NRF2 Promotes Tumor Maintenance by Modulating mRNA Translation in Pancreatic Cancer

Graphical Abstract

Highlights

- Nrf2 supports pancreatic tumor maintenance
- Nrf2 inhibits oxidation of specific translational regulatory factors
- Nrf2 promotes EGFR signaling to fuel cap-dependent mRNA translation
- Combined targeting of AKT and glutathione synthesis inhibits pancreatic cancer

Authors

Iok In Christine Chio, Seyed Mehdi Jafarnejad, Mariano Ponz-Sarvise, ..., Darryl J. Pappin, Nahum Sonenberg, David A. Tuveson

Correspondence
dtuveson@cshl.edu

In Brief
Pancreatic tumors are sustained through NRF2 which protects regulators of translation from oxidation and potentiates EGFR signaling, supporting a synergistic therapeutic effect of pro-oxidants and small molecules targeting the EGFR effector AKT.
NRF2 Promotes Tumor Maintenance by Modulating mRNA Translation in Pancreatic Cancer

Iok In Christine Chio,1,2 Seyed Mehdi Jafarnejad,3 Mariano Ponz-Sarvise,1,2,10 Youngkyu Park,1,2 Keith Rivera,1 Wilhelm Palm,4 John Wilson,1 Vineet Sangar,5 Yuan Hao,1 Daniel Öhlund,1,2 Kevin Wright,1,2 Dea Filippini,1,2 Eun Jung Lee,1,2 Brandon Da Silva,1,2 Christina Schoepfer,1,2 John Erby Wilkinson,6 Jonathan M. Buscaglia,7 Gina M. DeNicola,8 Herve Tiriac,1,2 Molly Hammell,1 Howard C. Crawford,8 Edward E. Schmidt,9 Craig B. Thompson,4 Darryl J. Pappin,1 Nahum Sonenberg,3 and David A. Tuveson1,2,*

1Cold Spring Harbor Laboratory, Cold Spring Harbor, NY 11724, USA
2Lustgarten Foundation Pancreatic Cancer Research Laboratory, Cold Spring Harbor, NY 11724, USA
3Department of Biochemistry and Goodman Cancer Research Centre, McGill University, Montreal, QC H3A 1A3, Canada
4Cancer Biology and Genetics Program, Memorial Sloan Kettering Cancer Center, New York, NY 10065, USA
5Institute of Systems Biology, 401 Terry Avenue N, Seattle, WA 98109, USA
6Departments of Molecular & Integrative Physiology and Internal Medicine, University of Michigan, Ann Arbor, MI 48109, USA
7Division of Gastroenterology, Department of Medicine, Stony Brook University School of Medicine, Stony Brook, NY 11794, USA
8Meyer Cancer Center, Weill Cornell Medical College, New York, NY 10021, USA
9Department of Microbiology and Immunology, Montana State University, Bozeman, MT 59718, USA
10Present address: Department of Oncology, Clinica Universidad de Navarra, CIMA, IDISNA, Pamplona 31008, Spain
*Correspondence: dtuveson@cshl.edu
http://dx.doi.org/10.1016/j.cell.2016.06.056

SUMMARY

Pancreatic cancer is a deadly malignancy that lacks effective therapeutics. We previously reported that oncogenic Kras induced the redox master regulator Nfe2l2/Nrf2 to stimulate pancreatic and lung cancer initiation. Here, we show that NRF2 is necessary to maintain pancreatic cancer proliferation by regulating mRNA translation. Specifically, loss of NRF2 led to defects in autocrine epidermal growth factor receptor (EGFR) signaling and oxidation of specific translational regulatory proteins, resulting in impaired cap-dependent and cap-independent mRNA translation in pancreatic cancer cells. Combined targeting of the EGFR effector AKT and the glutathione antioxidant pathway mimicked Nrf2 ablation to potently inhibit pancreatic cancer ex vivo and in vivo, representing a promising synthetic lethal strategy for treating the disease.

INTRODUCTION

Pancreatic ductal adenocarcinoma (PDA) is the fourth leading cause of cancer-related death in the United States, with incidence nearly matching mortality. This is reflected by a short median survival of 6 months and a 5-year survival of <5% (Siegel et al., 2015). The poor prognosis of PDA relates to the advanced disease stage at the time of diagnosis and to its profound resistance to therapies (Jemal et al., 2009). PDA exhibits a wide range of genetic and epigenetic alterations, including a high frequency (90%–95%) of activating KRAS mutations and inactivation of the tumor suppressors TP53, P16/INK4A, and SMAD4 (Jones et al., 2008). We previously found that oncogenic Kras expression induced an important regulator of redox control, the transcription factor nuclear factor erythroid-derived 2-like 2, Nfe2l2/Nrf2 (DeNicola et al., 2011). In response to oxidative stress, Nrf2 controls the fate of cells through transcriptional upregulation of antioxidant-response-element-bearing genes (Hayes and Dinkova-Kostova, 2014). In the context of oncogenic Kras, Nrf2 promotes pancreatic intraepithelial neoplasia by stimulating proliferation and suppressing senescence. Thus, Nrf2 is a key player in PDA initiation through the maintenance of redox homeostasis.

Although oxidative stress may disrupt biological functions, redox reactions in a cell are often tightly regulated and play essential physiological roles (Trachootham et al., 2008). The chemical properties of cysteine thiol groups render this amino acid exquisitely sensitive to changes in the cellular levels of reactive oxygen species (ROS) or reactive nitrogen species (RNS). Certain cysteine thiols can act as redox switches, since oxidative modifications may impact properties such as catalytic activity or conformation of a protein (Barford, 2004). Although the general concepts of redox signaling have been established, the identity and function of many regulatory switches remain unclear, particularly in the context of cancer. Given the elevated levels of Nrf2 in PDA and the ability of Nrf2 to potently lower ROS, we hypothesized that such redox switches may function as Nrf2 downstream effectors to support PDA progression.

