Emerging function of mTORC2 as a core regulator in glioblastoma: metabolic reprogramming and drug resistance

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ABSTRACT Glioblastoma (GBM) is one of the most lethal human cancers. Genomic analyses define the molecular architecture of GBM and highlight a central function for mechanistic target of rapamycin (mTOR) signaling. mTOR kinase exists in two multi-protein complexes, namely, mTORC1 and mTORC2. These complexes differ in terms of function, regulation and rapamycin sensitivity. mTORC1 is well established as a cancer drug target, whereas the functions of mTORC2 in cancer, including GBM, remains poorly understood. This study reviews the recent findings that demonstrate a central function of mTORC2 in regulating tumor growth, metabolic reprogramming, and targeted therapy resistance in GBM, which makes mTORC2 as a critical GBM drug target.

KEYWORDS Glioblastoma; mTOR; metabolic reprogramming; mTORC2; Warburg effect; PI3K

Introduction

Glioblastoma (GBM) is the most common malignant primary brain cancer of adults, accounting for more than 45% of malignant primary brain and CNS tumors. GBM is also one of the most lethal cancers, with a median survival of only 15 months for patients despite combined treatment, including surgery, radiotherapy, and chemotherapy.

GBM was one of the first cancer types profiled by The Cancer Genome Atlas project and is now one of the most genomically well-characterized forms of human cancer for which a central function of dysregulated growth factor receptor signaling has been demonstrated. Receptor tyrosine kinase gene amplification and mutations, PI3K catalytic and regulatory subunit genetic mutations, and PTEN gene deletion and mutation all result in constitutive PI3K pathway activation in the majority of GBMs, thus rendering the downstream effect or mechanistic target of rapamycin (mTOR) a compelling GBM drug target.

mTOR is a serine threonine kinase that exists in two distinct complexes, mTOR complexes I and II (mTORC1 and mTORC2), which differ in terms of their regulation, function, and responsiveness to the allosteric inhibitor, rapamycin. mTORC1, which contains mTOR kinase in complex with six known components including Raptor, links upstream growth factor receptor signaling to downstream protein translation and cell proliferation through PI3K. mTORC1 integrates growth factor receptor signaling into amino acid and energy status to ensure sufficient nutrients and ATP to enable tumor proliferation and cell growth. mTORC1 also regulates protein degradation, ribosome biogenesis, glucose, lipid and nucleotide metabolism, as well as autophagy. In contrast to that of mTORC1, the function of mTORC2 remains poorly understood. This study reviews mTOR signaling in GBM with a focus on the newly identified central function of mTORC2 in GBM pathogenesis.
localization, regulation, function, and sensitivity to the allosteric inhibitor, rapamycin. Structurally, both share four common components: mTOR, mLST8, Deptor, and Tti1/Tel2. In addition to the common components, mTORC1 contains two other proteins PRAS40 and Raptor, whereas mTORC2 has three other proteins, Rictor, mSIN1, and Protor-1.

mTORC1 signaling is activated in response to the growth factor receptor signaling and nutrient availability through complementary and independent mechanisms. This complex is activated in the majority of adult GBMs, partly as a consequence of growth factor receptor signaling through the PI3K-AKT pathway. TSC1/2 is the key regulator of mTORC1 and inhibits mTORC1 signaling by converting the mTORC1 activator Rheb to its inactive state. Growth factors stimulate PI3K/AKT activity, thereby inhibiting the tumor suppressors TSC1/2, thus resulting in mTORC1 activation. AKT also activates mTORC1 by phosphorylating PRAS40 and dissociating PRAS40 from mTORC1. mTORC1 signaling is regulated by the nutrient and energy levels. Amino acids act independently of TSC1/2 to activate mTORC1 through Rag GTases by promoting the translocation of mTORC1 to the lysosomal surface. The energy sensor, AMPK, also regulates mTORC1 by phosphorylating Raptor to inhibit mTORC1 activity.

Phosphatidic acid is required for the stability and activity of mTORC1. Thus, mTORC1 integrates growth factors, energy status, and amino acids to regulate numerous cellular processes, including protein translation, lipid synthesis, energy metabolism, lysosome biogenesis, and autophagy to promote tumor proliferation and cell growth. mTORC1 promotes protein translation by phosphorylating S6K1 and 4E-BP1, as well as controls proteasome-mediated protein degradation through an NRF1-dependent mechanism, thus balancing cellular protein synthesis and degradation. More importantly, mTORC1 is a key negative regulator of PI3K signaling that serves as a homeostatic rheostat. However, mTORC1, as a negative feedback loop, inhibits PI3K signaling by directly or S6K1-dependently phosphorylating IRS1 and promoting IRS1 degradation.

