Introduction

Mammalian MDH and its Role in Hydroxyglutarate Metabolism

This document is intended to support faculty who are interested in MDH-related CUREs using mammalian MDH. Each reviewed publication includes a summary and limited commentary. Interpretation of collections of publications are discussed as a mini review on a particular sub-topic. This work is meant to help lower the barrier in implementing a mammalian/human MDH CURE project.

Introduction to Hydroxyglutarate Metabolism

2-hydroxyglutaric acid (2-HG), or α-hydroxyglutaric acid, is an α-hydroxy form of glutaric acid with two enantiomers and is similar in structure to α-ketoglutaric acid (α-KG) (see Fig. 1). The conjugate base forms of each of these metabolites are α-hydroxyglutarate and α-ketoglutarate, respectively. These oncometabolites are critical small molecules defining the fate of many cancers. The D-(*R*)-2-HG enantiomer is an antagonist of α-ketoglutarate capable of binding to dioxygenases that control demethylases responsible for histone and DNA methylation, the activation of oncogenes, loss of tumor suppressing genes and cell differentiation in addition to promoting additional oncogenic mutations. This loss of epigenetic control is observed in disorders other than cancer including cardiac ischemia, hypoxia, mitochondrial dysfunction altering collagen maturation, T-cell differentiation and genetic instability.

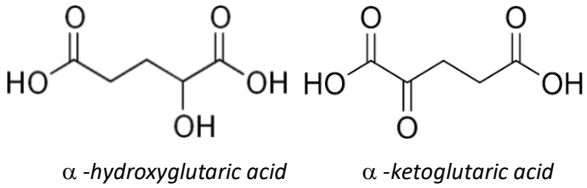

Figure 1: Structures of α-hydroxyglutaric acid and α-ketoglutaric acid (the latter is referred to as 2-oxoglutaric acid in some publications).

The D-2-hydroxyglutarate enantiomer is primarily generated by a mutated isocitrate dehydrogenase (IDH), the enzyme that catalyzes the conversion of isocitrate to α-KG (see Fig. 2). IDH1 (cytosolic) and IDH2 (mitochondrial) isoforms are the most commonly mutated metabolic genes in the cancer genome and are often found as early lesions in tumorigenesis. The mutations occur in a few hotspots that result in an enzyme capable of catalyzing the conversion of α-KG to 2-HG. Both IDH isoforms primarily produce the D enantiomer.

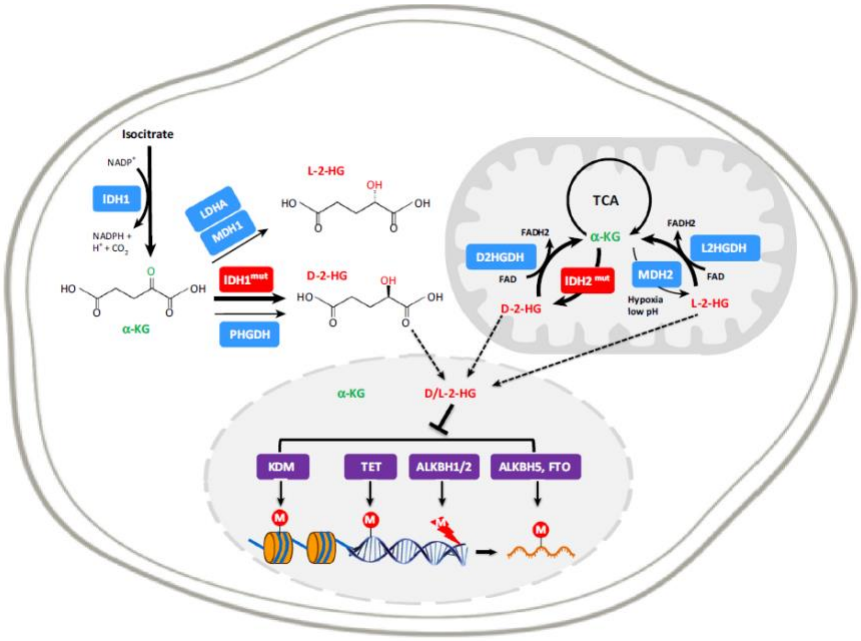

Figure 2: Metabolism and targets of 2-HG. From Ye, et al, (2018) © by the authors and published by Elsevier. All rights reserved.

The L-(*S*)-2-HG is primarily generated in hypoxic and acidic cellular conditions by promiscuous enzyme activities of malate dehydrogenase (MDH) and lactate dehydrogenase (LDH). These enzymes shift their affinities and catalytic functions from their normal substrates to α-KG, producing L-2-HG. These side reactions are normally physiologically irrelevant, but under certain conditions may influence enzyme evolution and may cause either harm or benefit to the cell. L-2-HG is involved in developmental pathologies and in the development of brain and kidney cancers. L-2-HG has been shown to inhibit α-KG dependent enzymes, and its role, while significant, is less well understood. 2-HG metabolism has recently been reviewed by Ye, et al (2018).

