Akt signal transduction induces coordinated increases in glycolysis and apoptosis resistance in a broad spectrum of cancers. Downstream of Akt, the FoxO transcription factors regulate apoptosis via Bim, but the contributions of FoxOs in regulating Akt-induced glycolysis are not well described. We find that FoxO3a knockdown is sufficient to induce apoptosis resistance in conjunction with elevated glycolysis. Glycolysis in FoxO3a-deficient cells was associated with increased S6K1 phosphorylation and was sensitive to rapamycin, an inhibitor of the mTORC1 pathway that has been linked to glycolysis regulation. We show that mTORC1-dependent glycolysis is increased in FoxO3a knockdown cells due to decreased expression of the TSC1 tumor suppressor that opposes mTORC1 activation. FoxO3a binds to and transactivates the TSC1 promoter, indicating a key role for FoxO3a in regulating TSC1 expression. Together, these data demonstrate that FoxO3a regulates glycolysis downstream of Akt through transcriptional control of Tsc1.

Bioenergetic regulation is emerging as a key target for cancer therapeutics. In many tumors, carcinogenesis is associated with apoptosis resistance that is fueled by glycolysis, rendering cancer cells vulnerable to interruptions in glucose metabolism (1–3). Oncogenic mutations frequently induce glucose-dependent survival through the Akt signal transduction pathway (3). The mechanisms that coordinate glycolysis and survival downstream of Akt may be important determinants of chemotherapeutic responses in cancer.

A key question is whether Akt coordinates metabolism and survival through separate effects on core components of the apoptotic and glycolytic machinery or whether these processes are coordinately regulated by shared elements of the Akt pathway. There is evidence for separate effects of Akt on core regulatory components of apoptosis versus glycolysis; Akt can phosphorylate and inhibit the proapoptotic BH3 protein Bad, and Akt has been shown to directly alter glucose uptake by promoting plasma membrane trafficking of Glut1 (4, 5).

Beyond direct effects of Akt on the core metabolic and apoptotic machinery, Akt may also coordinate metabolism and apoptosis by inhibiting downstream tumor suppressor proteins. Akt inhibits the tumor suppressor activity of the tuberous sclerosis complex proteins 1 and 2 (TSC1 and TSC2) through direct phosphorylation of TSC2 (6). The TSC1-TSC2 protein complex is a key regulator of signal transduction through mTOR complex 1 (mTORC1), an evolutionarily conserved kinase complex that integrates signals induced by cell-extrinsic nutrients and growth factors (6, 7). Decreased function of TSC1 and TSC2 is associated with activated mTORC1 and downstream kinases such as ribosomal protein S6 kinase 1 (S6K1) (6). Hypomorphic alleles of TSC1 or TSC2 cause both familial and sporadic tuberous sclerosis, a tumor growth syndrome that can affect the central nervous system, kidneys, and lungs (8). Although there is extensive evidence documenting tumor growth upon mutation of TSC1 or TSC2, little is known about transcriptional regulators of TSC1 or TSC2.

Although the TSC-mTORC1-S6K1 pathway appears to be critical for acute modulation of cellular metabolism in response to homeostatic and oncogenic signals, Akt can also mediate enduring alterations in cell metabolism and apoptosis through transcriptional effects. In mammalian systems, activated Akt regulates four related transcription factors of the Forkhead box subclass O family, designated FoxO1, FoxO3a, FoxO4, and FoxO6 (9–11). Akt phosphorylation of FoxOs inhibits their transcriptional activity by triggering nuclear exclusion and proteasomal degradation (12). In the unphosphorylated state, FoxOs are localized in the nucleus, where they regulate transcription of genes involved in cell cycle arrest, apoptosis, and stress responses (reviewed in Ref. 13). Activated FoxOs induce cell death by transcription of various proapoptotic genes such as Bim, Fasl, and 24p3 (14–17). FoxO1 can regulate circulating glucose homeostasis by promoting gluconeogenesis and repress expression of glycolytic enzymes in hepatocytes (18, 19). However, the mechanism of FoxO1 regulation of glycolytic enzymes, whether direct or through transcriptional intermediates, is not clear. These data suggest that chronic Akt activation downstream of oncogenes may establish durable increases in glycolysis and apoptosis resistance through inactivation of FoxO transcription factors.

