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Increased HIF-1α Expression in T Cells and Associated with Enhanced Th17 Pathway in Systemic Lupus Erythematosus

Chien-Sheng Wu
Far Eastern Memorial Hospital
Hsiu-Jung Liao
Far Eastern Memorial Hospital
I-Tsu Chyuan
Cathay General Hospital
Szu-Chieh Wang
National Taiwan University College of Medicine
Hua-Yi Lee
National Taiwan University College of Medicine
Ping-Ning Hsu (phsu8635@ntu.edu.tw)
National Taiwan University https://orcid.org/0000-0003-4479-3391

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Abstract

Recent emerging evidences indicate that dysfunction of metabolic remodeling underlies aberrant T cell immune responses in systemic lupus erythematosus (SLE). However, how these aberrant immune activation and metabolic dysfunction interact in lupus patients are not fully understood. This study was undertaken to investigate the expression of HIF-1α, a regulator of metabolic reprogramming, in T cells from SLE. Our results demonstrated that HIF-1α expression is increased in CD4 T cells from SLE patients both in intracellular staining and quantitative real-time PCR analysis. In addition, there is enhanced HIF-1α expression in Th17-skewing murine T cells, and lentivirus-mediated HIF-1α overexpression promotes Th17 differentiation. Moreover, HIF-1α gene expression is positively correlated with the expression of glycolysis- and IL-17-associated genes in SLE patients. These results indicate that HIF-1α expression is increased in T cells from SLE patients, and is associated with enhanced Th17 pathway, implicating HIF-1α contributes to the activation of Th17 cells in SLE, and represents a potential novel therapeutic target.

Key Messages

1. Increased expression of HIF-1α, a regulator of metabolic reprogramming, in CD4 T cells from SLE patients.

2. There is enhanced HIF-1α expression in Th17-skewing murine T cells, and lentivirus-mediated HIF-1α overexpression promotes Th17 differentiation.

3. HIF-1α gene expression is positively correlated with glycolysis- and Th17-associated pathways in SLE patients.

4. The results implicate that HIF-1α contributes to the activation of Th17 cells in SLE, and represents a potential novel therapeutic target.

Introduction

SLE is a systemic autoimmune disease characterized with production of autoantibody and dysregulation of T cell signaling and effector function [1]. Recent emerging evidences indicate that dysfunction of metabolic remodeling underlies many aberrant immune responses [2]. Metabolic remodeling is intrinsically linked to T cell activation, differentiation, and function. In addition, metabolism switching between glycolysis and oxidative phosphorylation is critical for T cell effector functions, and increased glycolysis leads to autoimmunity [3, 4]. However, how these aberrant immune activation and metabolic dysfunction interact in lupus patients are not fully understood.

HIF (hypoxia-inducible factor)-1α is first known as a sensor of oxygen and is induced in hypoxic environment [5, 6]. Recent studies reveal HIF-1α is regulated by various non-hypoxic conditions [7], including immune activation [8]. Induction of the HIF-1α transcription factor and glycolysis interferes the

apoptosis and differentiation of activated human T cells [9]. HIF-1α modulates immune responses within localized inflammatory lesions by orchestrating immune interplay regulating disease activity and progression. In addition, HIF-1α drives inflammation by controlling the Th17/Treg balance through enhancing the differentiation of CD4+ T cells to pro-inflammatory Th17 cells [10, 11]. HIF-1α is a key regulator of immune metabolism, mediates the metabolic switch from oxidative phosphorylation to glycolysis [12], and its role had been studied in experimental autoimmune encephalitis or lupus animal models [13-15]. Recent accumulating evidences have demonstrated that metabolic reprogramming determines T cell fate and function. To investigate whether HIF-1α affects immune metabolism and T cell function in SLE, we studied the expression of HIF-1α, a regulator of metabolic reprogramming, in T cells from SLE patients, and its association with Th17 pathway. Our results demonstrated HIF-1α expression is increased in T cells from SLE patients, and is positively correlated with glycolysis- and Th17- associated pathways, implicating HIF-1α contributes to the activation of Th17 cells in SLE.

Methods

Identification of candidate genes in Gene Expression Omnibus (GEO) dataset

GEO data GDS4719 and GSE13887 were selected for candidate genes analysis (https://www.ncbi.nlm.nih.gov/sites/GDSbrowser?acc=GDS4719 and https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE13887) [16]. This dataset included expression profiling of CD3-positive T cells isolated from SLE patients and healthy controls (HC). The gene expression data in Series Matrix File with TXT format were downloaded and then analyzed by GEO tools or R script and RStudio (Northern Ave, Boston, MA) [17].

