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Previews

R-2-Hydroxyglutarate as the Key Effector of *IDH* Mutations Promoting Oncogenesis

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The tumor-associated isocitrate dehydrogenase (IDH) mutants are unique in that they have lost their normal catalytic activity and gained a novel function to produce *R*-2-hydroxyglutarate (*R*-2-HG). A recent study now shows that *R*-2-HG can reversibly promote leukemogenesis in vitro, suggesting a therapeutic potential of targeting mutant IDH1 and IDH2.

Mutations in metabolic enzymes (isocitrate dehydrogenase 1 and 2 [IDH1/2], fumarate hydratase [FH], and succinate dehydrogenase [SDH]) have been found in human cancer (Oermann et al., 2012). *IDH1* and *IDH2* are the most frequently mutated metabolic genes identified in human cancers, commonly observed in secondary glioblastomas, cytogenetically normal acute myeloid leukemias (AML), cartilaginous tumors, and intrahepatic cholangiocarcinomas. The IDH enzymes normally catalyze the oxidative decarboxylation of isocitrate to produce α-ketoglutarate (α-KG) and NADPH. A remarkable feature shared by cancer-associated IDH mutations is the loss of IDH's normal catalytic activity to produce α-KG and the gain of a neomorphic function to produce the *R*-enantiomer of 2-hydroxylglutarate (*R*-2-HG) (Dang et al., 2009). IDH1/2 mutant tumor cells are thus expected to have a reduced α-KG level and an increased *R*-2-HG level, which, under normal physiological conditions, is present at extremely low concentrations, if any, but can accumulate to high levels (millimolars) in tumors. A key issue in studying IDH1/2 mutation-induced tumorigenesis is the pathophysiological function of *R*-2-HG.

2-HG is structurally similar to α-KG with the exception of the oxidation state on the carbon C-2 position, whereby a hydroxyl group in 2-HG replaces a ketone group in α-KG. This structural similarity suggests the possibility that 2-HG may act as a competitive inhibitor of α-KG by antagonizing the function of α-KG-dependent enzymes such as the α-KG-dependent dioxygenases (Loenarz and Schofield, 2008). These enzymes are involved in a wide range of cellular regulations from demethylation of DNA and histone to protein hydroxylation, including the hydroxylation and degradation of hypoxia inducible factor 1α (HIF-1α). Indeed, recent studies show that 2-HG can inhibit the activity of multiple α-KG-dependent dioxygenases (Chowdhury et al., 2011; Xu et al., 2011), among them, the JmjC domain-containing histone demethylases (KDMs) and the ten-eleven translocation (TET) family of DNA hydroxylases, which is a tumor suppressor and critically important for the demethylation of 5-methylcytosine (5mC) in DNA. 2-HG binds to the α-KG binding pocket in dioxygenases, thereby acting as a competitive inhibitor of α-KG (Xu et al., 2011). The inhibition of TET enzymes by 2-HG is particularly noteworthy because *TET2* is also frequently mutated in AML, in which *IDH1/2* mutations are common. Moreover, mutations of *IDH1/2* and *TET2* genes are mutually exclusive in AML (Figueroa et al., 2010), indicating that they may act in the same pathway. Furthermore, AML with either *IDH1/2* or *TET2* mutations display similar genomic DNA methylation and gene expression profiles, indicating that TET2 is a pathologically relevant target of 2-HG. Therefore, altered epigenetic modification is currently considered a major mechanism underlying the tumorigenesis associated with IDH1/2 mutations (Oermann et al., 2012) (Figure 1).

