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MINI-SYMPOSIUM: When Genetics Meets Epigenetics—A New Option for Therapeutic Intervention in Brain Tumors?

Metabolic Modulation of Epigenetics in Gliomas

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Abstract

Cancer metabolism and epigenetics are two relatively new areas of cancer research. Recent years have seen an explosion of studies implicating either altered tumor metabolism or epigenetic mechanisms in the pathogenesis or maintenance of brain tumors. A new paradigm is emerging in cancer biology that represents a convergence of these themes, the metabolic regulation of epigenetics. We discuss this interrelationship in the context of two metabolic enzymes that can influence the pathogenesis of gliomas by altering the epigenetic state. The first of these enzymes is isocitrate dehydrogenase 1 (IDH1), which is mutated in secondary glioblastomas and ~70% of grade II/III astrocytomas and oligodendrogliomas. Mutant IDH1 results in the production of a metabolite 2-hydroxyglutarate (2-HG) that can inhibit DNA and histone demethylating enzymes resulting in the glioma-CpG island phenotype (G-CIMP) and increased histone methylation marks. Pyruvate kinase M2 (PKM2), an enzyme that plays a critical role in the glycolytic pathway, is a second example of a metabolic enzyme that can affect histone modifications. In epidermal growth factor receptor (EGFR)-driven glioblastoma, PKM2 translocates to the nucleus and phosphorylates histone 3 at threonine 11 (H3-T11). This causes dissociation of HDAC3 from the CCND1 (Cyclin D1) and c-MYC promoters and subsequent histone acetylation, leading to transcription of Cyclin-D1 and c-MYC, and subsequent cell proliferation. Modification of the epigenetic state by alterations in metabolic enzymes is a novel phenomenon that contributes to the pathogenesis of gliomas and may help in the identification of new therapeutic targets.

INTRODUCTION

Gliomas are infiltrative brain tumors consisting primarily of astrocytomas and oligodendrogliomas classified into low-grade [World Health Organization (WHO) grades I and II] and high-grade (WHO grades III and IV) tumors. Glioblastomas (grade IV astrocytic tumors) are the most lethal and neurologically destructive of gliomas. Despite several decades of intensive research, the prognosis for glioblastomas and high-grade gliomas in general remains dismal. This underscores the importance of elucidating the pathogenesis of these tumors to effectively combat them. Recent years have uncovered many aspects of glioma biology including novel genetic and molecular alterations, leading to classification of gliomas into various subgroups. It is becoming increasingly evident that at least some of these genetic and molecular alterations result in changes in cellular metabolism.

Glioblastomas frequently exhibit increased glucose consumption and lactate production in the presence of oxygen, the Warburg effect (43). Activation of PI3K/AKT in glioblastoma cell lines leads to increased glucose uptake and glycolysis (5, 17, 43). Primary glioblastomas show various genetic alterations such epidermal growth factor receptor (EGFR) amplification, phosphatase and tensin homolog (PTEN) loss and platelet-derived growth factor receptor type A (PDGFRA) amplification resulting in enhanced signaling of receptor tyrosine kinases and deregulation of the PI3K/AKT pathway (1, 22), thus stimulating glucose uptake and aerobic glycolysis (5, 17). More recently, the $NADP^+$-dependent enzyme isocitrate dehydrogenase 1 (IDH1) was found to be mutated in ~70% of grade II and grade III astrocytomas and oligodendrogliomas, and secondary glioblastomas (4, 35, 50). IDH1 catalyzes oxidative decarboxylation of isocitrate to α-ketoglutarate (α-KG) in the cytosol. Mutant IDH1 alters cellular metabolism by generating the oncometabolite, 2-hydroxyglutarate (2-HG), from α-KG that can accumulate to millimolar concentrations (13, 45).

