDH to supply the substrates for glutathione and nucleotide production (Fig. 6), with regulation of this program synergizing with regulation of the pentose phosphate pathway to supply ribose for nucleotides24,25. NRF2 indirectly regulates ATP4 transcriptional activity via unknown mechanisms. Additionally, although ATF4 completely rescues PHGDH expression following NRF2 depletion, the partial rescue of other serine biosynthesis genes suggests that NRF2 regulates these genes combinatorially through ATF4 and additional factors. Furthermore, these findings suggest that multiple NRF2-regulated pathways coordinately contribute to tumorigenesis. Our work encourages the integration of metabolite tracing on large panels of cancer cell lines with gene expression analysis. This approach is a powerful tool for determining the mechanisms responsible for the differential regulation of metabolic pathways and may identify additional links between the activity of metabolic pathways and genetic alterations in cancers.

URLs. The Cancer Genome Atlas Research Network, http://cancergenome.nih.gov/.

METHODS

Methods and any associated references are available in the online version of the paper.

Note: Any Supplementary Information and Source Data files are available in the online version of the paper.

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AUTHOR CONTRIBUTIONS

G.M.D., R.J.D. and L.C.C. designed the study. G.M.D. and E.M. performed molecular biology experiments. G.M.D., P.H.C., E.M., J.A.S., Z.H. and J.M.A. performed metabolomics and isoform labeling and analyzed the data. D.W. performed xenograft experiments. H.T. and Y.X. performed bioinformatics analysis. K.E.H., I.L.W. and J.D.M. contributed highly annotated lung cancer cell lines. G.M.D., E.M. and L.C.C. wrote the manuscript. All authors commented on the manuscript.

COMPETING FINANCIAL INTERESTS

The authors declare competing financial interests: details are available in the online version of the paper.

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ONLINE METHODS

Animals. Male nude mice (CrTac:NCr-Foxn1nu) were obtained from Taconic and maintained under pathogen-free conditions. Experiments were performed according to Institutional Animal Care and Use Committee (IACUC) guidelines. Mice were injected at 8 weeks of age with 2 × 10⁶ NSCLC cells on each flank. shRNAs were randomized so they were evenly distributed across mice. As the animal study was exploratory, no statistical test was used to determine adequate sample size. No mice were excluded from the analysis. The study did not use blinding.

Cell culture. All NSCLC cell lines used in this study were obtained from the Hamon Cancer Center Collection (University of Texas–Southwestern Medical Center). Cells were maintained in RPMI-1640 (Life Technologies) supplemented with 5% or 10% FCS without antibiotics at 37 °C in a humidified atmosphere containing 5% CO₂ and 95% air. All experiments were performed in media containing serum and glycine except where otherwise noted. All cell lines have been DNA fingerprinted using the PowerPlex 1.2 kit (Promega) and tested for mycoplasma by the e-Myc (Boca Scientific) or MycoAlert (Lonza) kit. Although NCI-H135 is listed in the database of commonly misidentified cell lines, it was originally derived by J.D.M. and fingerprinted before use.

CellTox Green staining. Cells were incubated in RPMI supplemented with 10% FCS containing 1× CellTox Green (Promega) and 5 µM Syto 17 (Life Technologies) for 20 min, washed in PBS and imaged on an EVOS FL cell imaging system (Life Technologies).

Chromatin immunoprecipitation. Cells (5 × 10⁶) were fixed at 37 °C in RPMI with 1% formaldehyde for 10 min; lysed in 1% SDS, 10 mM EDTA and 50 mM Tris-HCl, pH 8.1, plus protease inhibitors and sonicated with a probe tip until DNA was an average of 1 kb in size. Input was saved, and lysate was diluted in immunoprecipitation buffer (1% Triton, 2 mM EDTA, 150 mM NaCl and 20 mM Tris-HCl, pH 8.1) and mixed with beads (Dynal Protein A, Invitrogen) that were prebound overnight with antibodies to Nrf2 (H-300, Santa Cruz Biotechnology), ATF4 (11815, Cell Signaling Technology) and RNA polymerase II (pS5, ab5131, Abcam) or with rabbit IgG (sc-2027, Santa Cruz Biotechnology). Chromatin was immunoprecipitated overnight, and beads were washed six times with RIPA buffer (50 mM HEPES, pH 7.6, 1 mM EDTA, 0.7% sodium deoxycholate, 1% NP-40 and 0.5 M lithium chloride) and twice with TE buffer. Beads were incubated with solution containing 1% SDS and 0.1 M NaHCO₃ for 30 min at room temperature, and cross-links were then reversed for both the input and the immunoprecipitate by heating overnight in a 65 °C water bath. DNA was purified with a QIAquick spin kit (Qiagen), and quantitative PCR was performed in triplicate with Fast SYBR Green Master Mix on a Step One Real-Time PCR system (all from Life Technologies). Primer sequences are listed in Supplementary Table 4.

