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Lysine hydroxylase 1 (PLOD1) regulates glucose metabolism and promotes Th17 cell differentiation in psoriasis

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Abstract
Psoriasis is a common and chronic inflammatory skin disease. To treat psoriasis, more effective molecular targets still need to be developed and the mechanisms elucidated. PLOD1, as one of the genes related to glycolysis, can promote the glycolysis of cells. However, it is not clear whether upregulation of PLOD1 plays a causal role or is directly involved in psoriasis. Herein, we have examined the PLOD1 expression in patients with psoriasis and analyzed its effect on the progression of psoriasis. We found that PLOD1 is highly expressed in psoriasis. Also, the role of PLOD1 in promoting Th17 cell differentiation was further revealed, and PLOD1 promotes T cell glycolysis. Mechanically, PLOD1 activates the PI3K/AKT/mTOR pathway and thereby affects the progression of psoriasis. In conclusion, PLOD1 regulates glucose metabolism and promotes Th17 cell differentiation, which affects the progression of psoriasis.

Introduction

Psoriasis is a common skin disease that afflicts about 2% of the global population.[1] It is characterized by excessive proliferation and abnormal differentiation of keratinocytes.[2] Previous studies have found that communication between keratinocytes and immune cells, such as T cells, can induce skin damage in psoriasis. The immune imbalance of T cells affects the progression of psoriasis.[3] When susceptible individuals are exposed to various factors, such as microbial infections, they can activate and polarize their Th cells toward differentiation by Th17, Th1, and Th22 cell subsets.[4] In turn, these pro-inflammatory cytokines act on keratinocytes and endothelial cells, leading to activation, proliferation, and production of chemokines. To treat psoriasis, more effective molecular targets still need to be developed and the mechanisms elucidated.

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Previous epidemiological studies have confirmed that psoriasis is related to abnormal glucose metabolism. Existing research supports an association between psoriasis and type 2 diabetes, with more severe the psoriasis, the higher the risk of developing diabetes.[5] A study showed that postprandial glucose homeostasis was disturbed in nonobese patients with moderate to severe psoriasis with normal glucose tolerance. Compared with healthy controls, these patients had impaired postprandial glucose tolerance and increased secretion of islet beta cells.[6]

PLOD1, encoded by the procollagen lysine 2-oxyglutarate 5-dioxygenase 1 (PLOD1) gene, is an enzyme mediating the hydroxylation of Xaa-Lys-Gly lysyl residues.[7] PLOD1, as one of the genes related to glycolysis, promotes the glycolysis of GC cells.[8] Hypoxia-induced PLOD1 overexpression promotes glioblastoma via the NF-κB pathway.[9] PLOD1 promotes tumorigenicity and metastasis in bone cancer, while dysregulation of miR-34C-induced PLOD1/Hippo pathway affects tumor progression, providing a potential therapeutic strategy for treatment.[10] However, it is not clear whether upregulation of PLOD1 is critical or is directly involved in the pathogenesis of psoriasis.

Herein, we examined the expression of PLOD1 in psoriasis patients and analyzed its effect on the progression of psoriasis. PLOD1 is highly expressed in psoriasis patients and promotes T cell glycolysis and Th17 cell activation by promoting the activation of the PI3K/AKT/mTOR pathway.

Materials and Methods

GEO database analysis

The GEO database analysis of healthy control and psoriasis patients was performed online using the CHIP (GSE192867, http://www.ncbi.nlm.nih.gov/geo/).

Cell culture

Peripheral blood $CD4^+$T cells were stimulated with CD3 and CD28 antibodies (2 μg/mL, ab16669, and 1 μg/mL, ab243228, respectively, bought from Abcam) in RPMI 1640 supplemented with 10% FBS. To induce Th17 cell differentiation, naive $CD4^+$T cells were activated in the presence of hIL-1β (50 nM) and hIL-23 (50 nM, all bought from Sigma). The PI3K agonist 740 Y-P (HY-P0203, MedChemExpress, USA) was dissolved in DMSO and added to the culture medium at a final concentration of 10 μM for 24 h prior to analysis.

Cell transfection

p-LKO.1-PLOD1 shRNA plasmids (shPLOD1) and p-LKO.1-vector shRNA plasmids were obtained from Addgene. $CD4^+$T cells were transfected with p-LKO.1-PLOD1 shRNA plasmids to deplete the expression of PLOD1. p-LKO.1-vector shRNA plasmids were used as control and transfected using lipofectamine 2000 (Invitrogen). A total of 100 μl of the mixture was slowly added to the six-well plate, and the cells were incubated for 6 h. Then, the transfection effect was verified for subsequent experiments.

