Leptin promoted glycolytic metabolism to induce DCs activation via STAT3-HK2 pathway

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Keywords: leptin dendritic cell glycolysis HK2 Th17 cell

Abbreviations:

DCs: Dendritic cells

PBMC: Peripheral blood mononuclear cells

STAT: signal transducer and activator of transcription

MAPK: mitogen-activated protein kinase

PI3K: phosphatidylinositide 3-kinases

RA: rheumatoid arthritis

SLE: systemic lupus erythematosus

MS: multiple sclerosis

APCs: Antigen-presenting cells

Th17: T helper 17

Treg: regulatory T

IL: Interleukin

LPS: Lipopolysaccharide

HK: hexokinase

PFK: phosphofructokinase

PKM: pyruvate kinase M

GLUT1: glucose transporter 1

LDHA: lactate dehydrogenase A

2-DG: 2-deoxyglucose

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- **Summary**:

Leptin is over-secreted in many autoimmune diseases, which can promote dendritic cells (DCs) maturation and up-regulate the expression of inflammatory cytokines, but the underlying mechanisms are not fully elucidated. Considering the major role of leptin in maintaining energy balance and the significant role of glycolysis in DCs activation, our study aims to investigate whether leptin promotes the activation of DCs via glycolysis and its underlying mechanisms. We demonstrated that leptin promoted the activation of DCs, including up-regulating the expression of co-stimulatory molecules and inflammatory cytokines, enhancing the proliferation and T helper 17 (Th17) cell ratio in peripheral blood mononuclear cells (PBMC) co-cultured with leptin-stimulated DCs. Leptin also enhanced DCs glycolysis with increased glucose consumption, lactate production, and the expression of hexokinase 2 (HK2). In addition, the activation of DCs stimulated by leptin could be inhibited by the glycolysis inhibitor 2-DG. To explore the signaling pathways involved in leptin-induced HK2 expression, we observed that only the inhibitors of STAT3 (NSC74859) could repress the enhancement of HK2 triggered by leptin stimulation. Therefore, our results indicated that leptin promoted glycolytic metabolism to induce DCs activation via STAT3-HK2 pathway.

- **Introduction**:

Leptin is an adipose-secreted hormone that plays an important role in both metabolism and immunity [1]. It plays a biological role by binding and activating the leptin receptor, which exists in a variety of immune cells, such as monocytes/ macrophages, T lymphocytes, B lymphocytes, NK cells and dendritic cells (DCs) [2]. Activation of leptin signal can lead to JAK trans-phosphorylation and activation of several intracellular signaling cascades including the signal transducer and activator of transcription (STAT), mitogen-activated protein kinase (MAPK), phosphatidylinositide 3-kinases (PI3K) and mTOR pathways [3]. Leptin not only acts as an adipokine, but also influences a multitude of physiological and pathological processes, including vascular function, inflammatory and immune response [6]. Moreover, many studies have shown that the prevalence of autoimmune diseases, such as rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), and multiple sclerosis (MS), is increased and associates with serum leptin levels [7].

Antigen-presenting cells (APCs), including DCs, deliver antigens and produce cytokines, stimulating naïve $CD4^+$ T cells to expand clonally and differentiate into proinflammatory T helper 1 (Th1), Th2, and Th17 or anti-inflammatory regulatory T (Treg) cells [10]. In RA patients, DCs are recruited in high quantity to joint synovial fluid and tissues [11]. These synovial DCs are generally mature and activated [12]. In addition, DCs can produce cytokines Interleukin (IL)-23, TGF-β and IL-6 to induce Th17 differentiation and participate in the pathogenesis of RA [12]. Therefore, it is vital to explore the mechanism of DCs activation in autoimmune diseases such as RA for disease development and treatment intervention. Studies have found that DCs express leptin receptor, and leptin is required for the activation and complete maturation of DCs [1]. However, the definite mechanism is still unclear.

There is a growing appreciation for the role of metabolic changes in the phenotype and function of DCs. Moreover, evidence is accumulating that many fundamental cellular processes in DCs are regulated by metabolic processes or the metabolites which they generate. The glycolysis metabolic capacity of DCs activated

by LPS is enhanced, which is manifested by the increase of glucose uptake and lactic acid production [13]. When the activated DCs enhance the glycolysis ability, the expression of three key rate-limiting enzymes: hexokinase (HK), phosphofructokinase (PFK), pyruvate kinase M (PKM) genes is increased. In addition, the expression of glucose transporter 1 (GLUT1) and lactate dehydrogenase A (LDHA) is also up-regulated [14]. Blocking glycolysis using 2-deoxyglucose (2-DG) (an inhibitor of HK) results in marked inhibition of DC activation, showing the importance of glucose as a substrate to support DC function [15]. Direct leptin signal may enhance the glycolysis of activated $CD4^+$ T cells, and the leptin signal is critical for glucose metabolism in effector Th17 cells. However, little is known about the role of leptin in DCs metabolism.

Our study aims to explore whether leptin can affect DC activation and the interaction between DC and T cells by changing the glycolysis ability of DCs as well as its molecular mechanism. We found that leptin was essential for DCs glycolysis and function by up-regulating HK2 expression through STAT3 signal. Our study further suggests that there may be an abnormal function of innate immune cells in the microenvironment of leptin over-expression such as autoimmune diseases. And the abnormal phenomenon may be related to the reprogramming of glucose metabolism. It provides potential ideas for the treatment of related diseases targeting leptin or energy metabolism.

