Akt Requires Glucose Metabolism to Suppress Puma Expression and Prevent Apoptosis of Leukemic T Cells

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The PI3K/Akt pathway is activated in stimulated cells and in many cancers to promote glucose metabolism and prevent cell death. Although inhibition of Akt-mediated cell survival may provide a means to eliminate cancer cells, this survival pathway remains incompletely understood. In particular, unlike anti-apoptotic Bcl-2 family proteins that prevent apoptosis independent of glucose, Akt requires glucose metabolism to inhibit cell death. This glucose dependence may occur in part through metabolic regulation of pro-apoptotic Bcl-2 family proteins. Here, we show that activated Akt relies on glycolysis to inhibit induction of Puma, which was uniquely sensitive to metabolic status among pro-apoptotic Bcl-2 family members and was rapidly up-regulated in glucose-deficient conditions. Importantly, preventing Puma expression was critical for Akt-mediated cell survival, as Puma deficiency protected cells from glucose deprivation and Akt could not readily block Puma-mediated apoptosis. In contrast, the pro-apoptotic Bcl-2 family protein Bim was induced normally even when constitutively active Akt was expressed, yet Akt could provide protection from Bim cytotoxicity. Up-regulation of Puma appeared mediated by decreased availability of mitochondrial metabolites rather than glycolysis itself, as alternative mitochondrial fuels could suppress Puma induction and apoptosis upon glucose deprivation. Metabolic regulation of Puma was mediated through combined p53-dependent transcriptional induction and control of Puma protein stability, with Puma degraded in nutrient-replete conditions and long lived in nutrient deficiency. Together, these data identify a key role for Bcl-2 family proteins in Akt-mediated cell survival that may be critical in normal immunity and in cancer through Akt-dependent stimulation of glycolysis to suppress Puma expression.

Growth factor stimulation or neoplastic transformation of quiescent cells requires increased generation of energy, reductive potential, and biosynthetic precursors to meet the demands of cell growth and proliferation (1). In both T cell activation and T cell acute lymphoblastic leukemia (T-ALL),2 glucose uptake and glycolysis in particular are highly elevated to support these metabolic requirements, and failure to increase glycolysis prevents proliferation and can lead to apoptosis (2, 3). This highly glycolytic phenotype has been termed aerobic glycolysis (4) and is strongly induced by the phosphatidylinositol 3-kinase (PI3K)/Akt signaling pathway (2, 3). When activated in normal T cells by engagement of the T cell receptor and co-stimulatory receptor, or in cancer cells via loss of the lipid phosphatase phosphatase or tensin homolog or activating mutation of PI3K (5), Akt can promote aerobic glycolysis through increased trafficking of the glucose transporter Glut1 to the cell surface (6), activation of phosphofructokinase and hexokinase activity (7, 8), and stimulation of mTOR (9). In addition to promoting glucose uptake and metabolism, Akt can be highly anti-apoptotic and can promote growth factor independence and is associated with resistance to chemotherapy in cancers, including T-ALL (10, 11). Importantly, it is now clear that the metabolic and cell survival effects of Akt function are linked, and unlike anti-apoptotic Bcl-2 family proteins, Akt requires abundant glucose to protect cells from death (12–14). The molecular nature of this metabolic checkpoint remains poorly understood, but it may play a critical role in the survival of highly glycolytic cells such as activated lymphocytes or cancer cells.

In principle, Akt may rely on glucose for several reasons. In one potential mechanism, Akt may prevent apoptosis through control of hexokinase localization to the mitochondrial outer membrane (11). In this case, Akt-driven glucose uptake is proposed to play a critical role to support generation of intracellular glucose 6-phosphate to bind hexokinase, promoting its interaction with the outer mitochondrial membrane protein VDAC. Association of hexokinase with VDAC may then inhibit recruitment of the pro-apoptotic protein Bax (15) or may prevent death via a pathway that is independent of Bcl-2 family proteins (14). Importantly, the effector metabolite in

2 The abbreviations used are: T-ALL, T cell acute lymphoblastic leukemia; MePyr, methyl-pyruvate; myrAkt, myristoylated Akt; PI, propidium iodide; ER, endoplasmic reticulum.

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The on-line version of this article (available at http://www.jbc.org) contains supplemental Figs. 1–10.
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this model is glucose 6-phosphate, and further glucose metabolism is not essential (13, 15).

Alternatively, metabolic control of Bcl-2 family proteins may play a critical role in Akt-mediated glucose addiction. Bcl-2 family members are important regulators of apoptotic cell death, and the BH3-only Bcl-2 family members Bim and Puma are essential for the death of many lymphocytes (16). A growing number of reports have now described metabolic regulation of Bcl-2 family proteins that may be sensitive to Akt-driven glucose metabolism (17–20). The pro-apoptotic protein Bad has been shown to associate with mitochondrial glucokinase to regulate its function and Bad-mediated apoptosis (21). Increased glucose metabolism can also initiate an intracellular nutrient signaling pathway that leads to inhibition of GSK-3 and stabilization of Mcl-1 (18). Conversely, Bcl-2 family proteins are also affected by insufficient glucose and are essential for cell death of nutrient-deprived cells. Even in the presence of growth factor, glucose deprivation leads to induction and activation of the pro-apoptotic Bcl-2 proteins Bim, Puma (19), and Bax (22, 23), as well as decreased Mcl-1 expression (20). These changes in the balance of anti- and pro-apoptotic Bcl-2 family members appear critical to elicit apoptosis, as expression of the anti-apoptotic protein Bcl-xL or deficiency of the pro-apoptotic proteins Bim, Puma, or Bax allows cells to survive glucose deprivation for prolonged periods (12, 19, 22, 23). In addition, the pro-apoptotic BH3-only protein Noxa is induced in T cell activation and may promote the death of glucose-deprived activated T cells (17). Despite the multiple mechanisms by which Akt acts to support cell survival, whether Akt requires glucose to regulate Bcl-2 family proteins to control cell survival remains uncertain.

We have previously shown that glucose deprivation can lead to transcriptional induction of Puma and Bim (19). As Akt and control of glucose metabolism have been implicated in T cell development (24), homeostasis (3, 25), activation and memory (2, 3, 26–28), and transformation (29, 30), we hypothesized Akt-driven glucose metabolism may support cell survival by metabolic regulation of Bcl-2 family proteins. Through analysis of Bcl-2 family members, these data show that constitutively active Akt drives glycolysis to inhibit expression of the pro-apoptotic BH3-only protein Puma, and this metabolic checkpoint is essential for Akt-mediated cell survival.