Quantitative PCR

RNA was extracted from 106 cells with the Trizol reagent (TaKaRa, Japan). Then, total RNA was reverse-transcribed into cDNA by RT reagent kit (Takara). Quantitative PCR assay was performed with the use of SYBR Ex Taq™ II (Takara). The levels of targeted genes were determined by using the $2^{-\Delta\Delta CT}$ method. Quantitative PCR was performed with the SYBR mixture (Takara). Primer sequence: PLOD1: Forward: 5'-AAGCCGGAGGACAACCTTTTA-3', Reverse: 5'-GCGAAGAGAATGACCAGATCC-3'; GAPDH: Forward: 5'-TGTGGGCATCAATGGATTTGG-3', Reverse: 5'-ACACCATGTATTCCGGGTCAAT-3'.

Immunoblotting

10^6 cells were harvested using the RIPA buffer (Beyotime, China). After centrifugation, the BCA protein assay kit (Beyotime Biotechnology) was used to detect the concentration of protein. Samples were separated by SDS-PAGE, and further transferred onto PVDF membranes. The proteins were blocked with 5% milk for 1 h, and then antibodies were added and incubated at 4°C overnight. Primary antibodies include PLOD1 (Thermo fisher, PA5-61892; 1:1000), GLUT1 (Abcam, ab115730; 1:1000), LDHA (Abcam, ab52488; 1:1000), AKT (Abcam, ab8805; 1:1000), p-AKT (phospho T308, Abcam, ab38449; 1:1000), PI3K (Abcam, ab182617; 1:1000), p-PI3K (phospho Y164, Abcam, ab138364; 1:1000), mTOR (Abcam, ab32028; 1:1000), p-mTOR (phospho S2448, Abcam, ab84400; 1:1000), and β-actin (Abcam, ab8226; 1:3000). This was followed by secondary antibodies being incubated for 1 h.

ECAR test

Extracellular acidification rate (ECAR) was detected on an XF24 Extracellular Flux Analyzer (Seahorse Bioscience, Billerica, MA). The inhibitors of ETC and OXPHOS were injected in 10^5 cells: glucose (1.5 μM), oligomycin (1 μM), and 2-DG (0.5 μM).

Flow cytometry

10^5 cells were fixed and incubated with IL-17A (rabbit, 1:200, ab302922, abcam, UK) and ROR-γT (mouse, 1:200, ab289552, abcam, UK) antibodies at 37°C for 30 min in dark. The cells were then incubated with secondary antibodies. The stained cells were then washed by centrifugation and resuspended and analyzed on a BD FACSCanto II (BD Biosciences).

Glucose intake, lactate production, and ATP production test

The glycolysis levels of 10^5 cells were detected using the glucose intake (ab136955), lactate production (ab65330, abcam), and ATP levels (ab83355), which were measured according to the relevant instructions.

ELISA

Human IL-17A (ab216167) was detected via ELISA (Abcam, UK). Briefly, samples were aspirated into wells. And, biotin-conjugated primary antibodies were added and followed by avidin-conjugated horseradish peroxidase (HRP). The enzyme substrate was then used for color reaction. The intensity of each well was measured with a microplate reader. Each group had five repeated independent samples. Standard reagents and samples were added and incubated for 20 h at 4°C. The OD450 value was measured by a microplate reader (Bio-Rad, CA).

Statistics

GraphPad 8.0 software was used and performed in this study. Data were represented as mean ± SD. $p<0.05$ was considered to be significant. Each cellular experiment was repeated three times.

Results

PLOD1 is highly expressed in patients with psoriasis

To uncover the possible effects of PLOD1 on the progression of psoriasis, we first detected its expression in patients with psoriasis. Through the analysis of the online GEO database (GSE192867), we found that the expression of PLOD1 was high in $CD4^+$T cells is patients with psoriasis, compared to the normal volunteers (Figure 1). Therefore, PLOD1 is highly expressed in patients with psoriasis.