- **Materials and Methods**:

1. DCs culture and treatment

Human DC cell lines were purchased from Wuhan Fine Biotech China. DCs were cultured at 37°C under 5% CO2 atmosphere in RPMI 1640 medium with 10% Foetal Bovine Serum, 100 U/mL penicillin/streptomycin, 2mM L-glutamine. DCs were treated with leptin (RD systems) at 100 ng/ml for 4, 6, or 12 hrs. In some experiments, DCs were pretreated with 2-DG (Beijing Solarbio Science) at 10 mM for 2 hrs, before stimulation with leptin. Then cells were collected for RT-PCR analysis,

protein extraction and flow cytometry analysis. Supernatants were collected for glucose assay, cytokines assay, lactate assay. To investigate the signal pathway induced by leptin, Akt and STAT3 signaling pathway inhibitors: LY294002, NSC74859 pretreated on DCs for 2 hrs, before treatment of leptin. Parallel culture without stimulation was used as control.

2. RT-PCR

DCs were cultured in media with different stimuli for 6 hrs before RNA extraction. RNA was extracted from cells using RNA Isoplus (Takara) according to the manufacturers' instructions, cDNA was synthesized with a reverse transcription kit (Takara) to be used as the template for PCR. The primers were listed as follows: β-actin (forward) CAT GTA CGT TGC TAT CCA GGC (reverse) CTC CTT AAT GTC ACG CAC GAT; LDHA (forward) TTG ACC TAC GTG GCT TGG AAG (reverse) GGT AAC GGA ATC GGG CTG AAT; HK2 (forward) TTG ACC AGG AGA TTG ACA TGG G (reverse) CAA CCG CAT CAG GAC CTC A; PKM1/2 (forward) ATG TCG AAG CCC CAT AGT GAA (reverse) TGG GTG GTG AAT CAA TGT CCA; IL-23 (forward) CTC AGG GAC AAC AGT CAG TTC (reverse) ACA GGG CTA TCA GGG AGC A; IL-6 (forward) CCT TCG GTC CAG TTG CCT TCT C (reverse) CCA GTG CCT CTT TGC TGC TTT C; TGF-β (forward) AGC GAC TCG CCA GAG TGG TTA (reverse) GCA GTG TGT TAT CCC TGC TGT CA; TNF-α (forward) CGA GTG ACA AGC CTG TAG C (reverse) GGT GTG GGT GAG GAG CAC AT. All primers were provided by Takara. Data were normalized to the expression of the housekeeping gene β-actin.

3. Isolation of Human PBMC and co-culture assay

PBMCs were isolated from the whole blood of healthy individuals by density gradient centrifugation over Ficoll-Hypaque. All human subject studies have been approved by the ethics committee of the Dalian Medical University, and informed consent was obtained from all subjects. DCs that had previously been cultured in

medium alone, leptin alone, 2-DG alone, leptin plus 2-DG for 12 hrs, were washed twice in PBS, then the DCs co-cultured with PBMC (5 × 104 DCs and 25 × 104 PBMC) in RPMI 1640 medium with anti-CD3 anti-body (3μg/ml, eBioscience) for 3 days. For carboxyfluorescein diacetate succinimidyl ester (CFSE)-based proliferation assay, PBMC were labeled with 1μM CFSE (eBioscience) before co-culture. PBMC were collected after 3 days for flow cytometry analysis.

For intracellular cytokine IL-17a staining, on the fourth day, DCs and PBMC co-culture systems were stimulated with 50ng/ml PMA plus 1μg/ml Ionomycin. After 2 hrs, Brefeldin A 10μg/ml (eBioscience) was added and kept for 2 hrs. PBMC were collected for flow cytometry analysis.

4. Flow cytometry analysis

The following fluorescently labeled antibodies were used for flow cytometry: Anti-Human CD80 PE (eBioscience), Anti-Human CD86 PE (eBioscience), anti-mouse/human CD11b PerCP/Cy5.5 (BioLegend), Anti-Human CD4 FITC (eBioscience), Anti-Human Foxp3 PE (eBioscience), Anti-Human IL-17a PE (eBioscience). Stimulated DCs as indicated were harvested and washed in PBS, stained with specific surface antibodies, and incubated at room temperature for 30 minutes. Then cells were washed twice in PBS for flow cytometry analysis.

For intracellular staining of Foxp3, PBMC were fixed, permeabilized by Foxp3 Fixation/Permeabilization reagent (eBioscience) for 60 min ，and labeled with Anti-Human Foxp3 for 30 min. Then cells were washed twice in PBS for flow cytometry analysis.

For intracellular staining of IL-17a, PBMC were fixed and permeabilized by Cell Fixation & Permeabilization Kit (FcmacsBiotech, china) as the instruction. Then cells were stained with Anti-Human CD4 and Anti-Human IL-17a for 30 min, after that cells were washed twice in PBS for flow cytometry analysis.

5. Metabolic assays

The levels of glucose and lactate in the culture medium of the DCs (1×10^5/well) were measured with the use of Glucose (HK) Assay Kit (Sigma) and Lactate Assay Kit (Beijing Solarbio Science) according to the manufacturer's instructions.

6. Western blotting

Total protein was extracted using RIPA lysis buffer (Beyotime, Beijing, China). Lysate proteins were separated by an 8% sodium dodecyl sulphate-polyacrylamide gel and electrophoretically transferred to polyvinylidene fluoride membranes. After blocking with 5% milk for 90 min, the membranes were incubated with antibody against leptin receptor or β-actin at the indicated dilutions for 12-16 hrs at 4°C. Membranes were washed and incubated for 2 hrs with HRP-coupled goat polyclonal anti-rabbit IgG. The membranes were scanned using an Odyssey Sa Imaging System (LICOR Biosciences, Lincoln, NE, USA).