Patients and healthy controls

A total of 43 SLE patients fulfilled the 1997 ACR revised classification criteria for SLE [18] and 20 HC from Far Eastern Memorial Hospital and National Taiwan University Hospital were recruited after obtaining informed consents. The study was approved by the Research Ethics Committee of the Far Eastern Memorial Hospital and National Taiwan University Hospital. Informed consent forms were obtained for all subjects. At each visit, major clinical manifestations of SLE, serology markers, and the SLEDAI-2K were documented [19], and 10 ml sodium heparinized venous whole blood were obtained peripherally from each of the participants for serum and cell isolation.

Mice

C57BL/6 mice were purchased from the Animal Center of National Taiwan University and National Laboratory Animal Center. Female mice at the age of 6-8 weeks were used in this study. All mice were maintained in the specific pathogen-free (SPF) facility at the College of Medicine, National Taiwan University. All the experimental procedures and the use of the animals were approved by the Institutional Animal Care and Use Committee (IACUC) in National Taiwan University Medical Center.

Preparation and enrichment of CD4 T cells from human and mice

Human peripheral blood mononuclear cells (PBMC) were isolated by Ficoll-Paque PLUS (GE, Uppsala, Sweden). Human CD4 T cells were separated by RosetteSep™ Human CD4 T Cell Enrichment Cocktail (Stemcell, Vancouver, BC, Canada). Cells were cultured in RPMI-1640 medium with L-glutamine (Corning, Newark, USA) and supplemented with 10% FBS (Corning Inc, Corning, NY) and antibiotics (Sigma-Aldrich, St Louis, Missouri).

For isolation of murine CD4 T cells, spleens isolated from mice were ground and passed through the 40 µm strainer. For enrichment of CD4 T cells, MojoSort™ $CD4^+$ T cell isolation kit (Biolegend, San Diego, CA) was used according to the manufacturer's instruction. Briefly, the lymphocyte suspension was centrifuged at 400 × g for 5 minutes and resuspended the pellet in 90 µl FACS buffer. CD4 biotin-antibody cocktail 10 µl was added for 15 minutes on ice. The suspension was washed with 4 ml FACS then centrifuge at 400 × g for 5 minutes to collect the pellet. The pellet was resuspended in 90 µl FACS buffer with 10 µl streptavidin-nanobeads for 15 minutes on ice. Suspension was diluted with 3 ml FACS buffer and non-T cells were removed by standing in the MojoSort™ magnet for 5 minutes.

RNA isolation and quantitative real-time polymerase chain reaction (qPCR).

Total RNA from human or mice CD4 T cells was isolated using a RNeasy Plus Mini kit (Qiagen) and then converted into complementary DNA (cDNA) using a SuperScript VILO cDNA Synthesis kit (Invitrogen Life Technologies). Afterward, quantitative amplification was performed with SYBR Green Master Mix by QuantStundio3 (Thermo Fisher Scientific, Vantaa, Finland). For the quantification of each PCR product, the threshold cycle (CT) was used. The delta CT value was calculated from the difference in the CT of the gene of interest and that of the housekeeping gene β-actin. The relative expression of target gene was expressed as $2^{-\Delta CT}$ normalized to β-actin. The primer sequences were as follows:

Human *HIFA1*

Forward:5'-GAAAGCGCAAGTCCTCAAAG-3'

Reverse:5'-TGGGTAGGAGATGGAGATGC-3'

Human *IFNG*

Forward:5'- GAGTGTGGAGACCATCAAGGAAG-3'

Reverse:5'- TGCTTTGCGTTGGACATTCAAGTC-3'

Human *IL17A*

Forward:5'-CGGACTGTGATGGTCAAC-3'

Reverse:5'-CAAGGTGAGGTGGATCGGTT-3'

Human *SLC2A1*

Forward:5’-AGGTGATCGAGGAGTTCTAC-3’

Reverse:5’-TCAAAGGACTTGCCCAGTTT-3’

Human *SOCS1*

Forward:5’-GTCCCCCTGGTTGTTGTAG-3’

Reverse:5’-AAGAGGTAGGAGGTGCGAG-3’

