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703.CELLULAR IMMUNOTHERAPIES: BASIC AND TRANSLATIONAL

Glycolysis-Driven IFN- γ Expression in T-Cell Via Histone Lactylation Promotes Acute Graft-Versus-Host Disease

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Background

Cell metabolism is a highly regulated process essential for sustaining life and driving evolution. Cellular metabolites and metabolic flux intricately govern post-translational modifications of proteins. In the context of acute graft-versus-host disease (aGVHD), T cells undergo enhanced glycolysis, resulting in increased lactate production. Consequently, lactate may act as a substrate for lysine lactylation (Kla), influencing gene expression by modulating histone biology. This study aims to assess T cell glucose metabolism during aGVHD, investigate the role of histone lactylation, and explore its impact on T cell functionality.

Methods

We conducted a comparative analysis of histone lactylation between aGVHD patients and individuals undergoing allo hematopoietic stem cell transplantation (control). Subsequently, we established a murine bone marrow transplant model and isolated T cells for analysis. Glucose metabolism status was assessed using extracellular acidification rate (ECAR) and oxygen consumption rate (OCR) assays, along with the 2-NBDG glucose uptake assay. To comprehensively investigate histone lactylation in T cells, we employed the cleavage under targets and tagmentation (CUT&Tag) technique to explore its regulatory effects on gene expression. To investigate the impact of lactylation on aGVHD pathology, we constructed an aGVHD model by applying T cells with a specific deletion of LDHA.

Results

By analyzing the serum lactate at the onset of aGVHD, we found that patients with grade III-IV aGVHD had significantly higher lactate levels compared to those with grade I-II aGVHD (**Figure 1A**). In light of this finding, we further examined the histone lactylation levels in T cells from aGVHD patients. The results demonstrated an enhanced histone lactylation in these T cells compared to controls (**Figure 1B**).

In the aGVHD mice, the ECAR of T cells exhibited a significant increase (**Figure 1C**). Intriguingly, the OCR were also significantly higher in the aGVHD mice compared to the bone marrow T cell-depleted (BM-TCD) mice (**Figure 1D**). Additionally, the 2-NBDG revealed a significantly higher fluorescence intensity in aGVHD mice (**Figure 1E**). Through analysis of T cell RNA-seq data, we discovered upregulation of most glycolytic enzymes in aGVHD mice similar to human data (**Figure 1F**). Notably, LDHA, glucose transporter 1 (*Slc2a1*), and monocarboxylate transporter 4 (*Slc16a3*) were highly upregulated (**Figure 1G**).

The upregulation of LDHA in T cells from aGVHD mice prompted us to investigate the presence of histone lactylation. The results revealed that histone in T cells from aGVHD mice exhibited a higher level of lactylation (**Figure 1H**). Because lactylation was particularly prominent at the H4K12 site, we conducted CUT&Tag experiments. We found promoters of key regulatory genes of the Th1 subset, such as *Tbx21* and *Ifng*, along with several genes associated with IFN- γ production, including *Stim2*, *Crtam*, *Il18rap*, *Fasl*, and *Phf11a*, *Cxcr3* were all lactylated (**Figure 1I**). Specifically, the Call Peak analysis confirmed the elevated H4K12la levels on the promoters *Tbx21* and *Ifng* (**Figure 1J**). The expression of IFN- γ and CXCR3 in CD4⁺ and CD8⁺ T cells of aGVHD mice was significantly higher than in BM-TCD mice (**Figure 1K**).

To confirm the role of histone lactylation in IFN- γ generation, we intervened with T cells using the p300 lactylation writer inhibitor (A485). The results demonstrated a significant reduction in histone lactylation upon A485 treatment, which was effectively restored by the addition of neutral lactate sodium (Nala) (**Figure 1L**). Notably, A485 treatment led to a decrease in IFN- γ ⁺ T cells, while Nala administration resulted in a substantial recovery of IFN- γ expression (**Figure 1M**). Additionally, the infusion of *Cd4*^{cre} *Ldha*^{fl/fl} T cells into recipient mice improved the survival of aGVHD mice (**Figure 1N**).

Conclusion

This study represents the first global investigation of T cell histone lactylation in aGVHD. Importantly, we have uncovered a novel link between histone lactylation and the regulation of IFN- γ -related gene expression in the context of aGVHD. Considering the heightened glycolysis and the pivotal role of IFN- γ in aGVHD pathology, it is plausible to postulate that T cell-produced lactate induces histone lactylation, thereby promoting the transcription of IFN- γ -related genes and contributing to the development of aGVHD.