In contrast to those of mTORC1, mTORC2 regulation and function, particularly in cancer, remain poorly understood. A recent work suggests that growth factor signaling through PI3K promotes mTORC2-ribosome binding to stimulate TORC2 kinase activity. In GBM, EGFRvIII and PTEN loss, both of which can potentially promote PI3K signaling, also stimulate mTORC2 kinase. PI3K-independent mechanisms of mTORC2 activation, including WNT-LRP5 signaling through the small GTPase, RAC1, during osteoblast differentiation, miR-29 regulation of YAP and Hippo pathway activation, and Notch signaling, have recently been described.

mTORC1 and mTORC2 are reciprocally regulated. Downstream of mTORC1, S6K1 inhibits mTORC2-dependent phosphorylation of AKT by phosphorylating Rictor on Thr1135 and mSIN1 on Thr86 and Thr398. Until recently, the majority of mTORC2 activity was believed to be mediated by its phosphorylation of AKT on Ser473, as well as by controlling the folding and stability of AKT protein, thus promoting maximal AKT signaling. Accordingly, mTORC2 can promote mTORC1 signaling in an AKT-dependent manner. However, mTORC2 activates the additional members of the AGC subfamily of kinases, including SGK1 and PKC-α, to regulate cell survival, metabolism, and cytoskeletal organization. Thus, a Drosophila model of EGFR-P13K-driven gliomas identified mTORC2 as a requirement for tumor formation, independent of AKT and mTORC1. mTORC2 promotes GBM growth, survival, and chemotherapy resistance through SGK1-dependent and NF-kB-dependent signaling. Therefore, both mTORC1 and mTORC2 should be inhibited to prevent GBM tumor growth.

The mTOR inhibitor rapamycin interacts with the FKBP12 protein and then binds to the FRB domain in mTOR to inhibit its kinase activity allosterically. mTORC1 is considered to be highly rapamycin sensitive, whereas mTORC2 is less sensitive, depending on the cell context. In GBM cells, rapamycin can strongly inhibit mTORC1 in the nanomolar range, but mTORC2 can remain insensitive to it, even at relatively high doses. Notably, rapamycin and its analogs may have limited activity against some mTORC1 effectors, including the limited capability to suppress 4E-BP1 phosphorylation in GBM cells. Therefore, the failure of rapamycin (sirolimus) and its analog CCI-779 (temsirolimus) to suppress mTORC2 signaling, as well as 4E-BP1 phosphorylation in GBM patients, may contribute to clinical resistance.

mTORC2 reprograms metabolism

Metabolic reprogramming is a central hallmark of cancer. Cancer cells need to reprogram their core cellular metabolism to generate sufficient ATP, adequate macromolecules, and appropriate cellular redox status for rapid cell growth, proliferation, and survival. Recent studies suggest that mTORC2 has a central function in metabolic reprogramming, thus contributing to GBM growth and drug resistance. mTORC2 appears to control the metabolism of cancer cells in at least three ways: modulating nutrition (glucose, lipid, amino acid) import, regulating the activity or expression of specific metabolic enzymes, and rewiring of metabolic networks (Figure 1).
Glycolytic metabolism

Cancer cells increase glucose uptake to meet the increased energetic and biosynthetic demands imposed by rapid tumor growth. Even in the presence of sufficient oxygen to support oxidative phosphorylation, tumor cells convert the majority of glucose into lactate. This biochemical adaptation, called “the Warburg effect”, is considered the classic metabolic phenotype of cancer, although it can also be utilized by non-neoplastic, rapidly proliferating cells.

The Warburg effect enables the rapidly proliferating cells to utilize the glucose-derived carbons for lipid, ribose, glycerol, serine, and glycine synthesis. Thus, such effect is an efficient method by which cells use glucose for anabolic metabolism while still obtaining sufficient ATP. However, the cells need to take up more glucose for such high cost demands because only two molecules of ATP are yielded per molecule of glucose. Recent studies also demonstrate that mTORC2 serves a central function in the Warburg effect.

First, mTORC2 regulates glycolytic metabolism through its activation of AKT. This complex directly phosphorylates AKT on Ser473 to ensure its maximal activity, thus stimulating the expression of the glucose transporter GLUT4 and activating two key glycolytic enzymes, hexokinase 2 (HK2) and phosphofructokinase-1 (PFK-1); such condition increases glucose uptake and glycolysis.

Consistent with this notion, hepatic Rictor knockout mice showed constitutive gluconeogenesis and impaired glycolysis attributed to the loss of AKT Ser473 phosphorylation and reduced glucokinase, which suggests a function of mTORC2 in glycolytic metabolism in normal and cancer cells.

Second, in GBM, mTORC2 promotes the Warburg effect, independent of AKT, by regulating c-Myc levels. A signaling cascade whereby mTORC2 controls the cellular level of c-Myc by inactivating the acetylation of FoxO1 and FoxO3 has recently been described.
been identified. The inactivation of FoxOs releases c-Myc from a suppressive miR-34c-dependent network, which targets the 3’-UTR of c-Myc mRNA and inhibits its translation. C-Myc consequently increases the expression of the key regulatory genes controlling glucose transport and glycolysis. These genes include GLUT1, HK2, pyruvate kinase M2 isoform (PKM2), lactate dehydrogenase A (LDHA), and pyruvate dehydrogenase kinase isozyme 1 (PDK1), which inhibits pyruvate dehydrogenase (PDH). Thus, c-Myc controls the expression of a repertoire of genes promoting the Warburg effect downstream of mTORC2.

