Another small molecule in the oncometabolite mix: L-2-Hydroxyglutarate in kidney cancer

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ABSTRACT

Alterations in metabolism are now considered a hallmark of cancer. One of the clearest links between metabolism and malignancy are oncometabolites. To date, several putative oncometabolites with transforming properties have been identified in the context of tumors due to both gain and loss of function mutations in genes encoding enzymes of intermediary metabolism. Through an unbiased metabolomics approach, we identified elevations of the metabolite 2- hydroxyglutarate (2-HG) in the most common histology of kidney cancer that is among the most common malignancies in both men and women. Subsequent analyses demonstrate that the predominant enantiomer of 2-HG elevated in renal cancer is the L(S) form. Notably, elevations of L-2HG are due in part to loss of expression of the L-2HG dehydrogenase (L2HGDH) which normally serves as an enzyme of "metabolite repair" to keep levels of this metabolite from accumulating. Lowering L-2HG levels in RCC through re-expression of L2HGDH mitigates tumor phenotypes and reverses epigenetic alterations known to be targeted by oncometabolites. These data add to the growing body of evidence that metabolites, similarly to oncogenes and oncoproteins, can play a role in tumor development and/or progression. As such, they represent a unique opportunity to utilize these findings in the clinic setting.

INTRODUCTION

Cancer-associated mutations in genes encoding key metabolic enzymes have established a direct link between altered metabolism and cancer. Loss-of-function mutations in genes encoding tricarboxylic acid (TCA) cycle enzymes fumarate hydratase (*FH*) and succinate dehydrogenase (*SDH*) lead to accumulation of fumarate and succinate, respectively, whereas gain-of-function mutations in isocitrate dehydrogenase (*IDH1/2*) cause increased levels of D-2-hydroxyglutarate (D-2HG) [1-4]. Mutant IDH1/2 forms a dimer with the wild-type IDH and obtains a neomorphic activity to catalyze the reduction of α-ketoglutarate (α-KG) directly to D-2HG in the presence of NADPH. This chiral molecule is structurally similar to α-KG and is normally present at low levels in both its D-/L-enantiomers [5]. The abnormal accumulation of these oncometabolites inhibits histone demethylation and DNA hydroxylation by competitively inhibiting the catalytic activities of α-KG-dependent enzymes including the Junomji histone demethylases and ten-eleven translocation enzymes (TETs 1-3), respectively [6,7]. The TETs convert 5-methylcytosine (5-mC) to 5-hydroxymethylcytosine (5-hmC), a reaction though to promote DNA demethylation via passive and/or active means. However, recent studies in acute myelogenous leukemia suggest that 5hmC may be a stable epigenetic mark [8]. Somatic mutations in *IDH1* and *IDH2* have been demonstrated in many human cancers including low grade glioma, glioblastoma, cholangiocarcinoma, chondrosarcoma, and acute myeloid leukemia as recently summarized [5].

The L-enantiomer of 2HG (L-2HG) is generally more potent at inhibiting α-KG-dependent dioxygenases as demonstrated by cell-free and *in vitro* studies [6,7,9]. In contrast to D-2HG produced by *IDH* mutations, L-2HG is likely formed from α-KG through the "off-target" activity of malate dehydrogenase (MDH) [10]. L-2HG dehydrogenase (L2HGDH) is present to counter this off-target reaction by converting L-2HG back to α-KG. As such, L2HGDH is referred to as an enzyme of metabolite repair [11]. L-2HG has previously been linked to L-2-hydroxyglutaric aciduria (L-2HGA), a rare metabolic disorder caused by a defect in

Table 1: Oncometabolite-related enzymes and associated tumor types			
Enzyme	**2-HG enantiomer**	**Tumor**	**Ref.**
IDH1	D-2HG	AML	Mardis *et al*. 2009
		Glioma	Yan *et al*. 2009
		Secondary Glioblastomas	Yan *et al*. 2009
		Chondrosarcoma	Amary *et al*. 2011
		Cholangiocarcinoma	Borger *et al*. 2012
		Melanoma	Shibata *et al*. 2011
		Prostate cancer	Kang *et al*. 2009
IDH2	D-2HG	AML	Ward *et al*. 2010
		Glioma	Yan *et al*. 2009
		Secondary Glioblastomas	Yan *et al*. 2009
		Chondrosarcoma	Amary *et al*. 2011
		Cholangiocarcinoma	Borger *et al*. 2012
		Angioimmunoblastic T-Cell Lymphomas (Aitls)	Cairns *et al*. 2012
PHGDH	D-2HG	Breast Cancer Cells	Fan *et al*. 2015
MYC	not specified	Breast Cancer	Terunuma *et al*. 2014
L2HGDH	L-2HG	Renal Cancer	Shim *et al*. 2014