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Novel Roles for MDH Activity in the Production of D- and L-2-HG

Hypoxia-mediated increases in L-2-hydroxyglutarate coordinate the metabolic response to reductive stress, Oldham et al. *Cell Metab* (2015) 22:291

Low oxygen (hypoxia) results in the increase in concentration of hypoxia inducible factor 1α (HIF1α), a transcription factor which drives epigenetic shifts in response to the hypoxic conditions including a shift away from citric acid cycle and oxidative phosphorylation and toward glycolysis and anerobic production of lactate. Under hypoxic conditions, α-KG and NADH accumulate as a result of citric acid cycle and electron transport chain dysfunction. Mitochondrial malate dehydrogenase (MDH2) catalyzes the conversion of the excess α-KG to L-2HG, the latter of which accumulates in a variety of cell types (see Fig. 3). In this study, the investigators knocked down both MDH1 and MDH2 activities and subjected two different cell types to hypoxic conditions. In both cases, the concentration of α-KG increased and L-2HG decreased without changing MDH expression. These results are consistent with those of Chowdhury et al., who showed that L-2-HG inhibits α-KG dependent enzymes involved in hydroxylation of HIFα, which targets it for degradation.

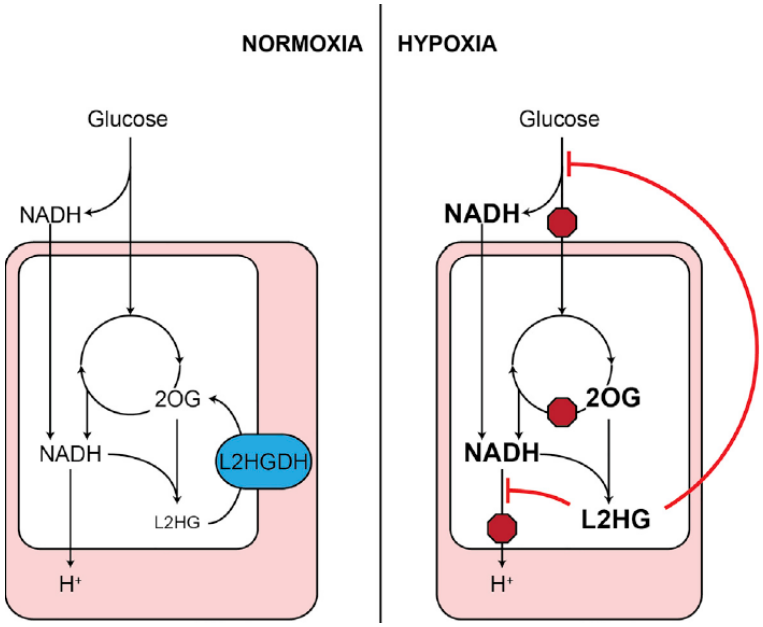

Figure 3: Alterations in glucose metabolism occur under hypoxic conditions. From Oldham, et al. (2018) © by Elsevier. All rights reserved.

Acidic pH is a metabolic switch for 2-hydroxyglutarate generation and signaling, Nadtochiy et al. *J Biol Chem* (2016) 291:20188

Extended hypoxia and other conditions such as growing tumors and cardiac ischemia lead to cellular acidification. In this study, artificially acidifying cells drove L-2-HG production in a pH-dependent manner within one hour of acidification. Using purified pig heart mitochondrial MDH protein, NADH and α-KG reacted to form 2-HG. The activity of this transformation was highest at pH 6.6-7.0, while the reciprocal pH dependence was observed for the transformation of oxaloacetate (OAA) to malate by mitochondrial MDH. LDH showed a 2-fold greater 2-HG production than MDH2 under the same acidic conditions. The authors concluded that acidification of intracellular pH could drive a stem or cancer like epigenetic change brought about by the L-isomer of 2-HG, making both LDH and MDH2, enzymes that produce L-2-HG, important targets.

Commentary: Note that there is a real pH dependence for mitochondrial (MDH2) versus cytosolic MDH (MDH1) and that only the mitochondrial MDH isoform was tested here, which is something that could be easily investigated. Both acidic and hypoxic conditions drive a number of post translational modifications that were not investigated here. It could be that phosphorylation or acetylation of the enzyme could shift one or more of the isoforms to a higher 2-HG producer; something that would be difficult to detect with multiple enzymes in a cellular system. Also as hypoxia (Oldham et al., 2018) (independent of acidification?) increases the NADH/NAD^+ ratio, it would also be interesting to investigate the kinetics of L-2-HG production with low and high NADH/NAD^+ ratios.

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L-2-hydroxyglutarate production arises from non-canonical enzyme function at acidic pH, Intlekofer et al. Nat Chem Biol (2017)13:494.