**EXPERIMENTAL PROCEDURES**

**Cell Culture**—Control, Bcl-xL, and myrAkt expressing FL5.12 cells were cultured as described previously (12, 18) in RPMI 1640 media (Mediatech) with 10% FBS (Gemini Bio-Products), 0.5 ng/ml IL-3 (PeproTech), and 55 μM β-mercaptoethanol (Invitrogen). FL5.12 cells were treated with 1 μg/ml doxycycline (Sigma) to induce myrAkt expression for 16–24 h, and IL-3 deprivation was accomplished by washing cells three times with PBS followed by culture ≤ IL-3. Jurkat, MOLT-4, and CCRF-CEM T-ALL cells (American Type Culture Collection) were cultured in RPMI 1640 media with 10% FBS. Cells were glucose-deprived by washing three times in PBS and cultured in glucose-free RPMI 1640 media (Invitrogen) with 10% diazoyl FBS (Gemini Bio-Products). For nutrient replacement, glucose (10 mM; Sigma), methyl pyruvate (10 mM or indicated dose; Sigma), fructose (10 mM; Sigma), or oleate and palmitate (0.5 mM each in BSA; Sigma) were added to glucose-free media. Tunicamycin (2 μg/ml; Sigma), rotenone (200 nM; Sigma), oligomycin (50 ng/ml; Sigma), and cycloheximide (25 μg/ml; Sigma) were used where indicated.

**T Cell Isolation and Culture**—T cells were isolated by negative selection (StemSep) from murine spleens. T cells were cultured in RPMI 1640 media with 10% FBS, 5 ng/ml IL-2 (PeproTech), and 55 μM β-mercaptoethanol (Invitrogen). T cells were stimulated on plates coated with 5 μg/ml anti-CD3ε and anti-CD28 antibodies (Pharmingen) for 48 h, and T cells were washed off the plates and cultured an additional 24 h with IL-2 + glucose before treatment.

**Transfections and Plasmids**—Transient transfections were conducted by nucleofection using the Amaza system (kit V; Amaza Biosystems). Mouse Puma, Bim (19), CHOP (31), and Foxo3a (32) were knocked down by shRNAi using previously described plasmids. The human Puma shRNAi was acquired commercially (clone TRCN0000033612; Open Biosystems). Noxa shRNAi was generated as described previously (33) using the target sequence 5′-AAGGACGAGTGTGCT-CACCTC. The Puma luciferase promoter construct was generated by cloning 2 kb upstream from the Puma transcription site into the pGL3 vector (Promega). Puma, Bim, or p53 overexpression was accomplished using WT mouse Puma, FLAG-tagged mouse Bim, or WT human p53 in the pEF6 (Puma and Bim) or pcDNA3.1 (p53) vectors (Invitrogen).

**Immunoblots**—Cells were lysed in radioimmunoprecipitation assay (RIPA) buffer with protease (Pharmingen) and phosphatase (Sigma) inhibitors, and protein concentrations were determined by bicinchoninic acid protein assay (Bio-Rad). Equal protein amounts were run on 10–20% SDS-polyacrylamide gels (Bio-Rad). Primary antibodies were as follows: Bcl-xL (Cell Signaling Technology), phospho-Akt (S473; Cell Signaling), total Akt (Cell Signaling), actin (Sigma), phospho-GSK3 (S9/21; Cell Signaling), GSK3β (Cell Signaling), phospho-Foxo3a (T32; Cell Signaling), Foxo3a (Cell Signaling), Puma (Cell Signaling), Bid (Cell Signaling), Noxa (ProSci Inc.), Bak (Cell Signaling), Bak (Millipore), BIP (Cell Signaling), CHOP (Santa Cruz Biotechnology), calnexin (Sigma), mouse Mcl-1 (BioLegend), human Mcl-1 (Sigma), mouse Bcl-2 (Pharmingen), and human Bcl-2 (Cell Signaling). Secondary antibodies were anti-rabbit (Cell Signaling) and anti-mouse (Pharmingen) horseradish peroxidase-labeled antibodies and anti-rabbit (Invitrogen) and antimouse (LiCor) fluorescent-labeled antibodies. Blots were imaged using Supersignal West Pico chemiluminescent substrate (Thermo Scientific) or the Odyssey infrared imaging system (LiCor). Images were uniformly contrasted, and some were separated digitally for ease of viewing (indicated by white spaces). For blots displaying multiple Bcl-2 family members, multiple gels were run with the same lysates due to the similar size of many Bcl-2 family members, and representative actin blots were displayed.

**Cell Death and Cycle Analysis**—For propidium iodide (PI) exclusion, cells were treated with 1 μg/ml propidium iodide and analyzed on a FACSscan flow cytometer (BD Biosciences).
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Triplet samples of a minimum of 5000 counts were analyzed using FlowJo software (TreeStar). Cell cycle and DNA content analysis was conducted as described (19), as were active Bax (31) and caspase activity assays (34).

Acylcarnitines and Metabolic Assays—Acylcarnitine profiles were determined by mass spectrometry after culturing cells in 1 μM L-carnitine (Sigma) for 36 h as described previously (31, 35). Glucose uptakes were conducted as described previously (6) using 2-deoxy-O-[3H]glucose (GE Healthcare). Glycolytic rate was measured as described previously (23). ATP was measured using the ATP bioluminescence assay kit CLS II (Roche Applied Science).

Luciferase Assays—Cells were transfected with the Puma luciferase promoter-reporter and a Renilla plasmid (phRG-TK; Promega) 24 h prior to culture ±IL-3 and/or glucose for 10 h. Cell lysates were analyzed using the Dual-Luciferase reporter assay system (Promega). Luciferase activity was normalized to Renilla activity as a transfection and loading control.

Quantitative Real Time PCR Analysis—Cells were harvested in TRIzol solution (Invitrogen), and mRNA was extracted and quantitated. Reverse transcription reactions were conducted with SuperScript II RT (Invitrogen), and quantitative real time PCR was conducted using IQ SYBR Green Supermix (Bio-Rad). Puma and β2-microglobulin primers used have been described previously (19).

Statistical Analysis—Statistical significance was determined by Student’s t test with p values as indicated with asterisks in the corresponding figure legends. For cell death curves, statistical significance was determined by two-way analysis of variance with replicates and is indicated with an asterisk.