Human *SOCS3*

Forward:5’-GTGGAGAGGCTGAGGGACTC-3’

Reverse:5’-GGCTGACATTCCCAGTGCTC-3’

Human *STAT3*

Forward:5’-GGCCCCTCGTCATCAAGA-3’

Reverse:5’-TTTGACCAGCAACCTGACTTTAGT-3’

Human *RORC*

Forward:5’-TTTTCCGAGGATGAGATTGC-3’

Reverse:5’-CTTTCCACATGCTGGCTACA-3’

Human *ACTB*

Forward:5’-TTAGTTGCGTTACACCCTTTCTTG-3’

Reverse:5’-TCACCTTCACCGTTCCAGTTT-3’

Mice *Hif1a*

Forward: 5’-CCTGCACTGAATCAAGAGGTTGC-3’

Reverse: 5’-CCATCAGAAGGACTTGCTGGCT-3’

Mice *Actb*

Forward: 5’-CATTGCTGACAGGATGCAGAAGG-3’

Reverse: 5’-TGCTGGAAGGTGGACAGTGAGG-3’

In vitro Th17 cell differentiation

To obtain murine Th17-skewed cells, purified $CD4^+$ T cells (10^6/well) were seeded into the 96-well plate and stimulated with anti-CD3 and anti-CD28 antibody (3 and 1 mg/well, respectively; Biolegend, San Diego, CA) under the Th17-skewing condition which consisted of RPMI medium supplemented with 10% FBS, 50 ng/ml IL-6, 50 ng/ml IL-23, 2.5 ng/ml TGF-β (Peprotech, Rehovot, Israel), and 10 μg/ml neutralizing antibodies against IFN-γ and IL-4 (Biolegend, San Diego, CA) [10, 11]. All cells were cultured at 37°C for 4 days.

Western blotting

Whole-cell protein lysate from mice or human T cells was extracted by using the PhosphoSafe Extraction Reagent (Merck Millipore, Darmstadt, Germany). Proteins were separated by 6-8% Tris-glycine SDS-acrylamide gels and transferred onto PVDF membrane. The protein was labeled with monoclonal primary Abs against HIF-1α and β-actin (Cell Signaling Technology Inc, MA). The secondary Ab was labeled with horseradish peroxidase, and electro-chemiluminescence was used to visualize the bands.

Overexpression of HIF-1α in murine T cells via lentivirus

For preparation of HIF-1α expressing vectors, 293T cells were transfected with pCMV-ΔR8.91, pMD.G, and HIF-1α-Green fluorescent protein (GFP) plasmids by using Lipofectamine 2000 to produce HIF-1α-GFP containing lentivirus. Lentivirus were harvested by PEG-it virus precipitation solution (System Biosciences Llc, Palo Alto, CA).

Murine T cells were harvested after cultured in Th17-skewing condition for 4 days and re-seeding into a new well pre-coated with a combination of anti-CD3/anti-CD28 Ab (Biolegend, San Diego, CA), and infected with HIF-1α-GFP containing lentivirus for 24 hours, then subjected to flow cytometry analyses.

Flow cytometry analysis

For flow cytometry analysis, enriched human CD4 T cells were treated with Fc receptor blocker (BD Biosciences, San Jose, CA) for 30 minutes on ice and then stained with surface marker specific antibody in FACS buffer for 30-60 minutes. Cells were labeled with anti-CD3, anti-CD4, anti-CD8, anti-GLUT-1-FITC (R&D Systems, Minneapolis, MN). After surface staining, cells were fixed with BD CytofixTM buffer overnight. After 24 hours, T cells were then treated with cell-fixation/cell permeabilization kit (Invitrogen, Carlsbad, CA) according to the method described by the manufacture in room temperature and stained with anti-HIF-1α-PE (BioLegend, San Diego, CA), anti-ROR-γt-BV421 (BD Biosciences, San Diego, CA). After permeabilization and intracellular staining, cells were resuspended in FACS buffer (2% FBS in PBS with EDTA) and analyzed by flow cytometer and FACSDiva software (BD Biosciences, San Diego, CA).

