Review

CellPress

mTORC2 in the center of cancer metabolic reprogramming

Kenta Masui[1,2], Webster K. Cavenee[1], and Paul S. Mischel[1]

[1] Ludwig Institute for Cancer Research, University of California San Diego, La Jolla, CA 92093, USA
[2] Laboratory of Neuropathology, Tokyo Metropolitan Institute of Medical Science, Setagaya-ku, Tokyo 156-8506, Japan

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 global transcription may yield new insights into tumor pathogenesis, and provide a new landscape of promising drug targets, while yielding important clues into mechanisms of resistance to the signal transduction inhibitors currently in use. We review here the recently identified central regulatory role for mechanistic target of rapamycin complex 2 (mTORC2), a downstream effector of many cancer-causing mutations, in metabolic reprogramming and cancer drug resistance. We consider the impact of mTORC2-related metabolism on epigenetics and therapeutics, with a particular focus on the intractable malignant brain tumor, glioblastoma multiforme (GBM).

Metabolic reprogramming in cancer – a coordinated effort

Metabolic reprogramming is a central hallmark of cancer [1]. Nearly 100 years ago Otto Warburg demonstrated that cancer cells convert the majority of glucose they take up into lactate even in the presence of sufficient oxygen to support oxidative phosphorylation. This biochemical adaptation, termed 'the Warburg effect,' has once again assumed a central role in framing cancer as a metabolic disease, spurring considerable interest in trying to understand the survival advantages conferred by this adaptation and the signaling pathways that regulate it [2].

Cancer cells increase glucose uptake to meet the increased energetic and biosynthetic demands imposed by rapid tumor growth. However, ratcheting up glucose uptake is not without risk to the cell. If all the glucose taken up by tumor cells were to be fully oxidized in the tricarboxylic acid (TCA) cycle, the levels of reactive oxygen species (ROS) generated could be catastrophic. The transfer of electrons from NADH and $FADH_2$ to molecular oxygen through the cellular respiratory chain is energy-efficient, yielding 36 ATP molecules per molecule of glucose, but superoxide anions are produced in this process, generating mitochondrial ROS [3–8]. Cancer cells have developed adaptations to allow them to: (i) utilize the glucose-derived carbons for lipid synthesis through the activity of ATP citrate lyase, (ii) leverage the glucose-derived carbons for production of ribose, glycerol, serine, and glycine, and (iii) secrete the excess glucose-derived carbons as lactate. These coordinated glycolytic adaptations enable tumor cells to meet their energetic and anabolic needs without suffering catastrophically high levels of ROS. However, they need to take up more glucose to achieve this because only two molecules of ATP are yielded per molecule of glucose.

The Warburg effect alone cannot account for the full spectrum of metabolic changes required for tumor growth [2,9]. Glutaminolysis, the catabolism of glutamine to support tumor cell proliferation, is also a central feature of cancer metabolic reprogramming, providing: (i) a source of nitrogen for nucleotide and amino acid synthesis, (ii) a mechanism to produce NADPH for lipid and nucleotide synthesis, and (iii) an alternative carbon source to supply TCA cycle intermediates [10]. Tumor cells also require large amounts of lipid for membrane biogenesis, signal transduction, and potentially as an energy source. *De novo* lipogenesis is a metabolic hallmark of cancer, which can be augmented by uptake of exogenous lipids [11–14].

The Warburg effect, glutaminolysis, and lipogenesis are not exclusive to cancer. They can all be activated in rapidly proliferating cells engaged in physiological processes such as the immune response or wound repair [15,16]. This raises the question of whether cancer metabolic reprogramming simply represents the enhanced use of biochemical adaptations available to rapidly proliferating cell types or whether the two differ in fundamental ways. One of the crucial differences between cancer cells and non-cancer cells lies in the inability of non-cancer cells to take up autonomously sufficient nutrients for anabolic metabolism [16]. In metazoans, the metabolism of individual cells is tightly regulated by balancing intrinsic and extrinsic molecular cues, thus instructing cells on how best to meet their demand for ATP generation, macromolecule biosynthesis, and maintenance of redox in the context of a multicellular organism [9]. By contrast, cancer cells meet their metabolic demands in an entirely cell-intrinsic fashion, enabling cell-autonomous growth, a *sine qua non* of cancer [16]. The specificity of cancer metabolic reprogramming may therefore lie in the coordination of responses that enable tumor cells to achieve what non-neoplastic cells cannot – that is, to meet all of their needs in an entirely cell-autonomous fashion.

Understanding how cancer-causing mutations cause coordinated engagement of cellular signaling pathways,

Corresponding author: Mischel, P.S. (pmischel@ucsd.edu).
Keywords: mTORC2; c-Myc; metabolic reprogramming; epigenetics; drug resistance; glioblastoma.

