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Novel Insight into 2-Hydroxyglutarate Production in Human Cells

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Abstract

Mutations in metabolic enzymes, especially Isocitrate dehydrogenase1/2, strongly implicate altered metabolism in tumorigenesis by generating 2-HG. 2-HG is an oncometabolite mostly identified in AML and glioma. Wild type IDH1 and IDH2 catalyze the interconversion of isocitrate and α-KG, which is a TCA cycle intermediate and an essential cofactor for many enzymes, while 2-HG produced by mutant of IDH1/2 functions as a competitive inhibitor of α-KG, leading to epigenetic alteration and disruption of PHDs-mediated protein hydroxylation. However how is 2-HG produced in non-IDH mutated cells is still not well defined. Recent studies demonstrated that the accumulation of 2-HG in non-IDH mutated cells might be due to many other cellular mechanisms, including MYC status, expression of IDH1/2, dysregulation of 2-HGDH and hypoxia. Here we review what is known about the molecular mechanisms of transformation by IDH mutations and the mechanisms of carcinogenic metabolites 2-HG accumulation in non-IDH mutated cells. We also discuss the strategies for separation of two enantiomers of 2-HG (D and L) and their implications for the identification of the cancer subtypes and the development of targeted therapies to treat different types of human malignancies.

Keywords: IDH1/2; 2-hydroxyglutarate; Non-mutation; Cancer; Hypoxia; Enantiomers separation

Introduction

Malignant tumor is a complex disease due to the varieties of the carcinogenic mechanism [1-4]. Metabolic abnormality has been considered to play an important role in tumorigenesis and tumor progression since the observation was made by Otto Warburg [5]. What he thought was cancer cells preferentially generate energy by metabolizing glucose to lactate under aerobic condition [5], indicating cancer cells switch from oxidative phosphorylation to glycolysis to generate ATP in adequate oxygen condition, also known as "Warburg effect" [6,7]. Although normal cells use 'aerobic glycolysis' for proliferation, "Warburg effect" supports the hypothesis that this metabolic transform is a crucial characteristic of the rapid proliferating cells [8]. However, whether this metabolic alteration is the cause of cancer or the response of accelerated cell proliferation is still not well defined.

Mutations of Isocitrate dehydrogenase1/2 (IDH1/2) greatly contribute to tumorigenesis in acute myelocytic leukemia (AML) and glioma. Eukaryotic cells express three different isoforms of IDH, IDH1, 2 and 3 [9]. IDH1 and IDH2 are homodimeric $NADP^+$-dependent enzymes that catalyze the oxidative decarboxylation of isocitrate to produce α-ketoglutarate (α-KG), NADPH, and CO_2. IDH3 is a structurally unrelated heterotetrameric NAD^+-dependent enzyme that similarly decarboxylates isocitrate and produces α-KG, NADH, and CO_2. The reactions catalyzed by IDH1 and IDH2 are reversible, and the directionality of the reactions therefore depends in large part on the relative *Km* values of the forward and reverse reactions and the relative levels of isocitrate and α-KG in the cell [10]. The reaction catalyzed by IDH3 is irreversible under physiologic conditions and is principally regulated by substrate availability and positive and negative allosteric effectors. IDH1 localizes to the cytoplasm and plays a key role in promoting the activity of the numerous cytoplasmic and nuclear dioxygenases that require α-KG as a cosubstrate [11]. IDH1 catalyzes the reductive carboxylation of α-KG to isocitrate, which can then be further metabolized to acetyl-CoA to support lipid biosynthesis [12-14]. IDH2 and IDH3, in contrast, localize to the mitochondrial matrix. IDH2-mediated reductive carboxylation of α-KG plays an important role in sustaining energy production and promoting cell proliferation during periods of hypoxia [15]. IDH2, as an important source of mitochondrial NADPH, also plays an important role in protecting cells from oxidative stress.