RESULTS

Akt Requires Glucose to Inhibit Apoptosis after Growth Factor Withdrawal—The PI3K/Akt pathway is activated in many cancers and can promote growth factor-independent glucose metabolism and survival. To investigate the role of glucose in Akt-mediated survival, we examined the IL-3-dependent hematopoietic precursor cell line, FL5.12, stably expressing either excess Bcl-xL or constitutively active Akt (myristoylated Akt; myrAkt) (Fig. 1A). FL5.12 cells provide a controlled and experimentally malleable model of the growth factor dependence and survival mechanisms downstream of Akt. Withdrawal from IL-3 led to sharply decreased glucose uptake and glycolysis of control and Bcl-xL-expressing cells (Fig. 1, B and C). Activated Akt, however, largely maintained glucose metabolism. With continued IL-3 deprivation, control FL5.12 cells underwent cell death, whereas Bcl-xL- and myrAkt-expressing cells survived for a prolonged period in normal media (Fig. 1D and supplemental Fig. 1) (12). If cells were deprived of glucose, however, myrAkt could no longer maintain survival, unlike Bcl-xL-expressing cells, which persisted in the absence of glucose. The death of myrAkt-expressing cells in glucose-deficient conditions appeared apoptotic in nature, as caspase activity increased (Fig. 1E), and cell death could be prevented by co-expression of Bcl-xL (Fig. 1, F–H). Further evidence that myrAkt inhibited apoptotic cell death in a glucose-dependent fashion was observed by the increased appearance of activated Bax (Fig. 1G) and subdiploidal (sub-G1) DNA content after both IL-3 and glucose deprivation, both of which could be prevented by co-expression of Bcl-xL.

Akt Requires Glucose to Suppress Puma Induction after Growth Factor Withdrawal—Because active Akt appeared to depend on the maintenance of glucose metabolism in the absence of growth factors to prevent apoptotic cell death, we sought to determine the potential role of metabolic regulation of Bcl-2 family proteins by Akt-dependent metabolism. We therefore examined the protein levels of a variety of Bcl-2 family members in myrAkt-expressing cells cultured in the presence and absence of IL-3 and/or glucose (Fig. 2A). To minimize complications due to rapid apoptosis of control cells cultured without IL-3 and glucose (supplemental Fig. 1), myrAkt-expressing cells were compared with cells expressing Bcl-xL. Control FL5.12 cells have been analyzed and found to show similar regulation of Bcl-2 family proteins as Bcl-xL-expressing cells (supplemental Fig. 2) (18, 19). In both myrAkt- and Bcl-xL-expressing cells, Bid, Noxa, Bcl-xL, Bcl-2, Bax, and Bak did not significantly change in response to treatment.

In contrast, Bim and Puma were tightly regulated by IL-3 and glucose (Fig. 2A). Both Bim and Puma were induced in Bcl-xL-expressing cells after withdrawal of either IL-3 or glucose. Similarly, Bim was induced in myrAkt-expressing cells after either IL-3 or glucose withdrawal. Puma induction, however, was suppressed after IL-3 withdrawal of myrAkt-expressing cells if glucose was present. If glucose was absent, myrAkt could no longer prevent Puma induction. Importantly, this up-regulation of Puma appeared to occur as a consequence of altered metabolism rather than due to altered signaling, as glucose withdrawal did not affect Akt phosphorylation or phosphorylation of the Akt substrates Foxo3a and GSK-3. Therefore, among the Bcl-2 family members tested, myrAkt appeared to uniquely regulate Puma expression in a glucose metabolism-dependent fashion.

Puma Expression Is Necessary and Sufficient for Efficient Apoptosis—Activation of the Akt signaling pathway protected growth factor-deprived cells from apoptosis in a manner that correlated with a glucose-dependent suppression of Puma induction. Bim, Noxa, and Puma may all contribute to cell death, however, leaving the specific role of Puma unclear. To directly determine the contribution of Puma and other BH3-only proteins to Akt-mediated cell survival or apoptosis, expression of Puma, Bim, and Noxa were suppressed using shRNAi, and cell death and Bax activation of myrAkt-expressing cells were observed after IL-3 and glucose deprivation (Fig. 2, B–D, and supplemental Fig. 3). Consistent with pro-apoptotic contributions of these proteins, reduction of Puma, Bim (19), or Noxa (17) expression each increased cell viability after glucose deprivation. Despite only a modest reduction in protein expression, however, Puma deficiency appeared to provide the strongest protection from Bax activation and cell death.

These data did not indicate if regulation of Puma expression was sufficient among the Bcl-2 family proteins to promote apoptosis of cells with active Akt. Therefore, control, myrAkt-, and Bcl-xL-expressing cells were transiently transfected with control, Puma, or Bim expression plasmids, and
cell death was observed (Fig. 2, E and F). Puma and Bim levels were highest in Bcl-xL-expressing cells, possibly due to association of Puma with Bcl-xL and protein stabilization. Bim and Puma each led to a similar extent of apoptosis in control cells, and Bcl-xL-expressing cells were strongly protected from cell death. Surprisingly, although myrAkt expression provided significant protection against Bim, it was unable to protect against Puma. This pattern of Bim and Puma toxicity is consistent with the ability of myrAkt-expressing cells to survive in the absence of IL-3 despite Bim expression if glucose is present to inhibit Puma induction (Figs. 1D and 2A). Therefore, Puma is both necessary and sufficient to cause apoptosis of myrAkt-expressing cells deprived of glucose. Furthermore, metabolic regulation of Puma expression is likely to be of particular importance, as Akt did not appear to efficiently suppress Puma-induced apoptosis.

Maintenance of Mitochondrial Metabolism in the Absence of Glucose Inhibits Puma Expression and Cell Death—A central function of glucose is to provide metabolic fuel through glycolysis and mitochondrial oxidation. Therefore, we sought
to quantitate mitochondrial metabolite levels of IL-3-deprived control, Bcl-xL-, and myrAkt-expressing FL5.12 cells in the presence or absence of glucose by observing acylcarnitines using a mass spectrometry-based metabolomics approach. Acylcarnitines are intermediates derived from a variety of metabolic substrates, including glucose, amino acids, and lipids that reflect carbon chains available for mitochondrial oxidation (35, 36). Unlike Bcl-xL-, myrAkt-expressing cells were highly dependent on glucose to maintain certain short (Fig. 3A) and long chain (Fig. 3B) acylcarnitines (complete profile in supplemental Fig. 4). Glucose-deprived control cells were beginning to undergo apoptosis at this time (supplemental Fig. 1) and resembled myrAkt-expressing cells with reduced mitochondrial activity. These data suggest that myrAkt-expressing cells are dependent on glucose for glycolytic flux and as a source of mitochondrial fuel that may be necessary to maintain survival.