Despite correlative evidence for the role of 2-HG in mediating the oncogenic effects of IDH1/2 mutations, 2-HG has not been formally proven to induce oncogenic transformation. Losman et al. (2013) now provide compelling evidence that 2-HG is indeed an oncometabolite capable of stimulating proliferation and suppressing differentiation, two properties obligatory for tumorigenesis in a cell culture leukemia model. The TF-1 human erythroleukemia cell line requires the cytokine granulocyte-macrophage colony-stimulating factor (GM-CSF) for proliferation and erythropoietin (EPO) for differentiation. Using this cell line model, the authors found that expression of the cancer-associated IDH1 R132H mutant, but not wild-type IDH1, promoted TF-1 cell proliferation even in the absence of GM-CSF and inhibited differentiation in response to EPO. Moreover, expression of an IDH1 R132H mutant unable to produce *R*-2-HG did not induce transformation of TF-1 cells. These data suggest that *R*-2-HG functions as an oncometabolite by promoting cytokine-independent growth and blocking EPO-induced differentiation.

Direct evidence for an oncogenic function of *R*-2-HG was obtained by treating TF-1 cells with a cell permeable *R*-2-HG analog (Losman et al., 2013). TF-1 cells passaged in the presence of the cell permeable *R*-2-HG gained cytokine-independent growth and no longer differentiated in response to EPO, phenotypes similar to those caused by the mutant IDH1 R132H expression. These observations show that *R*-2-HG is the key factor that mediates the oncogenic function of mutant IDH1 in TF-1 cells. It took those cells several passages to acquire both