Since α-KG is a TCA cycle intermediate and an essential cofactor for many enzymes, including JmjC domain-containing histone demethylases, ten-eleven translocation 1 5-methylcytosine hydroxylases (TET1) and EglN prolyl-4-hydroxylases(PHDs). 2-hydroxyglutarate (2-HG) is structurally similar to α-KG and generated by mutant IDH1/2, leading to epigenetic alterations and tumorgenesis. However, recently scientists found that 2-HG could also accumulate in non-IDH mutated cancer due to multiple cellular mechanisms. In this review, we will discuss the mechanisms of 2-HG production in non-mutated cancer cells and their implication in 2-HG associated human cancers.

Enantiomers of 2-HG

2-HG carries an asymmetric carbon atom in its carbon backbone and therefore occurs in two distinct forms, D-2-hydroxyglutarate (D-2-HG) and L-2-hydroxyglutarate (L-2-HG) (Figure 2). It is important to note that both D-2-HG and L-2-HG are found in human body [16]. Although the enantiomers of D L-2-HG are identical in their physical and chemical properties, these metabolites are entirely different entities in term of their biochemical properties. Routine analytical methods to detect 2-HG are not able to differentiate between D- and L-2-HG, and as a consequence the sum of the two metabolites is measured. Although

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the product caused by IDH1/2 mutations was demonstrated to be D-2-HG [17], numbers of reports measured total 2HG instead of D-2-HG [18-20]. As a consequence, minor increase of D-2-HG might be missed since endogenous levels of L-2-HG in healthy individuals is equal to or exceeds the level of D-2-HG. It is proposed that level of the IDH-specific D-2-HG rather than total 2-HG could increase the specificity to predict IDH1/2 mutations [17-21]. In addition, the accurate diagnosis of 2-HG related metabolic diseases relies on the determination of the configuration of the enantiomers, either D-2-HG or L-2-HG in patients [22,23].

In addition, a recent study suggested that IDH mutations lower 2-oxoglutarate production and hence EglN activity, leading to HIF-1α stabilization, although this finding has been disputed [24]. Early studies showed that D-2-HG cannot only reduce levels of α-ketoglutarate, but also inhibit activity of HIF1α-PHDs, leading to decreased HIF-1α degradation and an enhanced HIF-1α orchestrated "pseudohypoxic" response. Accordingly, HIF1α has been shown to be upregulated in cells treated with exogenous D-2-HG and in cells that overexpress mutant IDH1. Conversely, later studies suggested that in contrast to L-2-HG, D-2-HG stimulates the activity of this enzyme [25,26]. Accordingly, the expression of mutant IDH1 has been shown to enhance HIF-1α degradation and diminish HIF1α response levels, whereas the loss of HIF1α-PHD activity can block the transformation ability of mutant IDH. In certain cellular contexts, therefore, HIF1α or other specific targets of hydroxylation by HIF1α-PHD appear to suppress the oncogenic potential of D-2-HG. Thus, it remains somewhat unclear whether D-2-HG has an agonistic or antagonistic effect on HIF-1α-PHD at tumor-relevant concentrations and whether HIF1α could act as a tumor suppressor in some IDH-mutated tumors. In general, determination of D-2-HG and L-2-HG instead of total 2-HG is more persuasive and required for clinical and scientific research.

Diverse mechanisms of aberrant 2-HG accumulation

Generation of D-2-HG by IDH mutations in human cancers: The identification of cancer-associated mutations in three metabolic enzymes including fumaric acid dehydrogenase (FH), succinate dehydrogenase (SDH), and isocitrate dehydrogenase (IDH), which suggests that altered cellular metabolism and carcinogenesis are closely related. Loss-of-function mutations in FH and SDH have been identified in renal cell paragangliomas and carcinomas [27]. Mutations in two isoforms of IDH (IDH1 and IDH2), are common found in cancers, including gliomas, chondrosarcoma, cholangiocarcinoma, colorectal cancer, prostate cancer, lung cancer and some subtypes of acute myeloid leukemia [28-30]. As mentioned above, IDH, a key enzyme in TCA cycle, catalyzes isocitrate into α-KG, which plays an important role in biological material and energy metabolism. While the mutations alter the active site of the enzyme leading to high levels of a carcinogenic metabolite, 2-HG, which is normally found at very low levels in cells [31-33]. Wild-type IDH1 and IDH2 first catalyze isocitrate oxidation to oxalosuccinate, which is an unstable intermediate. In this reaction, NADPH will be produced. In the second step, oxalosuccinate transfer to α-KG, resulting in production of CO_2. Mutant IDH1 and IDH2 catalyze a single-step reaction. In this reaction, NADPH is oxidized to NADP+, with concomitant reduction of α-KG to 2-HG. The mutant enzymes are unable to catalyze the carboxylation of 2-HG and therefore cannot generate α-KG (Figure 1). Some research groups have demonstrated that 2-HG functions as an 'oncometabolite' by affecting a variety of signaling pathways related to cellular proliferation, transformation and differentiation, also 2-HG could modulate the activity of metabolic and epigenetic tumor suppressor enzymes that