Here we demonstrate that FoxO3a knockdown is sufficient to activate cellular glycolysis in conjunction with increased cell...
survival. Mechanistically, FoxO3a is required for transcriptional induction of TSC1, and FoxO3a deficiency triggers elevated S6K1 signaling. Rapamycin treatment prevents elevated glycolysis in cells lacking FoxO3a, demonstrating a critical role for mTORC1-S6K1 as positive mediators of glycolysis controlled by FoxO3a. Considering the importance of glycolysis in fueling tumor growth and survival, this finding suggests FoxO3a as an important determinant of tumor progression and chemotherapeutic response. These results advance the molecular mechanisms that can be exploited to restrain oncogenic glycolysis in cancers.

**EXPERIMENTAL PROCEDURES**

**Cell Lines and Constructs**—IL-3-dependent hematopoietic FL5.12 cells and immortalized murine embryonic fibroblasts were maintained as described (20). Cells were transduced with pKD-GFP retroviral constructs expressing a short hairpin targeting FoxO3a/FoxO1. In FL5.12 cells, this construct yielded consistent knockdown of both FoxO3a and FoxO1. The targeting sequence was: 5'-GTGCCCTACTTTCAAGGATAGG-3'. Where indicated, pKD-GFP was used as empty vector control. The silencing-resistant FoxO3a reexpression construct (srFoxO3a) was made by introducing silent mutations in the human FoxO3a cDNA to abrogate short hairpin RNA recognition: 5’-GGCGTTCCCTACTTTCAAGGACACAGAGG-3’. srFoxO3a was expressed from the MIT retroviral vector (21). Expression of exogenous FoxO3a was confirmed by flow cytometry and immunoblot. 75% bases of the TSC1 locus encompassing two FoxO-response elements were selected using 1 mg/ml G418.

**Quantitative Reverse Transcription-PCR**—RNA was extracted from 10 × 10^6 cells using a Qiagen RNeasy mini kit coupled with DNase I digestion. Gene-specific priming was employed on 1 μg of RNA to generate cDNA using the Invitrogen Thermoscript reverse transcription-PCR system. Finally, quantitative PCR was performed using Absolute Blue QPCR SYBR Green mix (Eppendorf MasterCycler). Primer sequences 5’ to 3’ are: gene-specific reverse transcriptase primers, actin, CAGGAGGCAATGATCTTG; FoxO3a, CGCTGTTGGCTGAAAGTGAGTGAGTCTTG; Tsc1, TGCTGCTGCACTGACTCAC. QPCR primers are: actin, CCTGTGCGATCCCATGAAACT, CACCAATGCCTTGTTACA; FoxO3a, GAACAG ACCAG-CACCTTCCTCT; TGAAGCAAGGCTTGGCAA; Tsc1, CAGGAGTTACAGACAAAGCTGG, AGCTTCTGAGAGCCTGCTG.

**Glycolysis**—To measure glycolytic rates, 1 × 10^6 cells were cultured in 500 μl of IL-3-free medium for a total of 3 h including 1 h of incubation at 37 °C with 5 μl of 5-[3H]glucose. The reaction was terminated using 500 μl of 0.2 N HCl. The rate of glycolysis is assessed by measuring the [3H]water released during the conversion of 2-phosphoglycerate to phosphoenolpyruvate in a protocol adapted from Ref. 24 as nmol of glucose converted/1 × 10^6 cells/h. Where indicated, rapamycin was added to cultures at a concentration of 10 nM for 48 h prior to assay.

