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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, Renner *et al*.[1] investigated the link between D-2-hydroxyglutarate (D-2HG) immunosuppressive effects and metabolic reprogramming in dendritic cells (DCs) 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 (2-HG). The enantiomer D-2HG is produced by cancer cells with gain-of-function mutations in isocitrate dehydrogenases (IDH) enzymes. While its role in tumorigenesis has been extensively described, recent studies are reporting D-2HG cell-nonautonomous functions and key role as a regulator of immunity, through metabolic crosstalk within the tumor microenvironment (TME).

In 2018, D-2HG was shown to fine tune the immune responses by affecting T cell metabolism[2]. Exogeneous D-2HG triggered HIF-1α protein destabilization 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 TCA cycle resulting in metabolic skewing towards oxidative phosphorylation (OxPHOS), increased regulatory T cell abundance, and reduced T helper 17 cells polarization. This comprises 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 level 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 ETC complex I. Therefore, CD8+ T cells treated with D-2HG displayed higher OxPHOS, while no change in glucose consumption was observed. Pharmacological inhibition of LDH recapitulated D-2HG effects, such as the decrease in IFNγ signaling, confirming the key role of LDH regulation and glycolysis flux for T cell functions.

Furthermore, Hammon, Renner *et al*.[1] focused on another important myeloid cell population, the dendritic cells (DCs), for which the link between D-2HG immunosuppressive effects and metabolic reprogramming was not yet investigated. Interestingly, they showed that human monocyte-derived DCs use a different mechanism compared to T cells. In DCs, D-2HG led to a decrease of the major histocompatibility class II (MHC II) expression (HLA-DP, HLA-DR) and function (INFγ and IL-12 secretion), thus reducing T cells stimulation and favoring AML immunoescape (Figure 1). Contrarily to T cells, D-2HG treatment increased glucose uptake, lactate production, LDHA expression, and delayed methylation in DCs, together with enhanced mitochondrial respiration. Treatment of DCs with exogenous lactate altered DC differentiation, mimicking D-2HG. Reactivation of the D-2HG target TET2 with Vitamin C (VitC) restored DNA demethylation and oxygen consumption but did not alter lactate level nor MHC II antigens expression. Finally, the addition of LDHA inhibitors to VitC decreased lactate level and partially restored MHC II antigens and DC markers expression, indicating promising opportunities for a dual approach targeting metabolic dependencies and epigenetic plasticity.

Interestingly, Everts et al. previously showed that Toll-like receptor (TLR)-induced activation of DCs depended on the glycolytic flux towards TCA cycle[4]. In this study by Hammon, Renner *et al.*[1], metabolomic analyses of the supernatant of D-2HG-treated DCs did not show a significant increase of glutamine or proline uptakes, leading to questioning whether the higher glucose consumption could also feed the TCA cycle, explaining the observed higher oxygen consumption. If it holds true, the partial rescue of differentiation could be explained by inhibiting LDH alone, without blocking the entry of pyruvate into the TCA cycle. Further investigations of the catabolic fates of nutrients following D-2HG treatment through isotopic profiling or genetic manipulations could help better understand the link between DCs activation and glycolysis adaptation.

Immunoescape 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 where the authors compared the effect of D-2HG on DCs 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 VitC significantly decreased AML viability, in particular in patients harboring IDH mutations, HLA-DR and HLA-DP expressions were increased independently from IDH status. Nevertheless, the therapeutic effect was mostly VitC-driven and the potential of LDHA inhibitors needs further investigation in the AML context.

Of particular interest, D-2HG has been shown to decrease aerobic glycolysis in a panel of D-2HG-sensitive leukemia cells and primary IDH-wild type AMLs 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 the 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 AMLs and D-2HG resistant cells, and led to the maintenance of glycolysis and OxPHOS following D-2HG[7]. In the current issue, Hammon, Renner, and colleagues[1] noted the induction of MYC expression in DCs following D-2HG exposure suggesting that these cells may be resistant to D-2HG. MYC direct targeting has proven to be challenging due to its role as transcriptional modulator. However, a strategy to attenuate its activity may become relevant for treating leukemias and simultaneously restoring DCs phenotype, counteracting immune escape.

Altogether, D-2HG effects on metabolic rewiring, in particular glycolysis and OxPHOS, are cell-type dependent and are strictly interconnected to the TME. Therefore, a better understanding of the role of D-2HG in reshaping the TME will be instrumental to develop better therapeutic strategies. In that direction, decreasing D-2HG level with IDH mutant inhibitor in gliomas, improved T-cell infiltration and anti-tumor efficacy of peptide vaccine[8], as well as activation and expansion of DCs enhancing tumor regression in combination with an anti-PDL1 immune checkpoint blockade[9]. Moreover, some metabolic determinants of IDH mutant AMLs such as increased fatty acid oxidation are not reversed by IDH mutant inhibitors and are thus D-2HG independent[10]. Improving the classification of the metabolic adaptations either as 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.