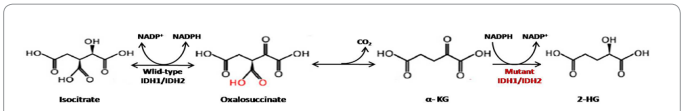

Figure 1: Reactions catalyzed by wild-type and mutant IDH1 and 2.

use α-KG as a cosubstrate [34-36]. α-KG and 2-HG differ only in the replacement of the "ketone" group in α-KG with a hydroxyl group in 2-HG. IDH1 and IDH2 mutations occur at different frequencies in different tumor types [37-58].

HG accumulation in non-IDH mutated cells

MYC-driven D-2-HG accumulation: Recently, Ambs' group demonstrated that D-2-HG accumulated at high levels in a subset of tumors and human breast cancer cell lines without IDH mutation [58]. They discovered an association between increased D-2-HG levels and MYC pathway activation in breast cancer. The aberrant accumulation of D-2-HG in a subset of human breast tumors with poor outcome and linked this observation to MYC activation and glutamine dependence. This absence of IDH mutations in breast tumors suggested a novel mechanism for D-2-HG accumulation in breast cancer. Further analyses by this group revealed globally increased DNA methylation in D-2-HG-high tumors and identified a tumor subtype with high tissue D-2-HG and a distinct DNA methylation pattern that was associated with poor prognosis and occurred with higher frequency in African-American patients. Tumors of this subtype had a stem cell–like transcriptional signature and tended to overexpress glutaminase, suggesting a functional relationship between glutamine and 2-HG metabolism in breast cancer [58].

Furthermore, D-2-HG is found frequently accumulated in tumors and cell lines of the basal-like/ mesenchymal subtype, reaching concentrations comparable to those in IDH-mutant gliomas and leukemias, despite the absence of IDH mutations.

Involvement of L-2-HG dehydrogenase in L-2-HG accumulation: There were also some evidence, showing that L-2-HG elevation is mediated in part by reduced expression of L-2-HG dehydrogenase (L-2-HGDH) [59]. L-2HGDH reconstitution in renal cell carcinoma (RCC) lowers L-2-HG and promotes 5hmC accumulation. In addition, L-2HGDH expression in RCC cells reduces histone methylation and suppresses *in vitro* tumor phenotypes, suggesting that L-2-HG is also capable to inhibit normal function of α-KG and contribute to tumorigenesis of kidney cancer.

Hypoxia-mediated accumulation of L-2-HG: An intratumoral hypoxia environment could be formed, due to the rapid proliferation of cancer cells. When cells are placed in a low oxygen environment they normally undergo a series of metabolic adaptations including an increase in glucose uptake and glycolysis and a decrease in oxidative phosphorylation. Conversely, the presence of oxygen is associated with a decrease in glycolysis and an increase in oxidative phosphorylation. The coupling of oxidative phosphorylation is mediated by the Hypoxia-inducible factors (HIFs) [60]. HIFs are transcription factors that respond to changes in available oxygen in the cellular environment, to be specific, to decreases in oxygen, or hypoxia [61]. HIF is a heterodimer consisting of an unstable alpha subunit and a stable beta subunit [62-64]. Under low oxygen conditions the HIF1α subunit is stabilized, dimerizes with a HIF beta subunit, translocates to the nucleus, and transcriptionally activates a suite of genes that increase glucose uptake, increase glycolysis, and decrease oxidative phosphorylation [62-64].