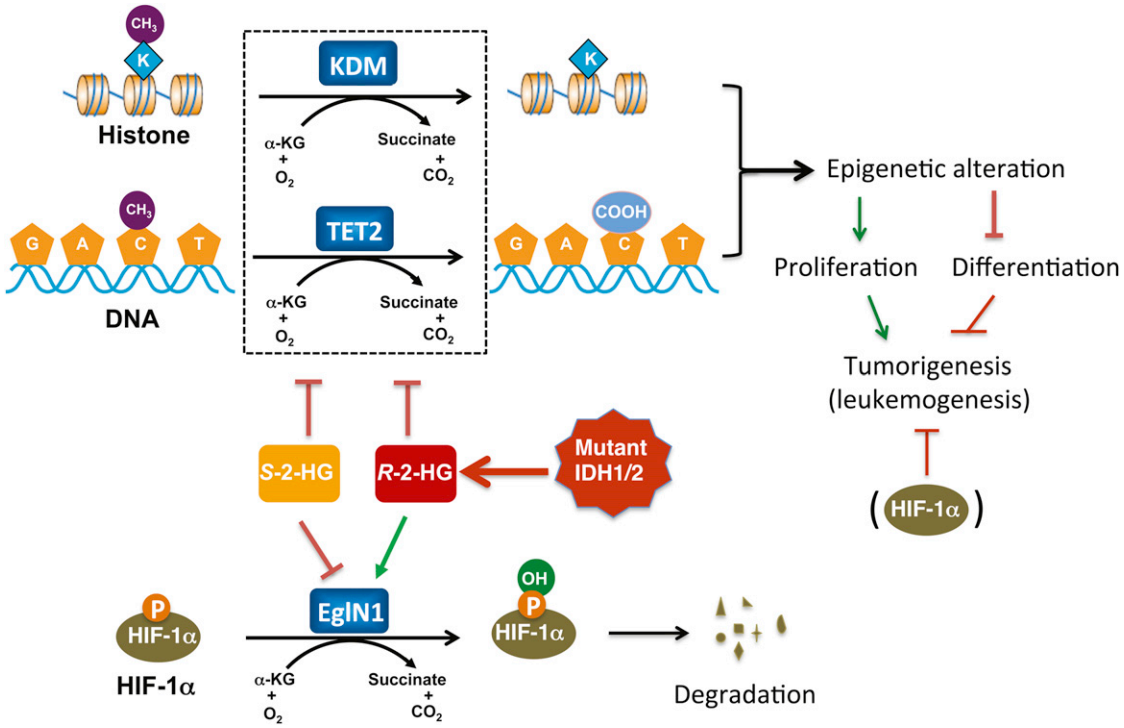

Figure 1. A Proposed Model for *R*-2-HG in Tumorigenesis

R-2-HG produced by the mutant IDH1/2 promotes tumorigenesis by inhibiting 5mC hydroxylase (TET2) and lysine demethylases (KDM), leading to the demethylation of DNA and histone, respectively. The epigenetic alterations associated with IDH1/2 mutations result in changes of gene expression and tumorigenesis. These processes can be recapitulated by *R*-2-HG, demonstrating *R*-2-HG as a true oncometabolite. *R*-2-HG does not inhibit, but rather stimulates EglN1, which promotes HIF-1α degradation by hydroxylation. HIF-1α might suppress leukemogenesis, but this may not apply to other cancer types with IDH1/2 mutations.

the cytokine independence and inability to differentiate, an observation consistent with epigenetic alterations caused by *R*-2-HG, which presumably alters both DNA and histone methylation. Interestingly, the oncogenic effects of *R*-2-HG on TF-1 cells are reversible. If *IDH1/2*-mutated tumors were similarly dependent on the continuous presence of *R*-2-HG, inhibition of mutant IDH1/2 could be an effective therapeutic treatment for these cancers.

To uncover which α-KG-dependent dioxygenase serves as the key target of *R*-2-HG in transformation, Losman et al. (2013) performed a small hairpin RNA knockdown screen of dioxygenase family members. Their data point to TET2 was likely a key α-KG-dependent dioxygenase responsible for the oncogenic function of *R*-2-HG among those tested.

There are two enantiomers of 2-HG: *R*-2-HG and *S*-2-HG. Only *R*-2-HG is produced by mutant IDH1/2. Both enantiomers inhibit many α-KG-dependent dioxygenases, with *R*-2-HG being significantly less potent than *S*-2-HG (Chowdhury et al., 2011; Xu et al., 2011; Koivunen et al., 2012). Surprisingly, Losman et al. (2013) found that *R*-2-HG, but not *S*-2-HG, promoted leukemic transformation in a dose- and passage-dependent manner. How then could *S*-2-HG, being a more potent inhibitor of TET2, be ineffective in promoting oncogenic transformation? Koivunen et al. (2012) had previously reported that *S*-2-HG inhibits EglN1, a member of the α-KG-dependent dioxygenases responsible for HIF-1α hydroxylation and degradation, whereas *R*-2-HG actually promotes EglN1 activity. The authors showed that inhibition of EglN1 by *S*-2-HG might suppress its ability to promote cytokine-independent growth and inhibit EPO-induced differentiation. This conclusion is surprising given the fact that HIF-1α is normally associated with tumor promotion rather than inhibition. The data by Losman et al. (2013) imply that inhibition of EglN1, and hence accumulation of HIF-1α, may be incompatible for leukemogeneis caused by TET2 inhibition by *R*-2-HG (Figure 1).

The function of *R*-2-HG in blocking differentiation is consistent with a previous report that supports the role of mutant IDH1 in suppressing hematopoietic differentiation (Figueroa et al., 2010). Besides altered DNA methylation, increased histone methylation has also been associated with the expression of cancer-associated mutant IDH1. Therefore, inhibition of the JmjC family of histone demethylases likely also contributes to tumorigenesis. A unified model for tumorigenesis caused by mutation in metabolic enzymes is that inhibition of α-KG-dependent dioxygenases leads to epigenetic alterations in both DNA and histone, therefore altering gene expression and oncogenic transformation (Figure 1). Consistent with this model, both KDM and TET enzymes are inhibited by succinate and fumarate, two metabolites that are structurally similar to α-KG and are accumulated in cells expressing cancer-associated mutant SDH and FH, respectively (Xiao et al., 2012). Further support for the epigenetic model comes from a mouse IDH1 R132H knockin study that reveals an increase of both DNA methylation and histone methylation (Sasaki et al., 2012). More studies are needed to demonstrate the functional significance of epigenetic

modification and expression of specific genes that are affected by cancer-associated metabolic enzyme mutations and the genetic interaction of *R*-2-HG with other oncogenes and tumor suppressor genes.

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