** Luciferase Assay**—293 cells were seeded in 96-well luminescence-compatible plates at 5 × 10^4 cells/well in a final volume of 100 μl of medium. The next day, using Lipofectamine 2000, they were co-transfected with firefly luciferase reporter construct (0.23 μg/well), FoxO3a-expressing vector (0.23 μg/well), and CMV-Renilla luciferase vector (0.13 μg/well) as internal transfection control. 24 h after transfection, luciferase reporter activity was assayed using the Dual-Glo luciferase assay system and read on a Glomax 96 microplate luminometer (Promega).

**Chromatin Immunoprecipitation**—100 × 10^6 HA-FoxO3a-expressing cells starved of IL-3 for 3 h were cross-linked in 1% formaldehyde and quenched in 125 mM glycine followed by washing two times in chilled phosphate-buffered saline. Cells were then lysed (1% SDS, 10 mM EDTA, 50 mM Tris, pH 8.0, and protease inhibitors) and sonicated for 12.5 min with 30-s pulses and 30-s idle time. Sheared chromatin was preclreated using protein A/G Plus-agarose and calf thymus DNA at 4 °C for 1 h. Preclreated chromatin was diluted 5-fold in dilution buffer (0.01% SDS, 1.1% Triton X-100, 1.2 mM EDTA, 16.7 mM Tris-HCl, pH 8.1, and 167 mM NaCl), and a portion of preclreated chromatin was saved as input. Immunoprecipitating antibodies were anti-RNA polymerase II (Millipore 05623), anti-HA (Cell Signaling 2367), or anti-iG (Jackson ImmunoResearch Laboratories 015-000-002). Immune complexes were collected on protein A/G Plus-agarose and washed in chilled low salt buffer (0.1% SDS, 1% Triton X-100, 2 mM EDTA, 20 mM Tris-HCl, pH 8.1, 150 mM NaCl), high

**Flow Cytometry**—To test viability ± FoxO3a, cells were washed in RPMI to deplete any residual IL-3 and then plated in complete medium lacking IL-3 (23). Survival was determined using propidium iodide exclusion in a flow cytometer. In some experiments, the Thy.1 expression marker was detected using anti-Thy.1 (BD Pharmingen 551401).

**Immunoblotting**—Cells were pelleted, washed in phosphate-buffered saline, and lysed in radioimmune precipitation buffer (150 mM NaCl, 1% Nonidet P-40, 0.5% sodium deoxycholate, 0.1% SDS, 50 mM Tris, pH 8.0) supplemented with protease and phosphatase inhibitors. After sonication, cell lysates were standardized for protein content prior to electrophoresis and transfer to nitrocellulose. The following antibodies were from Cell Signaling Technologies: FoxO3a (2497S), Tsc1 (4906), Tsc2 (3612), phospho-S6K1Thr389 (9234L), S6K1 (9021L), phospho-AktSer473 (4058L), Akt (9274S), HA-6E2 (2367), and phospho-S6Ser240/244 (4838). The anti-human FoxO1 antibody was obtained from Bethyl (A300-297A). The S6 antibody was a kind gift from Dr. George Thomas, and glyceraldehyde-3-phosphate dehydrogenase antibody was from Abcam (ab8245-100). Adjustments to brightness, contrast, levels, scale, and cropping were performed using Adobe Photoshop and Illustrator. Densitometry calculations were performed using ImageJ (National Institutes of Health).
FoxO3a Regulates TSC1

RESULTS AND DISCUSSION

FoxO3a Knockdown Triggers Elevated Glycolysis and Survival—To determine the function of FoxO3a in coordinately regulating glycolytic metabolism, we transduced IL-3-dependent murine hematopoietic FL5.12 cells with a retroviral short hairpin RNA that targets a conserved sequence in murine FoxO3a. At early time points of growth factor withdrawal prior to apoptosis commitment, FoxO3a-deficient cells sustained active glycolysis when compared with vector control cells (Fig. 1A). FoxO3a knockdown induced glycolysis in conjunction with increased apoptosis resistance (Fig. 1B). Restoring FoxO3a expression by overexpressing a silencing-resistant cDNA for FoxO3a suppressed growth factor-independent glycolysis and survival (Fig. 1, A and B). Interestingly, overexpression of FoxO3a was sufficient to accelerate the kinetics of apoptosis at early time points following growth factor withdrawal (Fig. 1B).