The cellular response to stress involves regulatory changes in many processes, including transcription, mRNA processing, and translation. Historically, increased cancer cell proliferation has been shown to require increased rates of protein synthesis and ribosome number (Johnson et al., 1975; Zetterberg et al., 1995). Mutations that deregulate mRNA translation are common events in human cancers (Bilanges and Stokoe, 2007), while...
enforced expression of certain translation factors transforms rodent fibroblasts and promotes tumorigenesis (Wendel et al., 2004). Small molecules that target these pathways suppress mRNA translation and have anti-tumor effects (Choo and Blenis, 2009). Thus, deregulation of translation is an important step in oncogenic transformation and tumor maintenance. Here, we show that Nrf2 directly stimulates mRNA translation by maintaining the reduced state of specific cysteine residues in multiple proteins that participate in translational regulation. In parallel, redox regulation by Nrf2 also promotes epidermal growth factor receptor (EGFR) autocrine signaling through AKT in KRAS mutant cells to fuel cap-dependent translation initiation. These functions converge to promote global protein synthesis in PDA. Combined inhibition of AKT signaling and synthesis of glutathione, a vital intracellular antioxidant, synergistically hampered the survival of PDA cells in vitro and in vivo, which presents an opportunity for therapeutic intervention.

RESULTS

Nrf2 Is a Critical Biological Dependency in Pancreatic Cancer

The organoid culture system supports the proliferation of normal and malignant primary pancreatic ductal cells of both mouse and human origins (Boj et al., 2015). Nrf2 protein was upregulated in human tumor (hT) organoids compared to normal counterparts (hN) (Figure S1A). While hN organoids tolerated NRF2 knockdown, this was detrimental in most hT organoids tested (three out of five) (Figure 1A). Upon Nrf2 knockdown (Figure S1B), the two surviving hT organoids exhibited substantial elevation of ROS (Figure 1B) and decreased cell proliferation that was out of five) (Figure 1A). Upon NRF2 knockdown (Figure S1B, ure S1C). Similar proliferative defects were also observed in the KRAS mutant cells, likely through a DNA-damage-independent mechanism.

Pancreatic organoids are typically maintained in complete media, which contain various growth factors, including epidermal growth factor (EGF) and NAC. Oncogenic Kras upregulated Nrf2 and its downstream target gene Nqo1 in K and KP organoids (Figures S1H and S1I). The induction of Nrf2 and Nqo1 in KP organoids was more prominent upon exclusion of both EGF and NAC from the media (reduced) (Figures S1J and S1K). While removal of EGF and NAC limited the passaging capacity of N organoids, they were dispensable for K and KP organoids (Figure S1L). Thus, all organoids described hereinafter were cultured and passaged in complete media, while experiments were all conducted in reduced media.

Oncogenic Kras activation in K and KP organoids resulted in a significant increase in intracellular glutathione (GSH) (Figure S1M) and decreased levels of ROS (Figure S1N), features reversed upon Nrf2 ablation (Figures 1F, 1G, and S1M). Notably, no increases were observed in the levels of RNS (Figure S1O) or in mitochondrial superoxides (Figure S1P), upon either chronic ablation or acute knockdown of Nrf2 in murine tumor organoids (T organoids) and hT organoids, respectively. This indicates that Nrf2 specifically modulates cytoplasmic ROS in pancreatic ductal cells. Deficiency of Nrf2 impeded proliferation of N, K, and KP organoids in culture and was largely corrected by NAC (Figure 1H), KPN organoids did not exhibit elevated DNA damage response (Figure S1Q). Therefore, Nrf2 regulates redox homeostasis and cell proliferation in Kras mutant pancreatic epithelial cells, likely through a DNA-damage-independent mechanism.

Global Cysteine Proteomics Reveal Redox-Dependent Regulation of the Translational Machinery

Cysteine residues are the most abundant cellular thiol. Alterations in cellular redox levels may cause reversible modifications on specific cysteine residues and alter the activity of their corresponding proteins (Paulsen and Carroll, 2010). Thus, reactive-cysteine-containing proteins are potential candidates to carry out the redox-sensitive effector functions of Nrf2. To decipher changes in the cysteine proteome, we devised a highly sensitive proteomic method using a selectively cleavable cysteine-reactive (ICAT) reagent to enrich for and identify reduced cysteine-containing peptides (Sethuraman et al., 2004). This was combined with a parallel analysis of the total proteome by using amine-reactive isobaric tags for relative and absolute quantification (iTRAQ; Ross et al., 2004) (Figure 2A). By normalizing cysteine peptide changes to that of the total proteome, oxidative alterations in the cysteine proteome were quantified independent of protein expression differences, an approach not applied previously in cysteine proteomics. To establish this method, murine T organoids were treated with an inhibitor of GSH synthesis, buthionine sulfoximine (BSO). In this experiment, 88% of the ICAT-enriched unique peptides contained cysteines, indicating the specificity of the technique. From a total of 4,089 proteins identified and quantified, 4,805 unique cysteine-containing peptides were detected. Upon BSO treatment, 1,192 unique cysteine-containing peptides were oxidized. Notably, cysteine...
residues oxidized by BSO included a previously reported redox-sensitive protein, Pkm2 (Anastasiou et al., 2011). The full list of oxidized peptides is shown in Table S1.

To identify Nrf2-dependent, redox-sensitive effector pathways, the cysteine proteomes of three pairs of N, Nn, K, and Kn and four pairs of KP and KPn organoids were normalized and merged. A total of 2,591 unique cysteine-containing peptides corresponding to 1,446 proteins were identified in all of the four 8-plex iTRAQ runs (MassIVE: MSV000079394). Of these, 56 cysteine-containing peptides were significantly oxidized when Nrf2 was deleted in N organoids, 49 in K organoids, and 255 in KP organoids (Figure 2B; Table S2). As in BSO-treated tumor organoids, Pkm2C358 was also substantially oxidized in KPn organoids. Global levels of reduced cysteine peptides were similar across the different genotypes (Figure S2A), indicating the absence of widespread changes in protein oxidation upon Nrf2 ablation. To determine whether the subset of proteins specifically oxidized in KPn cells represented any particular biochemical processes, the data were analyzed using DAVID (Huang et al., 2009) for pathway enrichment. Interestingly, proteins containing oxidized cysteines in KPn organoids were strongly enriched for participation in protein folding and in mRNA translation (Figure 2C). Nrf2-deficient
T organoids also exhibited a similar enrichment (Tables S3 and S4). Besides the classic translation pathway proteins curated in the DAVID database (ribosomal proteins and translation initiation and elongation factors), we identified additional proteins implicated in translational regulation to be oxidized in KPn organoids (Figures 2D and S2B), further coupling Nrf2 activation with translational control. Translation-related peptides exhibited an average of 20%–50% increase in oxidation in KPn organoids (Figure S2C), in line with studies conducted in yeast reporting that the majority of cysteine residues are only partially (~25%) oxidized (Brandes et al., 2011). Treatment of murine T organoids with BSO similarly led to significant oxidation of proteins in the translation machinery (Figures S2D and S2E). These genetic and pharmacological observations collectively support the redox-dependent regulation of the translation machinery through cysteine modifications, prompting us to further evaluate the functional role of Nrf2 in mRNA translation for pancreatic cancer cells.

