Cancer Biology and Signal Transduction

Mutant Ras Elevates Dependence on Serum Lipids and Creates a Synthetic Lethality for Rapamycin

Darin Salloum, Suman Mukhopadhyay, Kaity Tung, Aleksandra Polonetskaya, and David A. Foster

Abstract

The conversion of normal cells to cancer cells involves a shift from catabolic to anabolic metabolism involving increased glucose uptake and the diversion of glycolytic intermediates into nucleotides, amino acids, and lipids needed for cell growth. An underappreciated aspect of nutrient uptake is the utilization of serum lipids. We investigated the dependence of human cancer cells on serum lipids and report here that Ras-driven human cancer cells are uniquely dependent on serum lipids for both proliferation and survival. Removal of serum lipids also sensitizes Ras-driven cancer cells to rapamycin—indicating that the enhanced need for serum lipids creates a synthetic lethal phenotype that could be exploited therapeutically.

Introduction

An emerging hallmark of cancer is the "metabolic transformation" that occurs to accommodate the needs of a proliferating population of cells (1). The conversion of normal cells to cancer cells involves a shift from catabolic to anabolic metabolism involving increased glucose uptake and the diversion of glycolytic intermediates into nucleotides, amino acids, and lipids needed for cell growth (1–4). In addition to glucose, cancer cells utilize glutamine as a nitrogen source for nucleotides and as a carbon source (5). Cancer cells also need essential amino acids that mammalian cells cannot synthesize. An underappreciated aspect of nutrient uptake is the utilization of exogenously supplied fatty acids (6). Cells grown in culture are provided with media that is supplemented with glucose, essential amino acids, and glutamine as nutrients for cell growth. However, mammalian cell do not synthesize all of the unsaturated lipids needed for membrane biosynthesis—there are "essential fatty acids" that must also be present in the medium (6). Conventional growth media used for culturing mammalian cells do not contain lipids—they are provided in the serum that typically supplement culture media.

One of the emerging fields of cancer therapeutics is the possibility of targeting the special metabolic needs of cancer cells (7). There has been considerable enthusiasm about the possibility of interfering with both glucose (8) and glutamine (5) utilization as therapeutic options for human cancers. However, although interfering with fatty acid synthesis in cancer cells has received attention (9), there has been very little reported on the utilization of exogenously supplied lipids and the therapeutic options.

mTOR—the mammalian/mechanistic target of rapamycin—integrates signals that respond to nutrients and promotes cell-cycle progression and cell survival (10). We have previously reported that suppression of mTOR in the absence of serum results in apoptosis in cancer cells harboring mutant Ras genes (11–13). In this report, we identify an enhanced need for exogenously supplied serum lipids in Ras-driven human cancer cell lines that creates a synthetic lethality (14) for suppressing mTOR in the absence of serum. This finding suggests that the increased need for serum lipids by Ras-driven cancers may represent an Achilles’ heel that could be therapeutically targeted in what may be as many as 30% of all human cancers.

Materials and Methods

Cells, cell culture conditions

The MDA-MB-231, Calu-1, BJ, MCF7, BxPC3, T24, HT29, Panc-1, and HCT116 cell lines used in this study were obtained from American Type Culture Collection. No authentication was performed by the authors. Cell lines were maintained in Dulbecco’s Modified Eagle Medium (DMEM; Sigma) supplemented with 10% FBS.
BxPC3 cell line was maintained in RPMI(Sigma) medium supplemented with 10% FBS. Delipidated FBS was obtained from Gemini Bio Products (900-123).

Materials

Reagents were obtained from the following sources: antibodies against cleaved PARP, actin, Akt, P-Akt (Ser473), P-Akt (Thr308), S6 kinase, P-S6 kinase (Thr389), 4EBP1, P-4EBP1 (Thr37–46), fatty acid synthase (FASN), stearoyl-CoA desaturase-1 (SCD1), and ATP citrate lyase (ACL) were obtained from Cell Signaling; antibodies against KRas were obtained from Abcam. MTT reagent (ACL) were obtained from Cell Signaling; antibodies against cleaved PARP, actin, Akt, P-Akt (Ser473), P-Akt (Thr308), S6 kinase, P-S6 kinase (Thr389), 4EBP1, P-4EBP1 (Thr37–46), fatty acid synthase (FASN), stearoyl-CoA desaturase-1 (SCD1), and ATP citrate lyase (ACL) were obtained from Cell Signaling; antibodies against KRas were obtained from Abcam. MTT reagent was obtained from Sigma. Rapamycin was obtained from LC Laboratories, and 5-(N-ethyl-N-isopropyl) amiloride (EIPA) was obtained from Sigma.