Recent evidence suggests that activation of mTORC1 enhances the capacity of the cell for glycolytic metabolism (26). Interestingly, FoxO3a knockdown cells show evidence of increased mTORC1 activity as indicated by the phosphorylation of ribosomal protein S6, which is phosphorylated by S6K1 in response to mTORC1 signaling (Fig. 1C). This suggests a novel form of cross-talk between the FoxO transcription factors and mTORC1 in glycolysis control.

FoxO3a Regulates Glycolysis through mTORC1—To test whether augmented mTORC1-S6K1 signaling is required for elevated glycolysis in FoxO3a knockdown cells, we measured the effect of rapamycin on the rate of glycolysis. Rapamycin inhibits the activation of mTORC1 and suppresses phosphorylation of downstream signaling targets such as ribosomal protein S6. Elevated glycolysis in FoxO3a knockdown cells was largely suppressed by treatment with rapamycin, indicating an important role for mTORC1 in mediating elevated glycolysis (Fig. 2). This result is consistent with a recent report describing a role for mTORC1 signaling downstream of Akt in mediating glycolysis (26).

Previous studies in Drosophila have demonstrated a role for dFOXO in regulating growth factor receptor signaling through transcriptional control of the insulin receptor and 4EBP1 homologues (27, 28). From an analysis of the conserved promoter regions from sequenced mammalian genomes (29), we identified potential FoxO binding sites in the promoter of TSC1. Immunoblot analysis confirmed decreased TSC1 protein in FoxO3a knockdown cells (Fig. 3A). Decreased TSC1 expression correlated with decreased TSC2 levels, with concomitant increases in phosphorylation of S6K1 and S6. Decreased TSC2 protein is likely due to degradation upon failure to bind to TSC1, a required chaperone that preserves TSC2 stability. Importantly, reexpression of silencing-resistant FoxO3a was sufficient to restore TSC1-TSC2 expression and reduce the phosphorylation of S6K1 and S6 to levels comparable with control cells. Similarly, combined knockdown of FoxO3a and FoxO1 in murine embryonic fibroblasts and the human keratinocyte cell line (HaCat) correlated with decreased TSC1 expression (Fig. 3, B and C). These results are in agreement with a previous analysis that indicated functional redun-

salt buffer (as before, containing 500 mM NaCl), and LiCl wash buffer (0.25 mM LiCl, 1% Nonidet P-40, 1% deoxycholate, 1 mM EDTA, 10 mM Tris-HCl, pH 8.1), and washed two times in 10 mM Tris/5 mM EDTA. DNA was extracted from beads twice using 100 μl of elution buffer (1% SDS and 0.1 mM NaHCO3) and supplemented with 0.25 mM NaCl. After an overnight 65 °C incubation to reverse cross-links, samples were incubated for an additional hour at 65 °C with 10 μM EDTA, 40 μM Tris (pH 6.8), and 2 μg of proteinase K. DNA was purified using the QIAquick PCR purification kit for PCR. In some cases, qPCR chromatin immunoprecipitation (ChIP) results were calculated as described, introducing a cycle C value of 40 for reactions that did not produce product based on melting curve analysis (25). Primers are: BIM, 5'-GGGC-AGTG-3' and Tsc1 Set 1, 5'-GGGGTACATTCTGAGT-3'; Tsc1 Set 2, 5'-TCGAGACTCGTCACGCCA-3'; and Tsc1 Set 2, 5'-TCGAGACTCGTCACGCCA-3', 5'-CCG-TACCTACCCACCGTCTCC-3'.
dancy between FoxO3a and FoxO1 in the regulation of transcriptional targets and suggest a possible role for FoxO1 in regulating TSC1 expression (30). In FoxO3a knockdown FL5.12 cells, loss of FoxO3a expression correlated with decreased expression of endogenous TSC1 mRNA, and FoxO3a reexpression triggered a recovery in endogenous TSC1 mRNA (Fig. 4). Together, these data indicate a critical role for FoxO3a in expression of TSC1.