mRNA Translation Is Impeded in Nrf2-Deficient Cells
mRNA translation is a vital cellular process that regulates growth and metabolism (Sonenberg and Hinnebusch, 2009). In cancer cells, hyper-activated signaling pathways influence translation, supporting uncontrolled growth and survival (Mavrakis and Wendel, 2008). When compared to N organoids, KP organoids exhibited elevated levels of mRNA associated with translationally active polysomes and decreased levels of mRNA associated with translationally inactive monosomes (Figure 3A, left), consistent with the notion that translational upregulation plays an oncogenic role in cancer. While deletion of Nrf2 from N and K organoids did not noticeably alter the distribution of monosomes and polysomes (Figure S3A), deletion of Nrf2 from KP organoids led to a measurable decrease in polysomes, with a corresponding increase in monosomes (Figure 3A, right), suggesting a decrease in translation efficiency in cancer cells when Nrf2 is absent. Impaired translation in KP organoids was not due to eIF2 (eukaryotic initiation factor 2)-mediated activation of the unfolded protein response upon elevated ROS (Figure S3B) (Ling and Soll, 2010). When pulsed with [35S]-methionine ([35S]-Met) to measure the rate of nascent protein synthesis, a significant decrease in [35S]-Met incorporation was observed in Kn and KP organoids (Figures 3B and S3C). This translation defect was redox dependent (Figure 3B) and was observed in...
organoids and Suit2 harboring short hairpin NRF2 (shNRF2) (Figures 3B, S3D, and S3E). These effects were mitigated by NAC and other antioxidants such as trolox and GSH ethyl ester (Figures 3B and S3D–S3F). Of note, treatment of organoids with propargylglycine, an irreversible inactivator of γ-cystathionase, did not alter the difference in [35S]-Met incorporation into proteins in KP and KPN organoids (Figure S3G). This indicates that Nrf2-dependent decrease in [35S]-Met incorporation into proteins was not due to alterations in Met to cysteine exchange through the transsulfuration cycle in response to elevated oxidative stress (Belalcazar et al., 2014). Introduction of a heterotypic NRF2 mRNA in hT organoids expressing short hairpin RNA (shRNA) against endogenous NRF2-restored [35S]-Met incorporation (Figure S3H). Thus, Nrf2 regulates the activity of the translational machinery through the maintenance of redox homeostasis.

To further analyze the steps in mRNA translation that are impacted by Nrf2 in pancreatic cancer cells, a bicistronic reporter construct encoding Renilla luciferase under the simian virus 40 (SV40) promoter and firefly luciferase under the control of the IRES (internal ribosome entry site) sequences from the hepatitis C virus (HCV) was used. Interestingly, both cap-dependent translation of Renilla luciferase and cap-independent HCV-IRES-mediated translation of firefly luciferase were decreased in Suit2 cells bearing shNRF2 (Figure 3C), with no significant changes in the expression of the luciferase gene (Figure S3I). While HCV-IRES-mediated translation requires certain canonical initiation factors (Otto and Puglisi, 2004), the cricket paralysis virus IRES (CPV-IRES) (Wilson et al., 2000) enables translation of mRNAs independently of any translation initiation factors. We found that CPV-IRES-mediated translation was also impaired when Nrf2 expression was suppressed (Figure 3C). These translational defects were partially phenocopied by BSO treatment (Figure 3C) and were recapitulated in KPN organoids (Figure 3C). These results suggest that Nrf2 exercises redox-dependent control over multiple aspects of the translation machinery.

Since cysteine residues previously implicated as functional redox switches are reversibly oxidized, we evaluated the possibility that components of the translational machinery may also be redox switches that govern translation activity. Supplementation of KPN organoids with NAC reversed the oxidative cysteine changes in a number of translational regulatory proteins (Figure 3D). shRNA-mediated knockdown of selected initiation and elongation factors (Figure S3J) confirmed that they were independently essential for protein synthesis in PDA (Figure 3E).

Ectopic expression of these factors did not enhance protein synthesis in KP organoids (Figure S3K). However, ectopic expression of the cysteine to aspartic acid oxidation mimic (Perynmyakov et al., 2012) of the elongation factor eEF2 led to a significant decrease in the rate of nascent protein synthesis when compared to its wild-type counterpart (Figures 3F and S3L). A similar trend was also observed for the translation initiation factor elf3j but not for the Valyl-tRNA synthetase Vars (Figures 3F and S3L). These results suggest that eEF2Cys83 (and, possibly, additional components of the translation machinery) may function as a redox switch to modulate the activity of the elongation apparatus.

**Growth Factor Signaling Pathways Upstream of Cap-Dependent Translation Are Impaired in Nrf2-Deficient Cells**

Of the four phases of protein synthesis (initiation, elongation, termination, and recycling), initiation is considered to be the rate-limiting step in mRNA translation and is often exploited by cancer cells to support tumorigenesis (Pelletier et al., 2015). Based on our observation that cap-dependent mRNA translation was compromised in Nrf2-deficient cells (Figure 3C), and that the initiation factor elf3j may also be a candidate redox switch (Figure 3F), we extended our investigation on translation initiation biochemically. elf4E is the limiting initiation factor for mRNA translation, as it recognizes the m7GTP cap on mRNA and recruits elf4G (Gingras et al., 1999). In the absence of mitogenic signaling, elf4E is bound by the inhibitory 4E binding proteins (4EBPs), which sequester elf4E from interaction with elf4G. This process is critical for regulating mRNA translation in PDA organoids, as expression of a dominant active 4EBP1-4A mutant led to substantial decrease in nascent protein synthesis (Figure S4A) and cell proliferation (Figure S4B), phenocopying Nrf2 deficiency. The levels of elf4E, elf4G, and 4EBP1 were independent of Nrf2 status (Figure 4A; Figure S4C), and the oxidative states of these proteins as determined through iodoacetamide enrichment for reduced cysteine peptides were also similar in KP and KPN cells (Figure S4C). Despite these, the amount of inhibitory 4EBP1 co-precipitating with elf4E in m7GTP pull-downs was found to be elevated in Nn, Knn, and KPN organoids, with a concomitant decrease in elf4G co-precipitation (Figures 4A and S4D). This reflects a defective elf4F complex formation that would impart decreased cap-dependent translation initiation in Nrf2-deficient KP cells.