L2HGDH[12]. Intriguingly, brain tumors have been described in several L-2HGA cohorts from distinct geographical regions [13-17]. Despite these data, a clear connection between L-2HG and cancer remained to be established until our recent findings in kidney cancer [18]. We performed nontargeted metabolomics profiling in clear-cell renal cell carcinoma (ccRCC- the most common histology) and matched normal kidney. This pairwise analysis identified elevations of 2-HG in ccRCC. Subsequent enantiomeric resolution via tandem liquid chromatography-mass spectrometry (LC-MS) demonstrated that the predominant form of 2-HG elevated in ccRCC was the L(*S*) enantiomer in contrast to *IDH* mutant tumors. Interestingly, we found that elevated L-2HG tumors had reduced DNA 5-hmC levels compared with normal kidney and low L-2HG tumor consistent with the ability of this molecule to block TET activity. Consistent with prior studies, treatment of untransformed renal epithelial cells with cell-permeable L-2HG octyl ester treatment inhibited TET activity and reduced 5hmC levels. The elevations of L-2HG prompted us to examine L2HGDH expression. We analyzed L2HGDH expression in high L-2HG ccRCC tumors and RCC cell lines and confirmed that L-2HG levels are inversely correlated with L2HGDH expression. Notably, the *L2HGDH* locus is located at 14q, a region commonly lost in ccRCC and associated with worsened patient outcomes [19,20]. Knockdown or ectopic expression of L2HGDH modulated DNA hydroxymethylation and histone methylation in RCC cell lines. Furthermore, L2HGDH reconstitution in RCC cell lines suppressed *in vitro* tumor phenotypes. Together, these data suggest that L2HGDH has metabolic tumor suppressor activity and is an epigenetic regulator in kidney cancer.

Despite the relatively recent connection between *IDH* mutations and D-2HG, several clinical implications are emerging. In the context of glioma, the presence of *IDH* mutation confers a better prognosis for patients [21,22]. Moreover, recent studies indicate that D-2HG can be detected via magnetic resonance imaging (MRI)-based spectroscopy of the brain[23]. In the context of leukemia and cholangiocarcinoma, D-2HG may have the potential to be used as a biomarker given that it can be detected in the serum of patients with *IDH* mutant tumors [24,25]. Preclinical studies demonstrate the efficacy of inhibitors of mutant IDH for the treatment of leukemia. Indeed, small-molecule inhibitors against mutant forms of IDH1 and IDH2 have demonstrated reduction of D-2HG levels and led to increased differentiation and/or growth suppression of tumor cells [26,27]. This has led to the introduction of such agents into clinical trials. While *IDH* mutation is the most well characterized mechanism for D-2HG elevations in cancer, alternate mechanisms may promote D-2HG accumulation in malignancy. Phosphoglycerate dehydrogenase (PHGDH), an enzyme involved in the *de novo* synthesis of serine, has recently been shown to catalyze the NADH-dependent reduction of α-KG to D-2HG [28]. These findings are significant as amplification of PHGDH (located on chromosome 1p12) occurs in about 16% of all human cancers, including 6% of breast cancers and 40% of melanomas [29]. PHGDH overexpression in breast epithelial cells enhances the acquisition of malignant properties, whereas silencing of PHGDH inhibits growth of PHGDH-amplified cells identifying PHGDH as a potential therapeutic target in tumors. Whether the effects of PHGDH on tumorigenesis are D-2HG dependent remains to be determined. In addition, MYC overexpression in breast cancer promotes 2-HG accumulation in a glutamine dependent manner [30]. Our results demonstrate that

L-2HG is elevated in kidney cancer adds another layer of complexity to 2HG in cancer. Routine LC-MS analytical methods to detect 2HG do not distinguish between D-2HG and L-2HG [31]. Therefore, it is imperative to distinguish between these forms as there are distinct biochemical pathways for each enantiomer that mediate both synthesis as well as metabolism. Thus it appears that increases in 2HG levels (either D- or L-) can occur in cancer as the result of alteration in several genes (Table 1). If in fact 2-HG is tumor promoting in these settings, then differential strategies may be employed based on the underlying mechanism of 2-HG elevation. In addition, the chiral nature of 2-HG indicates that there are likely enantiomer-specific targets, which remains largely unexplored. Further research into how prevailing the L-2HG oncometabolite is in cancer and the identification of biologically significant targets is of paramount importance. Such studies should bolster the notion of cancer metabolism as a rational target for therapeutic approaches.

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CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

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