Metabolic stress caused by a lack of mitochondrial fuel may have stimulated Puma up-regulation to cause apoptosis of glucose-deprived cells with activated Akt. To test the contribution of mitochondrial metabolism in Akt-mediated survival, a cell permeate form of the glycolytic end product pyruvate, methyl pyruvate (MePyr), was supplied to provide mitochondrial fuel to glucose-deprived cells expressing myrAkt. Addition of MePyr efficiently protected myrAkt cells from apoptosis following glucose withdrawal (Fig. 3, C and E) and allowed myrAkt to suppress Puma induction after IL-3 withdrawal even in the absence of glucose (Fig. 3D). Importantly, MePyr did not affect the expression of Bim or other Bcl-2 family members tested (Fig. 3D). In addition, despite...
preventing apoptosis, MePyr could not replace glucose to promote cell cycle progression and proliferation, suggesting that MePyr did not rescue all glucose-derived metabolites necessary for proliferation (Fig. 3, E and F, and supplemental Fig. 5). Similar results were obtained with sodium pyruvate (data not shown). Neither glucose nor its immediate products are therefore uniquely required for Akt to prevent cell death.

Replacement of Glucose with Alternative Metabolic Fuels Can Rescue Akt-mediated Suppression of Puma and Death—Because it appeared that myrAkt-expressing FL5.12 cells required glucose as a source of mitochondrial fuel, inhibition of mitochondrial metabolic function may prevent the effects of MePyr on survival. To test this notion, we utilized the following two inhibitors of the electron transport chain: rotenone, which inhibits complex I, and oligomycin, which inhibits the F1/F0-ATP synthetase. Treatment of IL-3-deprived myrAkt-expressing cells with low levels of rotenone or oligomycin had little effect on survival in the presence of glucose but led to very rapid death when cells were cultured with MePyr in the absence of glucose (Fig. 4, A and B).

To further examine the role of mitochondrial metabolism in the regulation of Puma, myrAkt-expressing cells were pro-

FIGURE 3. Glucose deprivation of Akt-expressing cells causes mitochondrial metabolic stress, and addition of MePyr can rescue survival and Puma induction. A and B, control, Bcl-xL-, and myrAkt-expressing FL5.12 cells were cultured for 8 h in the absence of IL-3 and the presence or absence of glucose and harvested for mass spectrometric analysis of acetylcarnitine (A) and stearoylcarnitine (B). C–F, myrAkt-expressing FL5.12 cells were cultured as indicated. C, cells were assayed for cell death over time by PI exclusion. D, protein expression was by immunoblot after 10 h of culture; E, DNA content after 15 h to indicate cell cycle status; and F, cell accumulation after 72 h. E, black bars represent the gates used for cells with sub-G1, G1, or G2/M DNA content, and % of cells within these gates is summarized in the table below the histograms. Glu, glucose; MP, MePyr. Values represent the means ± S.D. of triplicate samples (A, B, E, and F) or triplicate experiments (C). C, Glu conditions were compared with Glu/MP for statistical analysis. Statistical significance is indicated with an asterisk, p < 0.05.
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T Cell Activation Causes Increased Glucose Uptake That Is Required to Prevent Induction of Puma and Bim and Apoptosis—Akt activation plays a key role to promote glucose metabolism and survival of primary activated lymphocytes. Therefore, we sought to determine whether the increased glucose metabolism of activated T cells (2) contributed to cell

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FIGURE 4. Alternative fuels prevent Puma induction and cell death in the absence of glucose. A and B, myrAkt-expressing FL5.12 cells were cultured in the absence of IL-3 with or without glucose or MePyr with either 200 nM rotenone (A) or 50 ng/ml oligomycin (B) as indicated and assayed for cell death over time by PI exclusion. C–F, myrAkt-expressing FL5.12 cells were cultured in the absence of IL-3 and glucose with or without oleate and palmitate (0.5 mM each) or fructose (10 mM). C and E, cells were assayed for cell death over time by PI exclusion. D and F, protein expression was determined by immunoblot. G and H, myrAkt-expressing FL5.12 cells were cultured in the absence of IL-3 with or without glucose and MePyr, oleate, or palmitate or fructose and assayed for Bax activation (G) and sub-G1 DNA content (H) after 16 h. G and H, black bar represents the gate used for cells with either active Bax or sub-G1 DNA content; % of cells within this gate is summarized in the table below the histograms. Glu, glucose; O/P, oleate and palmitate; Fru, fructose; MP, MePyr. Values represent the means ± S.D. of duplicate experiments (A, B, C, and E). Statistical significance is indicated with an asterisk, *p < 0.05.

Provisioned excess amounts of two physiologically relevant alternative metabolic fuels, the long chain fatty acids oleate and palmitate and fructose. Despite evidence that Akt can suppress lipid oxidation (37), but consistent with work showing that lipid oxidation can be forced in cells with activated Akt (38), we found that glucose could be replaced with a mixture of oleate and palmitate or fructose. These treatments prevented cell death and Puma induction (Fig. 4, C–F), Bax activation (Fig. 4G), and the appearance of sub-G1 DNA (Fig. 4H) in IL-3- and glucose-deprived cells expressing myrAkt. Similar to MePyr, however, neither fructose nor oleate and palmitate could rescue cell cycle arrest in the absence of IL-3 (data not shown). Akt-driven glucose metabolism therefore appeared to suppress Puma induction and apoptosis by a mechanism not unique to glucose or its immediate metabolic derivatives but rather by supporting mitochondrial metabolism.
survival through regulation of Puma. Glucose uptake was dramatically increased following primary murine T cell activation in an IL-2-dependent fashion that correlated with Akt phosphorylation (supplemental Fig. 6, A and B). Consistent with the findings that Akt requires glucose to prevent apoptosis, withdrawal of activated T cells from glucose led to cell death in a similar time course as withdrawal of cells from IL-2 (supplemental Fig. 6C).

Bim and Puma have both been implicated in the death of activated T cells after cytokine deprivation, with Bim appearing to play a particularly prominent role (39). To determine whether Akt-dependent glucose metabolism regulated these Bcl-2 family members in primary T cells, we examined Bcl-2 family protein expression in naive and activated T cells after 12 and 24 h of IL-2 or glucose deprivation (Fig. 5 A).