---

**Figure 3. Nrf2 Deficiency Impairs Protein Synthesis**

(A) Polysome profiles of N, KP, and KPN organoids treated with 300 μg/ml cycloheximide for 10 min. Absorbance light at 254 nm. Representative profiles from two biological replicates.

(B) [35S]-Met incorporation into protein from murine and human organoids grown in reduced media or in media containing 1.25 mM NAC. Data are means ± SEM (n = 3, Student’s t test). NS, not significant.

(C) Activities of Renilla and firefly luciferase in Suit2 cells bearing shScr or shNRF2 (left three graphs) or KP or KPN organoids (rightmost) transfected with the bicistronic reporter plasmid 24 hr prior. Data indicate percentage luciferase activity driven by SV40-CAP, HCV-IRES, or CPV-IRES. Data are means ± SD (n = 3, Student’s t test). Unt, untreated.

(D) Cysteine peptide counts of translational regulatory proteins in KP, KPN, and KPN organoids supplemented with 1.25 mM NAC (average of two biological replicates).

(E and F) [35S]-Met incorporation into protein from murine T organoids bearing shRNA (E) or V5-tagged wild-type (WT) or cysteine-mutated (CD) cDNAs of indicated proteins (F). Data are means ± SEM (n = 6, Student’s t test).

See also Figure S3.
Figure 4. Nrf2 Deficiency Impairs Mitogenic Signaling Pathways Governing eIF4F Complex Formation

(A) Lysates from KP and KPN organoids were subjected to 7-methyl-GTP pull-downs and analyzed for indicated proteins. WCL, whole-cell lysates.

(B and C) Immunoblot analysis for growth factor signaling pathway activation (B) and mRNA cap-binding proteins (C) in N, K, and KP organoids, as well as murine tumor (T) and hT (hM1, hT3) organoids with shScr or shNRF2. p, phospho.

(D and E) Immunoblot analysis of total (D) and EGFR tyrosine (Y) phosphorylation (E) in KP and KPN organoids. Red arrow indicates 150-kDa molecular-weight protein. 4G10 IP, total phospho-Y antibody immunoprecipitation.

(F) Protein array analysis for growth factors secreted into culture medium (Supernatant) and expressed in whole-cell lysates (WCL) in KP and KPN organoids. Red box indicates murine EGF.

(G) EGF ELISA of supernatant from N, Nn, KP, and KPN organoids after 3 or 6 days in culture. Data are means ± SD (n = 3, Student’s t test).

(H) Constitutive EGF shedding determined by alkaline phosphatase activity in the supernatant from KP organoids. Data are means ± SD (n = 3, Student’s t test).

(I and J) Activity of Adam10 from plasma membrane fractions of KP, KPN, and K Adam10 knockout (KAdamKO) organoids (I) or KP organoids expressing control vector versus Adam10 cysteine mutant (CD) (J), measured by cleavage and increase in 5-FAM (5-carboxyfluorescein) fluorescence of a FRET (fluorescence resonance energy transfer) probe.

(legend continued on next page)
PI3K (phosphatidylinositol 3-kinase)/AKT/mTOR signaling has been shown to promote phosphorylation of 4EBPs at multiple sites, resulting in the liberation of eIF4E from 4EBP1 and the stimulation of cap-dependent translation initiation (Ruggero and Sonenberg, 2005). Consistent with elevated 4EBP1 at the mRNA cap, phosphorylation of Akt and 4EBP1 were both decreased in Kn and KPN organoids, as well as in murine T organoids and hT organoids expressing shNRF2 (Figure 4B). pErk was moderately decreased in Kn and KPN cells and more clearly decreased in T organoids expressing shNRF2 (Figure 4B). The level of pS6 was not consistently downregulated in Kn and KPN cells, suggesting that these signaling alterations operate independently of S6 kinase (Figure 4B). Consistently, phosphorylation of eIF4G and eIF4E—substrates of Akt/mTOR (Raught et al., 2000) and Erk/Mnk signaling (Ueda et al., 2004)—respectively, were also decreased in Kn and KPN organoids, as well as in T organoids expressing shNRF2 (Figures 4C, S4E, and S4F). These signaling defects were not due to differential activation of oncogenic Kras (Figure S4G) or that of the IGF1 (insulin-like growth factor 1) receptor (Figure S4H) (Molina-Arcas et al., 2013). Neither the activity nor the expression level of PTEN (Maehama and Dixon, 1998) was upregulated in Nn and KPN organoids (Figure S4I). The signaling changes upon Nrf2 deficiency were most striking in the context of K and KP cells, reflecting critical Kras dependency.

A hallmark of Kras mutant cells is the role of autocrine and paracrine loops in amplifying oncogenic Kras signaling through AKT and ERK (extracellular signal-regulated kinase) (Ardivito et al., 2017). Decreased activation of AKT and MAPK in Nrf2-deficient cells was not due to oxidation of these kinases (Figure S4J). Immunoblot analysis of global tyrosine phosphorylation revealed decreased phosphorylation of a protein above 150 kDa (Figure 4D), which we then confirmed to be the EGFR (Figure 4E). By secretome arrays (Figure 4F) and ELISA (Figure 4G), we found that the release of EGF into culture media by KPN organoids was reduced when compared to that by KP organoids, while total intracellular levels of EGF protein (Figure 4F) and mRNA (Figure S4K) were not decreased. Indeed, induced (Figure S4L) and constitutive (Figure 4H) shedding of EGF—as determined by the expression of an EGF–alkaline phosphatase (AP) construct, followed by measurement of AP activity in the supernatant—revealed decreased EGF shedding in KPN organoids and in Nrf2-deficient tumor cells (Figure S4M). Consistent with reports showing that EGFR signaling is coupled to activation of cap-dependent translation in EGFR wild-type cells (Patel et al., 2013), EGFR supplementation was able to restore defective Akt and Erk activation in KPN organoids (Figure S4N). Addition of NAC alone also partially corrected these signaling defects (Figure S4N) and restored EGF release (Figure S4O). As with NAC supplementation, EGF was sufficient to mitigate the translational defects observed in Kn and KPN organoids (Figure S4P) and improved proliferation in KPN organoids (Figure S4Q). Doxycycline-induced knockdown of NRF2 in hT organoids led to decreased phospho-(p)EGFR and pERRB2 (Figure S4R). Similarly, the activity levels of EGFR in tumor lysates of Suit2 xenografts correlated with doxycycline-induced NRF2 knockdown (Figure S4S). In one shNRF2 tumor sample where NRF2 was reactivated, pEGFR was sustained at a high level (Figure S4S), further demonstrating a role of NRF2 in EGFR activation.