Lipid mix supplementation

Fatty acid mix was obtained from Invitrogen (11905) and was supplied to cells as 1:200 dilution complexed with 10% bovine serum albumin (BSA; Sigma) in 2:1 ratio for the final concentration of lipids in the media of 0.375 mg/L. The exact composition of the fatty acid mixture is provided in Table S1. Palmitic acid (Sigma) was diluted in 1-butanol (1-BtOH) was added for 20 minutes before lipids were collected. Lipids were extracted and separated by thin layer chromatography along with phosphatidylbutanol standard (Enzo Life Sciences, BML-ST401-0050). The phosphatidylbutanol fraction was identified through comigration with standards, and the levels of the PLD product [3H]-phosphatidylbutanol were determined by scintillation counting.

Western blot analysis

Proteins were extracted from cultured cells using M-PER (Thermo Scientific, 78501), and the Western blot analysis of extracted proteins was performed using enhanced chemiluminescence (Thermo Scientific) as described previously (15).

Flow cytometric analysis

Cells were washed and collected by trypsinization. Recovered cells were resuspended in a solution containing 7 mL of 2% BSA in PBS, 5 mmol/L EDTA, 0.1% NaN3, and were fixed by the dropwise addition of 3 mL of 100% ethanol. Fixed cells were collected and resuspended in 500 μL of sorting buffer containing 2% BSA in PBS, 0.1% Triton-X 100, 5 mmol/L EDTA, 40 μg/mL propidium iodide, 100 μg/mL RNAse A, and were incubated at 37°C for 30 minutes. The cells were filtered through a 70-μm mesh to remove cell aggregates. The DNA content was analyzed by flow cytometry (FACSCalibur; Becton Dickinson), and percentages of cells within each phase of the cell cycle were determined using WinCycle software (Phoenix Flow Systems).

Results

Lipid deprivation leads to increased PLD activity in Ras-driven human cancer cell lines

We previously reported that serum withdrawal led to an increase in PLD activity that was largely restricted to cancer cells harboring Ras mutations (11). PLD generates phosphatidic acid from phosphatidylcholine, which is required for the stability and activity of mTOR complexes (15, 16). mTOR complex 1 (mTORC1) is a sensor of nutrients that regulates both cell-cycle progression and survival (17). It has been suggested that activation of the mTOR signaling node is the most commonly dysregulated signal in human cancers (1, 18). Because phosphatidic acid is at the center of membrane phospholipid biosynthesis, we recently proposed that phosphatidic acid affects mTOR as an indicator of sufficient precursors for membrane synthesis in dividing cells and that cancer cells have coopted PLD to sustain cell proliferation and survival of cancer cells by providing the phosphatidic acid needed to keep mTOR active (4). Thus, we speculated that the increased PLD activity observed in Kras-driven cancer cells could be a response to insufficient lipids in serum. To test this hypothesis, we examined the impact of serum lipids on the PLD activity stimulated by the withdrawal of lipids from MDA-MB-231 breast, Calu-1 lung, and T24 bladder cancer cells—all of which harbor mutant Ras...
Ras-driven cancer cells are uniquely dependent on exogenous fatty acids for proliferation and survival