PLOD1 depletion suppressed Th17 cell differentiation

Subsequently, we detected the effects of PLOD1 on Th17 cell differentiation. To induce Th17 cell differentiation, naive $CD4^+$T cells were activated in the presence of hIL-1β (50 nM) and hIL-23 (50 nM). Interestingly, both qPCR and immunoblot assays confirmed the high mRNA and protein levels of PLOD1 in hIL-1β and hIL-23 treated $CD4^+$T cells (Figure 2A,B). Furthermore, PLOD1 shRNA plasmids were used to deplete the expression of PLOD1 in hIL-1β and hIL-23 treated $CD4^+$T cells. Immunoblot assays confirmed the downregulation of PLOD1 expression in PLOD1 shRNA transfected $CD4^+$T cells (Figure 2C). Further through FCM assays, we detected the percentage of $IL\text{-}17A^+ROR\text{-}\gamma t^+$ Th17 cells, and found that the depletion of PLOD1 suppressed the percentage of Th17 cells (Figure 2D). Similarly, ELISA also confirmed the decreased secretion of IL-17A in hIL-1β and hIL-23 treated $CD4^+$T cells upon PLOD1 ablation (Figure 2E). Collectively, PLOD1 depletion suppressed Th17 cell differentiation.

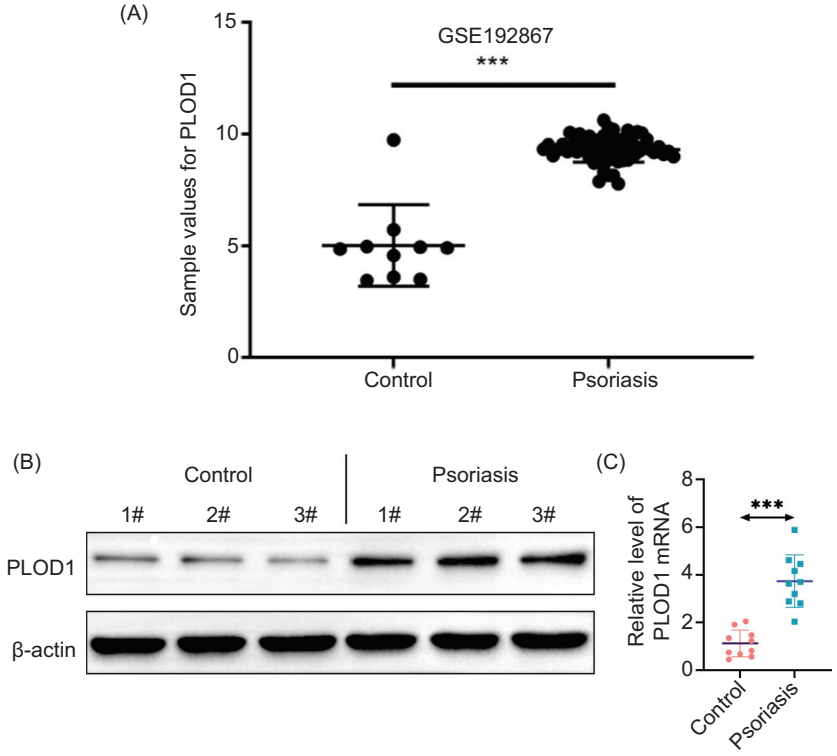

Figure 1 PLOD1 is highly expressed in patients with psoriasis. GEO database analysis (GSE192867) showed the expression of PLOD1 in peripheral blood from patients with psoriasis or healthy volunteers. ***$p<0.001$, psoriasis versus control.