Epigenetics encompasses heritable changes in DNA and DNA-associated proteins that are often accompanied by changes in gene expression. DNA methylation represents the most extensively studied epigenetic phenomenon in glioblastomas [recently reviewed in (15, 26, 33)]. In contrast, very little is known about histone modifications in gliomas. Histones are proteins around which DNA is organized into nucleosomes. Each nucleosome consists of ~147 DNA base pairs wrapped around a histone octomer composed of H2A, H2B, H3 and H4. Histones have amino acid tails that can undergo a variety of post-translational modifications such as acetylation, methylation, phosphorylation, ubiquitination and SUMOylation of arginine (R) and lysine (K) residues (38). This results in changes in DNA function and transcription by

regulating accessibility to cellular transcriptional machinery [reviewed in (3)]. To date, the most widely studied histone modifications are acetylation and methylation, which affect transcription differently. Histone acetylation of lysine residues usually activates transcription, while methylation can be an activator or repressor of transcription, depending on the histone residue that is methylated (3). For instance, methylation of H3K9, H3K27 and H4K20 is thought to be associated with silencing of transcription, and methylation of H3K4, H3K36 and H3K79 seems to be associated with activation of transcription [reviewed in (3, 9)]. Methylation of histone lysine residues is a complex phenomenon and is regulated by a variety of histone lysine methyltransferases (KMTs) and demethylases (KDMs) (3).

Recent studies have emerged implicating histone modifications in adult glioblastomas (16, 32, 51) and mutations in genes encoding histone proteins in pediatric glioblastomas (25, 36, 40, 48). This article focuses on the molecular connections between metabolism and epigenetics in gliomas using the two examples of IDH1 mutations in intermediate-grade gliomas and secondary glioblastomas, and PKM2 alterations in EGFR-driven primary glioblastomas to illustrate this phenomenon.

MUTATIONS IN IDH1 RESULT IN THE GLIOMA-CpG ISLAND METHYLATOR PHENOTYPE (G-CIMP)

Profiling of DNA promoter methylation in glioblastomas reveals a unique subset of cases collectively referred to as G-CIMP within the proneuronal subtype of glioblastomas that exhibit hypermethylation at a large number of loci (34). Glioblastomas and intermediate-grade gliomas (grade II and grade III) with G-CIMP are strongly associated with mutations in IDH1 (11, 16, 27, 34, 42). IDH1 mutations are the most common (specifically IDH1 R132H) of the IDH mutations in gliomas and account for more than 95% of cases (2, 6, 21). Astrocytic cell lines transfected with mutant IDH1 R132H and colon cancer cell lines with knock-in mutant IDH1 R132H show G-CIMP phenotypes very similar to IDH1 mutant gliomas (16, 42).

How mutations in IDH1 result in DNA methylation in gliomas is not entirely known. One hypothesis is that 2-HG inhibits α-KG-dependent enzymes as 2-HG is structurally similar to α-KG (10, 49). α-KG-dependent dioxygenases form a large family of enzymes influencing various functions in the cell such as carnitine synthesis, hypoxic sensing, collagen modifications and histone and DNA demethylation [reviewed in (29)]. Profiling experiments in samples from patients with acute myeloid leukemia showed mutual exclusivity between IDH1/2 and TET2 mutations (19). TET2 belongs to the TET family of enzymes that is dependent on α-KG to catalyze cytosine 5-hydroxymethylation (5hmC) and subsequent DNA demethylation (23, 41). Further astrocytic cell lines expressing mutant IDH1 R132H inhibit TET2-dependent 5hmC (42). However, no mutations in the TET family of proteins are reported in gliomas. Another factor to be considered is that histone methylation can promote DNA methylation and vice versa (18). In astrocytic cell lines expressing mutant IDH1 R132H, trimethylation of H3K9 occurs prior to DNA methylation, suggesting that histone methylation may contribute to DNA methylation and may provide an additional mechanism by which mutant IDH can contribute to G-CIMP (31).