As expected, Mcl-1, Bcl-xL, Bad, and Noxa were induced after T cell stimulation (17, 40). Puma, Bim, Bid, Bcl-2, Bax, and Bak were relatively unchanged by T cell activation. After glucose or IL-2 deprivation of activated cells, however, Puma and Bim were up-regulated. Glucose deprivation also reduced levels of Puma and Bim.

**FIGURE 5.** Addition of alternative mitochondrial fuel sources to activated murine primary T cells can prevent Puma induction after glucose deprivation, but Bim expression can mask rescue of cell survival. A, analysis of protein expression was by immunoblot for primary T cells. Cells were harvested immediately after isolation (Naive) or after activation and treatment in the absence of either glucose or IL-2 for 0, 12, and 24 h as indicated. B and C, wild type activated T cells were cultured in the presence of IL-2 with or without glucose, MePyr, fructose, or oleate and palmitate. B, cells were harvested for protein expression analysis after 12 h. C, cells were assayed for cell death over time by PI exclusion. Glu, glucose; MP, MePyr; Fru, fructose; O/P, oleate and palmitate. Values represent the means ± S.D. of triplicate experiments. Statistical significance is indicated with an asterisk, p < 0.05.

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phospho-Akt but did not appear to dramatically affect Noxa, Bcl-2, Bcl-xL, Bax, or Bak expression. Addition of MePyr to replace glucose inhibited Puma up-regulation but did not affect any other Bcl-2 family members tested, nor could it maintain phospho-Akt (Fig. 5B). Similarly, replacement of glucose with fructose or oleate and palmitate suppressed Puma expression. In contrast to MePyr, these nutrients also maintained phospho-Akt even in the absence of glucose.

Despite inhibiting Puma up-regulation, MePyr did not significantly rescue cell death of activated wild type T cells from glucose withdrawal (Fig. 5, C and D, and supplemental Fig. 7). In contrast, fructose or oleate and palmitate each readily suppressed T cell apoptosis upon glucose withdrawal (Fig. 5C). A key difference between MePyr and these treatments that may explain these distinct cell survival phenotypes in activated primary T cells was the ability of fructose and oleate and palmitate to maintain phospho-Akt. Because activated Akt suppressed Bim cytotoxicity (Fig. 2F), it was possible that failure of MePyr to maintain Akt activation in glucose-deprived cells allowed Bim-mediated apoptosis of glucose-deprived activated T cells despite reduced Puma expression. To test this, we examined the ability of MePyr to protect activated Bim- and Puma-deficient T cells from apoptosis upon glucose deprivation. Like wild type T cells, activated Puma−/− T cells were unaffected by the addition of MePyr upon glucose deprivation (Fig. 5E). Importantly, MePyr partially rescued the survival of glucose-deprived activated Bim−/− T cells (Fig. 5F). These data suggest that although MePyr can specifically inhibit Puma expression, both Puma and Bim play key roles in the apoptosis of glucose-deprived activated T cells.

**Glucose Metabolism Inhibits Puma Expression in T-ALL Cancer Cells**—Like activated T cells, T-ALL cells can be highly glycolytic (29, 30) and frequently have constitutively activated Akt. To determine whether glucose metabolism inhibits Puma expression in cancer cells, we withdrew glucose from three independent human T-ALL cell lines. Glucose deprivation of Jurkat cells did not affect phospho-Akt levels but did lead to a distinct induction of Puma and Bim (Fig. 6A). Addition of MePyr inhibited only Puma induction among the Bcl-2 family members tested (Fig. 6A and supplemental Fig. 8, A and B). Unlike activated primary T cells, Akt remained phosphorylated (Fig. 6A), and MePyr provided protection from cell death (Fig. 6B and supplemental Fig. 8, C and D). In addition, Bax activation (Fig. 6C) and caspase activation (Fig. 6D) were reduced by MePyr treatment. Rescue from apoptosis by MePyr was mitochondrially dependent, as cells underwent rapid cell death when sublethal doses of oligomycin were also provided with MePyr (Fig. 6E). Cell death upon glucose withdrawal was partially Puma-dependent, as Puma deficiency in Jurkat cells delayed cell death in the absence of glucose (Fig. 6F). Metabolic regulation of Puma may therefore contribute to survival of human cancer cells that have constitutively active Akt.

**Metabolic Control of Puma Transcriptional Induction and Protein Stability**—To test whether the metabolic checkpoint for control of Puma induction occurred at the transcriptional level, we examined metabolic regulation of a reporter construct consisting of 2 kb of the Puma promoter in Bcl-xL- or myrAkt-expressing FL5.12 cells (Fig. 7A). Upon IL-3 withdrawal in the presence of glucose, Puma promoter activity increased in both Bcl-xL- and myrAkt-expressing cells, although only modestly in myrAkt-expressing cells (Fig. 7A). In contrast, glucose deprivation enhanced Puma promoter activity in both Bcl-xL- and myrAkt-expressing cells equivalently, in a pattern similar to Puma protein expression (Fig. 2A). Interestingly, although MePyr reduced Puma protein levels (Fig. 3D), it did not significantly affect Puma promoter activity in either cell type. Consistent with this regulatory pattern for Puma transcription, Puma mRNA levels did not increase in myrAkt-expressing cells following IL-3 withdrawal but were strongly induced following glucose deprivation (Fig. 7B). Addition of MePyr, however, did not prevent the accumulation of Puma mRNA (Fig. 7B).

Transcriptional regulation of Puma in glucose deprivation may be mediated by p53, Foxo3a, and/or CHOP, each of which can induce Puma after a variety of cell stresses (41–44). CHOP responds to ER stress, and glucose withdrawal led to modest ER stress and CHOP induction that was not prevented by MePyr (supplemental Fig. 9). This ER stress was lower than that observed following treatment with the ER stress inducer tunicamycin that led to lesser induction of Puma than glucose withdrawal. Importantly, CHOP did not appear essential for transcriptional up-regulation of Puma upon glucose withdrawal, as shRNAi against CHOP had no effect of Puma protein or mRNA levels nor on survival of glucose-deprived cells (supplemental Fig. 10, A–C). Likewise, Foxo3a was not essential for Puma up-regulation or cell death upon glucose withdrawal, and instead it appeared to suppress Puma up-regulation (supplemental Fig. 10, D and E).