**FoxO3a DNA Binding Is Required to Transcribe Tsc1**—We identified two potential FREs in the TSC1 locus that conform to published FoxO binding sequences (31). To determine whether FoxO3a may bind to these sequences, we employed ChIP to test the binding of HA-tagged FoxO3a at the TSC1 locus in chromatin, using the Bim locus as a positive control (14). Epitope-tagged FoxO3a was employed for the assay to allow the use of ChIP-certified reagents for these experiments. Both the Bim and the TSC1 loci were detected in the FoxO3a and RNA polymerase II immunoprecipitations but not in the isotype control immunoprecipitation (Fig. 5A). To further characterize FoxO3a binding to the TSC1 locus, we expressed a constitutively active form of FoxO3a in which three Akt phosphorylation sites have been mutated to alanine (FoxO3a-TM for triple mutant) (9). As a control for DNA binding, we also expressed a form of FoxO3a-TM lacking a DNA binding domain (FoxO3a-TM ΔDBD) (Fig. 5B) (9). In addition to FoxO3a, we also found that FoxO1 can associate with the TSC1 locus (supplemental Fig. 1). Chromatin immunoprecipitation experiments showed that efficient FoxO3a association with the TSC1 locus requires the DNA binding domain, supporting the specificity of the ChIP results and indicating a role for direct DNA binding by FoxO3a. FoxO3a binding to the TSC1 promoter strongly suggests that FoxO3a can regulate transcription from the TSC1 promoter.

To directly test transcriptional regulation at the TSC1 locus, we tested FoxO3a transcriptional control of a 755-bp fragment from the TSC1 locus, containing both FREs, in a luciferase reporter construct in 293 cells. Constitutively active/nuclear FoxO3a-TM increased transcription ∼4-fold from the TSC1 reporter construct, similar to its effects on the positive control construct derived from the Fas ligand (FasL) promoter (Fig. 6) (9). Importantly, FoxO3a-TM ΔDBD was incapable of increasing transcription from either the TSC1 reporter or the FasL promoter, although expression levels were similar to FoxO3a-TM (Fig. 6). To test the specificity of FoxO3a-TM for the FREs in the TSC1 reporter, we measured luciferase production from a TSC1 reporter containing three altered nucleotides in the FREs (TSC1 FREmut). FoxO3a was unable to mediate increased transcription from this construct, indicating that the effects of FoxO3a on the TSC1 promoter are mediated through the FRE sites. Together, these data demonstrate that FoxO3a binds FREs to induce TSC1 transcription.

Altogether, regulation of endogenous TSC1 mRNA, DNA binding domain-dependent physical binding of the TSC1 promoter, and transcriptional regulation of the TSC1 reporter construct strongly supports a role for FoxO3a in regulating TSC1 expression. Nevertheless, the data do not rule out the possibility that FoxO3a also regulates TSC1 and/or TSC2 expression through parallel effects beyond direct mechanisms of transcriptional regulation.

**Restoration of TSC1 in FoxO3a-deficient Cells Suppresses Glycolysis**—To verify FoxO3a regulation of glycolysis via control of tumor suppressor TSC1, we expressed human TSC1...
using a retroviral construct in vector control and FoxO3a knockdown cells. Expression of TSC1 was confirmed by immunoblot analysis and correlated with increased Tsc2 expression (Fig. 7A). TSC1 reexpression in FoxO3a-deficient cells was sufficient to restore basal phosphorylation of the ribosomal protein S6, a downstream substrate of mTORC1 and S6K1. This indicates that FoxO3a regulation of mTORC1 activity is mediated through control of Tsc1 protein expression.

