Targeting glycolysis to rescue 2-hydroxyglutarate immunosuppressive effects in dendritic cells and acute myeloid leukemia

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In this issue of *Haematologica*, Hammon *et al.*[1] report their investigation of the link between the immunosuppressive effects of D-2-hydroxyglutarate (D-2HG) and metabolic reprogramming in dendritic cells and acute myeloid leukemia (AML).

The discovery that mutations in genes encoding key metabolic enzymes lead to the accumulation of oncometabolites has underscored a direct connection between altered metabolism and disease. Among the plethora of metabolites affecting tumorigenesis and the surrounding immune cell subsets, much interest has been invested in the metabolite 2-hydroxyglutarate (2HG). The enantiomer D-2HG is produced by cancer cells with gain-of-function mutations in isocitrate dehydrogenase (IDH) enzymes. While its role in tumorigenesis has been extensively described, recent studies are revealing D-2HG cell-nonautonomous functions and a key role as a regulator of immunity, through metabolic crosstalk within the tumor microenvironment.

In 2018, D-2HG was shown to fine tune immune responses by affecting T-cell metabolism.[2] Exogeneous D-2HG triggered destabilization of hypoxia-inducible factor 1α protein in T cells, resulting in the downregulation of the glycolytic enzymes lactate dehydrogenase A (LDHA) and pyruvate dehydrogenase kinase 1, thus decreasing lactate production. Glucose is directed to the tricarboxylic acid cycle resulting in metabolic skewing towards oxidative phosphorylation (OxPHOS), increased regulatory T-cell abundance, and reduced T helper 17 cell polarization. This is one of the strategies of AML cells to create a permissive environment promoting immune evasion. Recently, it was also reported that D-2HG reduces murine $CD8^+$ T-cell proliferation, cytotoxicity, interferon-γ (IFNγ) signaling, and directly inhibits LDHA/B.[3] Accordingly, in patients with IDH1-mutant gliomas, regions with high levels of 2HG correlate with lower lactate concentration and fewer $CD8^+$ T cells. LDH inhibition induced an altered NAD(H) balance, leading to an increased dependency on complex I of the electron transport chain. Therefore, $CD8^+$ T cells treated with D-2HG displayed higher OxPHOS, while no change in glucose consumption was observed. Pharmacological inhibition of LDH recapitulated the effects of D-2HG, such as the decrease in IFNγ signaling, confirming the key role of LDH regulation and glycolysis flux in T-cell functions.

Hammon *et al.*[1] focused on another important myeloid cell population, dendritic cells, for which the link between the immunosuppressive effects of D-2HG and metabolic reprogramming had not been previously investigated. Interestingly, they showed that human monocyte-derived dendritic cells use a different mechanism compared to T cells. In dendritic cells, D-2HG led to a decrease of major histocompatibility class II (MHC II) expression (HLA-DP, HLA-DR) and function (INFγ and interleukin-12 secretion), thus reducing T-cell stimulation and favoring the immune escape of AML cells (Figure 1). Contrarily to T cells, D-2HG treatment increased glucose uptake, lactate production, LDHA expression, and delayed methylation in dendritic cells, as well as enhancing mitochondrial respiration. Treatment of dendritic cells with exogenous lactate altered the differentiation of these cells, mimicking the effect of D-2HG. Reactivation of the D-2HG target, Tet methylcytosine dioxygenase 2 (TET2), with vitamin C restored DNA demethylation and oxygen consumption but did not alter lactate levels or MHC II antigen expression. Finally, the addition of LDHA inhibitors to vitamin C decreased lactate levels and partially restored the expression of MHC II antigens and dendritic cell markers, indicating promising opportunities for a dual approach targeting metabolic dependencies and epigenetic plasticity.