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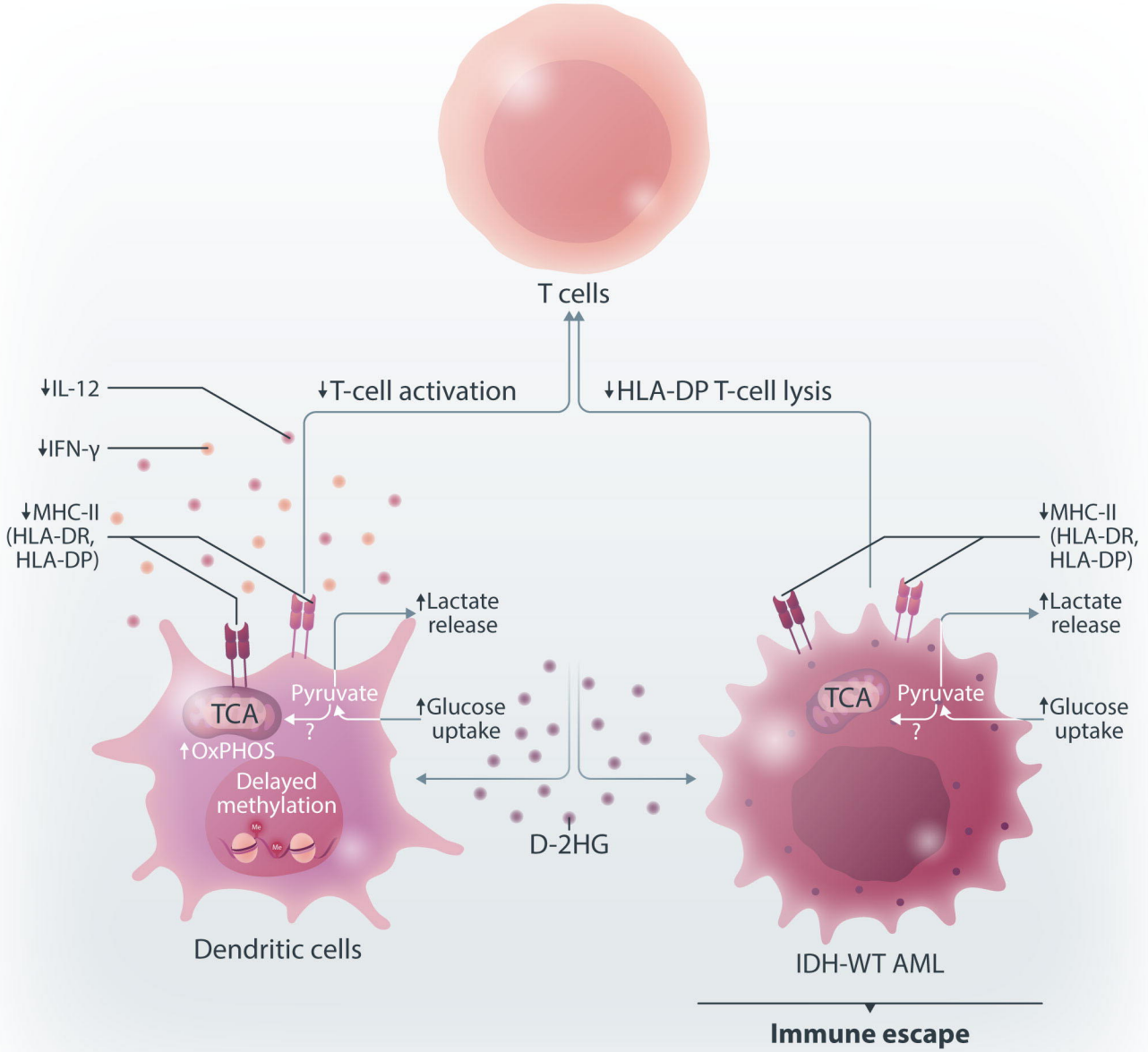
Figure 1. D-2HG-driven immune escape through metabolic reprogramming of DCs and AML. Exogeneous D-2HG treatment impairs DCs differentiation leading to a decrease of MHC II expression and T-cell activation. Metabolically, D-2HG increased glucose uptake and lactate release, as well as mitochondrial respiration. In IDH wild-type (WT) AML cells, exogeneous D-2HG treatment also enhanced glucose uptake and lactate production as well as lowered MHC II expression leading to reduced HLA-DP T cells lysis. Altogether, D-2HG drives immune escape in AML. Created with BioRender.com.


T cells
↓T-cell activation
↓HLA-DP T-cell lysis
↓IL-12
↓IFN-γ
↓MHC-II (HLA-DR, HLA-DP)
↑Lactate release
TCA
Pyruvate
?
↑Glucose uptake
↑OxPHOS
Delayed methylation
Me
Me
D-2HG
Dendritic cells
↓MHC-II (HLA-DR, HLA-DP)
↑Lactate release
TCA
Pyruvate
?
↑Glucose uptake
IDH-WT AML
Immune escape