7. Statistical analysis

Statistical significance was calculated with the Student *t*-test. *P*-value < 0.05 was considered statistically significant.

- **Results**:

1. Leptin promoted DCs activation

To study the effect of leptin on DCs, we first confirmed that CD11b (Fig. 1A) and the leptin receptor (Fig. 1B) were expressed in human DC cell lines. Activated DCs express high levels of the co-stimulatory molecules CD80, CD86 and secrete cytokines, which provide the signal that is required for triggering T cell response [17]. We found that DCs expressed higher levels of surface CD80, CD86 with stimulation of leptin (Fig. 1C). The mRNA expression of IL-6, IL-23, TNF-α, TGF-β and IL-1β were analyzed in DCs stimulated with leptin for 6 hrs. The results showed that the

expression of IL-6, IL-23, TNF-α was up-regulated by leptin. However, there was no significant difference in TGF-β or IL-1β (Fig.1D). Taken together, these data demonstrated that leptin promoted DCs activation.

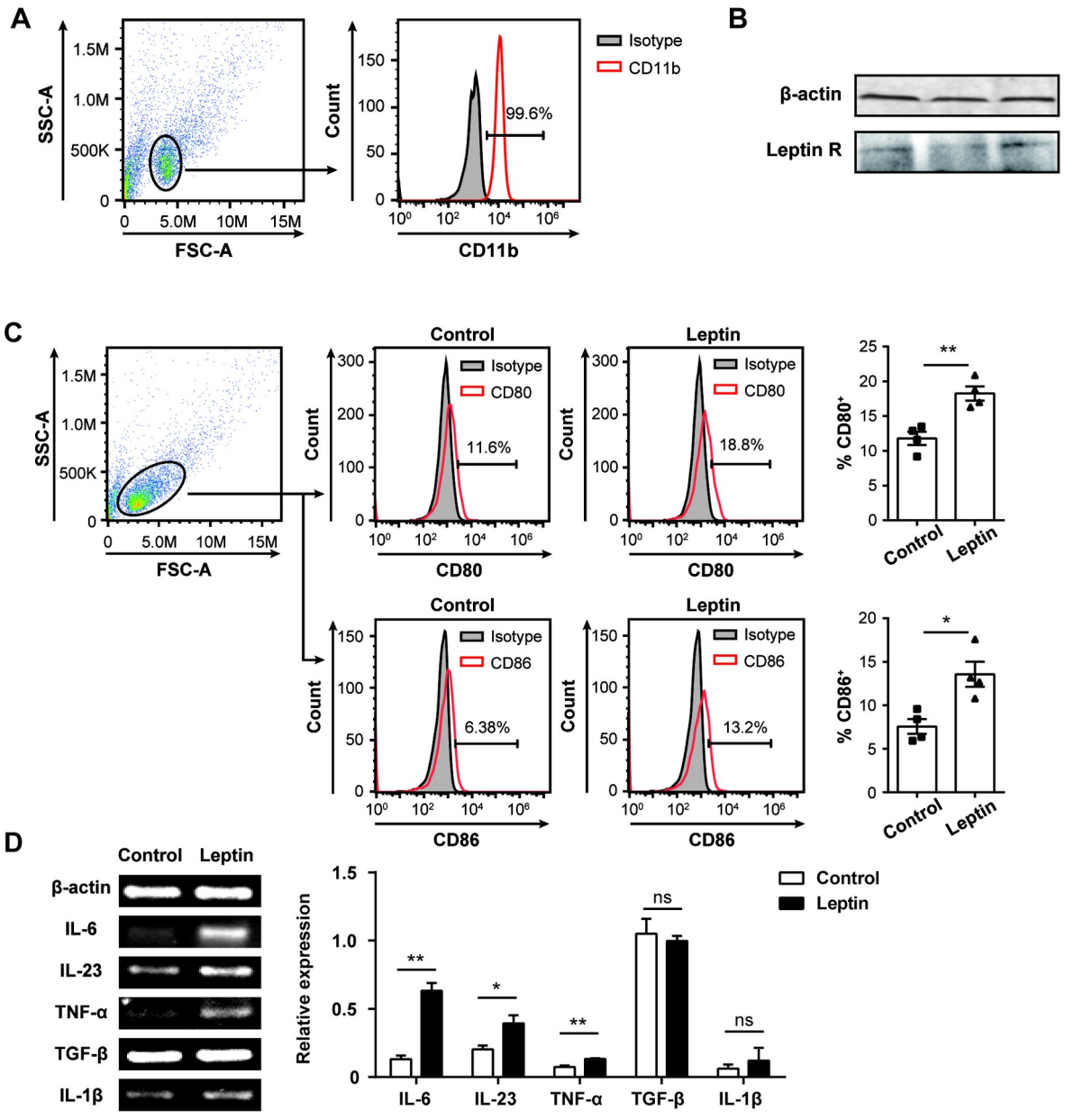


Fig.1 Leptin promoted DCs activation. (A) The expression of CD11b on DCs was analyzed by flow cytometry. (B) The expression of leptin receptor was tested by western blot. DCs were stimulated with leptin or not for 12 hrs (C) and 6 hrs (D). (C) The expression of CD80 and CD86 was analyzed by flow cytometry. (D) The mRNA expression of IL-6, IL-23, TNF-α, TGF-β, IL-1β were analyzed by RT-PCR. All data

are shown as the mean values ± SEM of 3 to 4 independent tests. Statistical significance was determined using the Student *t*-test, * $P < 0.05$, ** $P < 0.01$.