In contrast, activated T cells required p53 for maximal Puma induction upon glucose withdrawal, as p53−/− T cells showed only partial Puma mRNA and protein up-regulation (Fig. 7, C and D). This reduced level of Puma induction by glucose deprivation of activated p53−/− T cells could be further suppressed by treatment with MePyr, despite no rescue of Puma mRNA expression (Fig. 7, C and E). That p53 was important but not fully required for this metabolic checkpoint was confirmed in the p53-null T-ALL line Jurkat, in which re-expression of p53 promoted more rapid and greater accumulation of Puma protein upon glucose deprivation (Fig. 7F).

The ability of MePyr to suppress Puma protein but not mRNA upon glucose withdrawal of cells with activated Akt suggested regulation of Puma protein stability by MePyr may have also contributed to the metabolic suppression of Puma. To directly measure Puma protein half-life, myrAkt-expressing cells were IL-3- and glucose-deprived and treated with the protein synthesis inhibitor cycloheximide either in the presence or absence of MePyr. In the presence of glucose, insufficient Puma was detected to measure protein stability. Puma was readily detected and stable with ~70% remaining after 3 h of cycloheximide treatment and a half-life of over 4 h in the absence of glucose (Fig. 8). Addition of MePyr to glucose-deprived cells, however, greatly reduced Puma protein stability with less than 20% remaining after 3 h and a half-life of approximately 1 h. Therefore, Akt appears to promote cell survival by inhibiting Bim toxicity and stimulating glucose.
metabolism to control dual metabolic checkpoints that regulate Puma transcription and protein stability.

DISCUSSION

The PI3K/Akt pathway is commonly activated in activated T cells (45, 46) and in cancer cells (5) where it plays key roles to promote glucose metabolism and cell survival. Unlike anti-apoptotic Bcl-2 family proteins, however, it is now established that Akt cannot elicit its pro-survival functions when glucose is limiting (12, 38, 47). We have previously shown that Bcl-2 family proteins can be regulated by cell metabolism (18, 19, 48), and here we examined the potential role of Akt-driven glucose metabolism to control Bcl-2 family proteins. Importantly, Bim and Puma were both induced in glucose or growth factor withdrawal, but only Puma appeared to respond directly to mitochondrial metabolism. Thus, Akt appears to prevent cell death of growth factor-deprived cells through inhibition of Bim cytotoxicity and maintenance of glucose metabolism to suppress Puma up-regulation via metabolic checkpoints that control p53 activity and Puma protein stabilization.

The dependence of cells with activated Akt on glucose to suppress Puma induction was likely mediated through the role of glucose as a source of mitochondrial fuel. Akt can promote and maintain aerobic glycolysis while limiting alternative metabolic pathways, such as autophagy or lipid oxidation (31, 37). Cells expressing Bcl-xL, however, readily adapted and had only modest changes in mitochondrial metabolites when glucose-deprived. Importantly, this apparent addiction to glucose of cells with activated Akt was not absolute. Addition of exogenous alternative fuels to support mitochondrial metabolism, including pyruvate, long chain fatty acids, or fructose, could prevent Puma induction and apoptosis. In addition to possible direct regulation of cell death via hexokinase and glucose 6-phosphate (13, 49),
glucose and its immediate derivatives may not be uniquely required to prevent death of cells with activated Akt. Rather, activated Akt promotes dependence on glucose as a source of mitochondrial fuel that controls a metabolic checkpoint for Puma expression.

The mechanisms that control Puma expression remain uncertain. Transcription of Puma is increased partially through p53 activity, which may be activated by the AMP-activated protein kinase. Glucose deprivation and energy depletion have been shown to activate AMP-activated protein kinase, leading to p53 phosphorylation to promote cell cycle arrest (50), and AMP-activated protein kinase may also lead to apoptosis via this pathway with longer periods of glucose deprivation. The p53-independent transcriptional mechanism responsible for the remaining induction of Puma mRNA is not clear. Foxo3a and CHOP do not appear to be involved, but the p53-related transcription factor p73 has also been shown to regulate Puma (51) and may become activated upon glucose deprivation.

**FIGURE 7. Metabolism regulates p53-mediated Puma transcription.** A, Bcl-xL- and myrAkt-expressing FL5.12 cells were transfected with a Puma luciferase promoter-reporter construct, cultured in the presence or absence of IL-3 and/or glucose and MePyr as indicated for 10 h, and harvested to measure luciferase activity. B, Puma mRNA levels in myrAkt-expressing FL5.12 cells were measured by quantitative RT-PCR after 8 h of indicated IL-3, glucose, and MePyr treatments. Values represent the means ± S.D. of seven independent experiments. C–E, activated wild type and p53−/− primary T cells were cultured in IL-2 and in the presence or absence of glucose or MePyr for 12 h and assayed for Puma mRNA expression by quantitative RT-PCR analysis (C) and protein expression by immunoblot (D and E). Values in C represent the means ± S.D. of two independent experiments. F, Jurkat cells transfected with either control plasmid or wild type human p53 were cultured in the absence of glucose and harvested for immunoblot at the indicated times. MP, MePyr. Statistical significance is indicated with an asterisk, p < 0.05. N.S., not significant.

**FIGURE 8. Metabolic regulation of Puma protein stability.** myrAkt-expressing FL5.12 cells were cultured in the absence of both IL-3 and glucose with or without MePyr for 10 h at which time 25 μg/ml cycloheximide was added, and cells were harvested for protein analysis after additional indicated times. Puma protein levels were quantitated and normalized to actin to determine protein half-life (t1/2 = ln(2)/slope). Glu, glucose; MP, MePyr; CHX, cycloheximide. Values represented are means ± S.D. of three independent experiments. Statistical significance is indicated with an asterisk, p < 0.05.
Metabolic control of Puma expression was also mediated through regulation of Puma protein stability. The ability of diverse nutrients to control Puma protein expression suggests that mitochondrial metabolism may play a critical role to regulate Puma degradation. It remains unclear what proteolytic pathways or proteases may account for rapid Puma degradation in nutrient-rich conditions. Puma phosphorylation was recently described and such modification may impact proteolytic degradation (52). Ultimately, such nutrient regulation of proteolysis may provide an important mechanism to control Puma expression.