DNA methylation. DNA was extracted from cells in lysis buffer (10 mM Tris, 100 mM NaCl, 10 mM EDTA, 0.5% SDS and 0.4 µg/ml proteinase K, pH 8.0) by heating overnight at 65 °C. DNA was purified by precipitating protein with sodium chloride and precipitated with isopropanol. DNA (2 µg) was denatured in solution with 0.4 M NaOH and 10 mM EDTA at 95 °C for 10 min, and samples were neutralized by the addition of an equal volume of 2 M ammonium acetate (pH 7.0). DNA (200 ng) was spotted on a nylon membrane (GE Healthcare), cross-linked twice on Optimal mode in a Stratalinker oven and blocked in 10% FCS containing 1× CellTox Green (Promega) and 5 µM Syto 17 (Life Technologies) for 20 min, washed in PBS and imaged on an EVOS FL cell imaging system (Life Technologies).

Liquid chromatography and mass spectrometry measurement of total and 13C-labeled metabolites in A549 cells. Cells were plated the day before labeling at 2 × 10⁶ cells/10-cm dish, and the medium was changed to glucose-free RPMI containing 10% dialyzed serum and 10 mM [U-13C]glucose for the indicated time points. Metabolites were extracted in ice-cold 80% methanol and analyzed by targeted LC-MS/MS via selected reaction monitoring (SRM), as described. The following precursor ions were monitored: serine: m/z 106 (M+0) and 109 (M+3); cystathionine: m/z 223 (M+0) and 226 (M+3); glutathione: m/z 308 (M+0); glutathione (glycine): m/z 310 (M+0); homocysteine: m/z 136; IMP: m/z 249 (M+0), 354 (M+5) and 356 (M+7); inosine: m/z 367 (M+0), 272 (M+5) and 274 (M+7); AMP: m/z 248 (M+0), 353 (M+5) and 355 (M+7); dATP: m/z 390; dTTP: m/z 341; dTDP: m/z 323. The indicated amino acid for glutathione indicates which constituent amino acid was 13C-labeled. Samples were analyzed in triplicate. Data represent median-normalized values.

Luciferase assays. Luciferase assays were performed with the Dual-Glo Luciferase Assay System (Promega) according to the manufacturer’s instructions. NADPH/NADP+ ratios. NADP⁺ and NADPH concentrations were determined with the NADP/NADPH-Glo Assay kit (Promega) according to the manufacturer’s protocol.

NRF2 score calculation. Cell lines were grouped into NRF2-high and NRF2-low groups on the basis of the expression of five classic NRF2 target genes: NQO1, GCLC, GCLM, SLC7A11 and AKR1C1. The expression of each gene was normalized to the median across the cell lines, and the results for these five genes were then averaged together. NRF2 high versus NRF2 low was defined as the top quartile versus the bottom three quartiles. The top 20 overexpressed genes in the NRF2-high cell lines in comparison to the NRF2-low cell lines were used to calculate the NRF2 score: AKR1C1, AKR1C2, SPP1, ALDH3A1, LOC344887, AKR1C3, OSSN1, PGD, CYP4F1, AKR1B10, KIAA0319, SRXN1, NR0B1, SLC7A11, LOC100292680, ABC2C, CABYR, JAKMIP3, KNYU and PTGR1. Gene expression values were normalized to the median, and the results for these 20 genes were then averaged together to obtain the NRF2 score. Individual gene expression values and NRF2 scores are listed in Supplementary Table 3, and NRF2-high versus NRF2-low clustering is shown in Supplementary Figure 9.

Patient samples and survival analysis. The National Cancer Institute Director’s Challenge Consortium study (Director’s Consortium) and TCGA lung adenocarcinoma data were used in this study to evaluate the prognostic performance of gene signatures. The Director’s Consortium data set collected 442 resected lung adenocarcinomas at four US institutions and the TCGA
Research Network data include 203 patient samples for which gene expression and survival data are available. Unsupervised cluster analysis was used to group patients on the basis of the expression of PHGDH, PSAT1 and SHMT2, using average linkage clustering with the Spearman’s rank correlation distance metric. Clustering was performed with Cluster 3.0. Heat-map visualization was performed with JavaTreeView. Kaplan-Meier survival curves were used to determine the survival rate as a function of time, and survival differences were analyzed by a log-rank Mantel-Cox test using GraphPad Prism.