Recently, Intlekofer et al. and Oldham et al. showed mammalian cells selectively produce the L-2-HG under hypoxia in HIF-independent manner and L-2-HG arises from reduction of glutamine-derived a-KG [65]. During hypoxia, the resulting increase in L-2-HG is necessary and sufficient for the induction of increased methylation of histone repressive marks, including histone 3 lysine 9 (H3K9me3). L-2-HG regulates histone methylation levels and to help mitigate cellular reductive stress through inhibition of glycolysis and electron transport [65,66]. Furthermore, HIF1α and IDH1/2 independent L-2-HG induction by hypoxia may stabilize HIF1α, supporting a HIF1α dependent cell metabolic pattern in low oxygen condition.

Imbalanced IDH1/2 expression and D-2-HG overproduction: Base on above, a research group in Osaka University developed a novel methodology to computationally analyze gene expression in colorectal cancer (CRC), and revealed a novel and potential mechanism of CRC development, through overproduction of D-2-HG when there is an imbalance between IDH1 and IDH2 expression, resulting in decreased clearance of D-2-HG when the β-oxidization pathway is diminished [67]. Additional validation analysis with another gene expression dataset resulted in IDH1/2 imbalanced expression with a shorter disease-free survival (DFS) compared with balanced expression [67].

Separation of L-2-HG from D-2-HG

Considering that D-2-HG and L-2-HG enantiomers have identical physical and chemical properties, separation of the enantiomers is challenging. Two strategies have been developed to separate and quantify D/L-2HG by utilizing chiral column or chiral derivatization [68,69]. Rashed et al. used a ristocetin A glycopeptide antibiotic silica gel bonded chiral column combined with mass spectrometry analysis to detect D-2-HG and L-2-HG. While this strategy could not demonstrate perfect performance in real sample, such as metabolite products, especially small molecular metabolites with high polarity. In addition, the detection sensitivity of the method is very low due to the poor ionization efficiency of 2-HG in mass spectrometry. Chalmers et al. and Cheng et al. used chiral derivatization reagent to derivatize 2-HG followed by gas chromatography/mass spectrometry (GC/MS) or liquids chromatography/mass spectrometry (LC/MS) analysis respectively. However, the two-step derivatization procedure was tedious and the derivatized D-2-HG and L-2-HG were still not well separated in subsequent analysis [70,71]. Therefore, to develop a convenient and accurate approach to detect D-2-HG and L-2-HG enantiomers is highly desired for the diagnosis of 2-HG related metabolic diseases (Figure 2).

Conclusion

Accumulation of D-2-HG by IDH mutations greatly contributes to tumorigenesis in Glioma and AML through modulating DNA and histone methylation pattern, while the elevation of L-2-HG could be associated with MYC, 2-HGDH and hypoxia. In addition, L-2-HG has been shown to be capable to alter DNA methylation by impairing α-KG mediated enzyme activity, which is quite similar to D-2-HG. However, if so, is excessive aerobic exercise or anaerobic exercise associated with increased risk of cancer? How is L-D-2HG metabolized? Is there any other key molecules responsible for L-2-HG degradation? Does hypoxia promote cancer pathogenesis via L-2-HG? Further studies are required to elucidate the 2-HG related metabolic pathway, as well as the approaches for separation of these two enantiomers in human-derived specimens.

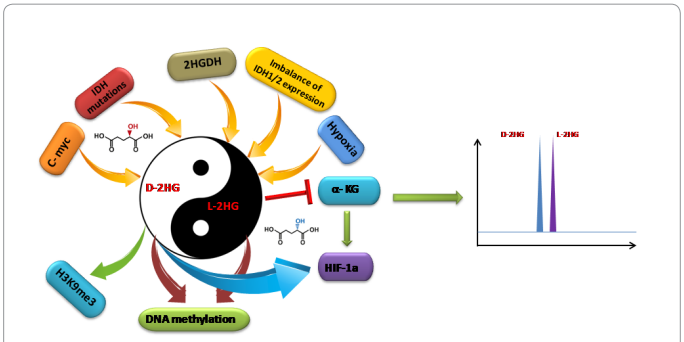

Figure 2: Schematic diagram for the generation and working mechanisms of 2-HG and the determination of D-2HG and L-2HG by derivatization strategy.

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