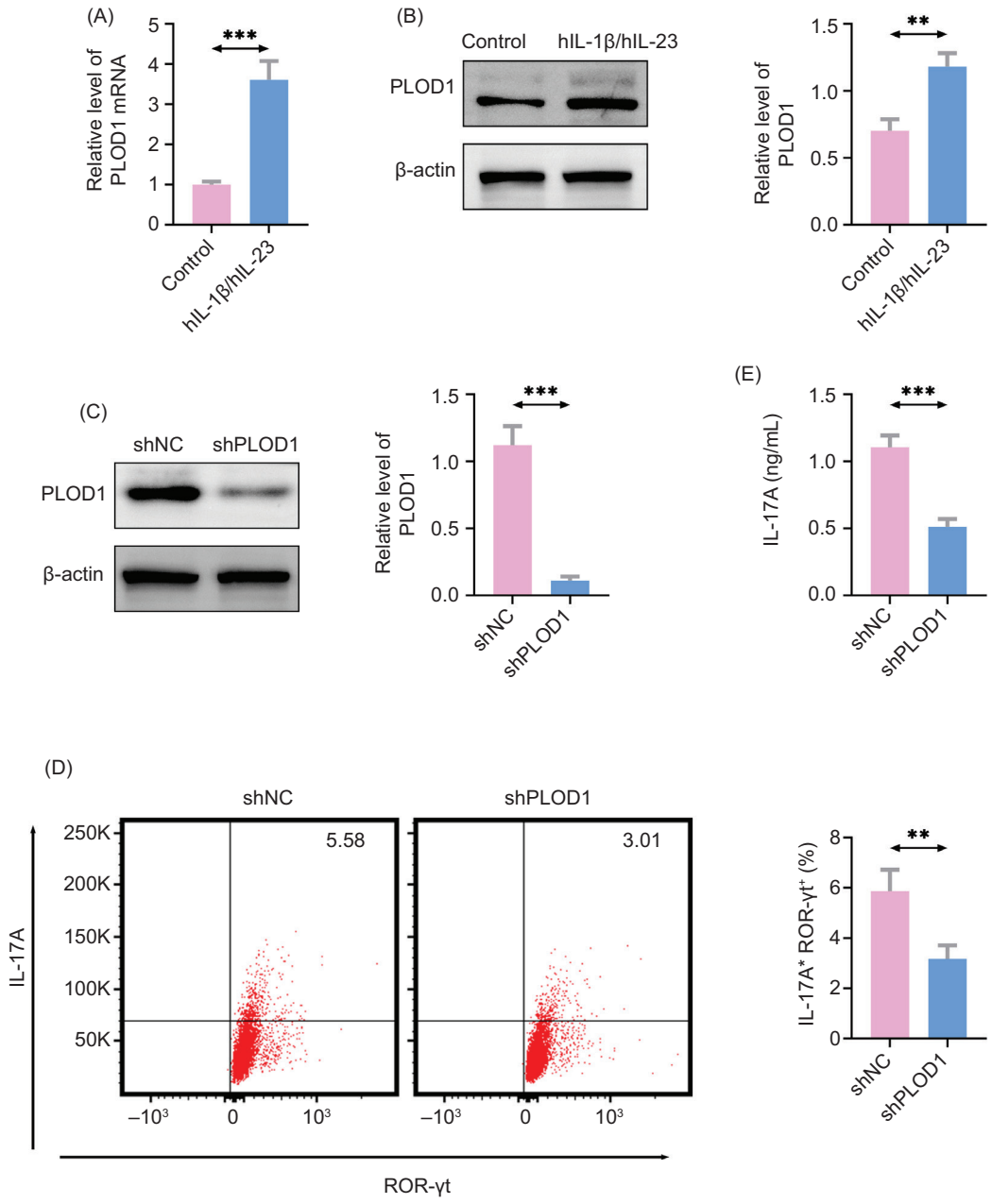

Figure 2 The role of PLOD1 in promoting Th17 cell differentiation. (A) Native CD4⁺T cells in healthy volunteers were treated with hIL-1β (50 nM) and hIL-23 (50 nM) to induce the differentiation of Th17 cells, and the mRNA levels of PLOD1 were detected in the indicated groups via qPCR assays. (B) Immunoblot assays showed the expression of PLOD1 in CD4⁺T cells from the indicated groups. (C) Immunoblot assays showed the expression of PLOD1 in hIL-1β (50 nM) and hIL-23 (50 nM) induced CD4⁺T cells upon the indicated transfection. (D) FCM assays showed the staining intensity of IL-17A and ROR-γt in hIL-1β (50 nM) and hIL-23 (50 nM) induced CD4⁺T cells upon the indicated transfection. (E) ELISA showed the secretion levels of IL-17A in hIL-1β (50 nM) and hIL-23 (50 nM) induced CD4⁺T cells upon the indicated transfection.**p<0.01, ***p<0.001.

PLOD1 promotes T cell glycolysis

We then detected the effects of PLOD1 on T cell glycolysis, which was important in the progression of psoriasis. Through the detection of glucose consumption, lactate production, and ATP levels, we noticed that PLOD1 depletion suppressed the levels of glucose consumption, the levels of lactate production, and ATP, suggesting the suppression of glycolysis in hIL-1β and hIL-23 treated CD4⁺T cells (Figure 3A). We further performed the ESAR assays, and the data confirmed that PLOD1 depletion suppressed the ECARs in hIL-1β and hIL-23 treated CD4⁺T cells, suggesting the inhibition of glycolysis (Figure 3B). Immunoblot assays further confirmed the decrease of GLUT1 and LDHA expression, key regulators in glycolysis, in hIL-1β and hIL-23 treated CD4⁺T cells (Figure 3C). Therefore, PLOD1 promotes CD4⁺T cell glycolysis.