2. Leptin-activated DCs promoted the proliferation of PBMC and up-regulated the percentage of Th17 cells

From the results above, we found that leptin-activated DCs expressed higher percentages of CD80, CD86 and mRNA levels of IL-6, IL-23, TNF-α. Of these, IL-6 and IL-23 play a critical role in promoting Th17 differentiation [18], and IL-6 can reduce Foxp3 levels of Treg [19]. We further used DCs activated with or without leptin to co-culture with PBMC in the presence of anti-CD3 antibody for 3 days, then examined the proliferation of PBMC and the percentages of Th17 and Treg in PBMC. As compared with the co-culture system with untreated DCs, the proliferation of PBMC was increased (Fig.2A) and the percentage of Th17 was up-regulated (Fig.2B) in the co-culture system with leptin-DCs. However, the percentage of Treg was unaffected in the co-culture system (Fig.2B). Overall, we suggested that leptin-DCs promoted the proliferation of PBMC and up-regulated the percentage of Th17 cells in PBMC, indicating that leptin was involved in T cells response induced by DCs.

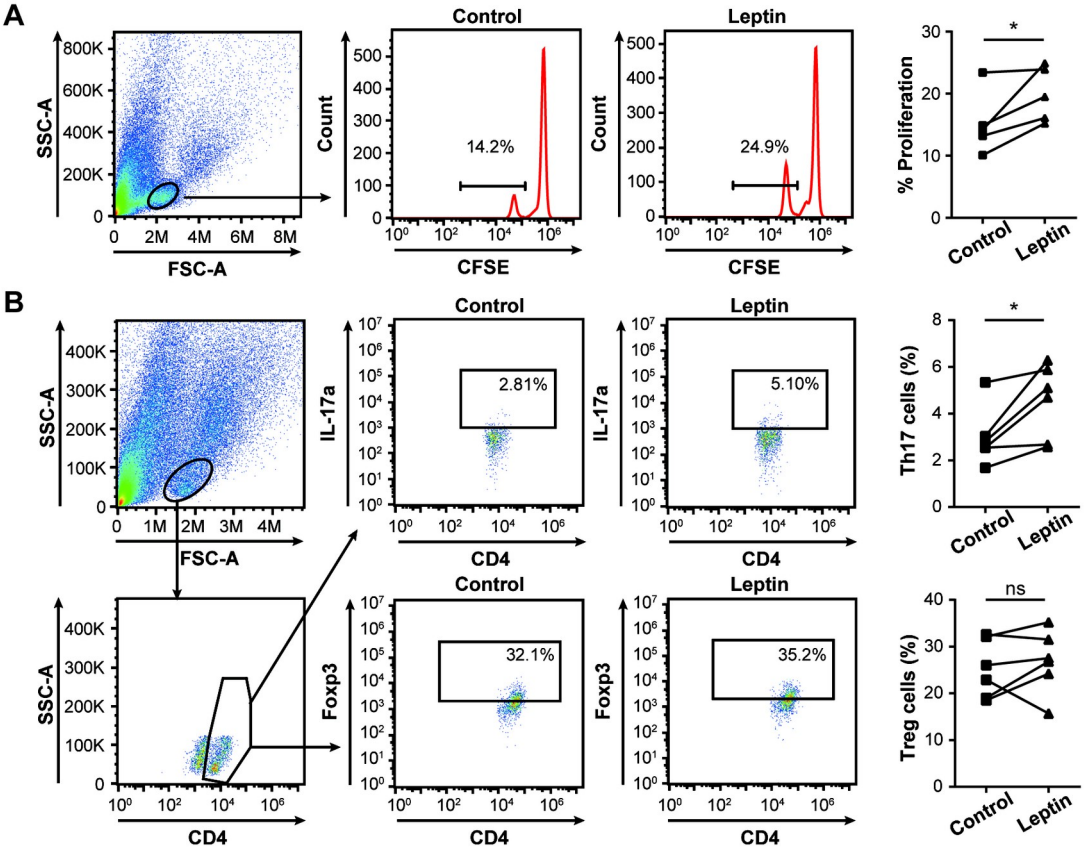
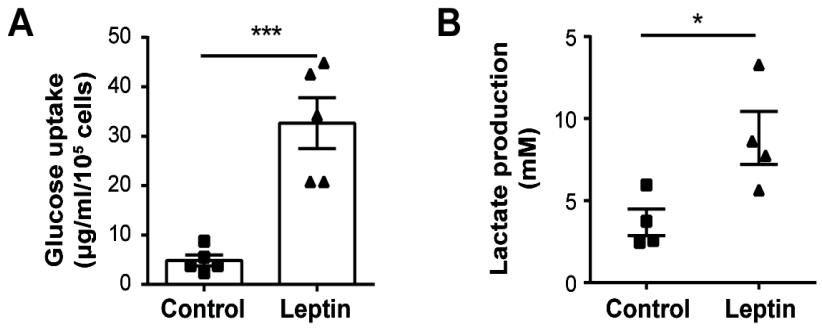


Fig.2 Leptin-activated DCs promoted the proliferation of PBMC and up-regulated the percentage of Th17 cells. CFSE-stained PBMC or untreated PBMC co-cultured with DCs had been pretreated with medium alone or leptin for 12 hrs in the presence of anti-CD3 antibody for 3 days. (A) CFSE-stained PBMC proliferation was measured by flow cytometry. The numbers above plots indicated percent $CFSE^+$ (proliferated) cells. Data are from one experiment representative of five experiments and shown as the mean values ± SEM. (B) The frequencies of $IL\text{-}17a^+$ and $Foxp3^+$ cells among co-culture were analyzed by flow cytometry on day 4. The percentage of each gate is shown. Data are from one experiment representative of six experiments. Statistical significance was determined using the paired Student *t*-test. * $P < 0.05$.