Elevated PLD activity promotes survival in response to stress in Ras-driven cancer cells (11). To test whether the increased PLD activity in Ras-driven cell lines yields a proliferative advantage, we investigated the lipid dependence of cell proliferation and viability for a subset of human cancer cell lines. MDA-MB-231 breast and Calu-1 lung cancer cells both harbor KRas mutations, whereas MCF7 breast cancer cells and the human diploid fibroblast BJ TERT cell line do not. Cells were plated and after 24 hours and shifted to the indicated conditions. After 5 days, viable and nonviable cells were counted. For all three cancer cell lines, there were very few viable cells after 5 days when put in a medium lacking FBS (Fig. 2A). In contrast, almost all of the BJ cells were still viable. There were fewer cells observed in the absence of serum than in the controls due to G1 cell-cycle arrest of these cells in the absence of serum (19). If the cells were put in medium containing 10% delipidated FBS, virtually all of the Ras-driven MDA-MB-231 and Calu-1 cancer cells were nonviable, whereas the MCF7 and BJ cells were mostly viable—indicating a significant difference in the way that Ras-driven cancer cells respond to the lack of serum lipids. Most strikingly, when the fatty acid mixture was provided to the KRas-driven MDA-MB-231 and Calu-1 cells, there was a dramatic increase in cell viability relative to the viability observed with delipidated FBS—indicating a greater dependence on lipids than growth factors for both proliferation and survival (Fig. 2A). Both the MCF7 and BJ cells had lower cell numbers with the fatty acid mixture than with the delipidated FBS—indicating a greater dependence on growth factors than lipids for proliferation. The combination of delipidated FBS and the fatty acid mixture restored full proliferation and survival to all cell lines. Of interest, a lipid mixture that contained only palmitic acid, the 16 carbon saturated fatty acids produced by de novo fatty acid synthesis, did not enhance either survival or proliferation—indicating that the longer chain unsaturated fatty acids present in the fatty acid mixture were critical. Similarly, BSA, which was included as a carrier for the fatty acids, did not improve the survival of the MDA-MB-231 or Calu-1 cells in 10% delipidated FBS. The pattern of a greater dependence of lipids than growth factors for Ras-driven cancer cells was also observed with a larger panel of human cancer cell lines (Supplementary Fig. S1).

We next compared the effect of serum deprivation in the absence and presence of fatty acids on cell-cycle progression using flow cytometry. All four cell lines (BJ, MCF7, MDA-MB-231, and Calu-1) had increased G1 DNA content when cells were shifted from 10% FBS to 0% FBS (Fig. 2B, left). However, if the cells were shifted to 0% FBS in the presence of the fatty acid mix, the BJ and MCF7 cells still arrested in G1, whereas the KRas-driven MDA-MB-231 and Calu-1 cells did not significantly accumulate in G1. Similarly, there was a reduction of cells in S-phase for all cell lines when placed in 0% FBS (Fig. 2B, right). However, if the fatty acid mix was provided, there was still a reduction in S-phase cells in the BJ and MCF7 cells, but not in the MDA-MB-231 and Calu-1 cells. These data demonstrate that the MDA-MB-231 and Calu-1 cells harboring KRas mutations continue to cycle in the absence of serum growth factors but need exogenously supplied lipids. In contrast, the MCF7 and BJ cells accumulated in G1 in the absence of growth factors and the addition of lipids did not make a difference: Thus, KRas-driven
cancer cells have a greater dependence on exogenously supplied lipids and a lesser dependence on serum growth factors than the BJ and MCF7 cells.

To further demonstrate that mutant Ras promotes a dependence on lipids, we transfected the BJ cells with a plasmid expressing oncogenic KRas (KRasV12G). Parental BJ cells transiently transfected with KRas-expressing and empty vector plasmids were shifted to a medium containing 0% FBS and 0% FBS containing the fatty acid mixture, and the relative cell numbers were determined 3 days later. The cells transfected with the KRasV12G plasmid had significantly fewer cells in the absence of FBS than the control BJ cells transfected with vector alone (Fig. 2C). However, if fatty acids were included with the medium lacking FBS, then the oncogenic Ras-transfected BJ cells grew as well as the parental BJ cells—indicating that the expression of KRasV12G sensitized the BJ cells to the lack of lipids in the media.

Oncogenic Ras prevents induction of stearoyl-CoA desaturase-1 levels upon serum and lipid withdrawal

The above results clearly indicate a differential response to lipid deprivation in cells expressing mutant Ras. Key enzymes in the generation of fatty acids needed for membrane biosynthesis include FASN, ACL, and SCD1; ref. 9). We examined the effect of serum and lipid withdrawal on the levels of these three enzymes in the BJ, MCF7, MDA-MB-231, and Calu-1 cells (Fig. 3A). Although no significant changes in expression levels of FASN and ACL were detected in response to serum or lipid deprivation, there was a dramatic increase in the level of SCD1 in the BJ and MCF7 cells. In contrast, the MDA-MB-231 and Calu-1 cells did not elevate SCD1 expression in response to serum or lipid deprivation (Fig. 3A). Similarly, if the KRasV12G-expressing plasmid was transfected into BJ cells, it suppressed the elevated expression of SCD1 observed in response to serum deprivation.