IDH1 MUTATIONS ARE ASSOCIATED WITH INCREASED HISTONE METHYLATION MARKS IN GLIOMAS

Immortalized human astrocytic cell lines or murine neurosphere cultures transfected with mutant IDH1 R132H show increases in H3K27me3, H3K9me3 (32, 42) and H3K36me3 (42) compared with cells overexpressing wild-type IDH1. Similarly, U87MG cells transfected with mutant IDH1 R132H show increased H3K9me2, H3K4me3, H3K27me2 and H3K79me2 compared with wild-type controls (49). In addition, heterozygous knock-in of IDH1 R132H in HCT116 colon cancer cell lines results in increased H3K9me3, H3K27me3 and H3K4me3 compared with the controls (16). The structural analogy between 2-HG and α-KG also comes into play in regulating histone methylation. The Jumonji C family of KDMs uses α-KG, Fe (II) and oxygen as cofactors to demethylate histone lysine residues [reviewed in (29)]. 2-HG inhibits these enzymes (10, 49) and inhibition of KDM4C by 2-HG decreases histone demethylation, resulting in increased methylation marks on H3K9 and H3K27 (32).

H3K9me3 is one of the methylation marks that showed a striking increase in cells expressing mutant IDH1 R132H (32). Methylation of H3K9 is thought to influence cell differentiation (8). Conditional deletion of the H3K9-specific KMT *Setdb1* causes lowered H3K9me3 and results in upregulation of lineage-specific differentiation markers (30). Conversely, increased H3K9me3 after knockdown of the KDM JmjD2A prevents neuronal crest cell induction (39). Cells with mutant IDH1 show suppressed glial differentiation. Neurospheres transfected with mutant IDH1 R132H show increased H3K9me3 accompanied by a decrease in glial fibrillary acidic protein (GFAP) and an increase in nestin expression compared with their IDH1 wild-type expressing counterparts (32, 42). Interestingly, in human glioma samples, the relationship between H3K9me3 and IDH1 R132H mutations varied between glioma subtypes and grades as assessed by immunohistochemistry (44). A robust relationship was observed between H3K9me3 staining and IDH1 mutations in all grades of oligodendrogliomas. However, grade III astrocytomas and glioblastomas showed positivity for H3K9me3 but did not show a significant relationship with IDH1 mutations (44). These data suggest that the roles played by IDH1 mutations and IDH1 mutant-derived 2-HG in H3K9 trimethylation may be context dependent and can vary between oligodendrogliomas and astrocytomas. While future experiments will address differences in the role played by IDH1 mutations in oligodendrogliomas vs. astrocytomas, the current working model of how IDH1 mutations and 2-HG can affect both DNA and histone methylation is presented in Figure 1.

PYRUVATE KINASE M2 REGULATES HISTONE MODIFICATIONS IN GLIOMAS

Pyruvate kinase is a key enzyme in the glycolytic pathway that catalyzes the conversion of phosphoenolpyruvate (PEP) to pyruvate. Four isoforms of pyruvate kinase exist (PKM1, PKM2, L and R). PKM2 is the main splice variant during brain development, while the major isoform in the adult brain is PKM1 (47). Cancer cells including glioblastoma cell lines show upregulated PKM2 (12, 24). Replacing PKM2 with PKM1 in lung cancer cell lines results in decreased lactate production and increased

