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MINI-SYMPOSIUM: CANCER METABOLISM IN BRAIN TUMORS

mTORC2 and Metabolic Reprogramming in GBM: at the Interface of Genetics and Environment

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Abstract

Metabolic reprogramming is a central hallmark of cancer, enabling tumor cells to obtain the macromolecular precursors and energy needed for rapid tumor growth. Understanding how oncogenes coordinate altered signaling with metabolic reprogramming and how cancer cells harness cellular metabolism and its metabolites for their survival may yield new insights into tumor pathogenesis. Here, we review the recently identified central regulatory role for mTORC2, a downstream effector of many cancer-causing mutations, in metabolic reprogramming and cancer drug resistance in glioblastoma. We further consider the emerging concept that mTORC2 may connect genetics with environmental alterations in brain cancer.

INTRODUCTION—GROWTH FACTOR RECEPTOR SIGNALING AND METABOLIC REPROGRAMMING IN BRAIN CANCER

Metabolic reprogramming is a central hallmark of cancer (13), including the highly lethal primary brain cancer glioblastoma (GBM). Tumor cells convert the majority of the glucose they take up into lactate even when sufficient oxygen is present to support oxidative phosphorylation (32). This adaptation, termed "the Warburg effect," enables cancer cells to use glucose-derived carbons to meet both the increased biosynthetic and energy demands imposed by rapid tumor growth. The Warburg effect alone cannot account for the full spectrum of metabolic changes in GBM. GBM cells also catabolize glutamine to support tumor cell proliferation (31) and engage in de novo lipogenesis (6, 12) to support tumor growth. Metabolic reprogramming may also exert some of its most important consequences by globally altering gene transcription and epigenetic landscape (14, 18), including through histone and non-histone protein acetylation (4, 16, 35, 37).

In cancer, including GBM, metabolic reprogramming is frequently a consequence of upstream mutations in the growth factor receptor–phosphoinositide 3-kinase (PI3K)–v-akt murine thymoma viral oncogene (Akt)–mechanistic target of rapamycin (mTOR) signaling network, although the underlying mechanisms are incompletely understood (30, 32). The genomic "portrait" of GBM reveals frequent genetic alterations of the key components of the growth factor receptor-PI3K–Akt signaling pathway that activate mTOR signaling (3, 24, 25), potentially suggesting a central role for metabolic reprogramming in GBM pathogenesis (5). Receptor tyrosine kinase (*RTK*) gene amplification and/or mutations are detected in 66% of the primary GBM samples analyzed by The Cancer Genome Atlas (TCGA), with *EGFR* amplification and/or mutation occurring in 57% of cases (3, 10). In addition, *PI3K* catalytic and regulatory subunit genetic mutations, and phosphatase and tensin homolog deleted on chromosome 10 (*PTEN*) gene deletion and mutation are common events in GBM (3), resulting in constitutive activation of the PI3K–Akt–mTOR hyperactivation (5).

In this mini-symposium article, we briefly outline a recent set of discoveries that point to a central role for mTOR complex 2 (mTORC2)-mediated metabolic reprogramming in GBM pathogenesis. Newly identified molecular mechanisms by which mTORC2 reprograms GBM cells are discussed. In addition, recent work demonstrating that elevated nutrient levels can drive resistance to targeted cancer treatments in GBM via its ability to maintain mTORC2 signaling is highlighted, nominating mTORC2 as a central node integrating altered growth factor receptor signaling with nutrient availability in GBM.

mTORC1 AND mTORC2—ESSENTIAL COMPONENTS IN METABOLIC REPROGRAMMING

mTOR is a serine/threonine kinase that exists in two distinct complexes, mTORC1 and mTORC2, which differ in their regulation,