3. Leptin facilitated glycolysis in DCs

Previous research has found that Leptin inhibits DCs apoptosis through the activation of the NF-κB and PI3K/Akt signaling pathways [20]. In addition, Akt plays a vital role in cell growth, proliferation, energy metabolism and survival [21]. Accordingly, we hypothesized that leptin could have an effect on glucose metabolism in DCs. When DCs were treated with leptin for 6 hrs, we found that glucose content in media was decreased, which means that the glucose uptake of DCs was increased, compared with untreated DCs (Fig.3A). In line with this, lactate production in media was also increased after stimulating with leptin (Fig.3B). Then we analyzed the expression of GLUT1 and glycolytic relative enzymes, including HK2, PKM1/2, and LDHA at the mRNA level. Compared with control cells, the expression of HK2 was up-regulated in leptin-stimulated DCs (Fig.3C). Taken together these results indicated that leptin facilitated glycolysis in DCs.



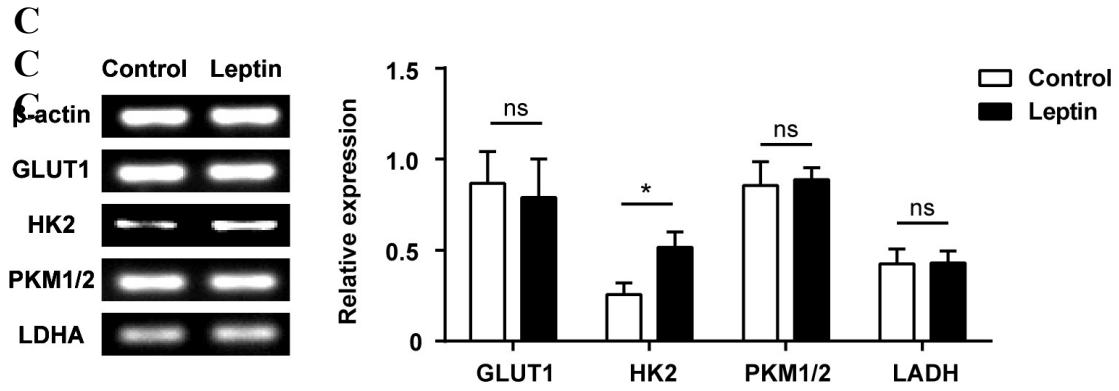


Fig. 3 Leptin facilitated glycolysis in DCs. DCs were stimulated with leptin or not for 6 hrs**.** (A) Glucose consumption and (B) lactate production by unstimulated or leptin-activated DCs. Bars represent the mean values ± SEM from 4 to 5 independent replicates. (C) Confirmation of differential mRNA expression of GLUT1, HK2, PKM1/2, LDHA in unstimulated and leptin-activated DCs was analyzed by RT-PCR and normalized to housekeeping gene β-actin expression. Bars represent the mean values ± SEM from 3 independent replicates. Statistical significance was determined using the Student *t*-test. * $P < 0.05$, *** $P < 0.001$.

4. DCs activation by leptin depended on glycolysis

Currently, some studies have found that glycolysis is important for DCs activation. Blocking glycolysis results in marked inhibition of DCs activation after exposure to TLR agonists [13]. Thus, we want to confirm the effect of glycolysis during activating DCs by leptin. To study whether suppression of glycolysis could affect DCs activation, DCs were treated with leptin after 2-DG inhibiting glycolysis. 2-DG is an inhibitor of hexokinase (HK), blocks the first and rate-limiting reaction of glycolysis, and competitively inhibits glucose uptake [22]. We observed that in leptin-induced DCs, the expression of CD80 and CD86 was impaired by 2-DG (Fig.4A). In addition, inhibition of glycolysis by 2-DG, the function of leptin-DCs to induce PBMC proliferation and promote the Th17 percentage was inhibited (Fig.4B) So we included that DCs activation by leptin depended on glycolysis.