Tsc1 reexpression triggered a decline in glycolysis, restoring the ability of cells to down-regulate glycolysis upon growth factor withdrawal (Fig. 7). These results are in line with the results in Fig. 1, confirming Tsc1 and mTORC1 as effectors regulating glycolysis downstream of FoxO3a.

Together, the data reveal dual control exerted by Akt over TSC tumor suppressor function. Previous analysis has shown direct phosphorylation and proteasomal degradation of TSC2, establishing a mechanism for acute regulation of the pathways by Akt. However, in tumors with sustained Akt signaling due to activation by upstream oncogenes, inactivation of FoxO3a can prevent Tsc1 transcription, resulting in chronic destabilization of Tsc2 due to unrestricted interaction with the E3 ubiquitin ligase HERC1 (32).

Interestingly, expression array analysis of large numbers of tumors has revealed decreased TSC1 expression in a number of tumor types characterized by frequent activation of Akt, including glioblastoma and melanoma (supplemental Table 1). Furthermore, TSC1 heterozygous mice share a common phenotype with FoxO-deficient mice. Tsc1 heterozygous mice develop liver hemangiomas, much like mice that develop uterine and liver hemangiomas upon somatic recombination of the FoxO1, FoxO3a, and FoxO4 loci (30, 33). The frequency of reduced TSC1 expression in cancer and the similarities in phenotype of TSC1+/− and FoxO-deficient mice suggest that FoxO inactivation may contribute to decreased TSC1 expression and increased mTORC1 signaling in cancer.

TSC1 and TSC2 mutations are detected in 80% of patients exhibiting symptoms of tuberous sclerosis (TS) (34). Disease severity in TS can vary greatly, and it is not clear whether disease severity is tied solely to the nature of the etiologic mutations. Our results suggest that alterations in FoxO activation may regulate TSC1 expression in TS patients, which in turn may influence the onset or severity of symptoms. Investigation of these possibilities is ongoing.

Recent results have revealed the therapeutic potential of drugs that can alter FoxO3a stability and activity, such as histone deacetylase inhibitors and AMP-activated protein kinase agonists (35–37). We propose that the therapeutic response to these drugs will depend, in part, on their ability to enhance

![Figure 6](image1.png)

**FIGURE 6.** FoxO3a regulates transcription from the TSC1 locus. A, luciferase activity was determined in 293 cells transfected with a TSC1 promoter construct containing FoxO response elements (TSC1 FRE), a TSC1 promoter construct lacking the FREs (TSC1 FREmut), or the Fas ligand (FasL) promoter as a positive control. FoxO3a-TM triggered ~4-fold induction of the TSC1 promoter but not the TSC1 FREmut construct. FoxO3a-TM-ΔBD did not activate the TSC1 promoter, indicating a requirement for DNA binding activity in FoxO3a-activated TSC1 transcription. Data are normalized to a CMV-Renilla luciferase transfection control. Error bars indicate S.D. B, expression control for 293 cells transfected with constitutively active FoxO3a (FoxO3a-TM) and activated FoxO3a lacking the DNA binding domain (FoxO3a-ΔBD). Vec., vector control. GAPDH, glyceraldehyde-3-phosphate dehydrogenase.