Like Nadtochiy et al., this work shows that both LDH and MDH catalyze the production of L-2-HG from α–KG. Purified recombinant LDH, MDH1 and MDH2 enzymes were able to produce L-2-HG, providing evidence for a direct link between LDH and MDH activities and production of L-2-HG. They also showed direct evidence for the production of D-2-HG from α-KG by mutant recombinant IDH1 and IDH2 enzymes, as well as 3-phosphoglycerate dehydrogenase (PHGDH). LDH production of L-2-HG from α-KG was found to be pH-sensitive (but LDH production of lactate from pyruvate was not, indicating that low pH could promote production of L-2-HG over lactate). MDH production of L-2-HG was also found to be pH sensitive, but to a lesser extent. At lower concentrations of α–KG (1-4 mM) MDH was pH sensitive (demonstrating greater activity at lower pH ~6.0-6.6) and the pH sensitivity was lost at 8 mM α–KG. Using a model docking program at different pH values, the investigators predicted that substitution of the Gln^{100} to an Arg residue in LDH would increase the rate of 2-HG production and would explain the pH sensitivity differences between pyruvate and α-KG in LDH. (Prior work by Wilks et al. showed that the Gln → Arg substitution in bacterial LDH converted the substrate specificity of LDH from pyruvate to OAA.) They found that the LDHA mutant reduced α-KG to 2-HG at a rapid rate at higher pH values and that there was no rate enhancement when the pH decreased. This occurs presumably because the LDH prefers to bind α-KG when its carboxylate "tail" is protonated (more is favored at low pH), which is less of an issue when the neutral Gln residue is substituted for the positively charged Arg residue.

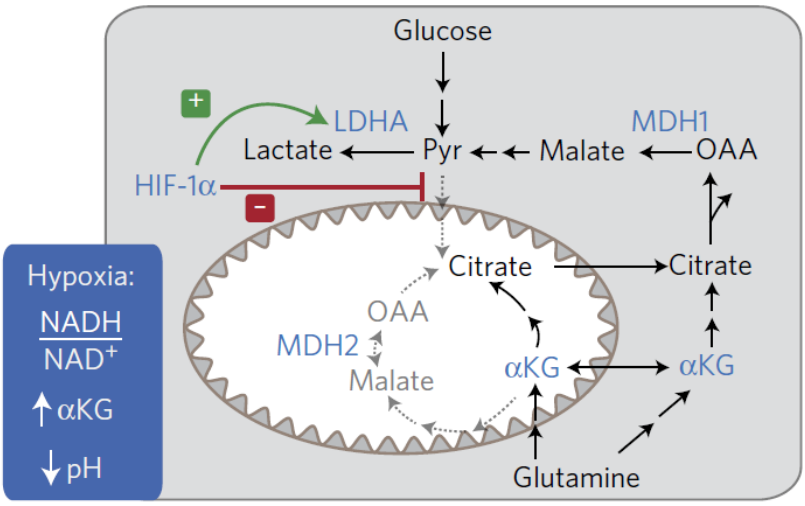

Figure 4: Metabolic changes that occur in hypoxic cells. LDHA, MDH1 and MDH2 are all able, using promiscuous activities, to catalyze the reduction of a-KG to 2-HG. From Intlekofer, et al. (2017) © by Nature America, Inc. All rights reserved.

They also show that L-2-HG stabilizes HIF1α independent of hypoxia. Loss of LDH or MDH in acidified cells decreased L-2-HG production and HIF response but not until both were knocked down did the 2-HG production decrease and HIF no longer remained stable.

Commentary: The investigators did not perform a site-directed mutagenesis Gln → Arg substitution for MDH as the OAA reaction catalyzed by MDH decreased with pH. They may have missed the point here. Post translational modification (PMT) regulation of the production would be interesting. The role of proton transporters regulating MDH and LDH activity in cancer cells is also something to discover in a cellular approach.

Appendix

Table A1: Cellular enzymes important in the study of MDH1 and MDH2

Enzyme name	Abbreviation	Alternative names or abbreviations	Cellular location	UniProt ID	UniProt accession number
NADP dependent malic enzyme	ME1		Cytosol	MAOX_HUMAN	P48163
Glutamate oxaloacetate transaminase	GOT1	aspartate transaminase (cAST, AST1) aspartate aminotransferase (cAAT, AAT1)		AATC_HUMAN	P17174
ATP citrate lyase	ACL		Cytosol	ACLY_HUMAN	P53396
Phosphoenol-pyruvate carboxykinase	PCK1, cPEPCK		Cytosol	PCKGC_HUMAN	P35558
Citrate synthase	CS		Mitochondrion	CISY_HUMAN	O75390
Glutamate oxidase transaminase	GOT2	aspartate transaminase (mAST, AST2) aspartate aminotransferase (mAAT and AAT2)	Mitochondrion	AATM_HUMAN	P00505
NAD dependent malic enzyme	ME2		Mitochondrion	MAOM_HUMAN	P23368
NADP dependent malic enzyme	ME3		Mitochondrion	MAON_HUMAN	Q16798
Fumarase	FH		Mitochondrion	FUMH_HUMAN	P07954
Aconitase	ACO2		Mitochondrion	ACON_HUMAN	Q99798
Phosphoenol-pyruvate carboxykinase	PCK2, mPEPCK		Mitochondrion	PCKGM_HUMAN	Q16822