Metalloproteases of the ADAM (a disintegrin and metalloprotease) family are thought to be responsible for the shedding of certain EGFR ligands, with Adam10 emerging as the main sheddase of EGF (Sahin et al., 2004). Although Adam10 was not detected in our initial cysteine proteomics experiment, mass spectrometric analysis of immunoprecipitated Adam10 revealed a substantial and specific decrease in the levels of a cysteine-containing peptide when Nrf2 was deleted (Figure S4T). Consistent with decreased EGF shedding, Adam10 was functionally impaired in KPN organoids (Figure 4I). Ectopic expression of a cysteine oxidation mimic of Adam10 (Figure S4U) inhibited the activity of endogenous Adam10 (Figure 4J) and led to a significant decrease in nascent protein synthesis in KP organoids (Figure 4K). These data suggest that redox-dependent regulation of Adam10 activity contributes to the maintenance of EGF autocrine signaling and cap-dependent translation in PDA.

**Nrf2 Supports Cap-Dependent Translation of Pro-survival Transcripts**

Enhanced eIF4F complex formation in cancer cells promotes cap-dependent mRNA translation and, thereby, elevates global protein synthesis rates. However, mRNAs vary widely in their inherent “translatability,” largely as a function of differences in the length and structure of their 5′ UTRs. Cellular mRNAs most sensitive to alterations in eIF4F complex formation are highly structured 5′ UTRs. These mRNAs often encode proteins that promote growth and transformation (Pelletier et al., 2015). In contrast, the majority of cellular mRNAs have relatively short, unstructured 5′ UTRs that enable efficient translation, even when eIF4F complex levels are limiting. We found that the levels of a number of pro-survival proteins were increased in KP compared to N organoids, with no changes in housekeeping proteins (Figure 5A; Figure S5A). Of the pro-survival proteins, Hif1α, Bcl2, and, more moderately, cyclin D1, cyclin D3, and c-Myc were decreased in KPN organoids (Figure 5A). Similar observations were made in murine T organoids bearing shNrf2 (Figure 5B). This is consistent with earlier reports showing a correlation between the expression of Nrf2 and some of these proteins (Fan et al., 2014; Ke et al., 2013; Malec et al., 2010; Niture and Jaiswal, 2012; Wang et al., 2016). qPCR of polysome fractions revealed an increase in the association of the mRNAs of these proteins with the translationally inactive monosome fractions (Figure 5C), while no differences were seen for Actin, Mcl-1, and, interestingly, Bcl2 mRNAs (Figure S5B). No decrease was observed in the total transcript level of these mRNAs (Figure S5C) or the half-life of the corresponding proteins (Figure S5D).

KL25covery energy transfer) substrate specific to Adam10. Activity was monitored over 2 hr at excitation, excitation/emission = 490 nm/520 nm. Data are means ± SD (n = 6, Student’s t test).

See also Figure S4.
Compromised Hif1α protein levels in KPN organoids corresponded with a decrease in the expression level of Hif1α target genes (Figure 5D), their inability to grow in hypoxia (Figure 5E), and heightened sensitivity to inhibition of glycolysis (Figure 5F) (Semenza, 2013). These data indicate that Nrf2-mediated protein synthesis in Kras mutant cells has a potent impact on the translation of certain proto-oncogenic mRNAs, thus supporting a role for Nrf2 in fully transformed PDA cells.

Combined Inhibition of AKT and GSH Synthesis Blunts Pancreatic Cancer Growth and Survival

The PI3K/AKT/mTOR pathway is believed to be a major pathway regulating global and mRNA-specific translation initiation (Ruggero and Sonenberg, 2005). We found that the basal phosphorylation status of 4EBP1 correlated strongly with the sensitivity of hT organoids to shNRF2 (Figure 6A). Inhibition of AKT using the pan-AKT inhibitor MK2206 markedly decreased the rate of protein synthesis (Figure 6B) and increased 4EBP1-elf4E interactions at the mRNA cap (Figure 6C) in KP organoids, the effects of which were potentiated in KPN organoids (Figures 6B and 6C). At a dose that suppressed KP intracellular GSH to a level found in KPN cells (Figure S6A), BSO bolstered the effect of MK2206 to further decrease the rate of protein synthesis (Figure 6D). Consistently, inhibition of 4EBP1 phosphorylation in both KP and hT organoids was most efficient in the presence of both inhibitors (Figure 6E).

Reactivation of EGFR and MAPK (mitogen-activated protein kinase) signaling upon inhibition of AKT is an important adaptive survival response, leading to drug resistance in Kras mutant cancer cells, and requires an intact autocrine EGFR signaling cascade to enact (Mendoza et al., 2011). Consistent with defective EGFR signaling in Kn and KPN organoids, these adaptive responses were attenuated (Figure S6B). While BSO treatment alone had minimal effects on the activity of these signaling proteins (Figure 6B and 6C), their inability to grow in hypoxia (Figure 5E), and heightened sensitivity to inhibition of glycolysis (Figure 5F) (Semenza, 2013). These data indicate that Nrf2-mediated protein synthesis in Kras mutant cells has a potent impact on the translation of certain proto-oncogenic mRNAs, thus supporting a role for Nrf2 in fully transformed PDA cells.

Combined Inhibition of AKT and GSH Synthesis Blunts Pancreatic Cancer Growth and Survival

The PI3K/AKT/mTOR pathway is believed to be a major pathway regulating global and mRNA-specific translation initiation (Ruggero and Sonenberg, 2005). We found that the basal phosphorylation status of 4EBP1 correlated strongly with the sensitivity of hT organoids to shNRF2 (Figure 6A). Inhibition of AKT using the pan-AKT inhibitor MK2206 markedly decreased the rate of protein synthesis (Figure 6B) and increased 4EBP1-elf4E interactions at the mRNA cap (Figure 6C) in KP organoids, the effects of which were potentiated in KPN organoids (Figures 6B and 6C). At a dose that suppressed KP intracellular GSH to a level found in KPN cells (Figure S6A), BSO bolstered the effect of MK2206 to further decrease the rate of protein synthesis (Figure 6D). Consistently, inhibition of 4EBP1 phosphorylation in both KP and hT organoids was most efficient in the presence of both inhibitors (Figure 6E).