A

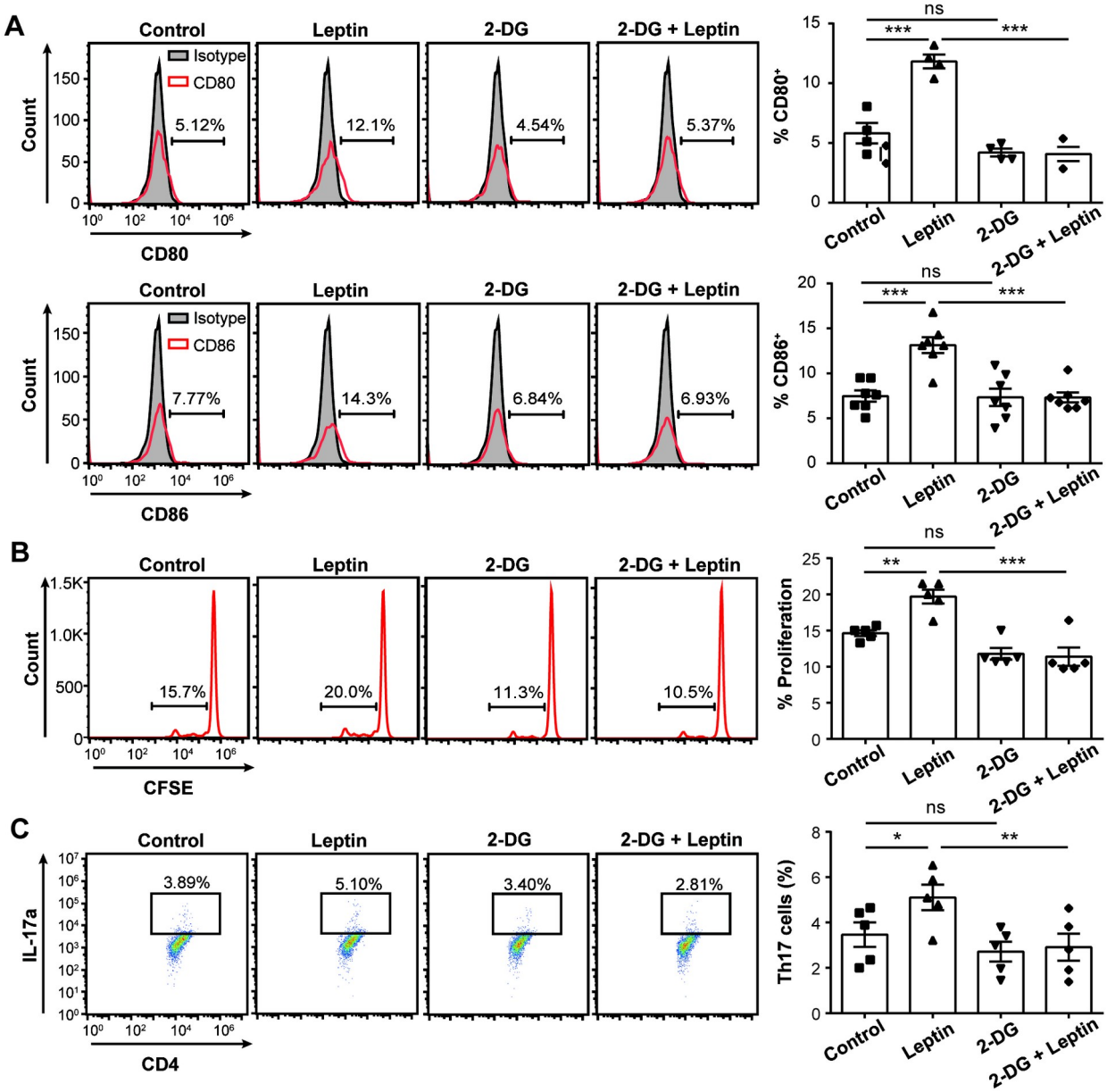


Fig.4 DCs activation by leptin depended on glycolysis (A) Stimulation of DCs with medium alone, leptin, leptin plus 2-DG for 12hrs, CD80 and CD86 expression was analyzed by flow cytometry. Bars represent the mean values ± SEM from 4 or more independent replicates. (B-C) CFSE-stained PBMC or untreated PBMC co-cultured with DCs had been pretreated with medium alone, leptin, leptin plus 2-DG for 12 hrs in the presence of anti-CD3 for 3 days. (B)CFSE-stained PBMC proliferative responses and (C) frequency of IL-17a^{+} cells were analyzed by flow cytometry. Bars

represent the mean values ± SEM from 5 experiments. Statistical significance was determined using the paired student *t*-test. * $P < 0.05$; ** $P < 0.01$, *** $P < 0.001$.

5. Leptin enhanced HK2 expression on DCs via leptin-STAT3 pathway.

Leptin interacts with its receptors, mainly through the JAK-STAT signaling pathway to transmit extracellular signals into cells [23]. In addition, leptin can activate the PI3K-Akt signaling pathway and protect DCs from apoptosis [20]. Our previous study found that leptin could up-regulate the expression of HIF-1α in fibroblast-like synoviocytes [24]. Studies have shown that Akt and HIF-1α play an important role in regulating the activity and expression of HK2 [13][25].To further understand the molecular mechanism of how leptin up-regulates HK2 expression, we detected the expression of HIF-1α. There was no significant difference in HIF-1α expression after leptin stimulated DCs (Fig.5A). Furthermore, we wanted to study the roles of Akt and STAT3 in leptin up-regulating HK2. We pretreated DCs with Akt or STAT3 signaling pathway inhibitors for 2 hrs, and then detected the effect of leptin on HK2 expression. Compared with the control group, the expression of HK2 mRNA was up-regulated after stimulation with leptin but was not affected by Akt inhibitor. What's more, the ability of leptin to promote the expression of HK2 mRNA was reversed when the STAT3 inhibitor was added in advance (Fig.5B). These results indicated that inhibition of STAT3 rather than Akt phosphorylation could repress leptin's up-regulation of HK2. Therefore, we suggested that leptin up-regulates the expression of HK2 in DCs through leptin-STAT3 signaling pathway.

