Blood Science

R-2-HG in AML . . . friend or foe?

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Cytosolic isocitrate dehydrogenase 1 (IDH1) and its mitochondrial counterpart, IDH2, are critical TCA cycle enzymes that catalyze the oxidative decarboxylation of isocitrate to produce alpha-ketoglutarate (α-KG). Mutations in IDH1/2 occur in ~80% of grade II-III gliomas and secondary glioblastomas,[1–3] and in 10% to 20% of acute myeloid leukemia (AML).[4–6] To date, all identified mutations in IDH1/2 are heterozygous, missense mutations, leading to the substitution of arginine 132 in IDH1 and arginine 172 or 140 in IDH2. Mutant IDH1/2 heterodimerize with wild type IDH proteins to facilitate their neomorphic activity, in which they utilize wild type product α-KG to produce the putative oncometabolite R(−)-2-hydroxyglutarate (R-2-HG).[7,8]

Because R-2-HG is structurally similar to α-KG, its accumulation in cells with IDH1/2 mutations leads to the competitive inhibition of α-KG-dependent dioxygenases.[9] Among these dioxygenases is the TET family of 5-methylcytosine (5mC) hydroxylases that convert 5mC to 5-hydroxylmethycytosine. In AML, R-2-HG has been shown to promote leukemogenesis through its inhibition of TET2 and the subsequent induction of histone- and DNA-hypermethylation.[10] Moreover, cell-based models have shown that cell permeable R-2-HG alone, in the absence of intracellular IDH1/2 mutations, is sufficient to induce a block in differentiation and promote hematopoietic cell transformation.[11] These findings and others provided the impetus to develop IDH inhibitors for the clinical treatment of *IDH1/2*-mutated cancers, which in AML patients have been shown to significantly reduce R-2-HG levels patient sera, reverse epigenetic changes due to its accumulation, and promote leukemia cell differentiation.[12]

In the most recent issue of Molecular Cell, Qing et al[13] follow up on their previously published work challenging the concept of R-2-HG as an oncometabolite. The authors hypothesize that because *IDH*-mutated glioma, glioblastoma, and AML patients have longer overall survival compared to *IDH*-wild type patients, and because only ≤40% of *IDH*-mutated AML patients respond to IDH inhibitor therapy, R-2-HG may instead act as anti-tumorigenic rather than in a tumor-promoting manner.

Previously, the authors demonstrated that R-2-HG induces cell cycle arrest and apoptosis in cell lines expressing wild type IDH1/2 by inhibiting the m^6A RNA demethylase fat mass and obesity-associated protein (FTO), an α-KG-dependent dioxygenase.[14] Here, the authors further dissect the functional consequence of R-2-HG-mediated inhibition of FTO, focusing on metabolic alterations induced by this signaling axis. The authors show that R-2-HG attenuates pro-leukemogenic glycolytic flux in AML cells by suppressing FTO-mediated up-regulation of two key glycolytic enzymes: phosphofructokinase platelet (*PFKP*) and lactate dehydrogenase B (*LDHB*).

This group previously screened 27 IDH1/2-wild type AML cell lines for their growth sensitivity to R-2-HG.[14] Here, they have selected one AML cell line whose growth was inhibited upon R-2-HG treatment in their original screen (NOMO1—"sensitive"), and one AML cell line whose growth was resistant to R-2-HG treatment (NB4—"resistant"). It is important to note that none of these cell lines expresses endogenous mutant IDH1/2, and thus would not naturally accumulate R-2-HG. Both cell lines were treated +/− an unknown concentration of a cell permeable form of R-2-HG prior to metabolomic analysis. Through this and subsequent metabolic assays in which cells are treated with two different concentrations of R-2-HG (50 and 300 μM), they identify glycolysis as a major metabolic pathway that is differentially suppressed by R-2-HG in "sensitive" versus "resistant" cell lines.

Having previously shown that FTO is a major inhibitory target for R-2-HG, the authors then go on to demonstrate that R-2-HG-mediated inhibition of FTO contributes to the suppression of glycolysis specifically in "sensitive" cells. Using shRNA-mediated knockdown and small molecule inhibition of FTO m^6A demethylase activity, they demonstrate that glycolysis is decreased in NOMO-1 cells upon genetic or pharmacological inhibition of FTO. To further dissect the metabolic target genes involved in R-2-HG-FTO-mediated suppression of glycolysis, the authors identify a subset of glycolytic genes that contain m^6A modifications and thus would be targets of FTO, and whose expression was suppressed by knockdown of *FTO* and by treatment with R-2-HG. Among these, two glycolytic genes, *PFKP* and *LDHB*, were found to be the most significantly downregulated in "sensitive" but not "resistant" cells treated with cell permeable R-2-HG, or subjected to induced expression of mutant IDH1 to promote endogenous production of R-2-HG. The authors go on to demonstrate that FTO directly binds to *PFKP* and *LDHB* via their m^6A sites, which are increased upon *FTO* knockdown or R-2-HG-treatment. Because m^6A plays an important role in regulating mRNA stability, the authors measured stability of *PFKP* and *LDHB* transcripts upon *FTO*