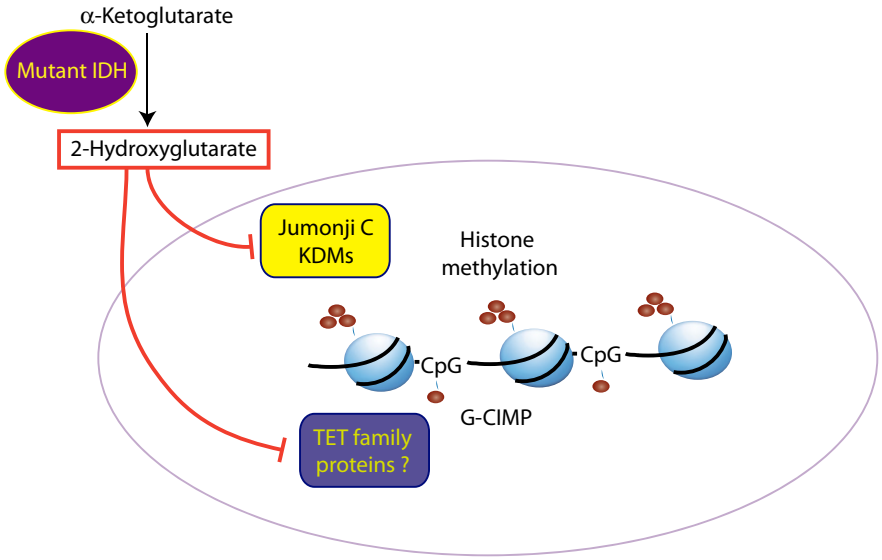

Figure 1. *Isocitrate dehydrogenase (IDH) mutations result in glioma-CpG methylation phenotype (G-CIMP) and histone methylation in gliomas.* Mutant IDH1 catalyzes the production of 2-hydroxyglutarate (2-HG) from α-ketoglutarate (α-KG). 2-HG is thought to inhibit α-KG-dependent dioxygenase enzymes as 2-HG is structurally similar to α-KG. The Jumonji C family of histone lysine demethylases (KDMs) and the TET group of DNA hydroxylases are α-KG-dependent dioxygenases. Inhibition of these enzymes may result in increased histone methylation marks and DNA methylation contributing to G-CIMP.

oxygen consumption, essentially reversing the Warburg effect and decreased xenograft proliferation in nude mice (12). Furthermore, PKM2 was identified as essential for survival of glioma stem-like cells using an unbiased RNAi screen (20). Knockdown of PKM2 in glioblastoma cell lines decreases survival and proliferation while lowering ATP levels (24). The effects of PKM2 on metabolic reprogramming in cancer cells are manifold and have been discussed elsewhere (7). More relevant to this review is the effect of PKM2 on histone modifications in EGFR-driven glioblastomas (51).

Upon EGFR activation, PKM2 translocates to the nucleus and phosphorylates histone 3 at threonine 11 (H3-T11) (51). This, in turn, leads to removal of the histone deacetylase 3 (HDAC3) from the CCND1 (Cyclin D1) and c-MYC promoter regions (51).