Disclosures No relevant conflicts of interest to declare.

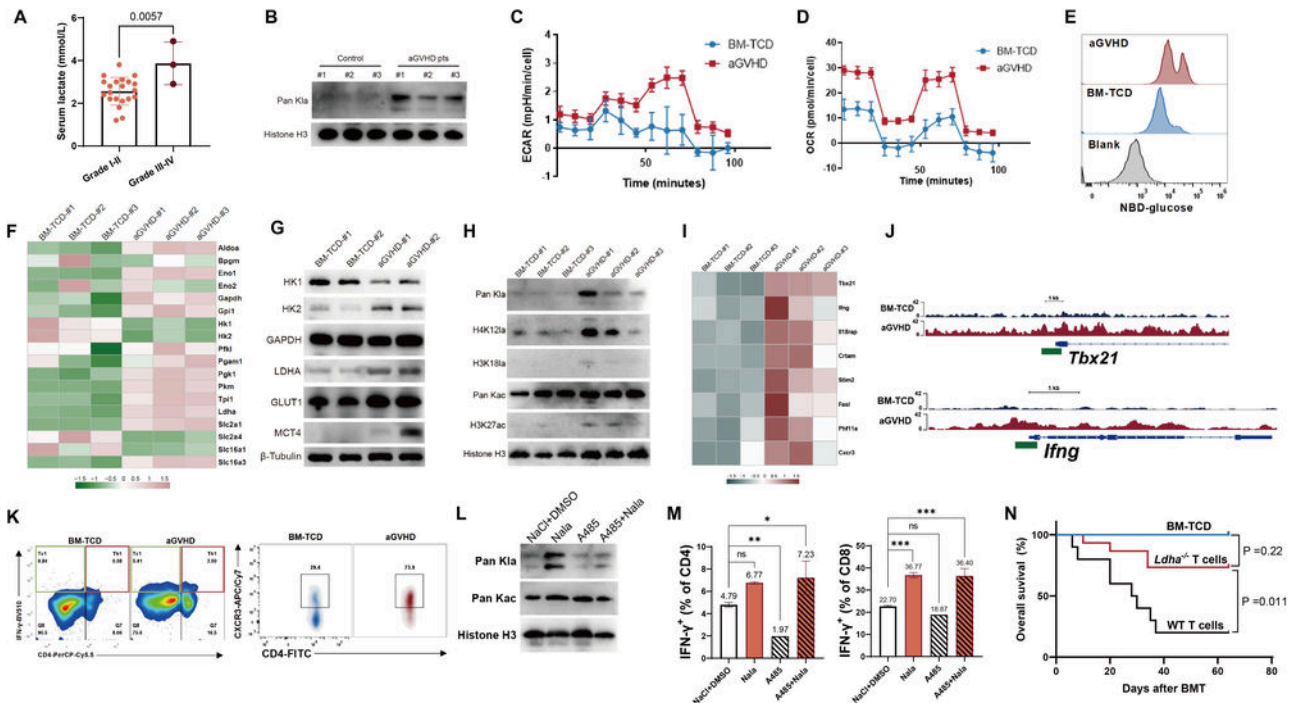


Figure 1. Heightened glycolysis of T cells driving histone lactylation to promote IFN- γ production. (A) Serum lactate of patients (pts) with grade I-II and III-IV aGVHD. (B) Western blotting analysis of Pan-histone lactylation in T cells from aGVHD patients and patients undergoing allogeneic hematopoietic stem cell transplantation without GVHD (control). (C) ECAR and (D) OCR of T cells were measured with Agilent Seahorse XF. (E) 2-NBDG glucose uptake assay of T cells. (F) The heatmap indicated the metabolic genes of the glycolytic pathway. (G) Confirmation of the expression of enzymes and transporters involved in the glycolytic pathway. (H) Histone K1a levels were examined in T cells. (I) Heatmaps displayed H4K12la-specific IFN- γ production-related genes. (J) Genome browser tracks depicted the CUT&Tag signal at the *Ifng* and *Tbx21* gene loci. (K) IFN- γ and CXCR3 expression in T cells from murine models. (L) Histone K1a levels in T cells after A485 and Na-lactate (Nala) interventions. (M) IFN- γ expression after A485 and Nala interventions. (N) Survival of BALB/c mice that received *Ldha*^{-/-} or wild type (WT) T cells from C57BL/6 donors.

Figure 1

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