1043-2760/
 http://dx.doi.org/10.1016/j.tem.2014.04.002

biochemical repertoires, and global transcription ensembles may yield crucial insights into the pathogenesis of cancer and shed new light on how tumor cells resist targeted therapies to which they should be vulnerable. In this light, it is not surprising that mutations in key regulators of phosphoinositide 3-kinase (PI3K)–AKT/protein kinase B (PKB)–mTOR signaling, and/or upstream receptor tyrosine kinases (RTKs), are found in the vast majority of cancers [17]. PI3K–AKT–mTOR signaling is the key mechanism that normal cells use to metabolize glucose in response to insulin [3]. Further, it is not surprising that c-Myc, a crucial regulator of glutaminolysis, is also amplified or mutated in some types of cancer [18], although co-occurrence of PI3K-activating mutations and c-Myc amplification appears to be the exception [17]. Understanding how tumors with PI3K–AKT–mTOR-activating mutations engage c-Myc signaling may provide important clues as to how tumor cells coordinate metabolic reprogramming to optimize growth. Mutations in metabolic enzymes such as isocitrate dehydrogenase 1/2 (IDH1/2) are highly informative because they provide a direct link between altered cellular metabolism and epigenetics [19,20]. How does metabolic reprogramming caused by more common cancer-causing mutations alter the epigenetic landscape of the cell? Does it do so through indirect regulation of enzymes that regulate histone acetylation and/or by regulating the level of intermediate metabolites such as acetyl-CoA whose levels directly influence epigenetic regulation [19]? This review focuses on a paradigmatic example which may have broad implications for understanding cancer metabolic reprogramming. Epidermal growth factor receptor (EGFR) is the most commonly activated oncogene in GBM, the highly lethal form of adult brain cancer [21]. In particular, EGFRvIII (Box 1), a constitutively active gain-of-function mutation resulting from an in-frame genomic deletion in the extracellular domain, has recently been shown to reprogram tumor cell metabolism, driving the Warburg effect [22–24], glutaminolysis [22,24], and lipogenesis [25]. We review here a set of recent discoveries involving EGFR-mutant GBM that highlight the integration of altered signaling, metabolic reprogramming, and epigenetic changes downstream of common cancer mutations, potentially providing new therapeutic opportunities.

Box 1. Epidermal growth factor receptor variant III (EGFRvIII)

Among numerous studies to decipher the interactions between growth factors and their cognate receptors, four members of the ErbB family receptors, particularly ErbB1 (epidermal growth factor receptor or EGFR), have been the most vigorously investigated. EGFR is a membrane-spanning glycoprotein consisting of an extracellular domain (ECD) and a cytoplasmic domain with multiple tyrosine residues which are phosphorylated upon ligand binding and receptor activation. EGFR is a chief regulator of epithelial cell growth, and its deregulation, often leading to the tumor formation, is the result of overexpression which is commonly associated with gene amplification and/or mutation [103]. Among the several reported tumorigenic mutations of EGFR, the most common, EGFRvIII (also known as de2–7 EGFR and ΔEGFR) which is characterized by an in-frame deletion of exons 2–7, and results in a constitutively active oncogenic form, occurs in the ECD [104]. As a result of the removal of 801 bp and subsequently 267 amino acids from the ECD, EGFRvIII exhibits a molecular weight of 145 kDa compared with that of 170 kDa for wild type EGFR [103], and can be detected by an antibody specific for EGFRvIII or PCR including an RT-PCR technique developed for EGFRvIII quantification in formalin-fixed paraffin-embedded samples [104]. EGFR amplification (or copy-number increases of chromosome 7p12, the site of the EGFR gene) is a hallmark of several cancers including primary GBM, and about 50% of EGFR-amplified GBM express the ligand-independent truncated variant EGFRvIII [21,104]. The ensuing strong and persistent activation of downstream PI3K/AKT signaling provides advantages for cell survival, proliferation, and motility. The pro-oncogenic effects of EGFRvIII are also mediated by several signaling pathways including Ras/MAPK and STAT3 [103]. Recently, EGFRvIII has been shown to activate mTORC2, which in turn activates NF-κB independently of AKT, causing resistance to chemotherapy [31]. The expression of EGFRvIII can affect the efficacy of cancer targeted therapies such as tyrosine kinase inhibitor (TKI) therapy. Expression of the constitutively active mutant EGFRvIII sensitizes tumors to EGFR inhibitors, but only if the PTEN tumor-suppressor protein is intact because PI3K signal flux is sustained by PTEN deficiency [105]. Recent single cell analyses using GBM patient-derived models and clinical samples revealed that resistance to EGFR TKI occurs by a surprisingly dynamic elimination and re-emergence of mutant EGFR (EGFRvIII) from extrachromosomal DNA (episomes), indicating a highly adaptive route by which cancers can circumvent therapies which target oncogenes [106].

mTORC1 and mTORC2 – essential partners in metabolic reprogramming

In many cancers, RTK amplification and mutations, PIK3CA mutations, and phosphatase and tensin homolog (PTEN) loss conspire to constitutively activate PI3K–AKT–mTOR signaling [17] and thereby to reprogram cellular metabolism. EGFRvIII mutation and PTEN loss, a common co-occurrence in GBM, play a central role both in tumorigenesis and in metabolic reprogramming through PI3K–AKT–mTOR activation [21,26]. mTOR is a serine/threonine protein kinase that integrates growth factor receptor signaling with cellular growth, proliferation, and survival through two distinct multiprotein complexes. mTORC1, a validated cancer drug target, regulates protein translation through its substrates S6K1 and 4E-BP1 as well as anabolic metabolism downstream of growth factor receptor-activated PI3K–AKT signaling and in response to amino acid nutrient levels [27–29].

mTORC2 is less well understood. mTORC2 has been considered to be insensitive to nutrient levels, but responsive to growth factor signaling, and to function mainly through activating AKT by phosphorylating it on Ser473 [30]. It can also phosphorylate other members of the protein kinases A, G, and C (AGC) family. Recent studies, however, suggest that mTORC2 may have an unexpectedly important role in cancer pathogenesis, promoting tumor growth and chemotherapy resistance in cancer cells [31], as well as controlling genome stability in yeast [32]. These effects appear to occur through AKT-independent signaling [31,32]. Both mTORC1 and mTORC2 are also necessary for the formation of EGFR–PI3K-driven gliomas in a *Drosophila* model [33], suggesting an important role for mTORC2 signaling that is independent of AKT–mTORC1 activation.

Structurally, both mTORC1 and mTORC2 contain mTOR, mLST8 and Deptor. The binding of Rictor to the HEAT repeats of the mTOR protein defines the mTORC2