Reactivation of EGFR and MAPK (mitogen-activated protein kinase) signaling upon inhibition of AKT is an important adaptive survival response, leading to drug resistance in Kras mutant cancer cells, and requires an intact autocrine EGFR signaling cascade to enact (Mendoza et al., 2011). Consistent with defective EGFR signaling in Kn and KPN organoids, these adaptive responses were attenuated (Figure S6B). While BSO treatment alone had minimal effects on the activity of these signaling proteins (Figure 6B and 6C), their inability to grow in hypoxia (Figure 5E), and heightened sensitivity to inhibition of glycolysis (Figure 5F) (Semenza, 2013). These data indicate that Nrf2-mediated protein synthesis in Kras mutant cells has a potent impact on the translation of certain proto-oncogenic mRNAs, thus supporting a role for Nrf2 in fully transformed PDA cells.

Combined Inhibition of AKT and GSH Synthesis Blunts Pancreatic Cancer Growth and Survival

The PI3K/AKT/mTOR pathway is believed to be a major pathway regulating global and mRNA-specific translation initiation (Ruggero and Sonenberg, 2005). We found that the basal phosphorylation status of 4EBP1 correlated strongly with the sensitivity of hT organoids to shNRF2 (Figure 6A). Inhibition of AKT using the pan-AKT inhibitor MK2206 markedly decreased the rate of protein synthesis (Figure 6B) and increased 4EBP1-elf4E interactions at the mRNA cap (Figure 6C) in KP organoids, the effects of which were potentiated in KPN organoids (Figures 6B and 6C). At a dose that suppressed KP intracellular GSH to a level found in KPN cells (Figure S6A), BSO bolstered the effect of MK2206 to further decrease the rate of protein synthesis (Figure 6D). Consistently, inhibition of 4EBP1 phosphorylation in both KP and hT organoids was most efficient in the presence of both inhibitors (Figure 6E).

Reactivation of EGFR and MAPK (mitogen-activated protein kinase) signaling upon inhibition of AKT is an important adaptive survival response, leading to drug resistance in Kras mutant cancer cells, and requires an intact autocrine EGFR signaling cascade to enact (Mendoza et al., 2011). Consistent with defective EGFR signaling in Kn and KPN organoids, these adaptive responses were attenuated (Figure S6B). While BSO treatment alone had minimal effects on the activity of these signaling proteins (Figure 6B and 6C), their inability to grow in hypoxia (Figure 5E), and heightened sensitivity to inhibition of glycolysis (Figure 5F) (Semenza, 2013). These data indicate that Nrf2-mediated protein synthesis in Kras mutant cells has a potent impact on the translation of certain proto-oncogenic mRNAs, thus supporting a role for Nrf2 in fully transformed PDA cells.

Combined Inhibition of AKT and GSH Synthesis Blunts Pancreatic Cancer Growth and Survival

The PI3K/AKT/mTOR pathway is believed to be a major pathway regulating global and mRNA-specific translation initiation (Ruggero and Sonenberg, 2005). We found that the basal phosphorylation status of 4EBP1 correlated strongly with the sensitivity of hT organoids to shNRF2 (Figure 6A). Inhibition of AKT using the pan-AKT inhibitor MK2206 markedly decreased the rate of protein synthesis (Figure 6B) and increased 4EBP1-elf4E interactions at the mRNA cap (Figure 6C) in KP organoids, the effects of which were potentiated in KPN organoids (Figures 6B and 6C). At a dose that suppressed KP intracellular GSH to a level found in KPN cells (Figure S6A), BSO bolstered the effect of MK2206 to further decrease the rate of protein synthesis (Figure 6D). Consistently, inhibition of 4EBP1 phosphorylation in both KP and hT organoids was most efficient in the presence of both inhibitors (Figure 6E).

Reactivation of EGFR and MAPK (mitogen-activated protein kinase) signaling upon inhibition of AKT is an important adaptive survival response, leading to drug resistance in Kras mutant cancer cells, and requires an intact autocrine EGFR signaling cascade to enact (Mendoza et al., 2011). Consistent with defective EGFR signaling in Kn and KPN organoids, these adaptive responses were attenuated (Figure S6B). While BSO treatment alone had minimal effects on the activity of these signaling proteins (Figure 6B and 6C), their inability to grow in hypoxia (Figure 5E), and heightened sensitivity to inhibition of glycolysis (Figure 5F) (Semenza, 2013). These data indicate that Nrf2-mediated protein synthesis in Kras mutant cells has a potent impact on the translation of certain proto-oncogenic mRNAs, thus supporting a role for Nrf2 in fully transformed PDA cells.
Figure 6. Inhibition of GSH Synthesis Sensitizes Pancreatic Cancer Cells to Pan-AKT Inhibition

(A) Immunoblot analysis of 4EBP1 activation status in hT organoids. VINCULIN, loading control. p, phospho.

(B) [35S]-Met incorporation into protein from KP and KPN organoids treated with DMSO or 1 μM MK2206 (MK) for 48 hr. Data are means ± SEM (n = 3, Student’s t test).

(C) Lysates from KP and KPN organoids treated with DMSO or 1 μM MK2206 were subjected to 7-methyl-GTP pull-downs and analyzed for indicated proteins. WCL, whole-cell lysates.

(D) [35S]-Met incorporation into protein from KP organoids treated with 1 μM MK2206 (MK), 100 μM BSO, or both for 48 hr. Data are means ± SEM (n = 3, Student’s t test). NS, not significant.

(E) Immunoblot analysis of AKT and 4EBP1 activation status in murine (left) and human hT1 (right) T organoids treated with 1 μM MK2206 (MK), 100 μM BSO, or both for 48 hr. p, phospho.

(F) Adaptive response in KP organoids (top) and hT organoids (bottom) upon treatment with vehicle only, with 1 μM MK2206, 100 μM BSO, or in combination for 48 hr. p = phospho.

(G) Cell viability of N, K, and KP organoids and the corresponding Nrf2-deficient organoids over increasing concentrations of MK2206 for 72 hr. Dotted lines indicate 95% confidence intervals (n = 5).

(H) EC50 (half maximal effective concentration) values of MK2206 in murine organoids.

(legend continued on next page)
cascades, BSO suppressed the reactivation of EGFR (Figure S6C) and MAPK (Figure 6F) upon treatment with MK2206. Combined treatment of MK2206 with BSO led to a higher level of ROS compared to single agents alone (Figure S6D), further supporting the premise that EGFR autocrine activation in Kras mutant cells is redox dependent.