Interestingly, Everts *et al.* previously showed that Toll-like receptor-induced activation of dendritic cells depended on

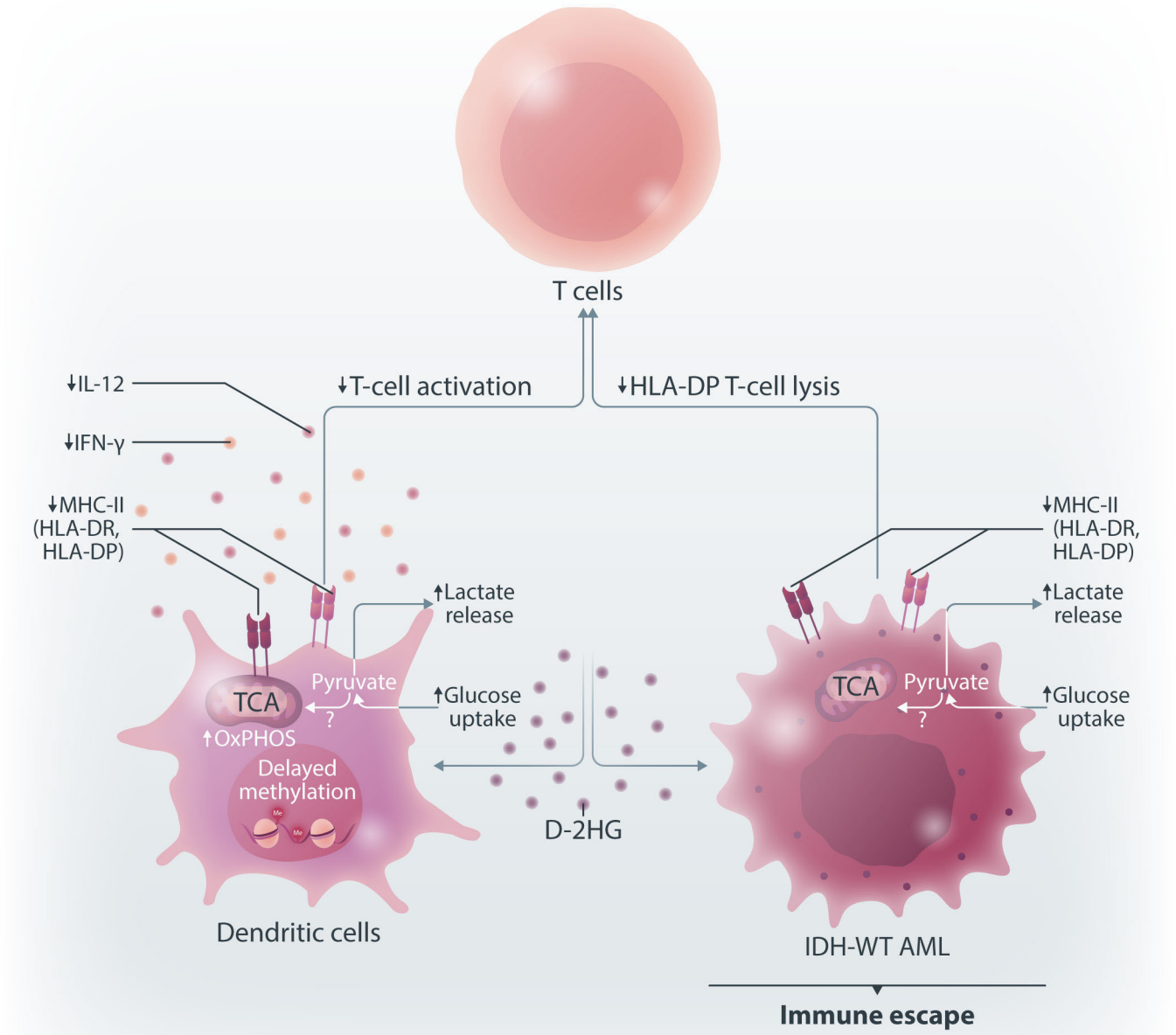

Figure 1. D-2-hydroxyglutarate-driven immune escape through metabolic reprogramming of dendritic cells and acute myeloid leukemia cells. Exogeneous D-2-hydroxyglutarate (D-2HG) treatment impairs dendritic cell differentiation leading to a decrease of major histocompatibility class (MHC) II expression and T-cell activation. Metabolically, D-2HG increases glucose uptake and lactate release, as well as mitochondrial respiration. Exogeneous D-2HG treatment also enhances glucose uptake and lactate production as well as lowering MHC II expression in IDH wild-type acute myeloid leukemia (AML) cells, leading to reduced HLA-DP T-cell lysis. Altogether, D-2HG drives immune escape in AML. TCA: tricarboxylic acid; IDH: isocitrate dehydrogenase; WT: wild-type; OxPHOS; oxidative phosphorylation; IFN: interferon; IL: interleukin.