Remarkably, mTORC1 and mTORC2 have been shown to converge on c-Myc through interlacing and complementary pathways. In EGFRvIII-expressing GBMs, mTORC1 functionally regulates the oncogenic activity of c-Myc by inducing the hnRNA1-dependent alternative splicing of the c-Myc-interacting protein, Delta Max. This dual regulation of c-Myc through AKT-mTORC1 and mTORC2 has significant implications on targeted therapy resistance in GBM, as will be discussed below.

Third, mTORC2 potentially suppresses tumor gluconeogenesis by inhibiting the transcriptional level of gluconeogenic genes, thus providing more carbons for the synthesis of macromolecules by cancer cells.

**Lipid metabolism**

Along with the reprogramming of glycolytic metabolism, altering the lipid metabolism is increasingly being recognized as another indicator of cancer cells. Tumor cells require abundant amounts of different lipids (including fatty acids, phospholipids, cholesterol, glycerolipids, and sphingolipids) for the synthesis of membranes and signaling molecules. Although mTORC2 is a key regulator in lipogenesis, lipolysis, and adipogenesis in normal cells, its functions in cancer lipid metabolism have not been well established.

Cancer cells are believed to increase de novo lipid synthesis and lipid uptake to meet the demands of the rapid proliferation and cell growth. Sterol regulatory binding proteins (SREBPs) are the master transcriptional regulators of lipid uptake and de novo lipid synthesis. After being transported from the ER to the Golgi, SREBPs process a series of cleavages on the Golgi membrane, thereafter releasing the helix-loop-helix domain of SREBPs to the nucleus. mTORC1 regulates SREBP function through several mediators, including S6K1 and lipin-1. In GBM, SREBP1 cleavage is activated by the mutant EGFR signaling but is insensitive to rapamycin, which suggests an emerging key function of mTORC2, or is rapamycin-resistant, despite such mTORC1-dependent mechanisms as 4E-BP1 signaling in GBM lipid metabolism reprogramming.

mTORC2 appears to mediate SREBP1 cleavage through AKT-dependent or AKT-independent pathways, thus activating the transcription of key genes in fatty acid and cholesterol de novo synthesis. These key genes include acetyl-CoA carboxylase (ACC), fatty acid synthase (FASN), and acyl-CoA synthetases (ACS). mTORC2 also up regulates cholesterol uptake by increasing the expression of LDLR through SREBP1.

Various lipid compositions may confer specific mechanical properties on membranes, as well as necessary oncogenic signals for tumor growth and survival. A recent lipidomic analysis combined with a systematic RNAi screening of lipid biosynthetic enzymes in HeLa cells showed that 11 lipids with specific chemical structures that accumulate in dividing cells are required for cell division. Cancer cells may actively regulate their cellular lipid composition in a similar manner. In yeast, TORC2 senses cellular sphingolipid levels and controls sphingolipid synthesis by activating serine: palmitoyl-coenzyme A transferase (SPT) through the yeast SGK1 homologue Ypk1, which suggests a function of mTORC2 in controlling membrane dynamics. Yeast TORC2 also controls ceramide biosynthesis by regulating the activity of ceramide synthase through Ypk2, the yeast homologue of SGK1.

The mRNA level of lysophospholipase (lysoPLD), which cleaves lysophospholipids to lysophosphatic acid (LPA), is significantly increased in glioma cells. Cellular phosphatidic acid has been shown to be an activator of mTORC1 and mTORC2. Whether mTORC2 contributes to the control of a specific lipid composition and how mTORC2 signaling regulates the phospholipid metabolism in cancer cells remain unclear.

Acetyl-CoA, the substrate for de novo fatty acid synthesis, as well as histone acetylation, serves an important function in the epigenetic regulation of cancer cells. This substrate may be regulated by mTORC2 signaling through the control of ATP citrate lyase (ACLY) expression, which catalyzes the first step of fatty acid synthesis that converts citrate to acetyl-CoA. The genetic depletion of rictor in the My5 precursor cells gives rise to myocytes, brown adipose tissue, and a subset of white adipose tissue, thus significantly reducing the level of ACLY in brown fat adipose tissue and protecting against the development of obesity in mice fed with a high-fat diet under thermoneutral conditions.

In cancer cells, increased glycolysis provides sufficient citrate from the TCA cycle for acetyl-CoA synthesis through ACLY. Thus, mTORC2 may potentially regulate histone and protein acetylation in GBM by controlling ACLY levels.

