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RESEARCH HIGHLIGHT

The mechanisms of *IDH* mutations in tumorigenesis

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Tumor-associated mutations in the isocitrate dehydrogenase 1 and 2 (*IDH1* and *IDH2*) genes result in the loss of normal catalytic activity, the production of α-ketoglutarate (α-KG), and gain of a new activity, the production of an oncometabolite, *R*-2-hydroxylglutarate (*R*-2-HG). New evidence supports previous findings that *R*-2-HG acts as an antagonist of α-KG to competitively inhibit the activity of multiple α-KG-dependent dioxygenases, including both histones and DNA demethylases involved in epigenetic control of gene expression and cell differentiation, and also reveals an intriguing new facet of *R*-2-HG in tumorigenesis.

The $NADP^+$-dependent isocitrate dehydrogenase IDH1 and IDH2 catalyze the oxidative decarboxylation of isocitrate to α-ketoglutarate (α-KG). IDH1 and IDH2 are localized in the cytoplasm and mitochondria, respectively, and represent by far the most frequently mutated metabolic enzymes in human cancer [1]. The tumor-derived mutants of both IDH1 and IDH2 lose their activity in producing α-KG [2, 3], and gain a surprising new catalytic activity, the production of *R*-2-hydroxyglutarate (*R*-2-HG) by reduction of α-KG [4]. Previous studies have shown that *R*-2-HG acts as an antagonist of α-KG to competitively inhibit a number of α-KG-dependent dioxygenases, including the JmjC domain-containing histone demethylases (KDMs) and the TET (ten-eleven translocation) family of DNA hydroxylases that catalyze the sequential oxidation of 5-methlycytosine (5mC) to 5-hydroxymethylcytosine (5hmC), 5-formylcytosine (5fC), and 5-carboxylcytosine (5caC), leading to eventual DNA demethylation (Figure 1) [5, 6]. Three papers recently published in *Nature* provide additional evidence that α-KG-dependent dioxygenases are the pathophysiological targets of mutant IDH1/2, and further underscore the presumptive role of *R*-2-HG as the first oncometabolite in contributing to tumorigenesis after *IDH1/2* mutations.

A subset of glioblastoma, known as the proneural subgroup, has previously found to display hypermethylation at a large number of loci and is enriched with *IDH1* mutations [7]. In one of the three *Nature* papers, Turcan *et al*. [8] determined whether *IDH1* mutation alone is sufficient to cause the hypermethylation phenotype by ectopic expression of $IDH1^{R132H}$ mutant in immortalized primary human astrocytes, a cell type from which glioblastoma is believed to develop. The authors found that introduction of mutant *IDH1* induced extensive DNA hypermethylation, altered the methylation of specific histones, and reshaped the methylome in a fashion that mirrors the changes observed in *IDH1*-mutated low-grade gliomas. The observed hypermethylation of DNA and histones can be explained by the direct inhibition of TET methylcytosine hydroxylases and JmjC family histone demethylases by *R*-2-HG, respectively. In keeping with the notion that TET hydroxylases directly regulate genomic DNA methylation levels and can be inhibited by the *R*-2-HG accumulated in *IDH1/2*-mutated cells, Turcan *et al*. also showed that ectopic expression of TET2 in cultured astrocytes decreased 5mC and increased 5hmC, and that both changes were inhibited by the co-expression of TET2 with mutant IDH1. These results are consistent with the findings made in acute myeloid leukemia (AML) in which *IDH1/2* and *TET2* genes are mutated in a mutually exclusive manner [9]. Moreover, Turcan *et al*. found that expression of wild-type *IDH1* decreased the average DNA methylation level in the genome, supporting the notion that the concentration of α-KG may be a rate-limiting factor of TET-catalyzed DNA demethylation [5].

In the second paper, Lu *et al*. [10] reported that ectopic expression of tumor-derived mutant *IDH1/2* or feed-

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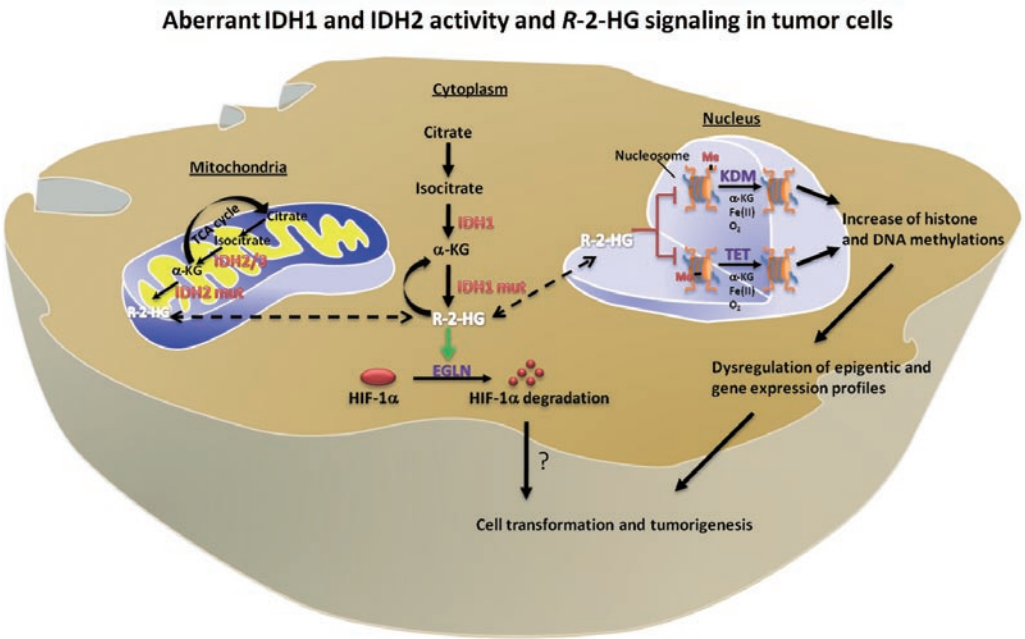