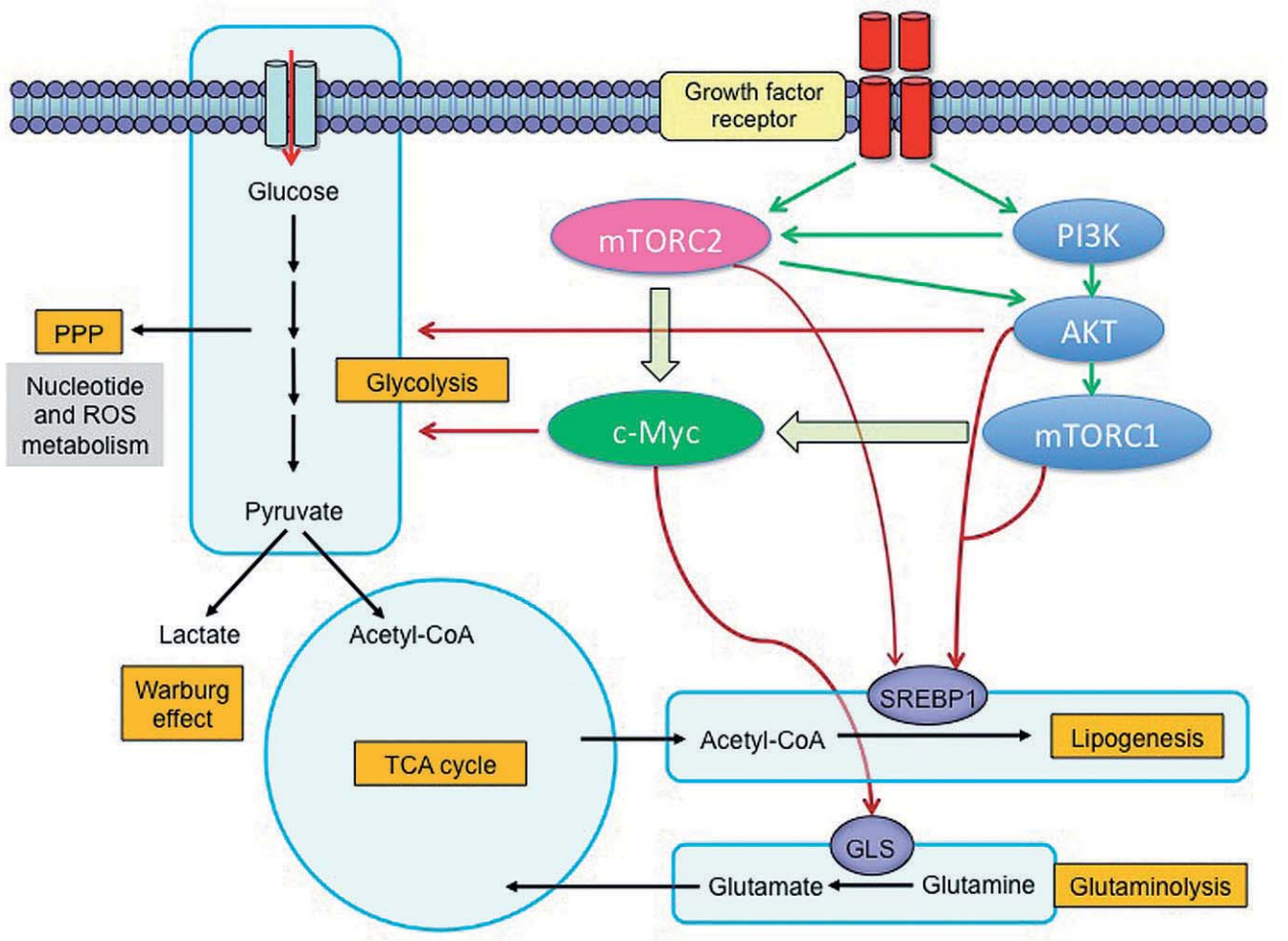

Figure 1. Mechanistic target of rapamycin complex 2 (mTORC2) signaling controls metabolic reprogramming in glioblastoma (GBM): mTORC2 reprograms the glycolytic metabolism, lipid, glutamine, nucleotide and ROS metabolism mainly through Akt and c-Myc. Akt and c-Myc promote glycolysis and Warburg effect to generate sufficient energy and macromolecules for rapid tumor growth. mTORC2 activates SREBP1 in an Akt-dependent and Akt-independent manner to promote lipogenesis, providing lipids for the synthesis of membrane and signal molecules. mTORC2 also regulates glutaminolysis by activating c-Myc. GBM with an activated mutant form of EGFR engages c-Myc signaling at least by two complementary steps, including mTORC1 and mTORC2. GLS = glutaminase; PPP = pentose phosphate pathway; ROS = reactive oxygen species; SREBP = sterol regulatory binding protein; TCA = tricarboxylic acid.

function and responsiveness to the allosteric inhibitor, rapamycin (36). mTORC1 contains mTOR kinase in association with Raptor, PRAS40, mLST8, Deptor and Tti1/Tel2 and is a validated cancer drug target (15). mTORC1 links upstream growth factor receptor signaling with downstream protein translation and cell proliferation through its substrates S6K1 and 4E-BP1. mTORC1 promotes anabolic metabolism downstream of activated PI3K–Akt signaling and in response to amino acid nutrient levels (15, 20). mTORC1 also regulates protein degradation, ribosome biogenesis, glucose, lipid and nucleotide metabolism, and autophagy (15, 36) (Figure 1).

mTORC2 contains mTOR kinase in association with Rictor, mSIN1, Protor1/2, mLST8, Deptor and Tti1/Tel2 (15). mTORC2 is activated in response to RTK–PI3K activation and possibly by association with ribosomes (38). mTORC2's most well-recognized function is the phosphorylation of Akt on Ser473 to promote Akt's maximal