**Glutamine and ROS metabolism**

mTORC2 controls glutamine uptake by regulating the cell surface amino acid transporters, SNAT2 and LAT1, through
AKT and SGK activation. This complex also up regulates glutaminase levels to promote glutaminolysis through c-Myc. The high levels of reactive oxygen species (ROS) derived from rapid proliferation can damage cancer cells. Reduced nicotinamide adenine dinucleotide phosphate (NADPH) and glutathione (GSH), two of the most abundant antioxidants, are responsible for controlling the increase in cellular ROS levels to maintain cellular redox status in cancer cells. mTORC2 regulates the production of NADPH and GSH in a c-Myc-dependent manner by promoting the expression of enzymes in NADPH and GSH synthesis.

**mTORC2 is a central regulator of drug resistance pathways**

mTORC2 can potentially contribute to cancer drug resistance via several pathways. First, mTORC2 may contribute to chemotherapy resistance directly through its activation of AKT. Second, mTORC2 can promote resistance through SGK-1-dependent and NF-κB-dependent signaling. Interestingly, the activation of the NF-κB pathway in this case is not AKT-dependent, though AKT has been shown to regulate NF-κB signaling in various other circumstances. This finding suggests that GBMs have developed additional chemotherapy resistance mechanisms because AKT inhibition alone will be insufficient to sensitize tumors to chemotherapy.

Several studies suggest a link between dysregulated metabolism and cancer drug resistance. As an example, LDHA, which regulates aerobic glycolysis, has been associated with taxol and trastuzumab resistance; silencing LDHA is capable of which regulates aerobic glycolysis, has been associated with metabolism and cancer drug resistance. As an example, LDHA, which regulates aerobic glycolysis, has been associated with metabolism and cancer drug resistance. As an example, LDHA, which regulates aerobic glycolysis, has been associated with metabolism and cancer drug resistance. As an example, LDHA, which regulates aerobic glycolysis, has been associated with metabolism and cancer drug resistance. As an example, LDHA, which regulates aerobic glycolysis, has been associated with metabolism and cancer drug resistance. As an example, LDHA, which regulates aerobic glycolysis, has been associated with metabolism and cancer drug resistance. As an example, LDHA, which regulates aerobic glycolysis, has been associated with metabolism and cancer drug resistance.

**Targeting mTOR signaling for cancer therapy**

Rapamycin and its analogues (rapalogues) that allosterically inhibit mTORC1 exhibit limited activity against mTORC2, although some inhibitory effect has been documented in certain cell types with prolonged rapamycin treatment. This condition highlights the need for a strategy to interrupt mTORC2 specific protein-protein interactions. In contrast to allosteric inhibitors, the ATP-competitive mTOR inhibitors that directly inhibit mTOR kinase activity by competing with ATP for binding to the kinase domain of mTOR have the potential to inhibit both mTORC1 and mTORC2. In GBM, the mTOR kinase inhibitors CC214-1 and CC214-2 inhibit rapamycin-resistant mTORC1 and mTORC2 signaling, thus blocking protein translation, cell proliferation, and tumor growth. Interestingly, EGFRvIII expression and PTEN loss sensitized tumor cells to CC214 compounds, thereby implying that GBM cells with higher levels of PI3K signaling may be more sensitive to the interruption of mTOR signaling.

The similarity of the kinase domains between mTOR and PI3Ks and the importance of PI3K signaling dependent of mTOR can cause ATP-competitive PI3K/mTOR kinase inhibitors to serve a key function in targeted cancer treatment. Pharmacokinetic failure appears to be a major cause of resistance to mTOR and dual PI3K/mTOR kinase inhibitors in patients. Considerable effort will be needed to improve the pharmacokinetic properties of these drugs in vivo and to optimize the therapeutic dosing regimens to enable patients to overcome the resistance caused by insufficient drug exposure and to prevent the failure to inhibit mTOR signaling sufficiently. Doses may be sufficient to block mTOR signaling in circulating white blood cells but are inadequate to inhibit intratumoral mTOR signaling in patients.
Cloughesy et al.\textsuperscript{82} have reported that the inhibition of GBM cell proliferation is correlated with the magnitude of mTOR inhibition in a Phase I rapamycin trial of GBM patients. However, even the concentrations of rapamycin in most GBM tissues were above the level known to confer anti-proliferative activity in PTEN-null cell lines \textit{in vitro} (typically 1 nM), not every patient showed corresponding biological responses (determined by Ki-67), partially because of inadequate mTOR signaling inhibition (determined by S6 phosphorylation). One of the possible mechanisms is that rapamycin can be sequestered in red blood cells\textsuperscript{83}, thus making the actual concentration in tumor cells inadequate for target inhibition. Rapamycin is not the only targeted therapy to fail in sufficiently suppressing intratumoral drug targets in patients; such failure has also been noted for the EGFR inhibitors erlotinib and lapatinib\textsuperscript{84}.