Figure 2. Ras-driven cancer cells are uniquely dependent on exogenous fatty acids. A, MDA-MB-231, Calu-1, MCF7, and BJ cells were plated and shifted to media conditions as indicated and the cell number was determined 5 days later. BSA, the carrier protein for the fatty acid (FA) mix and palmitic acid (PA), was included as a control. Each measurement was normalized against the cell number for cells grown in 10% FBS, which was given a value of 100%. Attached viable cells are in blue; detached nonviable cells are in red. Error bars, SD values for at least two independent experiments. B, changes in G1- and S-phase cell population were evaluated by flow cytometry. MDA-MB-231, Calu-1, MCF7, and BJ cells were plated at 30% confluence in 10% FBS and shifted to 0% FBS with or without BSA–fatty acid mixture. DNA content per cell was evaluated 48 hours later using flow cytometric analysis as described in Materials and Methods. Values were normalized to the cell-cycle profile in full serum condition, and relative difference is plotted as indicated. C, BJ cells were transfected with constitutively active KRas (V12G) or empty vector as described in Materials and Methods, and shifted to full growth serum media or to 0% serum media with or without BSA–fatty acid mixture 18 hours after transfection. Seventy-two hours later, cell number was determined. Each measurement was normalized against the relative cell number in 10% FBS, which was given a value of 100%. Error bars, SD values for at least two independent experiments.
withdrawal (Fig. 3B). Although the effect is more pronounced for serum withdrawal than for lipid withdrawal, the lack of response in cells with oncogenic KRas indicates that these cells have a disabled response to serum and lipid deprivation.

**Withdrawal of serum lipids creates synthetic lethality for rapamycin in cancer cells harboring mutant KRAs**

We previously reported that in the absence of serum, MDA-MB-231 cells, as well as most other cancer cells, are killed by rapamycin doses (20 μmol/L) capable of suppressing phosphorylation of the mTORC1 substrate eukaryotic initiation factor 4E-binding protein-1 (4E-BP1; refs. 12, 13). The high doses of rapamycin are required for complete dissociation of mTOR from its companion protein Raptor. At conventional nanomolar doses, mTOR is only partially dissociated from Raptor such that mTORC1 can still phosphorylate 4E-BP1, but cannot recognize and phosphorylate S6 kinase (20). The effect of high-dose rapamycin treatment was shown not to be due to off-target effects (12, 15, 21). In the presence of serum, MDA-MB-231 cells were protected by TGF-β present in serum, which prevented apoptosis by inducing G1 cell-cycle arrest (13, 20). However, although the MDA-MB-231 cells could be partially protected from the apoptotic effect of rapamycin by TGF-β alone, these cells still displayed significant subgenomic DNA indicating some level of apoptosis (13). Thus, there was apparently something else in the serum that along with TGF-β contributed to survival. We, therefore, examined the effect of serum lipids with rapamycin on the cell viability of MDA-MB-231, Calu-1, BJ, and MCF7 cells. As reported previously, in the absence of serum, rapamycin (20 μmol/L) induced cleavage of the caspase-3 substrate PARP in the MDA-MB-231 and Calu-1 cells, indicating apoptotic cell death (Fig. 4A). PARP cleavage was not observed in the presence of 10% FBS. PARP cleavage was not observed with rapamycin treatment in the BJ or MCF7 cells in either the presence or absence of serum. Most significantly, if the MDA-MB-231 or Calu-1 cells were deprived of lipids, by incubating in 10% delipidated serum, rapamycin still induced PARP cleavage, indicating that the lack of lipids created a synthetic lethal phenotype for rapamycin treatment. If the fatty acid mixture was provided in the absence of FBS, rapamycin still induced apoptosis, consistent with our previous observation that TGF-β was required for suppressing rapamycin-induced apoptosis in the presence of serum. If the fatty acid mixture was combined with the delipidated serum, PARP cleavage was suppressed. Stimulation of apoptosis, indicated by induction of PARP cleavage in the delipidated serum, required the high dose of rapamycin (Fig. 4B) that causes a complete dissociation of mTOR from the mTORC1 companion protein Raptor and suppresses 4E-BP1 phosphorylation (12). We also examined the effect of rapamycin on MDA-MB-231 cells deprived of lipids on cell viability/growth using the MTT assay. The high-dose rapamycin reduced cell viability in the absence of FBS (Fig. 4C), in delipidated FBS, and in the presence of the fatty acid mixture—but not in the presence of delipidated FBS + fatty acid mixture. The cytotoxic effect of rapamycin on MCF7 and BJ cells was substantially less than that observed for the MDA-MB-231 cells (Fig. 4C). The loss of cell viability, like PARP
cleavage, required a high-dose rapamycin treatment (Fig. 4D). Thus, the KRas-driven cancer cells are sensitized to rapamycin by depriving cells of either lipids or growth factors.