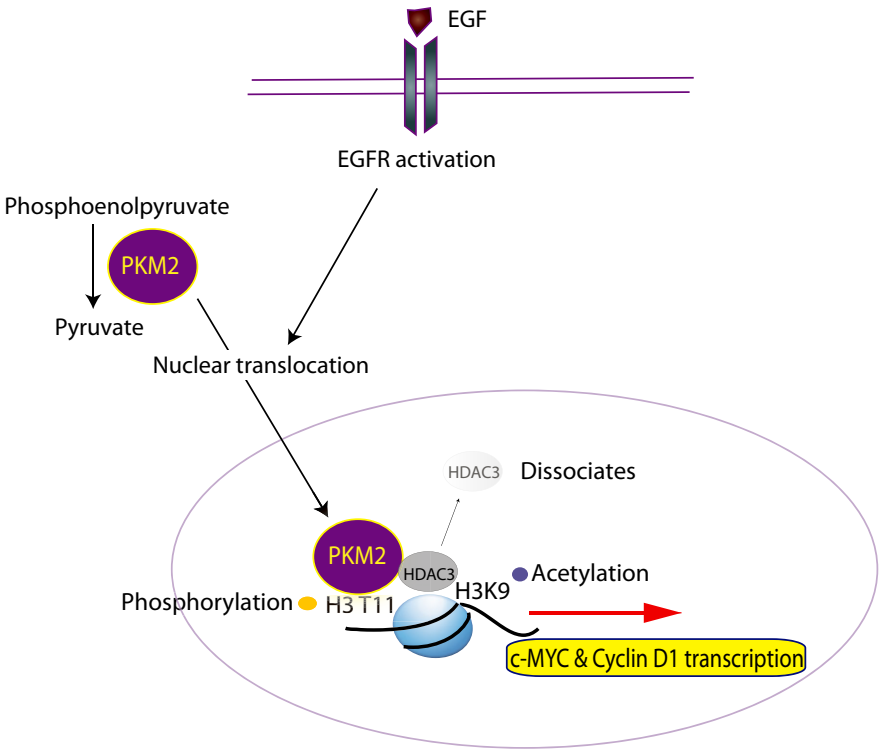

Figure 2. *Pyruvate kinase M2 (PKM2) regulates histone modifications in epidermal growth factor receptor (EGFR)-driven glioblastomas.* PKM2 catalyzes the conversion of phosphoenolpyruvate (PEP) to pyruvate in the glycolytic pathway. EGFR activation results in PKM2 translocation to the nucleus and phosphorylation of histone 3 at the threonine 11 residue (H3-T11). This leads to removal of the histone deacetylase 3 (HDAC3) from the CCND1 (Cyclin D1) and c-MYC promoter regions, acetylation of H3K9 and activation of transcription of Cyclin D1 and c-MYC.

PKM2 then forms a complex with β-catenin, which binds to the CCND1 and c-MYC promoter regions, leading to acetylation of H3K9 and activation of transcription (51, 52) (Figure 2). Reconstitution of cells with a mutant form of H3 [where threonine is replaced with an alanine residue (H3-T11A) that cannot be phosphorylated] in place of wild-type H3 prevented HDAC3 removal from the CCND1 and c-MYC promoters. Cells expressing mutant H3-T11A implanted into the mouse brains fail to form xenografts compared with their wild-type controls. Furthermore, phosphorylated EGFR correlated with both nuclear PKM2 and phosphorylated H3T11 levels in human glioma samples. Phosphorylated H3T11 levels were higher in 45 grade IV glioblastomas when compared to 30 diffused astrocytoma cases (51). These data suggest that PKM2 may play an important role as a regulator of histone phosphorylation and acetylation in EGFR-driven gliomas.

SUMMARY

The complex interplay between the metabolic state of a cancerous cell and its epigenetic machinery represents a novel mechanism by which the normal control of cell proliferation/differentiation can be disrupted. Epigenetic marks can integrate metabolism with nuclear transcription to allow cancer cells to coordinate their response to intrinsic and extracellular signals. Recent studies of IDH1 mutations in intermediate-grade gliomas and secondary glioblastomas, and PKM2 in EGFR-driven glioblastomas illustrate disruptions of this process. These tumorigenic events are not restricted to gliomas and other metabolic pathways likely contribute to the regulation of the epigenetic machinery. For example, the enzyme ATP-citrate lyase (ACL) drives nuclear/cytosolic acetyl-CoA synthesis and a significant defect in histone acetylation is seen when ACL is knocked down in cell lines (46). Sir2 belonging to the NAD^+-dependent sirtuin family of histone deacetylases extends lifespan in yeast on calorie restriction (28). Deacetylation of histone 4 at the K14 residue contributes to this effect on yeast lifespan (14). In mouse embryonic stem cells, threonine contributes to the synthesis of s-adenosylmethionine, which functions as a substrate for histone methylation reactions. Depletion of threonine or threonine dehydrogenase reduced trimethylation of H3K4 (37). While it remains to be determined if these pathways contribute to glioma pathology, these examples underscore the variety and complexity of mechanisms that link metabolism to epigenetics. Unraveling the dynamics and intricacies of these processes may help us develop a better understanding of gliomas to enable the development of novel therapeutic targets to effectively combat these highly aggressive tumors.

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