To overcome such intrinsic resistance, the pharmacokinetic properties of the drugs should be improved, and the dosing regimens should be optimized to achieve higher concentrations in tumor cells. Das Thakur et al.\textsuperscript{85} demonstrated that the continuous dosing of vemurafenib in BRAF-mutated melanoma mice over an extended period resulted in the emergence of resistant tumors. By contrast, intermittent dosing can re-sensitize tumors to vemurafenib \textit{in vivo}\textsuperscript{85}. An attractive question worthy of further exploration is whether discontinuous treatment with higher doses of targeted drugs will achieve more sufficient inhibition and yield better clinical outcomes for GBM patients.

**Conclusion**

In this review, we emphasized the important functions of mTORC2 in GBM, with a focus on its contributions to metabolic reprogramming and drug resistance. mTORC2 appears to be essential in GBM pathogenesis and is thus worth serious consideration as a drug target. However, the knowledge about mTORC2 signaling remains limited. Many questions still remain on how exactly is mTORC2 being regulated and on what is the best method to suppress it. Other questions include those on the key partners in the metabolic reprogramming of GBM and the significance of the function of mTORC2 in different types of cancer. Future mechanistic studies are required to further elucidate the function of mTORC2 in cellular growth and metabolism, given the emergence of mTORC2 as a compelling clinical drug target along with mTORC1.

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**Conflict of interest statement**

No potential conflicts of interest are disclosed.

**References**

1. Thakkar JP, Dolecek TA, Horbinski C, Ostrom QT, Lightner DD, Barnholtz-Sloan JS, et al. Epidemiologic and Molecular Prognostic Review of Glioblastoma. Cancer Epidemiol Biomarkers Prev 2014;23:1985-1996.
2. Cancer Genome Atlas Research Network. Comprehensive genomic characterization defines human glioblastoma genes and core pathways. Nature 2008;455:1061-1068.
3. Lawrence MS, Stojanov P, Mermel CH, Robinson JT, Garraway LA, Golub TR, et al. The somatic genomic landscape of glioblastoma. Cell 2013;155:462-477.
4. Brennan CW, Verhaak RG, McKenna A, Campos B, Noushmehr H, Salama SR, et al. The somatic genomic landscape of glioblastoma. Cell 2013;155:462-477.
5. Wullschleger S, Loewith R, Hall MN. TOR signaling in growth and metabolism. Cell 2006;124:471-484.
6. Laplante M, Sabatini DM. mTOR signaling in growth control and disease. Cell 2012;149:274-293.
7. Zhang Y, Nicholatos J, Dreier JR, Ricoult SJ, Widenmaier SB, Hotamisligil GS, et al. Coordinated regulation of protein synthesis and degradation by mTORC1. Nature 2014;513:440-443.
8. Jefferies HB, Fumagalli S, Dennis PB, Reinhard C, Pearson RB, Thomas G. Rapamycin suppresses S6' TOP mRNA translation through inhibition of p70s6k. EMBO J 1997;16:3693-3704.
9. Laplante M, Sabatini DM. mTOR signaling in growth control and disease. Cell 2012;149:274-293.
10. Ben-Sahra I, Howell JJ, Asara JM, Manning BD. Stimulation of de novo pyrimidine synthesis by growth signaling through mTOR and S6K1. Science 2013;339:1323-1328.
11. Nazio F, Strappazzon F, Antonioli M, Bielli P, Cianfanelli V,
Bordi M, et al. mTOR inhibits autophagy by controlling ULK1 ubiquitylation, self-association and function through AMBRA1 and TRAF6. Nat Cell Biol 2013;15:406-416.

12. Laplante M, Sabatini DM. mTOR Signaling. Cold Spring Harb Perspect Biol 2012;4.

13. Cloughesy TF, Cavenee WK, Mischel PS. Glioblastoma: from molecular pathology to targeted treatment. Annu Rev Pathol 2014;9:1-25.

14. Inoki K, Li Y, Zhu T, Wu J, Guan KL. TSC2 is phosphorylated and inhibited by Akt and suppresses mTOR signalling. Nat Cell Biol 2002;4:648-657.

15. Tee AR, Manning BD, Roux PP, Cantley LC, Blenis J. Tuberous sclerosis complex gene products, Tuberin and Hamartin, control mTOR signaling by acting as a GTPase-activating protein complex toward Rheb. Curr Biol 2003;13:1259-1268.

16. Kim E, Goraksha-Hicks P, Li L, Neufeld TP, Guan KL. Regulation of TORC1 by Rag GTPases bind raptor and mediate amino acid signaling to mTORC1. Science 2008;320:1496-1501.

17. Sancak Y, Peterson TR, Shaul YD, Lindquist RA, Thoreen CC, Bar-Peled L, et al. The Rag GTPases bind raptor and mediate amino acid signaling to mTORC1. Science 2008;320:1496-1501.

18. Gwinn DM, Shackelford DB, Egan DF, Mihaylova MM, Mery A, Vasquez DS, et al. AMPK phosphorylation of raptor mediates a metabolic checkpoint. Mol Cell 2008;30:214-226.