Suppression of macropinocytosis sensitizes Ras-driven cancer cells to rapamycin

The sensitivity of Ras-driven cancer cells deprived of serum lipids to rapamycin suggests a means to target the many cancers that harbor Ras mutations. Although ridling the serum of lipids in a human is problematic, it could be possible to block the uptake of serum lipids. Barsagi and Feramisco reported previously that mutant Ras stimulates macropinocytosis (22). We, therefore, wanted to examine whether blocking macropinocytosis would also sensitize the KRas-driven MDA-MB-231 and Calu-1 cells to rapamycin. Macropinocytosis can be suppressed by EIPA (23, 24). We, therefore, examined the effect of EIPA on the rapamycin sensitivity of MDA-MB-231, Calu-1, MCF7, and BJ cells in the presence of 10% FBS. EIPA treatment made the MDA-MB-231 and Calu-1 cells sensitive to the apoptotic effect of rapamycin (Fig. 5A), while having little or no effect on the MCF7 or BJ cells. We also examined whether EIPA could sensitize the BJ cells expressing activated KRas cells to rapamycin treatment. The BJ-KRas cells displayed PARP cleavage when treated with the combination of EIPA and 20 μmol/L rapamycin (Fig. 5B). Another indicator of apoptotic cell death is the appearance of subgenomic DNA. We compared the levels of subgenomic DNA in the BJ and MDA-MB-231 cells treated with EIPA and rapamycin. The combination of EIPA and rapamycin induced a substantial increase in subgenomic DNA in the MDA-MB-231, but not the BJ cells (Fig. 5C). The increase in subgenomic DNA observed in Fig. 5C corresponded with large drops in the percentage of cells in S-phase and G2–M cells relative to the drop in G1 cells (Table S2)—which is consistent with our previous work in which we demonstrated that the cells killed by rapamycin were in S-phase (13). The data provided in Fig. 5 demonstrate that EIPA, like lipid deprivation, creates a synthetic lethal phenotype for rapamycin in KRas-driven cancer cells—demonstrating that it could be possible to exploit the apparent acute need of lipids of KRas-driven cancer cells.

Discussion

The data presented here reveal an enhanced requirement for lipids in human cancer cells harboring activating Ras mutations. Depriving these cells of lipids...
leads to what we would call a "replicative cell death"—continued attempts to proliferate in the absence of sufficient nutrients. Whereas most cells are capable of synthesizing fatty acids for membrane phospholipids from glucose (9), the Ras-driven cancer cells apparently have a greater need for exogenously supplied lipids.

This unique property of Ras-driven cancer cells is apparently an Achilles’ heel for Ras-driven cancer cells in that suppressing mTOR in these cells induces apoptosis if the uptake of lipids is suppressed.

Of significance, two very recent reports have also identified the need for exogenously supplied protein as an amino acid supply (24) and for lipids (25) in Ras-transformed cells. The uptake of albumin served as a source of glutamine for needed for cell growth (24). We used albumin as a carrier for exogenously supplied lipids to promote cell survival and rapamycin resistance. The use of albumin alone did not promote survival by itself (Fig. 2), indicating that although Ras-transformed cells depend on scavenged proteins as an amino acid source for cell growth, it was the lipids that were critical for survival and resistance to rapamycin.

The lipid requirement for exogenous lipids by Ras-transformed cells was dependent on an unsaturated fatty acid (25). Consistent with this, the saturated fatty acid palmitic acid was not able to substitute for the mixture of fatty acids used in this study, which consisted with several unsaturated fatty acids.