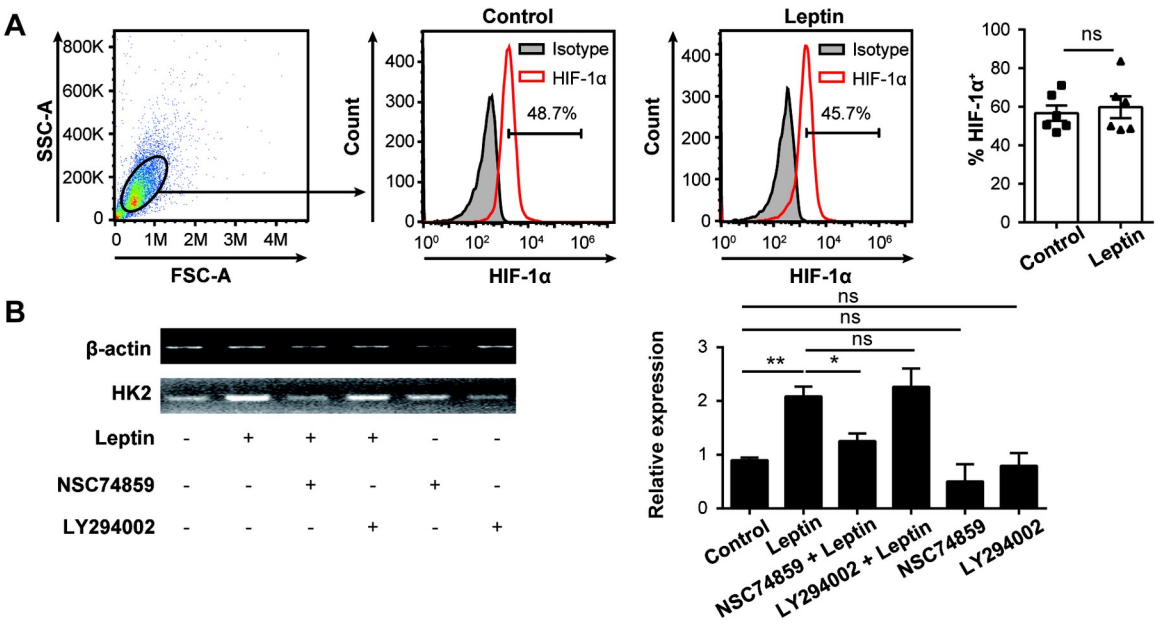


Fig.5 Leptin enhanced HK2 expression on DCs via leptin -STAT3 pathway. (A) DCs were treated by leptin for 4 hrs, then the expression of HIF-1α was assessed by flow cytometry. Bars represent the mean values ± SEM from 6 independent experiments. (B) DCs were treated for 2 hrs with Akt and STAT3 signaling pathway inhibitors: LY294002, NSC74859 or medium prior to stimulation with leptin for 6 hrs. mRNA expression of HK2 was analyzed by RT-PCR and normalized to housekeeping gene β-actin expression. Mean values ± SEM from 3 independent experiments are shown. Statistical significance is determined using the paired Student *t*-test. * $P < 0.05$, ** $P < 0.01$.

- **Discussion**:

Increasing evidence established that the inflammatory effect of leptin and the abnormal activation of DCs were involved in the pathogenesis of autoimmune diseases. It has been reported that leptin can promote the function of DCs, but its mechanism has not been clearly expounded. A better understanding of the mechanism of leptin promoting DCs function may become an important target for the treatment of autoimmune diseases. In this study, we investigated that leptin could enhance the immune function and glycolysis of DCs. When DCs glycolysis is inhibited, the effect of leptin on enhancing DCs immune function can be reversed. Therefore, we believe that leptin enhances DCs immune function in a glycolysis-dependent manner. Furthermore, we found that leptin might up-regulate the expression of HK2 by activating the STAT3 signaling pathway, thereby enhancing the glycolysis ability of DCs.

Leptin is not only involved in appetite and energy metabolism but also in the immune response. It can regulate both innate and adaptive immunity mainly through pro-inflammatory effects [26]. In innate immunity, leptin promotes the secretion of inflammatory cytokines and the activation of macrophages, neutrophils, and natural killer cells [5][27]. Functions of leptin in adaptive immunity include thymic homeostasis, naïve $CD4^+$ cell proliferation, promotion of Th1 responses, and suppression of $CD4^+$

$CD25^{high}$ regulatory T cells (Tregs) [5][27]. Consequently, leptin can contribute to the onset and progression of several autoimmune diseases, including Crohn's disease [28], rheumatoid arthritis [30][26], multiple sclerosis [32][31], and autoimmune hepatitis [33]. Leptin exerts its biological actions via signal transduction pathways activated following the binding of leptin receptors, which are expressed on a variety of immune cells, such as monocytes, neutrophils, DCs, and T and B lymphocytes [36]. Moreover, it has been reported that leptin promotes DCs maturation. DCs are the key professional antigen-presenting cells (APCs) that connect the innate and adaptive immune responses [38]. Of critical importance is their ability to rapidly switch from a resting state to an activated state in response to environmental danger signals associated with infection or cellular damage [39]. After DCs activation, the expression of costimulatory molecules on DCs and the production of cytokines are up-regulated, which is crucial to driving naïve T cell priming and differentiation. Emerging data suggest a role for DCs in initiating autoimmune attacks. Direct analysis of DCs phenotypes and DCs–Tcells interactions in human autoimmune diseases should shed light on how the pathogenesis occurs [40].

In this study, we firstly confirmed that the DC cell line used in the experiment expressed leptin receptor, then we found that leptin could up-regulate the expression of costimulatory molecules CD80 and CD86 on the surface of DCs in the early stage. Moreover, we observed leptin could up-regulate the expression of DCs inflammatory cytokines IL-6, IL-23 and TNF-α. And the ability of DCs to promote the proliferation of PBMC was enhanced after leptin treatment, along with the increased proportion of Th17 cells in PBMC co-cultured with DCs. IL-17-producing T helper (Th17) cells have been implicated in the pathogenesis of many inflammatory and autoimmune diseases while Treg cells can inhibit these phenomena and maintain immune homeostasis [41]. Thus, the Th17/Treg cell balance is of great importance in the pathogenesis of autoimmune diseases [42]. Therefore, our study showed that leptin could enhance the function of DCs and especially promote the proportion of Th17 cells in PBMC co-cultured with DCs. This result provides a new idea for targeted

leptin therapy of Th17 related diseases.