19. Foster DA. Phosphatidic acid and lipid-sensing by mTOR. Trends Endocrinol Metab 2013;24:272-278.

20. Tzatsos A, Kandror KV. Nutrients suppress phosphatidylinositol 3-kinase/Akt signaling via raptor-dependent mTOR-mediated insulin receptor substrate 1 phosphorylation. Mol Cell Biol 2006;26:63-76.

21. Um SH, Frigerio F, Watanabe M, Picard F, Joaquin M, Sticker M, et al. Absence of S6K1 protects against age- and diet-induced obesity while enhancing insulin sensitivity. Nature 2004;431:200-205.

22. Zinzalla V, Stracka D, Oppliger W, Hall MN. Activation of mTORC2 during osteoblast differentiation. Cell Metab 2013;17:745-755.

23. Tanaka K, Babic I, Nathanson D, Akhavan D, Guo D, Gini B, et al. Oncogenic EGFR signaling activates an mTORC2-NF-xB pathway that promotes chemotherapy resistance. Cancer Discov 2011;1:524-538.

24. Esen E, Chen J, Karner CM, Okunade AL, Patterson BW, Long F. WNT-LRP5 signaling induces Warburg effect through mTORC2 activation during osteoblast differentiation. Cell Metab 2013;17:745-755.

25. Tumaneng K, Schlegelmilch K, Russell RC, Yimlamai D, Basnet H, Mahadevan N, et al. YAP mediates crosstalk between the Hippo and PI(3)K–TOR pathways by suppressing PTEN via miR-29. Nat Cell Biol 2012;14:1322-1329.

26. Perumalsamy LR, Nagala M, Banerjee P, Sarin A. A hierarchical cascade activated by non-canonical Notch signaling and the mTOR-Rictor complex regulates neglected-induce death in mammalian cells. Cell Death Differ 2009;16:879-889.

27. Lee K, Nam KT, Cho SH, Guadapati P, Hwang Y, Park DS, et al. Vital roles of mTOR complex 2 in Notch-driven thymocyte differentiation and leukemia. J Exp Med 2012;209:713-728.

28. Julien LA, Carriere A, Moreau J, Roux PP. mTORC1-activated S6K1 phosphorylates Rictor on threonine 1135 and regulates mTORC2 signaling. Mol Cell Biol 2010;30:908-921.

29. Liu P, Gan W, Inuzuka H, Lazorchak AS, Gao D, Arojo O, et al. Sin1 phosphorylation impairs mTORC2 complex integrity and inhibits downstream Akt signalling to suppress tumorigenesis. Nat Cell Biol 2013;15:1340-1350.

30. Sarbassov DD, Guertin DA, Ali SM, Sabatini DM. Phosphorylation and regulation of Akt/PKB by the rictor-mTOR complex. Science 2005;307:1098-1101.

31. Alessi DR, Andjelkovic M, Audwells B, Cron P, Morrice N, Cohen P, et al. Mechanism of activation of protein kinase B by insulin and IGF-1. EMBO J 1996;15:6541-6551.

32. Facchinetti V, Ouyang W, Wei H, Soto N, Lazorchak A, Gould C, et al. The mammalian target of rapamycin complex 2 controls folding and stability of Akt and protein kinase C. EMBO J 2008;27:1932-1943.

33. Oh WJ, Wu CC, Kim SJ, Facchinetti V, Julien LA, Finlan M, et al. mTORC2 can associate with ribosomes to promote cotranslational phosphorylation and stability of nascent Akt polypeptide. EMBO J 2010;29:3939-3951.

34. García-Martínez JM, Alessi DR. mTOR complex 2 (mTORC2) controls hydrophobic motif phosphorylation and activation of serum- and glucocorticoid-induced protein kinase 1 (SGK1). Biochem J 2008;416:375-385.

35. Ikenoue T, Inoki K, Yang Q, Zhou X, Guan KL. Essential function of TORC2 in PKC and Akt turn motif phosphorylation, maturation and signalling. EMBO J 2008;27:1919-1931.

36. Read RD, Cavenee WK, Furnari FB, Thomas JB. A drosophila model for EGFR-Ras and PI3K-dependent human glioma. Plos Genet 2009;5:e1000374.

37. Masri J, Bernath A, Martin J, Jo OD, Vartanian R, Funk A, et al. mTORC2 activity is elevated in gliomas and promotes growth and cell motility via overexpression of rictor. Cancer Res 2007;67:11712-11720.

38. Weiler M, Blaes J, Pusch S, Sahm F, Czabanka M, Luger S, et al. mTOR target NDRG1 confers MGMT-dependent resistance to alkylating chemotherapy. Proc Natl Acad Sci U S A 2014;111:409-414.

39. Benjamin D, Colombi M, Moroni C, Hall MN. Rapamycin passes the torch: a new generation of mTOR inhibitors. Nat Rev Drug Discov 2011;10:868-880.
40. Sarbassov DD, Ali SM, Sengupta S, Sheen JH, Hsu PP, Bagley AF, et al. Prolonged rapamycin treatment inhibits mTORC2 assembly and Akt/PKB. Mol Cell 2006;22:159-168.