In congruence with our biochemical observations, Kn and KPn organoids were substantially more sensitive to MK2206 (Figures 6G, 6H, and S6E) and the multi-targeted PI3K inhibitor PI-103 (Figure S6F) than K and KP counterparts. Consistently, BSO exhibited a synergistic effect with MK2206 in K and KP organoids (Figures 6I, 6J, S6G, and S6H), as well as in human PDA cells (Figures 6I, 6J, S6I, and S6J). Of note, the sensitivity of N organoids toward MK2206 was not heightened by either BSO treatment (Figures 6I and 6J) or Nrf2 deficiency (Figures 6G and 6H), reflecting a potential therapeutic index.

Contrary to our observations with AKT inhibition, KPn organoids were not more sensitive to mTOR (mammalian target of rapamycin) inhibitors such as rapamycin and Torin1 (Figures S6K and S6L). We posit this to be due to mTOR-mediated growth suppression in cells that rely on extracellular proteins as an amino acid source (Palm et al., 2015), as in the case of Kras mutant cells (Bar-Sagi and Feramisco, 1986). Consistent with the notion that Nrf2 modulates Akt/mTOR activity downstream of mitogenic signaling, elevated levels of lysosomal hydrolysis were observed in KPn organoids (Figure S6M), and this process was redox dependent (Figures S6N and S6O). We posit that the proliferative advantage obtained in mTOR-inhibited Kras mutant cells may outweigh the suppressive effects from AKT and GSH inhibition. Thus, this renders direct antagonism of AKT to be more growth suppressive than that of mTOR in this context. Sensitivity of Nrf2-deficient cells was selective toward inhibition of PI3K/AKT, as similar vulnerability was absent toward the MEK (MAPK/ERK kinase) inhibitor AZD6244 (Figures S6P and S6Q) (Davies et al., 2007) or toward a panel of commonly used chemotherapeutics (Figure S6R).

Encouraged by our in vitro observations on MK2206 and BSO, we tested this strategy in the KPC mouse model (Higori ani et al., 2005). Mice were randomly assigned to treatments with vehicle, BSO, MK2206, or the combination of MK2206 and BSO. MK2206 and BSO effectively suppressed levels of pAkt (Figure S7A) and GSH (Figure S7B), respectively. While monotherapy did not markedly impact tumor growth, combination treatment was significantly growth suppressive (Figures 7A and 7B). Long-term treatment of KPC mice with this combination diminished neoplastic cell proliferation (Figure S7C) and modestly increased median survival when compared to treatment with MK2206 alone (Figure S7D). The synergistic effect of MK2206 and BSO was also observed in Suit2 xenograft models in terms of tumor kinetics (Figure 7C) and neoplastic cell proliferation (Figure 7D). Our results suggest that pro-oxidants may further augment the effectiveness of AKT inhibition in suppressing PDA growth through combined inhibition of mRNA translation and EGFR-dependent mechanisms of resistance (Figure S7E).

DISCUSSION

The elevated levels of free radicals and oxidative stress contributes to DNA damage and predisposition to cancer has led to the recommendation of antioxidant use as prophylaxis against neoplasia (Khansari et al., 2009). However, Nrf2 activators (Talalay et al., 2007) and various antioxidants have either failed to reduce cancer incidence or have, instead, promoted cancer in clinical and preclinical testing (Sayin et al., 2014). We present the alternative hypothesis that Nrf2’s antioxidant function stimulates pancreatic cancer, in part, by promoting mRNA translation and mitogenic signaling. Indeed, our findings may reflect cellular responses to a number of redox perturbants demonstrated previously to exhibit efficacy in Ras mutant cells. This includes erastin (Yang and Stockwell, 2008), dehydroascorbic acid (oxidized vitamin C) (Yun et al., 2015), and platinum-based cytotoxics (Socinski, 2004).

The synthetic lethal interaction we observed between Nrf2 loss and oncogenic Kras in PDA may also reflect the proto-oncogenic mRNAs that are exquisitely translationally upregulated in Kras mutant cells. For example, Hif1α, an important transcription factor that mediates adaptive responses to intratumoral hypoxia and malignant progression in PDA (Zhao et al., 2014), was markedly decreased in Nrf2-deficient Kras mutant cells. Hif1α likely contributes to the glycolytic phenotype of PDA (Ying et al., 2012), working in parallel with Nrf2 transcriptional target genes that are metabolic enzymes governing the pentose phosphate pathway (Mitsuishi et al., 2012) to promote metabolic rewiring in cancer cells.

Regarding the potential clinical implications of this work, AKT inhibitors have already shown some preliminary activity in clinical trials (Molife et al., 2014; Yap et al., 2011), and MK2206 is currently being investigated in patients with pancreatic cancer (https://ClinicalTrials.gov IDs NCT01783171 and NCT01658943). Our present study demonstrates that the combination of AKT inhibitors with oxidizing agents is active in PDA, and ongoing work will determine whether the investigational agent BSO (https://ClinicalTrials.gov IDs NCT00002730, NCT00005835, and NCT00661336) or alternative agents that target different aspects of redox regulation will be most attractive for rapid clinical translation.

EXPERIMENTAL PROCEDURES

Human Specimens

All human experiments were approved by the Institutional Review Boards of Memorial Sloan Kettering Cancer Centre, MD Anderson Cancer Centre, Weill Cornell Medical College, Stony Brook University, and Cold Spring Harbor laboratory. Written informed consent from the donors for research use of tissue in this study was obtained prior to acquisition of the specimen. Samples were confirmed to be tumor or normal based on pathological assessment.

(i) Cell viability of N, K, and KP organoids and Suit2 cells over increasing concentrations of MK2206 in the presence or absence of BSO for 72 hr. Dotted lines indicate 95% confidence intervals (n = 5).

(ii) EC50 values of MK2206 on pancreatic organoids and cell lines in the presence or absence of BSO. See also Figure S6.
Analysis of Global Protein Synthesis

Organoids or Suit2 cells were incubated for 30 min in media containing 10 μCi of [35S]-Met (NEG709A005MC, PerkinElmer). Cell lysates were prepared using standard procedures, and equal amounts of total protein were separated on a 4%–12% Bis-Tris polyacrylamide gel and transferred to PVDF (polyvinylidene fluoride) membrane. Membranes were exposed to autoradiography film for 12–48 hr and then developed. For liquid scintillation, equal amounts of radio-labeled total protein were TCA (trichloroacetic acid) precipitated, washed two times in acetone, and air dried at room temperature. The amount of [35S]-Met incorporated into protein was measured using a Beckman LS6500 Scintillation counter. Total protein content was determined by bicinchoninic acid (BCA) assay (Bio-Rad).

