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ADHFE1 is a MYC-linked oncogene that induces metabolic reprogramming and cellular de-differentiation in breast cancer

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ABSTRACT

The oncometabolite, *D*-2-hydroxyglutarate, accumulates in various cancers because of acquired mutations in isocitrate dehydrogenase 1 & 2. Here, we describe a new mechanism for *D*-2-hydroxyglutarate accumulation in breast cancer. It involves c-Myc signaling and alcohol dehydrogenase, iron-containing protein 1 (ADHFE1) and leads to metabolic reprogramming, de-differentiation, and increased mammary tumorigenesis.

During malignant transformation, signaling by the c-Myc/MYC proto-oncogene protein or the loss of the tumor suppressor, *TP53* (also known as p53), can rewire metabolic pathways to support tumor growth and metastasis.[1] We previously examined breast tumors and adjacent non-cancerous tissue from 67 patients and described the differential abundance of more than 200 metabolites.[2] One of them was 2-hydroxyglutarate, which was elevated up to 100-fold in tumors that were primarily estrogen receptor-negative. This accumulation of 2-hydroxyglutarate was closely associated with the co-occurrence of a c-Myc signaling signature in the tumors. *D*-2-hydroxyglutarate is a oncometabolite that accumulates in gliomas, leukemias, and other cancers because of acquired gain-of-function mutations in either cytosolic isocitrate dehydrogenase (*IDH1*) or mitochondrial *IDH2*.[3] However, our investigations did not detect these mutations in the studied breast tumors, indicating that an alternative mechanism must lead to increased 2-hydroxyglutarate in breast cancer.

Both *D*- and *L*-2-hydroxyglutarate are oncometabolites[4,5] and their accumulation – much like the increase in succinate and fumarate because of loss-of-function mutations in the succinate and fumarate dehydrogenase genes – inhibits α-ketoglutarate-dependent dioxygenases. Inhibition of these enzymes leads to DNA and histone hypermethylation, cellular de-differentiation, and predisposition to cancer.[6,7] Because the investigations by *Terunuma et al.*[2] neither identified the enantiomeric form of 2-hydroxyglutarate or the cause for its accumulation, we continued to interrogate the relationship between c-Myc signaling and increased 2-hydroxyglutarate in breast tumors. In the follow-up study by *Mishra et al.*,[8] we now show that human breast tumors produce *D*-2-hydroxyglutarate as the predominant enantiomer whereas increased *L*-2-hydroxyglutarate occurs in some tumors in association with lactate dehydrogenase B expression. Furthermore, we describe *D*-2-hydroxyglutarate-producing alcohol dehydrogenase, iron-containing protein 1 (ADHFE1) as a breast cancer oncoprotein that is associated with disease survival. We show that ADHFE1 promotes a reductive glutamine metabolism with increased *D*-2-hydroxyglutarate and mitochondrial reactive oxygen species (ROS) formation (Figure 1). This leads to cellular de-differentiation and enhanced epithelial-mesenchymal transition (EMT), thereby phenocopying alterations in *IDH*-mutant cancer cells with high *D*-2-hydroxyglutarate. We also show that *D*-2-hydroxyglutarate by itself induces EMT and alters metabolism in mammary epithelial cells, including upregulation of both reductive carboxylation of glutamine-derived α-ketoglutarate and mitochondrial ROS.