41. Gini B, Zanca C, Guo D, Matsutani T, Masui K, Ikegami S, et al. The mTOR kinase inhibitors, CC214-1 and CC214-2, preferentially block the growth of EGFRvIII-activated glioblastomas. Clin Cancer Res 2013;19:5722-5732.

42. Chang SM, Wen P, Cloughesy T, Greenberg H, Schiff D, Conrad C, et al. Phase II study of CC1-779 in patients with recurrent glioblastoma multiforme. Invest New Drugs 2005;23:357-361.

43. Reardon DA, Desjardins A, Vredenburgh JJ, Gururangan S, Friedman AH, Herndon JE 2nd, et al. Phase 2 trial of erlotinib plus sirolimus in adults with recurrent glioblastoma. J Neurooncol 2010;96:219-230.

44. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. Cell 2011;144:646-674.

45. Ward PS, Thompson CB. Metabolic reprogramming: a cancer hallmark even warburg did not anticipate. Cancer Cell 2012;21:297-308.

46. Cairns RA, Harris IS, Mak TW. Regulation of cancer cell metabolism. Nat Rev Cancer 2011;11:85-95.

47. Warburg O. On the origin of cancer cells. Science 1956;123:309-314.

48. Deprez J, Vertommen D, Alessi DR, Hue L, Rider MH. Phosphorylation and activation of heart 6-phosphofructo-2-kinase by protein kinase B and other protein kinases of the insulin signaling cascades. J Biol Chem 1997;272:17269-17275.

49. Gottlob K, Majewski N, Kennedy S, Kandel E, Robey RB, Hay N. Inhibition of early apoptotic events by Akt/PKB is dependent on the first committed step of glycolysis and mitochondrial hexokinase. Genes Dev 2001;15:1406-1418.

50. Kohn AD, Summers SA, Birnbaum MJ, Roth RA. Expression of a constitutively active Akt Ser/Thr kinase in 3T3-L1 adipocytes stimulates glucose uptake and glucose transporter 4 translocation. J Biol Chem 1996;271:31372-31378.

51. Hagiwara A, Cornu M, Cybulski N, Polak P, Betz C, Trapani F, et al. Hepatic mTORC2 activates glycolysis and lipogenesis through Akt, glucokinase, and SREBP1c. Cell Metab 2012;15:725-738.

52. Masui K, Tanaka K, Akhavan D, Babic I, Gini B, Matsutani T, et al. mTOR complex 2 controls glycolytic metabolism in glioblastoma through FoxO acetylation and upregulation of c-Myc. Cell Metab 2013;18:726-739.

53. Masui K, Cavenee WK, Mischel PS. mTORC2 in the center of cancer metabolic reprogramming. Trends Endocrinol Metab 2014;25:364-373.

54. Babic I, Anderson ES, Tanaka K, Guo D, Masui K, Li B, et al. EGFR mutation-induced alternative splicing of Max contributes to growth of glycolytic tumors in brain cancer. Cell Metab 2013;17:1000-1008.

55. Lamming DW, Sabatini DM. A Central role for mTOR in lipid homeostasis. Cell Metab 2013;18:465-469.

56. Yuan M, Pino E, Wu L, Kacergis M, Soukas AA. Identification of Akt-independent regulation of hepatic lipogenesis by mammalian target of rapamycin (mTOR) complex 2. J Biol Chem 2012;287:29579-29588.

57. Cybulski N, Polak P, Auwerx J, Ruegg MA, Hall MN. mTOR complex 2 in adipose tissue negatively controls whole-body growth. Proc Natl Acad Sci U S A 2009;106:9902-9907.

58. Jones KT, Greer ER, Pearce D, Ashrafi K. Rictor/TORC2 regulates Caenorhabditis elegans fat storage, body size, and development through sgk-1. PLoS Biol 2009;7:e60.

59. Soukas AA, Kane EA, Carr CE, Melo JA, Ruvkun G. Rictor/TORC2 regulates fat metabolism, feeding, growth, and life span in Caenorhabditis elegans. Genes Dev 2009;23:496-511.

60. Yao Y, Suraokar M, Darnay BG, Hollier BG, Shaiken TE, Asano T, et al. BSTA promotes mTORC2-mediated phosphorylation of Akt1 to suppress expression of FoxC2 and stimulate adipocyte differentiation. Sci Signal 2013;6:ra2.

61. Menendez JA, Lupu R. Fatty acid synthase and the lipogenic phenotype in cancer pathogenesis. Nat Rev Cancer 2007;7:763-777.

62. Currie E, Schulze A, Zechner R, Walther TC, Farese RV Jr. Cellular fatty acid metabolism and cancer. Cell Metab 2013;18:153-161.

63. Ye J, DeBose-Boyd RA. Regulation of cholesterol and fatty acid synthesis. Cold Spring Harb Perspect Biol 2011;3.

64. Guo D, Prins RM, Dang J, Kuga D, Iwamani A, Soto H, et al. EGFR signaling through an Akt-SREBP-1-dependent, rapamycin-resistant pathway sensitizes glioblastomas to antilipogenic therapy. Sci Signal 2009;2:ra82.