MePyr treatment rescued the survival of leukemic lymphocytes but not nontransformed activated lymphocytes when glucose was limiting, and this may have been due to distinct regulations of Akt itself. Events such as loss of PTEN (53) lead to aberrant and constitutive PI3K/Akt activation in cancer cells, whereas primary T cells did not maintain Akt activity upon glucose withdrawal. Differential Akt activity may impact Bcl-2 proteins to control apoptosis. The Bcl-2 family proteins Noxa, Bad, Bim, and Puma each contributed and set a threshold for apoptosis for glucose-deprived cells (16, 17), but only Bim and Puma were strongly induced and critical to initiate apoptosis. Although direct comparison of Bim and Puma expression is not possible, each was induced and similarly functional to promote apoptosis of control cells. If glucose was present, activated Akt could both suppress Bim-induced cell death and prevent induction of Puma. Akt has been reported to directly and indirectly lead to Bim phosphorylation (54, 55), and this may allow active Akt cells to inhibit Bim toxicity. Puma, however, was highly cytotoxic to cells regardless of Akt activity, and protection required suppression of Puma expression. MePyr may fail to protect glucose-deprived activated T cells despite inhibition of Puma expression because Akt becomes inactive and Bim is no longer inhibited. Cancer cells, in contrast, maintain Akt activity to inhibit Bim. Thus cancer cells may be particularly dependent on metabolic inhibition of Puma expression and sensitive to mechanisms that induce Puma.

This metabolic checkpoint for Puma expression appears to be an essential element of Akt-mediated cell survival and may play an important role to promote cell survival in cancer and immunity. Such a checkpoint may ensure that cells that fail to obtain sufficient nutrients undergo apoptosis rather than necrosis with its accompanying inflammation (56). Importantly, Puma induction was only partially p53-dependent. Thus, it may be possible to exploit metabolic regulation of Puma to promote apoptosis in a wide range of tumors. Consistent with this notion, pharmacological inhibitors of Akt have recently been shown to induce Puma and cell death in chronic lymphocytic leukemia cells irrespective of p53 status (57). This metabolic checkpoint for Puma expression may therefore play a key role in approaches to exploit cancer cell metabolism and elicit apoptosis.

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REFERENCES

1. Fox, C. J., Hammerman, P. S., and Thompson, C. B. (2005) Nat. Rev. Immunol. 5, 844–852
2. Frauwirth, K. A., Riley, J. L., Harris, M. H., Parry, R. V., Rathmell, J. C., Plas, D. R., Elstrom, R. L., June, C. H., and Thompson, C. B. (2002) Immunity 16, 769–777
3. Jacobs, S. R., Herman, C. E., Maciver, N. J., Wofford, J. A., Wiemer, H. L., Hammen, J. J., and Rathmell, J. C. (2008) J. Immunol. 180, 4476–4486
4. Warburg, O. (1956) Science 123, 309–314
5. Yuan, T. L., and Cantley, L. C. (2008) Oncogene 27, 5497–5510
6. Wiesman, H. L., Wofford, J. A., and Rathmell, J. C. (2007) Mol. Biol. Cell 18, 1437–1446
7. Deprez, J., Vertommen, D., Alesix, D. R., Hue, L., and Rider, M. H. (1997) J. Biol. Chem. 272, 17269–17275
8. Miyamoto, S., Murphy, A. N., and Brown, J. H. (2008) Cell Death Differ. 15, 521–529
9. Düvel, K., Yecies, J. L., Menon, S., Raman, P., Lipovský, A. I., Souza, A. L., Triantafellou, E., Ma, Q., Gorski, R., Cleaver, S., Vander Heiden, M. G., MacKeigan, J. P., Finan, P. M., Clisb, C. B., Murphy, L. O., and Manning, B. D. (2010) Mol. Cell 39, 171–183
10. Gutiérrez, A., Sarda, T., Greblianaute, R., Carracedo, A., Salmena, L., Ahi, Y., Dahlberg, S., Neuberg, D., Moreau, L. A., Winter, S. S., Larson, R., Zhang, J., Protopopov, A., Chiu, L., Pandolfi, P. P., Silverman, L. B., Hunger, S. P., Sallan, S. E., and Look, A. T. (2009) Blood 114, 647–650
11. Plas, D. R., and Thompson, C. B. (2002) Trends Endocrinol. Metab. 13, 75–78
12. Plas, D. R., Talapatra, S., Edinger, A. L., Rathmell, J. C., and Thompson, C. B. (2001) J. Biol. Chem. 276, 12041–12048
13. Gottlob, K., Majewski, N., Kennedy, S., Kandel, E., Robey, R. B., and Hay, N. (2001) Genes Dev. 15, 1406–1418
14. Majewski, N., Nogueira, V., Bhaskar, P., Coy, P. E., Seek, J. E., Gottlob, K., Chandel, N. S., Thompson, C. B., Robey, R. B., and Hay, N. (2004) Mol. Cell 16, 819–830
15. Pastorino, J. G., Shulga, N., and Hoek, J. B. (2002) J. Biol. Chem. 277, 7610–7618
16. Strasser, A. (2005) Nat. Rev. Immunol. 5, 189–200
17. Alves, N. L., Derks, I. A., Berk, E., Spijker, R., van Lier, R. A., and Eldering, E. (2006) Immunity 24, 703–716
18. Zhao, Y., Altman, B. J., Cololf, J. L., Herman, C. E., Jacobs, S. R., Wierman, H. L., Wofford, J. A., Dimascio, L. N., Ilkayeva, O., Kelekar, A., Reyna, T., and Rathmell, J. C. (2007) Mol. Cell. Biol. 27, 4328–4339
19. Zhao, Y., Cololf, J. L., Ferguson, E. C., Jacobs, S. R., Cui, K., and Rathmell, J. C. (2008) J. Biol. Chem. 283, 36344–36353
20. Pradelli, L. A., Bénéteau, M., Chauvin, C., Jacquin, M. A., Marchetti, S., Muñoz-Pinedo, C., Aubeger, P., Pende, M., and Ricci, J. E. (2010) Oncogene 29, 1641–1652
21. Danial, N. N., Gramm, C. F., Scorrono, L., Zhang, C. Y., Krauss, S., Ranger, A. M., Datta, S. R., Greenberg, M. E., Licklider, L. J., Lowell, B. B., Gigni, S. P., and Korsmeyer, S. J. (2003) Nature 424, 952–956
22. Chi, M. M., Pingsterhaus, J., Carayannopoulos, M., and Moley, K. H. (2000) J. Biol. Chem. 275, 40252–40257
23. Vander Heiden, M. G., Plas, D. R., Rathmell, J. C., Fox, J. C., Harris, M. H., and Thompson, C. B. (2001) Mol. Cell. Biol. 21, 5899–5912
24. Ciofani, M., and Zúñiga-Pflucker, J. C. (2005) Nat. Immunol. 6, 881–888
25. Rathmell, J. C., Vander Heiden, M. G., Harris, M. H., Frauwirth, K. A., and Thompson, C. B. (2000) Mol. Cell 6, 683–692
26. Cham, C. M., and Gajewski, T. F. (2005) J. Immunol. 174, 4670–4677
27. Cham, C. M., Driessen, G., O’Keefe, J. P., and Gajewski, T. F. (2008) Eur. J. Immunol. 38, 2438–2450
28. Pearce, E. L., Walsh, M. C., Cezar, P. J., Harms, G. M., Shen, H., Wang, L. S., Jones, R. G., and Choi, Y. (2009) Nature 460, 103–107
29. Barata, J. T., Silva, A., Brandao, J. G., Nadler, L. M., Cardoso, A. A., and Bousso, V. A. (2004) J. Exp. Med. 200, 659–669
30. Palomero, T., Dominguez, M., and Ferrando, A. A. (2008) Cell Cycle 7, 965–970
31. Altman, B. J., Wofford, J. A., Zhao, Y., Cololf, J. L., Ferguson, E. C., Wie-
Akt Prevents Apoptosis by Metabolic Inhibition of Puma