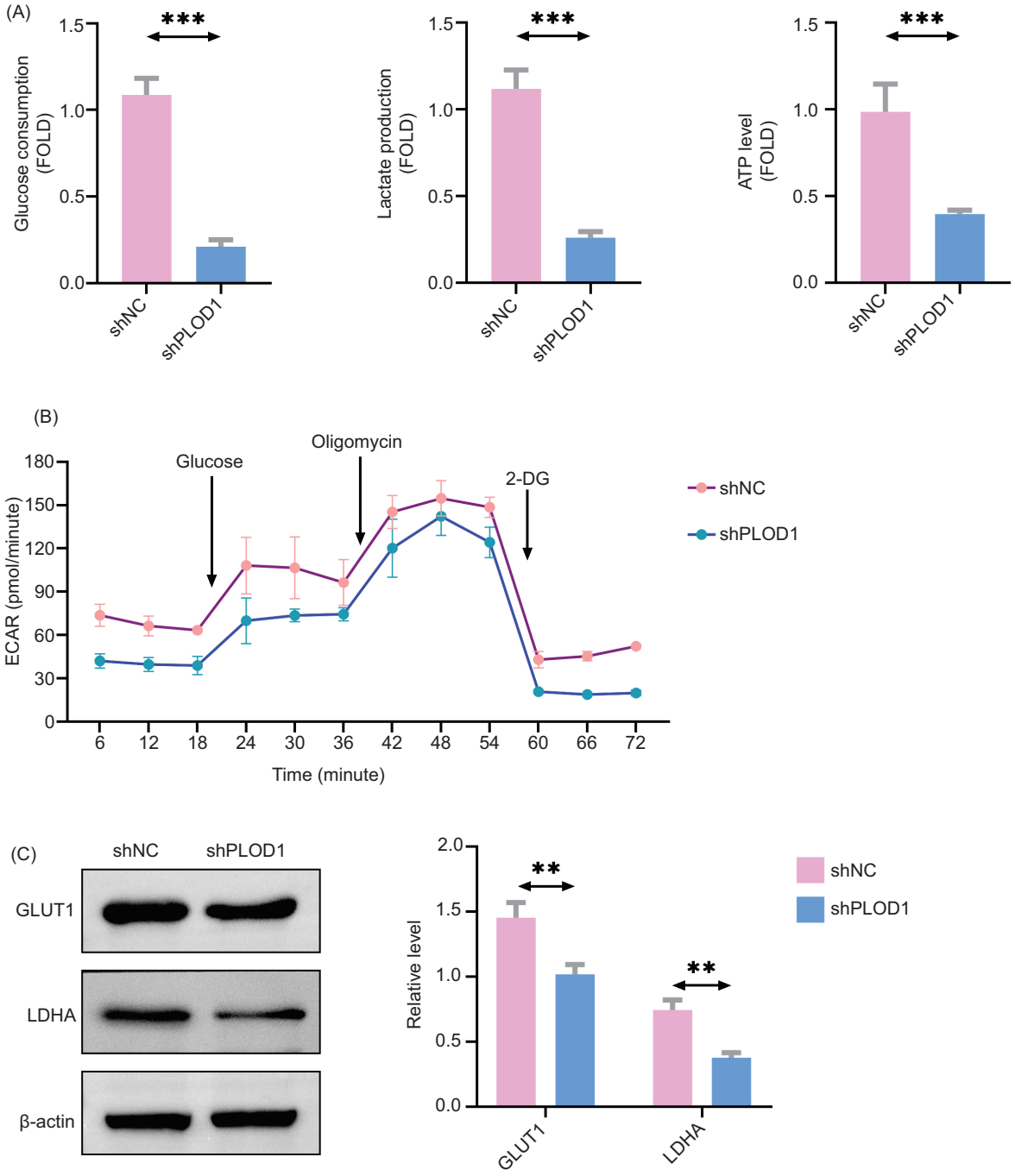

Figure 3 PLOD1 promotes T cell glycolysis. (A) The glucose consumption, lactate production, and ATP levels were detected by the corresponding kits in hIL-1β (50 nM) and hIL-23 (50 nM) induced CD4⁺T cells upon the indicated transfection. (B) ESAR assays showed the ECARs of hIL-1β (50 nM) and hIL-23 (50 nM) induced CD4⁺T cells upon the indicated transfection. (C) Immunoblot assays showed the expression of GLUT1 and LDHA in hIL-1β (50 nM) and hIL-23 (50 nM) induced CD4⁺T cells upon the indicated transfection. $^{**}p<0.01$, $^{***}p<0.001$.