The dependence of Ras-transformed cells on exogenously supplied nutrients over standard de novo synthetic pathways appears to be a Ras-driven program shift. In response to the lack of serum lipids, cells lacking mutant Ras showed a dramatic increase in the level of SCD1 (Fig. 3). This is consistent with the recent report from the Rabinowitz and Thompson laboratories where they showed that Ras-transformed cells were resistant to inhibition of SCD1 (25). Although they did not look at SCD1 protein levels, their study also demonstrated that SCD1 was not important for the growth of Ras-transformed cells. It is likely that the lack of increased SCD1 expression in the Ras-transformed cells is a reflection of the apparent scavenger program stimulated by Ras that involves macropinocytosis (22). In this regard, the elevated PLD activity observed in KRas-transformed cancer cells in response to lipid deprivation may be of significance. We have proposed that the phosphatidic acid requirement for mTOR complex stability and activity represents a means for sensing the presence of sufficient lipids for cell growth (4), consistent with the role of mTORC1 in sensing other nutrients such as essential amino acids and glucose (10). Phosphatidic acid is at the center of membrane phospholipid biosynthesis and is an ideal indicator of lipid sufficiency. The major pathway for phosphatidic acid synthesis from fatty acids is through the acylation of glucose-derived glycerol-3-phosphate (4). In the absence of de novo lipid biosynthesis and exogenously supplied lipids, you create a need for phosphatidic acid from a different source. We have proposed previously that the elevated PLD activity in cancer cells deprived of serum is an attempt to promote survival by keeping mTOR complexes intact and active (4, 11, 15). The KRas-driven cancer cells may be especially dependent on elevated PLD activity because of the deactivated de novo fatty acid synthesis machinery.

The rapamycin sensitivity of Ras-transformed cells deprived of lipids or with suppressed macropinocytosis...

Figure 5. Blockage of macropinocytosis mimics lipid deprivation and sensitizes Ras-driven tumors to rapamycin (rapa). A, MDA-MB-231, Calu-1, MCF7, and BJ cells were plated overnight and shifted to 10% FBS conditions containing EIPA (10 μmol/L) or rapamycin (20 μmol/L) as indicated. Cell lysates were collected 18 hours after treatment and levels of cleaved PARP were determined as in Fig. 4A. B, BJ cells were transfected with constitutively active KRas (V12G) or empty vector and treated with EIPA or rapamycin in the 10% FBS condition as described in A. Cell lysates were collected 18 hours after treatment, at which time the levels of cleaved PARP were determined as in A. Data in A and B are representative of least two independent experiments. C, MDA-MB-231 and BJ cells were plated at 40% confluence and treated as in B for 48 hours, after which these were collected and subjected to flow cytometric analysis. Total subgenomic DNA is plotted as indicated. Error bars, SD values for at least two independent experiments.
may be a reflection of the impact of lipid deprivation on cell-cycle progression. We reported previously that in the absence of serum, rapamycin induced apoptosis in the MDA-MB-231 cells, but in the presence of serum, rapamycin induced a TGF-β-dependent G₁ cell-cycle arrest that protected the cells from apoptosis (13, 26). However, if cells were synchronized in early S-phase, then the cells were killed by rapamycin—even in the presence of serum/TGF-β. These studies indicated that if cells get past the TGF-β-dependent G₁ cell-cycle checkpoint and enter S-phase, then the suppression of mTORC1 activates an apoptotic program. Consistent with our previous studies (13), the increase in subgenomic DNA observed in Fig. 5C corresponded with large drops in the percentage of cells in S-phase and G₂–M cells relative to the drop in G₁ cells (Table S2). Although these data are too preliminary to draw any firm conclusions about why rapamycin kills KRas-driven cancer cells when deprived of lipids, the data are consistent with an apoptotic effect on cells that have progressed into S-phase. It is possible that depriving KRas-driven cancer cells, where utilization of de novo synthesized fatty acids is suppressed (25), of serum lipids leads to the arrest or slowed progression through S-phase. Once a cell has entered S-phase, the cell has committed to replicating its genome and doubling its mass. If mTORC1 is suppressed in S-phase cells—telling the cell that there are not sufficient raw materials to finish the job—then a default apoptotic program is activated rather than try to remedy the situation at this phase of the cell cycle. Although there is still much to be learned about the impact of mTORC1 suppression on cells in S-phase, it is clear that in KRas-driven cancer cells, depriving cells of lipids creates a synthetic lethal phenotype for rapamycin treatment that could create therapeutic strategies for targeting the large number of human cancers that harbor Ras mutations.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

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Development of methodology: D. Salloum, S. Mukhopadhyay, D.A. Foster
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Writing, review, and/or revision of the manuscript: D. Salloum, D.A. Foster
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): D.A. Foster
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