activation (27). In GBM, *EGFRvIII*, the most common epidermal growth factor receptor (EGFR) mutation, and PTEN loss potently activate mTORC2 (29). mTORC2 can also activate other protein kinase A/protein kinase G/protein kinase C (AGC) subfamilies, including Akt/PKB, PKCα, and serum and glucocorticoid-inducible kinase 1 (SGK1). In a Drosophila model of EGFR–PI3K-driven gliomas, mTORC2, but not Akt or mTORC1, was required for tumor formation (26), suggesting a critical, Akt-independent role for mTORC2 in GBM pathogenesis. Recent studies suggest that mTORC2 can promote GBM growth and chemotherapy resistance in cancer cells (29), as well as controlling genome stability (28) and tumor metabolism including glycolysis, glutaminolysis, lipogenesis, and nucleotide and reactive oxygen species (ROS) metabolism (22) (Figure 1). These effects appear to occur through Akt-dependent and Akt-independent signaling.

METABOLIC REPROGRAMMING BY mTORC2—C-MYC, A MASTER REGULATOR OF mTOR-RELATED METABOLISM

We recently identified an unexpected Akt-independent role for mTORC2 in GBM metabolic reprogramming through its ability to control c-Myc protein level (21). mTORC2 markedly increases glycolysis in GBMs, which is mediated by regulating the intracellular level of c-Myc, a crucial regulator of the Warburg effect (8). mTORC2 signaling through PKCα, independent of Akt, promotes inactivating phosphorylation of class IIa HDACs (23), leading to constitutive acetylation of FoxO1 and 3, a family of negative regulators of c-Myc that reduce c-Myc protein levels through a microRNA-dependent process (11). Persistent mTORC2 signaling relieves GBM cells of a miR-34c-dependent blockade of c-Myc, potently promoting glycolysis to drive tumor growth. More importantly, activated mTORC2/acetylated FoxO/c-Myc expression was associated with significantly shorter survival in GBM patients (21).