PLOD1 activates the PI3K/AKT/mTOR pathway

Finally, the mechanism was studies. Through immunoblot assays, we noticed that the depletion of PLOD1 in hIL-1β and hIL-23 treated CD4⁺T cells decreased the phosphorylation levels of PI3K, AKT, and mTOR, revealing the inhibition of PI3K/AKT/mTOR axis caused by PLOD1 depletion (Figure 4). We concluded that PLOD1 activates the PI3K/AKT/mTOR pathway in CD4⁺T cells.

PLOD1 regulates glucose metabolism and promotes Th17 cell differentiation via the PI3K/AKT/mTOR pathway

To further confirm the mechanism of PLOD1 in regulating glucose metabolism and promoting Th17 cell differentiation, we performed rescue assays using PI3K agonist

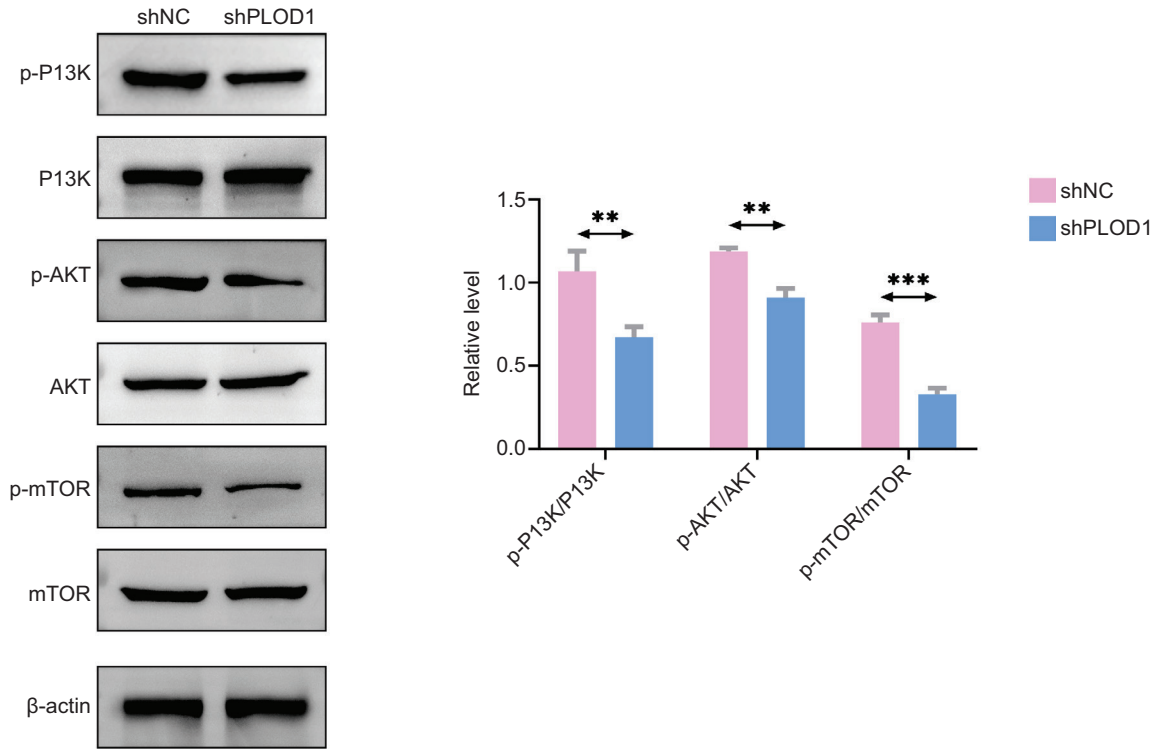

Figure 4 PLOD1 activates the PI3K/AKT/mTOR pathway. Immunoblot assays showed the expression and phosphorylation levels of PI3K, AKT, and mTOR in hIL-1β (50 nM) and hIL-23 (50 nM) induced CD4+T cells upon the indicated transfection. $^{**}p<0.01$, $^{***}p<0.001$.