Given the importance of metabolism in directing cell fate and functions, further investigation about cell metabolism is needed to provide alternative novel strategies for disease immunotherapy. Evidence is accumulating that the metabolic requirements of an activated DCs are also distinct from those of a quiescent DCs [13]. There is a growing appreciation for the role of metabolic changes in the phenotype and function of DCs. The ligation of Toll-like receptors (TLR) leads to rapid activation of DCs. Recent research showed that TLR agonists promoted a profound metabolic switch in resting immature DCs from mitochondrial β-oxidation and OXPHOS to glycolysis. It is similar to the Warburg effect of tumor cells [16]. 2-DG, a competitive inhibitor of glucose metabolism, has been proved to substantially impair the LPS-induced surface expression of the costimulatory receptor CD40 and costimulatory molecule CD86 and MHC class I and class II and production of interleukin 6, IL-12p70 and TNF in GM-DCs [43]. It is confirmed that glycolysis plays an important role in DC activation. In a study about human monocyte-derived DCs (MoDCs), the expression of HK2, a key enzyme of glycolysis, was up-regulated after activation of TLR4 agonist (LPS). In particular, they found that LPS-induced HK2 expression required HIF-1α accumulation and transcriptional activity [43]. In the past few years, studies on leptin suggested that leptin could be used as a regulator to link nutrition, metabolism and immune homeostasis. Researchers have found that leptin could up-regulate the expression of glucose transporter GLUT1 and increase glucose uptake in activated T cells [44].In the current study, we demonstrated that that leptin signal activation of DCs cell lines could also induce glycolysis with an increased glucose consumption and lactate production. Leptin strongly enhanced the expression of HK2, whereas the expression of other rate-limiting glycolytic enzymes was not significantly increased. The expression of HIF-1α in DC cells stimulated by leptin has not changed.

Leptin receptor clustering results in JAK2 transphosphorylation and activation of several intracellular signaling cascades including the STAT, PI3K, and AKT pathways [25]. Consequently, we further used Akt and STAT3 signaling pathway inhibitors to

explore the signaling pathways involved in leptin-induced glycolysis. And we found that only the inhibitors of STAT3 reduced the expression of HK2 triggered by leptin stimulation. In addition, we also observed that the effect of leptin on DCs function was inhibited when 2-DG was used to inhibit glycolysis. The addition of 2-DG contributed not only to the descending expression of costimulatory molecules CD80 and CD86 on the surface of DCs but also the decreased proliferation ability of PBMC co-cultured with DCs. Intriguingly, the percentage of Th17 cells decreased as well. These results confirmed that glycolysis played a key role in the activation of DC by leptin.

In summary, we identified that leptin could enhance the immune function of DCs in vitro, including up-regulating the expression of costimulatory molecules CD80 and CD86 as well as promoting the secretion of inflammatory cytokines. Moreover, leptin could promote the glycolysis ability of DCs. More importantly, the effect of leptin on DCs was glycolysis-dependent, which was mediated by leptin-STAT3-HK2 pathway (Fig.6). Importantly, our studies preliminarily clarified the effect of leptin on energy metabolism and immune function of DCs and analyzed its possible mechanism, which provided a new idea for the mechanism research and targeted therapy of autoimmune diseases and other related diseases. While our study provides compelling evidence that leptin signaling selectively promotes DCs function by promoting glycolysis, this novel concept is limited to cell lines and has not been validated in vivo. Whether leptin can change the function and phenotype of immune cells by reprogramming in autoimmune diseases is worthy of further study.

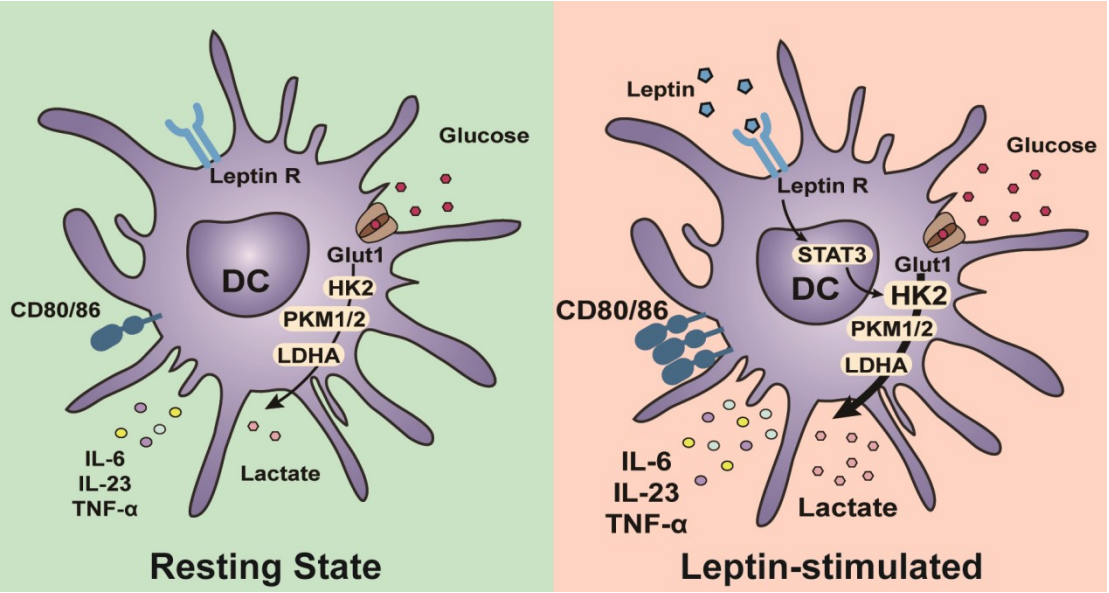


Fig.6 A Model for the Role of Leptin in Modulating the DC activation and glycolysis. Leptin promotes DCs activation via changing glucose metabolism, showing an increase in consumption of glucose and production of lactate. This process is controlled by the leptin/stat3/HK2 pathway.

- **Acknowledgements**:

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- **Disclosure:**

The authors declare no conflicts of interest.

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