![Figure 7](image2.png)

**FIGURE 7.** Tsc1 restoration suppresses glycolysis in FoxO3a-deficient cells. A, TSC1 reexpression in FoxO3a-deficient cells suppresses phospho-S6 (pS6) in growth factor-deprived cells. The same phospho-S6 blot is shown in short and long exposures. Densitometry calculations are based on the phospho-S6 long exposure normalized to the total S6 immunoblot. TSC1 o/ex, TSC1 overexpression; shFoxO3a, retroviral vectors expressing FoxO3a short hairpin RNA; GAPDH, glyceraldehyde-3-phosphate dehydrogenase. B, vector control and shFoxO3a cells + Tsc1 were cultured in the absence of growth factor for 2 h prior to glycolysis measurement. Restoration of Tsc1 expression was sufficient to suppress glycolysis in cells lacking Tsc1. Similar results were obtained in three experiments. Error bars indicate S.E.
FoxO3a activity in repressing glycolysis downstream of the TSC1-mTORC1-S6K1 pathway.

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REFERENCES

1. Kaplan, O., Navon, G., Lyon, R. C., Faustino, P. J., Straka, E. J., and Cohen, J. S. (1990) Cancer Res. 50, 544–551
2. Cay, O., Radnell, M., Jeppsson, B., Åhrén, B., and Bengmark, S. (1992) Cancer Res. 52, 5794–5796
3. Elstrom, R. L., Bauer, D. E., Buzzai, M., Karnaukas, 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
4. Datta, S. R., Dudek, H., Tao, X., Masters, S., Fu, H., Gotoh, Y., and Greenberg, M. E. (1997) Cell 91, 231–241
5. Wieman, H. L., Wolford, J. A., and Rathmell, J. C. (2007) Mol. Biol. Cell 18, 1437–1446
6. Inoki, K., Li, Y., Zhu, T., Wu, J., and Guan, K. L. (2002) Nat. Cell Biol. 4, 648–657
7. Nobukuni, T., Joaquin, M., Roccio, M., Dann, S. G., Kim, S. Y., Gulati, P., Byfield, M. P., Backer, J. M., Natt, F., Bos, J. L., Zwartkruis, F. J., and Thomas, G. (2005) Proc. Natl. Acad. Sci. U.S.A. 102, 14238–14243
8. Kriwacki, D. J., and Manning, B. D. (2005) Hum. Mol. Genet. 14, R251–R258
9. Brunet, A., Bonni, A., Zigmond, M. J., Lin, M. Z., Joo, P., Hu, L. S., Anderson, M. J., Arden, K. C., Blenis, J., and Greenberg, M. E. (1999) Cell 96, 857–868
10. Tang, E. D., Nuñez, G., Carr, F. G., and Guan, K. L. (1999) J. Biol. Chem. 274, 16741–16746
11. Plas, D. R., and Thompson, C. B. (2003) J. Biol. Chem. 278, 12361–12366
12. Biggs, W. H., 3rd, Meisenhelder, J., Hunter, T., Cavenee, W. K., and Arden, K. C. (1999) Proc. Natl. Acad. Sci. U.S.A. 96, 7421–7426
13. Salih, D. A., and Brunet, A. (2008) Curr. Opin. Cell Biol. 20, 126–136
14. Gilley, J., Coffin, P. J., and Ham, J. (2003) J. Cell Biol. 162, 613–622
15. You, H., Pellegrini, M., Tsuchihiara, K., Yamamoto, K., Hacker, G., Ehrlicher, M., Villunger, A., and Mak, T. W. (2006) J. Exp. Med. 203, 1657–1667
16. Park, S., Guo, J., Kim, D., and Cheng, J. Q. (2009) J. Biol. Chem. 284, 2187–2193
17. Devireddy, L. R., Teodora, J. G., Richard, F. A., and Green, M. R. (2001) Science 293, 829–834