These results have an intriguing implication; GBM cells are addicted to c-Myc. c-Myc is recognized as a central player in cancer, both through c-Myc's broad transcriptional activities (17) and through its critical role in metabolic reprogramming (8). However, *c-Myc* is rarely amplified or mutated in GBM (3), despite its potential importance in GBM pathogenesis. How do the mutations in growth factor receptor signaling pathways, such as *EGFR* mutations, cooperate with c-Myc to promote tumorigenesis? Recent work from our laboratory identifies a set of interlacing molecular mechanisms by which EGFRvIII co-opts c-Myc to reprogram cellular metabolism and drive tumor proliferation. First, EGFRvIII promotes the oncogenic activity of c-Myc by inducing mTORC1-dependent, hnRNPA1-dependent splicing of the c-Myc interacting protein MAX, to form the gain of function variant Delta MAX (1). Second, EGFRvIII upregulates c-Myc protein levels through mTORC2-dependent acetylation of FoxO1 and 3 (21). Taken together, along with a work indicating that Akt can control c-Myc levels via phosphorylation of FoxO1 and 3 (9, 11), these studies identify multiple interlacing complementary mechanisms by which EGFRvIII promotes metabolic reprogramming and tumor cell proliferation via its control of c-Myc (Figure 1). This tightly integrated, multi-pronged control mechanism suggests potentially targetable points of therapeutic intervention, but it also raises challenges for molecularly targeted therapies because any of these mechanisms may be sufficient to maintain c-Myc levels to promote drug resistance. More importantly, failure to inhibit mTORC2, which appears to be harder to target than mTORC1, even with ATP-competitive kinase inhibitors, may cause tumor cell resistance to PI3K or Akt-targeted therapies by maintaining elevated levels of c-Myc.

mTORC2—A CENTRAL PLAYER IN DRUG RESISTANCE

mTORC2 thus appears to be a central player in cancer drug resistance through several pathways. First, mTORC2 may contribute to chemotherapy resistance directly through its activation of Akt. Second, mTORC2 can promote resistance through NF-κB-dependent signaling (29). Interestingly, the activation of the NF-κB pathway in this case is not Akt-dependent, although Akt has been shown to regulate NF-κB signaling in other circumstances (2, 7). Third, Akt-independent mTORC2 signaling has also been shown to promote O-6-methylguanine-DNA methyltransferase (MGMT)-dependent resistance to one of the most used alkylating chemotherapy in GBM, temozolomide, through N-Myc downstream regulated gene 1 (NDRG1) (33). These findings suggest that Akt inhibition alone will be insufficient to sensitize tumors to chemotherapy. Fourth, mTORC2 might affect the sensitivity of cancer to chemotherapy as well as radiation through its regulation of genome stability (28, 34). More importantly, TORC2 has also been shown to regulate genome stability in budding yeast (28). To date, the role of mTORC2 in regulating genome instability in human cancer, including GBM, remains unproven and future studies are needed to examine this possibility. Fifth, failure to suppress mTORC2 may promote resistance to PI3K or Akt-targeted therapies through acetylation of FoxO1 and 3 and maintenance of elevated c-Myc levels, as described earlier. Thus, mTORC2 may lead to tumor cell resistance to therapy through multiple molecular mechanisms, including metabolic reprogramming.

EMERGING INSIGHT—WHEN GENETICS AND ENVIRONMENTS CONVERGE

Cancer metabolic reprogramming is a consequence of upstream mutations in the growth factor receptor–PI3K–Akt–mTOR signaling network, resulting in changes in intracellular nutrient levels. However, do cancer cells also adapt their signaling in response to extracellular nutrient availability? We recently made the surprising discovery that glucose or acetate, two "fuel sources" that are widely available in the brain and readily taken up by tumor cells (19), are required to activate mTORC2 and promote tumor growth (23). Glucose or acetate promoted growth factor receptor signaling through acetyl-CoA-dependent acetylation of Rictor, a core component of the mTORC2 signaling complex. Remarkably, in the presence of elevated glucose levels, Rictor acetylation is maintained to form an auto-activation loop of mTORC2 even when the upstream components of the growth factor receptor signaling pathway are no longer active, thus rendering GBMs resistant to EGFR, PI3K or Akt-targeted therapies (Figure 2). These results demonstrate that elevated nutrient levels can drive resistance to targeted cancer treatments and nominate mTORC2 as a central node for integrating growth factor signaling with nutrient availability in GBM (23).