Polysome Fractionation

Organoids were treated with 300 μg/ml cyclohexamide (Sigma) in media for 30 min at 37°C and then harvested on ice in PBS containing 300 μg/ml cyclohexamide. Cells were pelleted and lysed in 10 mM Tris-Cl (pH 8), 140 mM NaCl, 1.5 mM MgCl₂, 0.25% NP-40, 0.1% Triton X-100, 50 mM DTT, 150 μg/ml cyclohexamide, and 640 U/ml RNasin for 30 min. Lysates were cleared, separated on a 10%–50% sucrose gradient by ultracentrifugation, and fractionated using a Teledyne Isco gradient fractionation system.

Figure 7. Combined Inhibition of AKT and GSH Synthesis Suppresses Human and Mouse Pancreatic Tumor Growth In Vivo

(A) Tumor volumes of KPC mice treated daily with vehicle (methylcellulose), BSO, MK2206, or the combination for 7 days. Tumor volumes were determined by ultrasound imaging on the indicated days.

(B) Relative tumor volume of KPC mice treated with MK2206 or in combination with BSO on day 7. Student’s t test (n = 11). Data are means ± SEM, Student’s t test.

(C) Relative growth of subcutaneously xenografted Suit2 tumors in mice treated with vehicle, MK2206, or MK2206 + BSO for 7 days (left) or 14 days (right). Data are means ± SEM, Student’s t test.

(D) Phospho-histone H3 IHC of representative Suit2 tumors from treated mice (left). Quantification of pH3 positivity (right). Data are means ± SEM (n ≥ 5 fields of view), Student’s t test.

See also Figure S7.

Analysis of Global Protein Synthesis

Organoids or Suit2 cells were incubated for 30 min in media containing 10 μCi of [35S]-Met (NEG709A005MC, PerkinElmer). Cell lysates were prepared using standard procedures, and equal amounts of total protein were separated on a 4%–12% Bis-Tris polyacrylamide gel and transferred to PVDF (polyvinylidene fluoride) membrane. Membranes were exposed to autoradiography film for 12–48 hr and then developed. For liquid scintillation, equal amounts of radio-labeled total protein were TCA (trichloroacetic acid) precipitated, washed two times in acetone, and air dried at room temperature. The amount of [35S]-Met incorporated into protein was measured using a Beckman LS6500 Scintillation counter. Total protein content was determined by bicinchoninic acid (BCA) assay (Bio-Rad).

Polysome Fractionation

Organoids were treated with 300 μg/ml cyclohexamide (Sigma) in media for 30 min at 37°C and then harvested on ice in PBS containing 300 μg/ml cyclohexamide. Cells were pelleted and lysed in 10 mM Tris-Cl (pH 8), 140 mM NaCl, 1.5 mM MgCl₂, 0.25% NP-40, 0.1% Triton X-100, 50 mM DTT, 150 μg/ml cyclohexamide, and 640 U/ml RNasin for 30 min. Lysates were cleared, separated on a 10%–50% sucrose gradient by ultracentrifugation, and fractionated using a Teledyne Isco gradient fractionation system.

Therapeutic Intervention Studies in Mice

Upon detection of a mass during weekly palpation, KPC mice were subjected to high-contrast ultrasound imaging using the Vevo 2100 System with an MS250 13- to 24-MHz scanhead (Visual Sonics). Mice with tumor diameters of 7–9 mm were randomized and enrolled 1 day after scanning. MK2206 (Merck) was formulated in 0.5% methylcellulose. BSO (Sigma) was formulated in saline. Mice were administered methylcellulose vehicle or 100 mg/kg MK2206 every day via oral gavage and saline or 10 mmol/kg BSO via intraperitoneal injection. Tumor volume was monitored on days 4 and 7 after initial scan. The same dosing regimen was applied to athymic nude mice bearing Suit2 tumor cells. Drug administration was initiated 3 weeks post-transplantation of 10⁵ cells subcutaneously.

ACCESSION NUMBERS

The accession number for the cysteine proteomic dataset reported in this paper is MassIVE: MSV000079394.

SUPPLEMENTAL INFORMATION

Supplemental Information includes Supplemental Experimental Procedures, seven figures, and five tables and can be found with this article online at http://dx.doi.org/10.1016/j.cell.2016.06.056.
AUTHOR CONTRIBUTIONS

ACKNOWLEDGMENTS
We thank J.R. Prigge, Montana State University, for developing the anti-Nrf2 antibody and Dr. L. Baker for critical review of the manuscript. This work was performed with the CSHL Mass Spectrometry, Animal and Tissue Imaging, and Bioinformatics Shared Resources, which are supported by Cancer Center Support grant 5P30CA045508. D.A.T. is a distinguished scholar of the Lustgarten Foundation (LF) and director of the LF-designated Laboratory of Pancreatic Cancer Research. D.A.T. is supported by the CSHL Association; the NIH (5P30CA045508-26, 5P50CA101955-07, 1U10CA180944-01, 5U01CA168409-3, and 1R01CA190092-01); the Caraford Foundation; PCUK; the David Rubinstein Center for Pancreatic Cancer Research at MSKCC; Stand Up to Cancer/KWF; the STARR Foundation (7-AT-718), the DOD (W81XWH-13-PRCRP-IA); and the Precision Medicine Research Associates. We are also grateful for support from the following: the Damon Runyon Cancer Research Foundation (Shirley Stein fellow, DRG-2165-13, to I.I.C.C.); the Human Frontier Science Program (LT000190/2013 to I.I.C.C.); the Cancer Research Society and CCSRI (702317, N.S.); the Canadian Institute of Health Research (to S.M.J.); NIH grants AG040020 and CA152559 to E.E.S.; grant R01 CA159222 to H.C.C.; the Swedish Research Council (937-2013-7277 to D.O.); the Sociedad Española de Oncología Médica (SEOM; to M.P.-S.); and the Hope Funds for Cancer Research Fellowship (to W.P). This work was performed with the MSKCC Molecular Cytology Core, which is supported by Cancer Center Support grant P30CA008748. C.B.T. is a founder of Agios Pharmaceuticals and a member of its scientific advisory board. C.B.T. also serves on the board of directors of Merck.

Received: November 29, 2015
Revised: May 5, 2016
Accepted: June 29, 2016
Published: July 28, 2016

REFERENCES


Malec, V., Gottschald, O.R., Li, S., Rose, F., Seeger, W., and Hänze, J. (2010). HIF-1 alpha signaling is augmented during intermittent hypoxia by induction of...