18. Altmont, J., Richter, A., Harbaran, S., Suriawinata, J., Nakae, J., Thung, S. N., Meseck, M., Accili, D., and Dong, H. (2003) Am. J. Physiol. Endocrinol. Metab. 285, E718–E728
19. Zhang, W., Patil, S., Chauhan, B., Guo, S., Powell, D. R., Le, J., Klotzas, A., Matika, R., Xiao, X., Franks, R., Heidenreich, K. A., Sajan, M. P., Farese, R. V., Stoltz, D. B., Tso, P., Koo, S. H., Montminy, M., and Unterman, T. G. (2006) J. Biol. Chem. 281, 10105–10117
20. Vander Heiden, M. G., Chandel, N. S., Williamson, E. K., Schumacker, P. T., and Thompson, C. B. (1997) Cell 91, 627–637
21. Mitchell, T. C., Hildeman, D., Kedl, R. M., Teague, T. K., Schafer, B. C., White, J., Zhu, Y., Kappler, J., and Marrack, P. (2001) Nat. Immunol. 2, 397–402
22. Pymar, L. S., Platt, F. M., Askham, J. M., Morrison, E. E., and Knowles, M. A. (2008) Hum. Mol. Genet. 17, 2006–2017
23. Plas, D. R., Talapatra, S., Edinger, A. L., Rathmell, J. C., and Thompson, C. B. (2001) J. Biol. Chem. 276, 12041–12048
24. Ashcroft, S. J., Weerasinha, L. C., Basset, J. M., and Randle, P. J. (1972) Biochem. J. 126, 525–532
25. Chakrabarti, S. K., James, J. C., and Mirmira, R. G. (2002) J. Biol. Chem. 277, 13286–13293
26. Bhaskar, P. T., Nogueira, V., Patra, K. C., Jeon, S. M., Park, Y., Robey, R. B., and Hay, N. (2009) Mol. Cell Biol. 29, 5136–5147
27. Puig, O., Marr, M. T., Ruhf, M. L., and Tjian, R. (2003) Genes Dev. 17, 2006–2020
28. Jünger, M. A., Rintelen, F., Stocker, H., Wasserman, J. D., Végh, M., Radimerski, T., Greenberg, M. E., and Hafen, E. (2003) J. Biol. 2, 20
29. Xie, X., Mikkelsen, T. S., Gnirke, A., Lindblad-Toh, K., Kellis, M., and Lander, E. S. (2007) Proc. Natl. Acad. Sci. U.S.A. 104, 7145–7150
30. Paik, J. H., Kollipara, R., Chu, G., Ji, H., Xiao, Y., Ding, Z., Miao, L., Tothova, Z., Horner, J. W., Carrasco, D. R., Jiang, S., Gilliland, D. G., Chin, L., Wong, W. H., Castrillon, D. H., and DePinho, R. A. (2007) Cell 128, 309–323
31. Barthel, A., Schnoll, D., and Unterman, T. G. (2005) Trends Endocrinol. Metab. 16, 183–189
32. Chong-Kopera, H., Inoki, K., Li, Y., Zhu, T., Garcia-Gonzalo, F. R., Rosa, J. L., and Guan, K. L. (2006) J. Biol. Chem. 281, 8313–8316
33. Kriwacki, D. J., Zhang, H., Bandura, J. L., Heiberger, K. M., Glogauer, M., el-Hashemite, N., and Onda, H. (2002) Hum. Mol. Genet. 11, 525–534
34. Crino, P. B., Nathanson, K. L., and Henske, E. P. (2006) N. Engl. J. Med. 355, 1345–1356
35. Göttlicher, M., Minucci, S., Zhu, P., Krämer, O. H., Schimpf, A., Giavara, S., Sleeman, J. P., Lo Coco, F., Nervi, C., Pellicci, P. G., and Heintzel, T. (2001) EMBO J. 20, 6969–6978
36. Atadja, P., Hsu, M., Kwon, P., Trogani, N., Bhalla, K., and Remisiewski, S. (2004) Novartis Found. Symp. 259, 249–266; discussion 266–268, 285–288
37. Greer, E. L., Oskoui, P. R., Banko, M. R., Maniar, J. M., Gygi, M. P., Gygi, S. P., and Brunet, A. (2007) J. Biol. Chem. 282, 30107–30119