740 Y-P. Knockdown of PLOD1 in CD4+T cells significantly reduced IL-17A secretion (Figure 5A) and decreased the proportion of IL-17A+RORγt+ cells (Figure 5B). In addition, metabolic analysis revealed that depletion of PLOD1 led to a marked decrease in glucose consumption, lactate production, and ATP levels (Figure 5C). Importantly, these effects were reversed by treatment with the PI3K agonist 740 Y-P, which restored IL-17A secretion, the proportion of IL-17A+RORγt+ cells, and metabolic activity (Figure 5A-C). These findings collectively suggest that PLOD1 plays a crucial role in regulating IL-17A production, T cell differentiation, and metabolic reprogramming in CD4+T cells via activation of the PI3K/AKT/mTOR signaling pathway.

Discussion

Psoriasis is a common, chronic, inflammatory, and immune skin disease. Its pathogenesis is closely related to genetic, immune, and environmental factors, and there is no significant difference in its incidence between men and women.[11] The typical clinical manifestations of the disease are well-defined squamous erythema or plaques with localized or widespread lesions.[12] At present, more and more scholars regard psoriasis as a more complex clinical syndrome, which can be accompanied by a variety of comorbidities, of which the most common are metabolic diseases. Studies have shown that disorders of glycolipid metabolism are more common in psoriasis patients.[13] Herein, CD4+T cells were isolated by magnetic beads, which are crucial in the progression of psoriasis, and we found that PLOD1 regulates glucose metabolism and promotes Th17 cell differentiation in psoriasis. We therefore think that PLOD1 could serve as a target of psoriasis.

Th17 cells play a very important role in the pathogenesis of psoriasis. They are differentiated from lymphocytes, which mainly secrete pro-inflammatory factors such as IL-17 and participate in the immune defense of the body.[14] Under normal circumstances, both the number and function of Th17 cells are strongly suppressed and regulated to ensure a normal immune state. However, in diseases such as psoriasis, the number and activity of Th17 cells increase significantly, leading to inflammatory responses and dermal keratin metabolism disorders. Specifically, Th17 cells promote inflammation, keratinocyte proliferation, and angiogenesis by expressing a variety of inflammatory genes and signaling pathways.[15,16] Interestingly, by performing qPCR as well as immunoblot assays, we found the role of PLOD1 in promoting Th17 cell differentiation. Therefore, we thought PLOD1 affected psoriasis progression via mediating Th17 cell differentiation.

PI3K/AKT/mTOR axis is a key pathway to mediate glycolysis, and mTOR is a key intracellular signal regulator.[17] PI3K is located upstream of the mTOR signal pathway and participates in the regulation of cell proliferation, apoptosis, immunity, metabolism, and other processes, which play an important role in psoriasis.[18] When cells in the skin are stimulated, Akt is involved in regulating angiogenesis by phosphorylating multiple substrates. The level of Akt in keratinocytes is positively correlated with the severity