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knockdown, and found decreased stability of both. They then go on to show that YTHDF2, which is well known to destabilize m^6A containing mRNA transcripts, binds directly to *PFKP* and *LDHB* in *FTO* knockdown cells, leading to their downregulation

To evaluate how clinically targetable the R-2-HG-FTO-YTHDF2-PFKP/LDHB axis is, the authors utilize primary CD34+ hematopoietic stem/progenitor cells (HSPCs) to test the functional effects of modulating this pathway. Compared to "sensitive" AML cell lines, proliferation and survival of CD34+ cells were both unaffected by knockdown of *FTO*, *PFKB*, or *LDHB*. The authors therefore suggest that targeting this pathway would have minimal off-target toxicity by sparing normal cells and only affecting AML cells. They go on to use xenotransplantation animal models to demonstrate that loss of *FTO*, *PFKB*, and *LDHB* all attenuate leukemic disease progression *in vivo*, suggesting they are viable targets for AML therapy.

Although this work has provided a novel perspective on the pleiotropic role of R-2-HG in AML, several outstanding questions must be addressed. First, the foundation of this work is based on the authors' use of cell lines with wild type IDH1/2 that are already transformed by intrinsic genetic backgrounds that differ from IDH mutations to assess the effects of R-2-HG on proliferation and survival. Because R-2-HG is only produced to accumulating levels in the presence of mutant IDH1/2, this does not represent a physiologically relevant model of R-2-HG+ AML. This makes it difficult to glean the clinical and therapeutic implications of understanding how a metabolite is produced in the context of oncogenic mutation cells that do not harbor that mutation.

Secondly, the physiological concentrations of R-2-HG vary widely—in *IDH*-mutated AML patient sera, R-2-HG levels have been shown to range from 10 to 30,000 ng/mL.[15] However, the intracellular concentrations of R-2-HG in primary leukemia cells from these *IDH*-mutated AML patients were not determined. It is therefore difficult to correlate whether this serum range of R-2-HG levels would correlate with an intracellular range of R-2-HG in AML cells in these patients, and whether at either extreme of that range, R-2-HG has differing or opposite effects on AML cell transformation. In studies where extracellular treatment with cell-permeable R-2-HG has been shown to exhibit oncometabolite activity, concentrations range from high μM to mM range. For instance, in demonstrating that R-2-HG is a competitive inhibitor of α-KG-dependent dioxygenases, Xu et al[9] utilize 50 mM R-2-HG. Losman et al[11] demonstrate a dose-dependent increase of cytokine-independent proliferation in TF-1 cells treated with R-2-HG concentrations that range from 100 to 500 μM, where concentrations above 250 μM have the most significant effect on cytokine-independent proliferative ability. Here, the authors use 50 and 300 μM to demonstrate dose-dependent suppression of glycolysis and consequently cell proliferation. However, all of the aforementioned studies did not determine the intracellular concentrations of R-2-HG that correlate with differing or opposite effects on different cellular responses in different cell lines, and whether this intracellular range of R-2-HG accumulated in cells treated with cell-permeable R-2-HG is within the physiological range of R-2-HG levels in primary AML cells harboring *IDH* mutations. A comprehensive dose-effect study on how different intracellular concentrations of R-2-HG, within the physiological range, affect AML cell differentiation, proliferation, and survival, is thus needed.

Finally, the AML cell lines used reflect cells that are frozen in time, rather than leukemic cells that are differentiating from stem cell to blast. It is possible that R-2-HG differentially affects leukemia stem cells, blast cells, and more fully differentiated cells, and that R-2-HG has pleiotropic effects, perhaps depending on concentration, over the course of this evolution. This level of nuance is impossible to dissect when using cell line models only. Furthermore, the authors use primary HSPCs to test the effects of targeting this pathway on normal, healthy tissue. HSPCs, including leukemia HSPCs, are a quiescent cell population that is not glycolytic like the AML cell lines they use as a comparison, so are unlikely to be affected by inhibition of glycolysis. Better control for this comparison would be glycolytic tissue from healthy donors. More studies are warranted to better understanding how a range of R-2-HG concentrations affects leukemic cells as they evolve from stem to progenitor to blast cell.

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