**Proliferation assays.** Cells were seeded at 500–10,000 cells/well in 96-well plates on day −1 and infected on day 0 with lentivirus. Alternatively, cells were switched into RPMI supplemented with 10% dialyzed FBS containing full amino acids, lacking serine, or lacking serine and glycine. Plates were fixed on the indicated days with 4% paraformaldehyde, stained with crystal violet, washed and dried. Crystal violet was solubilized in 10% acetic acid, and OD600 was measured.

**Pulse labeling with 4-thiouridine.** Cells were labeled in 10-cm dishes at 70% confluence with 200 µM 4-thiouridine (4sU, Sigma-Aldrich) for 60 min as described. RNA was extracted with TRIzol. 4-thiouridine–containing mRNA molecules were biotinylated with biotin-HPDP (EZ-Link Biotin-HPDP, Pierce, 21341) and purified with the µMACS streptavidin kit (Miltenyi Biotec). RNA was eluted in 100 mM DTT, cDNA was synthesized and ATF4 levels were quantified and normalized to ACTB levels.

**Reagents.** Serine and glycine were purchased from Sigma-Aldrich. RPMI media lacking serine and/or glycine were custom prepared by Life Technologies. Serine and glycine were purchased from Sigma-Aldrich. RPMI media lacking serine and/or glycine were custom prepared by Life Technologies.

**RT-PCR.** RNA was isolated using an RNeasy kit (Qiagen). cDNA was synthesized using Superscript VILO Master Mix and analyzed by quantitative PCR using Fast SYBR Green Master Mix on a Step One Real-Time PCR system. Target gene expression was normalized to ACTB expression and is shown relative to expression in control samples. Primer sequences are listed in Supplementary Table 4.

**siRNA transfection.** 100,000 cells/well were reverse transfected in 800 µl of growth medium in 12-well dishes. DharmaFECT Duo (2 µl) was combined with 100 pmol of siRNA in a final volume of 200 µl, according to the manufacturer’s instructions, which was then added to the cells. Cells were analyzed after 2 d. Dharmacon ON-TARGETplus non-targeting siRNA (D-001810-10) and NFE2L2 (L-003755-00-0005) pools were used.

**Soft agar assays.** Soft agar assays were performed in triplicate in six-well dishes. A 1-ml base layer of 0.8% agar in RPMI was plated and allowed to solidify, and 5,000 cells/well were then plated in 0.4% agar on top. RPMI (1 ml) was added the following day to each well, and the medium was changed as needed. (Note that RPMI contains serine and glycine.) Soft agar assays were stained with 0.01% crystal violet in 4% paraformaldehyde in PBS and imaged in a ChemiDoc system (Bio-Rad). Colonies were quantified with ImageJ software.

**Immunoblotting.** Protein lysates were prepared using RIPA lysis buffer and separated on 4–12% NuPAGE gels (Invitrogen), transferred onto a nitrocellulose membrane (Millipore) and incubated with the following antibodies: monoclonal antibodies to actin (ab6276) and NRF2 (EP1808Y) (both Abcam) and antibodies to NQO1 (HPA007308) and PHGDH (HPA021241) (both Sigma), PSAT1 (PA5-22124, Pierce) and ATF4 (11815, Cell Signaling Technology). Alternatively, nuclear extracts were prepared as described. Histone extracts were prepared with the Histone Extraction kit according to the manufacturer’s instructions (Abcam, ab113476). Histone extracts were probed with antibodies to the following histones: H3K4me3 (9727, Cell Signaling Technology), H3K27me3 (07-449, Millipore) and total histone H3 (4499, Cell Signaling Technology).

**Statistical analysis.** Data were analyzed using a two-sided unpaired Student’s t test, and the Mantel-Cox test was used for survival analyses. For all statistical analyses, GraphPad Prism 6 software was used, and values of P < 0.05 were considered statistically significant (*P < 0.05, **P < 0.01, ***P < 0.001). The mean ± s.e.m. of at least three independent experiments performed in triplicate is reported. For all experiments, similar variances between groups were observed. Normal distribution of samples was not determined.

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Erratum: NRF2 regulates serine biosynthesis in non–small cell lung cancer
Gina M DeNicola, Pei-Hsuan Chen, Edouard Mullarky, Jessica A Sudderth, Zeping Hu, David Wu, Hao Tang, Yang Xie, John M Asara, Kenneth E Huffman, Ignacio I Wistuba, John D Minna, Ralph J DeBerardinis & Lewis C Cantley
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In the version of this article initially published, the colors of the lines in the key in the top right corner of Figure 5h were incorrect. The line labeled “High” should be red and the line labeled “Low” should be blue. The error has been corrected in the HTML and PDF versions of the article.