Figure 1 Summarization of reported mechanisms linking *IDH* mutation to tumorigensis. Regulation of α-KG-dependent dioxygenases by *R*-2-HG is likely to play a major role in the pathophysiology of tumors with *IDH* mutation.

ing cells with cell-permeable *R*-2-HG increases histone demethylation and results in blockade of the differentiation of 3T3-L1 adipoblasts to adipocytes. These results indicate that mutation of *IDH1/2* and accumulation of *R*-2-HG can broadly impair cell differentiation beyond the cell types in which *IDH1/2* mutations are found to associate with tumorigenesis. The authors further confirmed that *IDH1*-mutated gliomas have elevated levels of histone methylation compared with gliomas retaining the wild-type *IDH1* [5, 6]. As previously reported [5, 6], multiple KDMs that are inhibited by 2-HG, including KDM4C/JMJD2C, which causes repressive histone H3K9 di- and trimethylation and, when suppressed by RNA interference, blocks the 3T3-L1 adipogenesis. It remains to be determined whether collective inhibition of multiple KDMs or a few individual ones, such as KDM4C, is responsible for altering cell differentiation in *IDH1/2*-mutated cells. The authors also noted that expression of mutant *IDH1* increased histone methylation prior to the increase of DNA methylation, raising an intriguing possibility that histone methylation status may affect DNA methylation.

In the third paper, Koivunen *et al*. [11] proposed an enantiomer-specific mechanism of 2-HG in tumorigenesis. The authors reported two surprising findings. They showed first that immortalized human astrocytes stably expressing tumor-derived $IDH1^{R132H}$ mutant proliferate faster during late passages than those expressing either wild-type IDH1 or $IDH1^{R132H/3DN}$ mutant that lacks 2-HG-producing activity. Ectopic expression of R132H mutant IDH1 has previously been reported to decrease the growth of D54 glioblastoma cells [12], raising an intriguing possibility that the mutation of *IDH1/2* may exhibit different effects on cell growth in a cell context-dependent manner. More surprisingly, they found that *R*-2-HG, but not its enantiomer *S*-2-HG, substitutes for α-KG as a co-substrate, as opposed to an inhibitor, of EGLN, an α-KG-dependent prolylhydroxylase responsible for promoting the degradation of hypoxia inducible factor 1α (HIF-1α) (Figure 1). As the result of stimulating EGLN, accumulation of *R*-2-HG was found to associate with diminished, instead of increased, HIF-1α levels in cells expressing mutant IDH1/2. At first glance, these observations appear to be at odds with the generally accepted role of both enantiomers of 2-HG as inhibitors of α-KG-dependent dioxygenases, and HIF-1α as an oncogene in tumorigenesis, but may at least in part explain the apparent selection for *IDH* mutations to produce *R*-, but not *S*-2-HG in cancer. This data, also for the first time, reveals a qualitatively different property of two 2-HG enantiomers with respect to α-KG-dependent dioxygenases. It will be interesting to determine the strutural basis of this enantimoer-specific effect of 2-HG toward different α-KG-dependent dixoygenases. The observation that ectopic increase of *R*-2-HG reduces HIF-1α suggests that endogenous α-KG is limiting for HIF-1α hydroxylation by EGLN. The study by Koivunen *et al*. also suggests the complexity of EGLN regulation by *R*-2-HG and subsequent downregulation

of HIF-1α. It remains to be determined genetically whether a reduction or fluctuation of HIF-1α levels contributes to gliomagenesis in *IDH1/2*-mutated cells, because elevated HIF-1α generally contributes to cancer development. The only piece of genetic evidece—*IDH1/2* mutation occurs in a mutually exclusive manner with *TET2* mutation in AML—supports the notion that epigenetic alteration plays a direct and perhaps a key role in *IDH1/2* mutation-associated tumorigenesis.

IDH1/2 mutation has rapidly emerged as a favorable diagnostic and prognostic marker for certain tumors, such as low-grade gliomas and benign cartilaginous tumors. While the full mechanism linking *IDH* mutation to tumorigenesis is incompletely understood, regulation of α-KG-dependent dioxygenases by 2-HG is likely to play a major role in the pathophysiology of tumors with *IDH* mutation. These recent reports also highlight the impact of altered metabolism and metabolites on the epigenetic modification of cell differentiation and tumorigenesis.

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