man, H. L., Day, A. E., Ilkayeva, O., and Rathmell, J. C. (2009) Mol. Biol. Cell 20, 1180–1191
32. Khatri, S., Yepiskoposyan, H., Gallo, C. A., Tandon, P., and Plas, D. R. (2010) J. Biol. Chem. 285, 15960–15965
33. Fox, C. J., Hammerman, P. S., Cinalli, R. M., Master, S. R., Chodos, L. A., and Thompson, C. B. (2003) Genes Dev. 17, 1841–1854
34. Nutt, L. K., Margolis, S. S., Jensen, M., Herman, C. E., Dunphy, W. G., Rathmell, J. C., and Kornbluth, S. (2005) Cell 123, 89–103
35. An, J., Muoio, D. M., Shiotani, M., Fujimoto, Y., Cline, G. W., Shulman, G. I., Koves, T. R., Stevens, R., Millington, D., and Newgard, C. B. (2004) Nat. Med. 10, 268–274
36. Ferrara, C. T., Wang, P., Neto, E. C., Stevens, R. D., Bain, J. R., Wenner, B. R., Ilkayeva, O. R., Keller, M. P., Blasiole, D. A., Kendziorski, C., Yandell, B. S., Newgard, C. B., and Attie, A. D. (2008) PLoS Genet. 4, e1000034
37. Deberardinis, R. J., Lum, J. J., and Thompson, C. B. (2006) J. Biol. Chem. 281, 37372–37380
38. Buzzai, M., Bauer, D. E., Jones, R. G., Deberardinis, R. J., Hatzivassiliou, G., Elstrom, R. L., and Thompson, C. B. (2005) Oncogene 24, 4165–4173
39. Erlacher, M., Labi, V., Manzl, C., Böck, G., Tzankov, A., Häcker, G., Michalak, E., Strasser, A., and Villunger, A. (2006) J. Exp. Med. 203, 2939–2951
40. Boise, L. H., Minn, A. J., Noel, P. J., June, C. H., Accavitti, M. A., Lindsten, T., and Thompson, C. B. (1995) Immunity 3, 87–98
41. Yu, J., Zhang, L., Hwang, P. M., Kinzler, K. W., and Vogelstein, B. (2001) Mol. Cell 7, 673–682
42. Nakano, K., and Vousden, K. H. (2001) Mol. Cell 7, 683–694
43. You, H., Pellegrini, M., Tsuichiha, K., Yamamoto, K., Hacker, G., Erlacher, M., Villunger, A., and Mak, T. W. (2006) J. Exp. Med. 203, 1657–1663
44. Ishihara, T., Hoshino, T., Namba, T., Tanaka, K., and Mizushima, T. (2007) Biochem. Biophys. Res. Commun. 356, 711–717
45. Jameson, S. C. (2002) Nat. Rev. Immunol. 2, 547–556
46. Plas, D. R., Rathmell, J. C., and Thompson, C. B. (2002) Nat. Immunol. 3, 515–521
47. Elstrom, R. L., Bauer, D. E., Buzzai, M., Karnauskas, R., Harris, M. H., Plas, D. R., Zhuang, H., Cinalli, R. M., Alavi, A., Rudin, C. M., and Thompson, C. B. (2004) Cancer Res. 64, 3892–3899
48. Mason, E. F., Zhao, Y., Goraksha-Hicks, P., Coloff, J. L., Gannon, H., Jones, S. N., and Rathmell, J. C. (2010) Cancer Res. 70, 8066–8076
49. Majewski, N., Nogueira, V., Robey, R. B., and Hay, N. (2004) Mol. Cell. Biol. 24, 730–740
50. Jones, R. G., Plas, D. R., Kubek, S., Buzzai, M., Mu, J., Xu, Y., Birnbaum, M. J., and Thompson, C. B. (2005) Mol. Cell 18, 283–293
51. Melino, G., Bernassola, F., Ranalli, M., Yee, K., Zong, W. X., Corazzari, M., Knight, R. A., Green, D. R., Thompson, C., and Vousden, K. H. (2004) J. Biol. Chem. 279, 8076–8083
52. Fricker, M., O’Prey, J., Tolkovsky, A. M., and Ryan, K. M. (2010) Cell Death Dis. e59; doi:10.1038/cddis.2010.38
53. Stambolic, V., Suzuki, A., de la Pompa, J. L., Brothers, G. M., Mirtsos, C., Sasaki, T., Ruland, J., Penninger, J. M., Siderovski, D. P., and Mak, T. W. (1998) Cell 95, 29–39
54. Qi, X. J., Wildey, G. M., and Howe, P. H. (2006) J. Biol. Chem. 281, 813–823
55. Biwas, S. C., and Greene, L. A. (2002) J. Biol. Chem. 277, 49511–49516
56. Sauter, B., Albert, M. L., Francisco, L., Larsson, M., Somersan, S., and Bhardwaj, N. (2000) J. Exp. Med. 191, 423–434
57. de Frias, M., Iglesias-Serret, D., Costall, A. M., Coll-Mulet, L., Santidrián, A. F., Gonzalez-Girones, D. M., de la Banda, E., Pons, G., and Gil, J. (2009) Haematologica 94, 1698–1707