We focused our investigations on ADHFE1 because *ADHFE1* and *MYC* locus amplifications co-occur in breast tumors, as shown by *Mishra et al.*.[8] Additional data indicated the existence of a regulatory feedback loop between c-Myc signaling and ADHFE1 protein expression. The function of *ADHFE1* as a candidate oncogene was corroborated when MCF7 human breast cancer cells overexpressing *ADHFE1*, *MYC*, or both *ADHFE1* and *MYC* were injected into the mammary gland of mice. ADHFE1 and c-Myc expression not only enhanced tumor growth in a synergistic manner but also increased intratumor levels of 4HB and 2-hydroxyglutarate. 4HB is a substrate for ADHFE1 to produce *D*-2-hydroxyglutarate. c-Myc signaling can increase 4HB formation through upregulation of the enzyme, Aldo-Keto Reductase
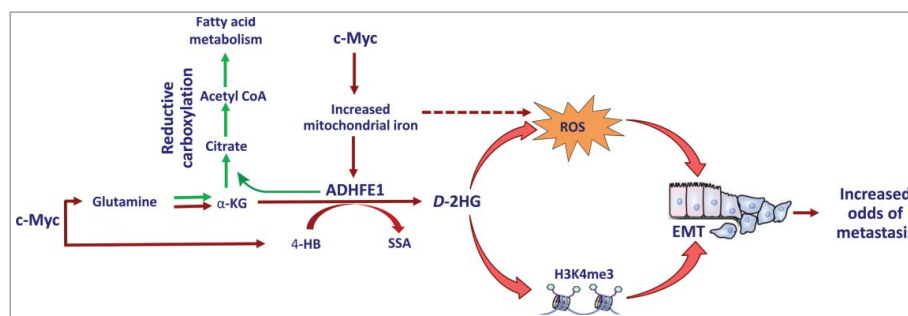
Figure 1. Upregulation of ADHFE1 promotes accumulation of *D*-2-hydroxyglutarate in breast tumors. c-Myc signaling increases *D*-2-hydroxyglutarate (*D*-2HG) by increasing the availability of both the substrates [4-hydroxybutyrate (4-HB) and α-ketoglutarate (α-KG)] and iron for the ADHFE1 enzyme. ADHFE1 and *D*-2HG up-regulate reductive carboxylation of glutamine-derived α-KG, reactive oxygen species (ROS) formation, and histone methylation at H3K4me3, leading to increased acetyl coenzyme A (acetyl CoA) and fatty acid synthesis and epithelial-mesenchymal transition (EMT). SSA, succinic semialdehyde; H3K4me3, histone trimethylation at histone H3, lysine 4.

Family 7 Member A2, thereby enhancing *D*-2-hydroxyglutarate production in breast tumors. While *Mishra et al.* provided preliminary data to support this hypothesis, additional research is needed to elucidate the potentially oncogenic function of 4HB synthesis in breast cancer biology.

ADHFE1 enzymatic activity may have an important function in fatty acid metabolism. Its upregulation in MCF10A and MCF12A mammary epithelial cells induced prominent changes in metabolism including reductive carboxylation of α-ketoglutarate, which is a metabolic pathway that promotes fatty acid synthesis in the absence of oxygen. Accordingly, *ADHFE1*-overexpressing cells acquired a growth advantage when cultured under hypoxic conditions. ADHFE1 also induced a moderate but significant increase in mitochondrial ROS. The latter, but not reductive carboxylation of α-ketoglutarate, was found to be a key factor to the induction of EMT by ADHFE1. Currently, it appears that the accumulation of *D*-2-hydroxyglutarate accounts for most of the phenotypes associated with ADHFE1 (e.g., increased reductive carboxylation, ROS, and EMT). Yet, additional investigations should test whether ADHFE1 promotes oncogenic phenotypes independent of *D*-2-hydroxyglutarate.

ADHFE1 is an iron-sulfur enzyme and its expression depends on iron availability, as *Mishra et al.* showed. c-Myc is a key regulator of iron metabolism and up-regulates genes in this pathway.[9] Since we did not find that c-Myc induces ADHFE1 through promoter activation, the effect of c-Myc on iron metabolism is likely the primary driver of c-Myc-induced ADHFE1. Moreover, an increased expression of iron metabolism genes has been reported for breast cancer cell lines with an aggressive mesenchymal phenotype,[10] which tend to have aberrant c-Myc signaling. Little is known about the function of ADHFE1 in these cells. Future studies may test whether 2-hydroxyglutarate and ROS, together with increased iron, account for at least some of the aggressive biology of mesenchymal breast cancer cell lines. Another key finding of our study is the observation that ADHFE1 significantly upregulates intracellular *D*-2-hydroxyglutarate under hypoxic conditions. Because of the additional finding that *ADHFE1*-overexpressing cells efficiently secrete *D*-2-hydroxyglutarate into the extracellular space, we speculate that an increased ADHFE1 enzymatic activity may not only affect cell biology but also the tumor microenvironment through *D*-2-hydroxyglutarate-mediated effects, particularly under hypoxic conditions.

In conclusion, our study shows that human breast tumors produce oncogenic *D*-2-hydroxyglutarate using a distinct pathway that involves c-Myc signaling and the mitochondrial enzyme, ADHFE1 (Figure 1). The upregulation of ADHFE1 and *D*-2-hydroxyglutarate similarly induced EMT, stemness, and invasion of mammary cells through increased ROS and changes in histone and DNA methylation. Because 2-hydroxyglutarate accumulates more so in basal-like breast tumors than other breast cancer subtypes, as shown by *Terunuma et al.*,[2] inhibition of ADHFE1 may offer a new therapeutic strategy for basal-like and triple-negative breast cancers.

Disclosure of potential conflict of interest

The authors declare no conflict of interest.

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