These results have a number of potentially important and unanticipated implications. If sufficient nutrients are present, GBM cells maintain mTORC2 signaling to drive cell proliferation and survival through acetylation-dependent feedforward activation of mTORC2, suggesting an interplay between oncogenic signaling and the environment. Further, extracellular nutrients can maintain oncogenic downstream growth factor receptor signaling even after tumor cells are treated with inhibitors that target key upstream components of the growth factor receptor signaling system to which they are "addicted," making the novel prediction that GBM cells, and possibly other cancer types, may use nutrients to escape targeted therapies (23). From a broader perspective, this work begs the question of how lifestyle changes, including diet, can potentially alter tumor cell metabolism, and if so, in what ways. Indeed,

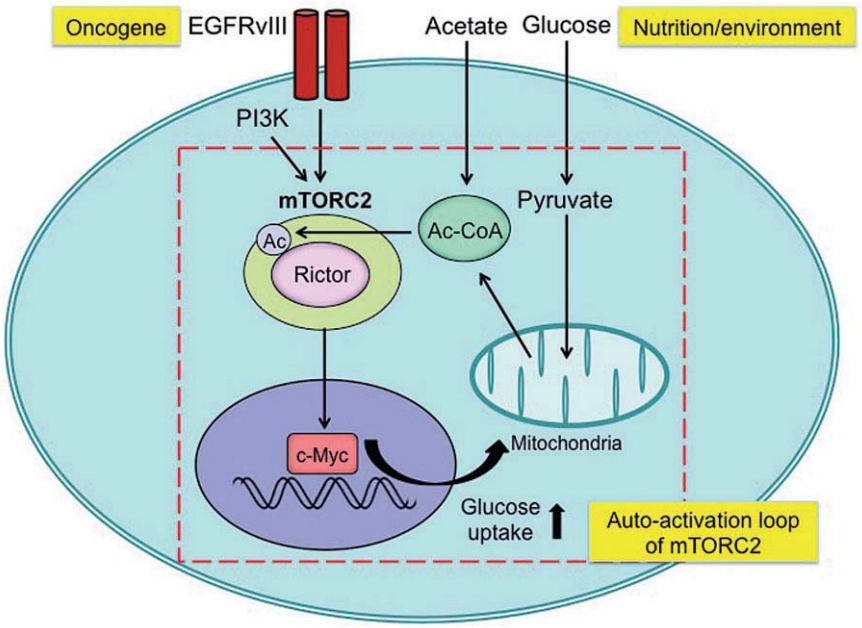

Figure 2. Oncogenic signaling and nutrient environment can interact through mechanistic target of rapamycin complex 2 (mTORC2): mTORC2 forms an auto-activation loop (i) by promoting glucose/acetate uptake and acetyl-CoA production through its downstream pathways of c-Myc (21) and (ii) by an activation of mTORC2 through acetyl-CoA-dependent acetylation of Rictor (23). By these mechanisms, glioblastoma (GBM) cells with activated mTORC2 are resistant to targeted therapies toward their upstream stimulators, including epidermal growth factor receptor (EGFR) and phosphoinositide 3-kinase (PI3K) as well as their downstream effector Akt.

there may be more interplay between oncogenic signaling and the environment than previously thought.

FUTURE PERSPECTIVES

Cancer cells reprogram their cellular metabolism to meet the biosynthetic and energetic demands imposed by rapid tumor growth and this process appears to be central to GBM pathogenesis. In GBM, mTORC2 appears to be a central node regulating this process, contributing to tumor growth and drug resistance. Signal transduction inhibitors hold the promise of much more effective, much less toxic treatments for GBM patients. However, that promise is unlikely to be realized until the consequences of cancer-causing mutations on metabolic reprogramming are understood, including the flexible ways in which tumor cells adapt to changing conditions to coordinately maintain the activity of downstream effectors necessary for tumor growth. Unraveling these important questions may point the way toward the more effective targeted cancer treatments, including for patients with GBM.

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