Acidosis & 2-Hydroxyglutarate Signaling by Mitochondria

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ABSTRACT

2-Hydroxyglutarate (2HG) is an epigenetic regulator, with potential roles in hypoxic/ischemic signaling. While D-2HG is an oncometabolite generated from α-ketoglutarate (αKG) by mutant isocitrate dehydrogenase (IDH), L-2HG is generated in hypoxic cells by lactate dehydrogenase (LDH) and malate dehydrogenase (MDH). Since acid pH is a common feature of hypoxia, we hypothesized pH may regulate cell 2HG levels. In HEK293T cells, cytotoxic acidification under normoxia moderately elevated 2HG, and boosting substrate αKG levels further stimulated this. Studies on isolated LDH and MDH revealed 2HG generation by both enzymes was stimulated by acid. Acids also inhibited the mitochondrial L-2HG removal enzyme L-2HGDH (L-2HGDH). Using cells stably expressing (12α) L-2HGDH to disrupt 2HG signaling, we found 2HG is necessary for acid induced HIF activation. In addition we synthesized a cell-permeable L-2HG dimethyl ester to investigate potential 2HG protective signaling, and found it to be protective in a perfused heart model of ischemia-reperfusion injury. These results lead to a prediction that pH alters substrate preference by α-ketoacid dehydrogenases, and in this regard we found that acid permits LDH to use exocacetate as a substrate to produce malate (i.e. to perform a reaction usually catalyzed by MDH). Finally, α-hydroxybutyrate is an important biomarker of mitochondrial dysfunction, and our data suggest that acid may play a role in driving α-HB generation from α-ketobutyrate. In summary, acidosis is an overlooked mediator of 2-Hydroxyglutaric signaling by mitochondria.

DISCUSSION & CONCLUSIONS

Exposure of cells to hypoxia is known to stimulate L-2-HG generation by LDH [1][2]. However, Figure 1 shows this is not the case for isolated LDH enzyme. Ergo, another change in hypoxic cells may be the trigger for 2-HG generation. We hypothesized this may be a pH (from lactic acid generated by anaerobic glycolysis). Figure 2 shows that cytotoxic acidification (alone (by inhibiting H+ export by NHE-1)) is sufficient to stimulate 2-HG generation. Increasing the availability of substrate α-ketoacid (with the α-KGDH inhibitor KMW) further enhances acid-stimulated 2HG generation. Figure 3 shows that acidic pH stimulates 2HG generation by purified LDH and MDH, with no effect on their native enzymatic reactions. Figure 4 shows the activity of L-2HG dehydrogenase (L-2HGDH) was inhibited by acidic pH. Together these data suggest a coordinated metabolic response to elevate L-2HG in response to acidosis.

Another metabolic reaction stimulated by hypoxia is reversal of isocitrate dehydrogenase (i) (i.e., reductive carboxylation of α-KG to citrate), and Figure 5 shows this reaction is also stimulated by acidic pH (not surprising since the reaction consumes a proton). Examining the common reaction mechanism of α-keto acid dehydrogenases (Figure 6A) leads to a hypothesis that acid pH may allow larger α-keto acids to enter the substrate binding pocket of LDH. An ensuing prediction, is that LDH should be able to catalyze the MDH reaction (OAA to malate) under acidic conditions. Figure S8A shows this is indeed the case. These findings, along with the well-known occurrence of 2-hydroxybutyrate as a biomarker of mitochondrial dysfunction (α-KGDH) (commonly associated with metabolic acidosis) suggest that α-keto to α-hydroxy acid conversion is a common metabolic signature of acidosis.

2-HG is an epigenetic signal, and an inhibitor of α-KGDH-dependent dioxygenases [7][8], including JmjC domain histone demethylases, TET 5-methylcytosine hydroxylases, AKB homolog DNA/RNA demethylases, and EGLN prolyl-hydroxylases that regulate hypoxia inducible factor (HIF). It is also known that acid pH can activate HIF in normoxia [9]. To test the role of 2-HG in this signaling pathway, we generated a cell line stably over-expressing L-2HGDH (Figure 7), and these cells showed blunted 2HG generation with acid+KMW. Furthermore, Figure 8 shows that acid induction of HIF was abrogated in L-2HGDH over-expressing cells. These data indicate a role for 2-HG in acid induction of HIF.

We previously showed 2HG is generated in the heart under the cardioprotective paradigm of ischemic pre-conditioning [10]. Thus we hypothesized 2HG may play a protective role against ischemia-reperfusion (IR) injury. Figure 9 shows that a cell-permeable di-methyl-L-2HG ester conferred cardioprotection in a perfused mouse heart model of IR injury. Signaling targets engaged by 2HG to afford this protection are currently under investigation.