the glycolytic flux towards the tricarboxylic acid cycle.[4] In the study by Hammon *et al.*[1] metabolomic analyses of the supernatant of D-2HG-treated dendritic cells did not show a significant increase of glutamine or proline uptake, leading to questioning whether the higher glucose consumption could also feed the tricarboxylic acid cycle, explaining the observed higher oxygen consumption. If this holds true, the partial rescue of differentiation could be explained by inhibiting LDH alone, without blocking the entry of pyruvate into the tricarboxylic acid cycle. Further investigations of the catabolic fates of nutrients following D-2HG treatment through isotopic profiling or genetic manipulations could help a better understanding of the link between dendritic cell activation and glycolysis adaptation.

Immune escape of leukemic cells in relapsing AML patients is also driven by the downregulation of MHC II genes and proteins (HLA-DP, HLA-DR).[5] Thus, another interesting point is raised in the second part of the study in which the authors compared the effects of D-2HG on dendritic cells and AML cells, showing major similarities (Figure 1). Indeed, D-2HG increased glucose uptake and lactate production in primary AML blasts, and lactate concentration inversely correlated with HLA-DP or HLA-DR levels. Accordingly, lysis by HLA-DP-specific T cells was reduced in IDH-mutant AML primary cells. While the combination of LDH inhibitors and vitamin C significantly decreased AML viability, in particular in patients harboring IDH mutations, HLA-DR and HLA-DP expression was increased independently of IDH status. Nevertheless, the therapeutic effect was driven mostly by vitamin C and the potential of LDHA inhibitors

needs further investigation in the context of AML.

Of particular interest, D-2HG has been shown to decrease aerobic glycolysis in a panel of D-2HG-sensitive leukemia cells and cases of primary IDH-wildtype AML through epitranscriptomic regulation mediated by fat-mass- and obesity-associated protein (FTO).[6] Inhibition of FTO by D-2HG increased global N6-methyladenosine (m6A) RNA modification and suppressed the expression of critical glycolytic genes including *LDHB*, leading to inhibition of the glycolytic flux with no impact on mitochondrial respiration. Low levels of FTO and hyperactivation of MYC signaling were observed in IDH-mutant AML and D-2HG-resistant cells, and led to the maintenance of glycolysis and OxPHOS following treatment with D-2HG.[7] In their study published in this issue, Hammon *et al.*[1] noted the induction of MYC expression in dendritic cells following exposure to D-2HG, suggesting that these cells may be resistant to D-2HG. Direct targeting of MYC has proven to be challenging due to its role as a transcriptional modulator. However, a strategy to attenuate its activity may become relevant for treating leukemias and simultaneously restoring dendritic cell phenotype, counteracting immune escape.

Altogether the effects of D-2HG on metabolic rewiring, in particular glycolysis and OxPHOS, are cell-type-dependent and are strictly interconnected with the tumor microenvironment. Therefore, a better understanding of the role of D-2HG in reshaping the tumor microenvironment will be instrumental to developing better therapeutic strategies. In that direction, decreasing D-2HG levels with IDH mutant inhibitors in gliomas, improved T-cell infiltration and anti-tumor efficacy of peptide vaccines,[8] as well as activation and expansion of dendritic cells enhancing tumor regression in combination with anti-PDL1 immune checkpoint blockade.[9] Moreover, some metabolic determinants of IDH-mutant AML, such as increased fatty acid oxidation, are not reversed by IDH mutant inhibitors and are thus independent.[10] Improving the classification of metabolic adaptations as either D-2HG-dependent or -independent in IDH mutant-driven cancers will be critical to design more efficient clinical strategies and improve the efficacy of IDH mutant inhibitors alone or in combination.

Disclosures

No conflicts of interest to disclose.

Contributions

Both authors contributed equally.

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