65. Holthuis JC, Menon AK. Lipid landscapes and pipelines in membrane homeostasis. Nature 2014;510:48-57.

66. Attila-Gokcumen GE, Muro E, Relat-Goberna J, Sasse S, Bedigian A, Coughlin ML, et al. Dividing cells regulate their lipid composition and localization. Cell 2014;156:428-439.

67. Roelants FM, Breslow DK, Muir A, Weissman JS, Thorner J. Prolonged rapamycin treatment inhibits mTORC2 assembly and Akt/PKB. Mol Cell 2006;22:159-168.

68. Aronova S, Wedaman K, Aronov PA, Fontes K, Ramos K, Bedigian A, et al. Regulation of ceramide biosynthesis by TORC2 regulates fat metabolism, feeding, growth, and life span. Proc Natl Acad Sci U S A 2011;108:19222-19227.

69. Aronova S, Bedigian A, Coughlin ML, et al. Dividing cells regulate their lipid composition and localization. Cell 2014;156:428-439.

70. Roelants FM, Breslow DK, Muir A, Weissman JS, Thorner J. Prolonged rapamycin treatment inhibits mTORC2 assembly and Akt/PKB. Mol Cell 2006;22:159-168.

71. Roelants FM, Breslow DK, Muir A, Weissman JS, Thorner J. Prolonged rapamycin treatment inhibits mTORC2 assembly and Akt/PKB. Mol Cell 2006;22:159-168.
reprograms brown fat metabolism and protects mice against obesity and metabolic disease. Cell Rep 2014;8:256-271.

72. Wellen KE, Hatzivassiliou G, Sachdeva UM, Bui TV, Cross JR, Thompson CB. ATP-citrate lyase links cellular metabolism to histone acetylation. Science 2009;324:1076-1080.

73. Boehmer C, Okur F, Setiawan I, Bröer S, Lang F. Properties and regulation of glutamine transporter SN1 by protein kinases SGK and PKB. Biochem Biophys Res Commun 2003;306:156-162.

74. Rosario FJ, Kanai Y, Powell TL, Jansson T. Mammalian target of rapamycin signalling modulates amino acid uptake by regulating transporter cell surface abundance in primary human trophoblast cells. J Physiol 2013;591:609-625.

75. Bai D, Ueno L, Vogt PK. Akt-mediated regulation of NFkappaB and the essentialness of NFkappaB for the oncogenicity of PI3K and Akt. Int J Cancer 2009;125:2863-2870.

76. Dan HC, Cooper MJ, Cogswell PC, Duncan JA, Ting JP, Baldwin AS. Akt-dependent regulation of NF-{kappa}B is controlled by mTOR and Raptor in association with IKK. Genes Dev 2008;22:1490-1500.

77. Zhao Y, Liu H, Liu Z, Ding Y, Ledoux SP, Wilson GL, et al. Overcoming trastuzumab resistance in breast cancer by targeting dysregulated glucose metabolism. Cancer Res 2011;71:4585-4597.

78. Zhou M, Zhao Y, Ding Y, Liu H, Liu Z, Fodstad O, et al. Warburg effect in chemosensitivity: targeting lactate dehydrogenase-A re-sensitizes taxol-resistant cancer cells to taxol. Mol Cancer 2010;9:33.

79. Liu H, Liu Y, Zhang JT. A new mechanism of drug resistance in breast cancer cells: fatty acid synthase overexpression-mediated palmitate overproduction. Mol Cancer Ther 2008;7:263-270.

80. Dang CV. MYC, metabolism, cell growth, and tumorigenesis. Cold Spring Harb Perspect Med 2013;3.

81. Gan B, Lim C, Chu G, Hua S, Ding Z, Collins M, et al. FoxOs enforce a progression checkpoint to constrain mTORC1-activated renal tumorigenesis. Cancer Cell 2010;18:472-484.

82. Cloughesy TF, Yoshimoto K, Nghiemphu P, Brown K, Dang J, Zhu S, et al. Antitumor activity of rapamycin in a Phase I trial for patients with recurrent PTEN-deficient glioblastoma. Plos Med 2008;5:e8.

83. Yatscoff R, LeGatt D, Keenan R, Chackowsky P. Blood distribution of rapamycin. Transplantation 1993;56:1202-1206.

84. Vivanco I, Robins HI, Rohle D, Campos C, Gronemmes C, Nghiemphu PL, et al. Differential sensitivity of glioma- versus lung cancer-specific EGFR mutations to EGFR kinase inhibitors. Cancer Discov 2012;2:458-471.

85. Das Thakur M, Salangsang F, Landman AS, Sellers WR, Pryer NK, Levesque MP, et al. Modelling vemurafenib resistance in melanoma reveals a strategy to forestall drug resistance. Nature 2013;494:251-255.

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