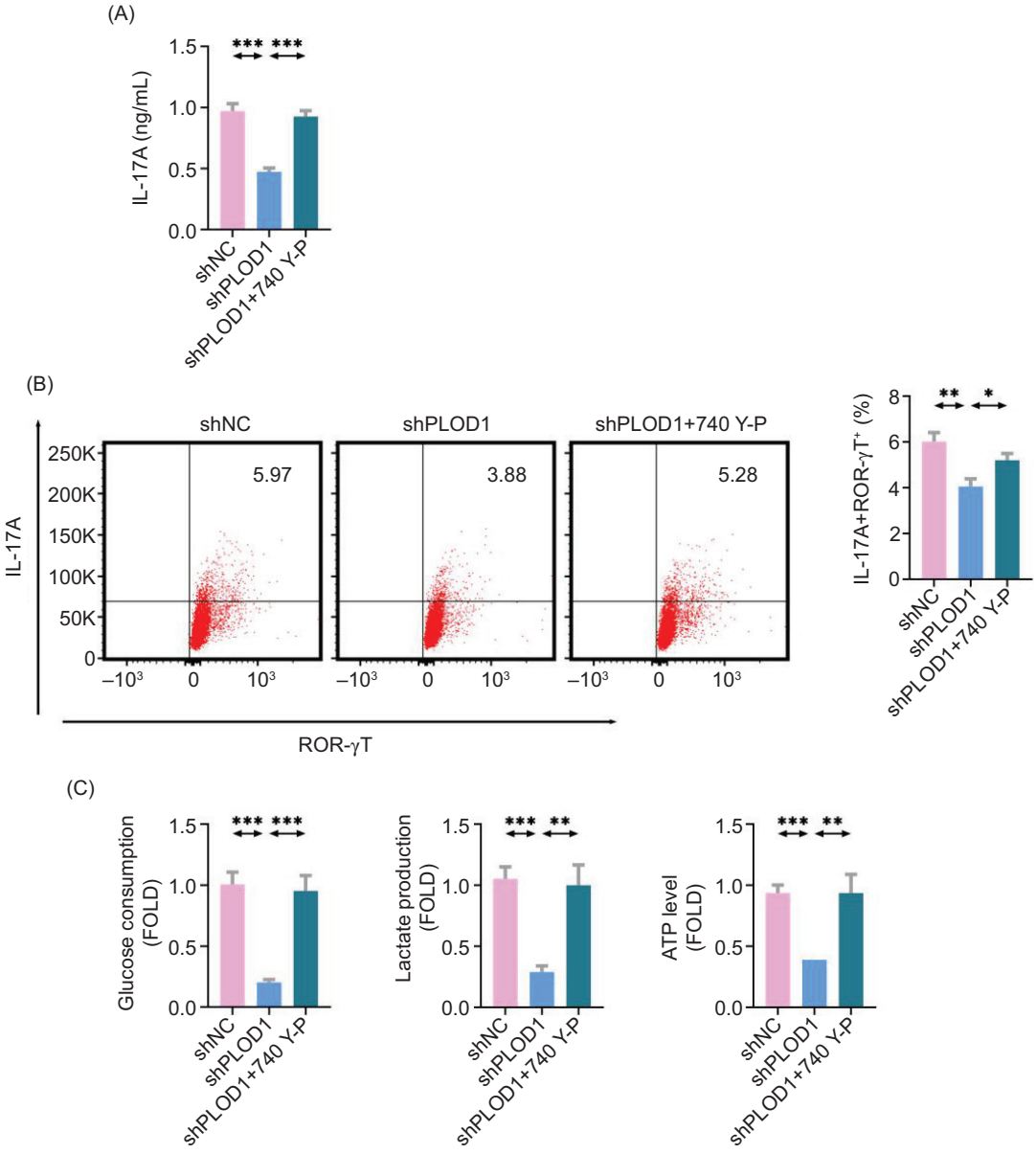

Figure 5 PLOD1 regulates glucose metabolism and promotes Th17 cell differentiation via the PI3K/AKT/mTOR pathway. (A) ELISA analysis of IL-17A secretion in CD4⁺T cells transduced with shNC or shPLOD1, with or without treatment with the PI3K agonist 740 Y-P. (B) Flow cytometry analysis of IL-17A+RORγt+ cell proportions in CD4⁺T cells transduced with shNC or shPLOD1, with or without 740 Y-P treatment. Quantification of IL-17A+RORγt+ cells is shown on the right. (C) Metabolic analysis of glucose consumption, lactate production, and ATP levels in CD4⁺T cells transduced with shNC or shPLOD1, with or without 740 Y-P treatment. Data are presented as mean ± SD. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

of psoriasis. At the same time, inhibition of the PI3K/Akt signaling pathway can reduce symptoms in patients with psoriasis.[19] Here, we revealed that PLOD1 affected the progression of psoriasis via PI3K/AKT/mTOR axis. Our data confirmed that this pathway could serve as a target of psoriasis. However, the precise mechanism needs further study.

Glucose metabolism is vital in psoriasis. Patients with psoriasis are more likely to develop diabetes, atherosclerosis, and other diseases.[20] On the one hand, hypoglycemic drugs can not only regulate abnormal blood glucose but also improve the skin condition of patients with psoriasis.[21] On the other hand, anti-psoriasis drugs can also have different effects on blood glucose. This study suggests that if abnormal glycolysis is found, timely intervention should be carried out to slow down the development of the disease.

In summary, we revealed that PLOD1 regulates glucose metabolism and promotes Th17 cell differentiation in psoriasis via mediating the PI3K/AKT/mTOR axis. Therefore, PLOD1 could serve as a target of psoriasis.

Acknowledgements

Not applicable.

Ethics Approval

This article does not contain any studies with human participants or animals performed by any of the authors.

Data Availability

The authors declare that all data supporting the findings of this study are available within the paper and any raw data can be obtained from the corresponding author upon request.

Authors Contributions

Zhen Yue and Congjun Jiang designed and performed the study; Zhen Yue, Wanlu Zhang, Gege Zhu, and Huiya Sun supervised the data collection, analyzed the data, and interpreted the data; Zhen Yue and Congjun Jiang prepared and reviewed the manuscript for publication. All authors have read and approved the final manuscript.

Conflicts of Interest

The authors state that there are no conflicts of interest to disclose.

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