Before staining, murine T cells were treated with GolgiSTOP (BD Biosciences, San Diego, CA) for 4 hours. For intracellular staining, harvested cells were fixed with Invitrogen Fixation/Permeabilization buffer for 30 minutes at room temperature. After permeabilization, cells were stained with fluorescent-conjugated

anti-IL-17A-APC (BD Bioscience, San Diego, CA), anti-GLUT1-Alexa Flour 488 (Abcam, Cambridge, UK), anti-RORγt-BV421 (BD Bioscience, San Diego, CA), or anti-HIF-1α- Alexa Flour 647 (Abcam, Cambridge, UK).

Statistical analyses

For the categorical parameters, Chi-square statistics was used. For a comparison between two groups of samples, Student's t-test or non-parametric Mann-Whitney U test was used as appropriate. Pearson's correlation or Spearman's rank-order correlation test was used to detect significant correlations between two parameters of interest. Statistical analyses were performed by MedCalc (MedCalc Software Ltd, Ostend, Belgium) or Prism 7 (GraphPad software Inc, San Diego, CA).

Results

Increased HIF-1α gene expression in transcriptome profiles of T cells from patients with SLE in GEO database

To screen for the candidate metabolic genes associated with SLE, we analyzed Gene Expression Omnibus (GEO) data GDS4719 and GSE13887 compared the expression of target genes in T cells from SLE patients and healthy control (HC) [16]. After analysis of the dataset, the results in Fig. 1 demonstrated that the expression of HIF-1α gene (*HIF1A*) was significantly increased in T cells from patients with SLE, indicating HIF-1α, the essential regulator of metabolic switch, is upregulated in T cells from lupus patients, which implies that HIF-1α may contribute to metabolic reprogramming of T cells in the development and disease activity of lupus.

Increased HIF-1α expression in CD4 T cells from patients with SLE

To confirm the results derived from the database, we further investigated the expression of HIF-1α in T cells from SLE patients. CD4 T cells were isolated from SLE patients, and the protein expression of HIF-1α was analyzed with intracellular staining in flow cytometry and Western blot. The gene expression of HIF-1α in T cells was measured with quantitative real-time PCR. The results in Fig. 2 demonstrated increased HIF-1α protein expression in CD4 T cells from SLE patients in Western blot and intracellular staining ($p < 0.001$ by Mann-Whitney U test) (Fig. 2a). In addition, the gene expression level of HIF-1α was also significantly higher in patients with SLE when compared with HC (Fig. 2b). We further compared the gene expression level of HIF1-α between active (SLEDAI >6) and inactive (SLEDAI ≤ 6) SLE; but there is no significant difference between these two groups. Taken together, all these results demonstrate increased HIF-1α expression in CD4 T cells from patients with SLE.

Enhanced HIF-1α expression in Th17-skewing murine T cells

Previous studies demonstrated that elevated HIF-1α expression level is strongly associated with increased disease activity in a variety of inflammatory diseases. In addition, HIF-1α drives inflammation

by controlling the Th17/Treg balance through enhancing the differentiation of CD4 T cells to pro-inflammatory Th17 cells [10, 11]. To further confirm HIF-1α expression is enhanced in Th17 cells and to study the impacts of HIF-1α on T cell differentiation, we cultured murine CD4 T cells in Th17-skewed condition and analyzed the expression of HIF-1α. The results in Fig. 3 demonstrated in Th17-skewing condition (Fig. 3a), the HIF-1α expression was increased in Th17 cells in intracellular staining (Fig. 3b). The HIF-1α mRNA expression level is also significantly elevated in Th17 cells in quantitative real-time PCR analysis (Fig. 3c). These results indicate HIF-1α expression is enhanced in Th17 cells, implicating elevated HIF-1α expression may drive inflammation by controlling the Th17 differentiation.

Overexpression of HIF-1α promotes Th17 differentiation in murine T cells

To further investigate the effects of increased HIF-1α expression on the differentiation of CD4 T cells to Th17 cells, we overexpressed HIF-1α in murine CD4 T cells under Th17- skewed condition via transducting T cells with HIF-1α-GFP-containing lentivirus. The results in Fig. 4 demonstrated that lentivirus-mediated HIF-1α overexpression in T cells significantly increased IL-17 producing T cells when compared with vector control. These results indicate that HIF-1α overexpression promotes differentiation of CD4 T cells to Th17 cells.

HIF-1α gene expression is positively correlated with glycolysis- and Th17-associated genes in SLE patients

HIF-1α has been demonstrated to promote glycolysis reprogramming and the differentiation of Th17 in mice [10, 11]. To study whether HIF-1α gene expression is associated with metabolic or Th17-assocaited genes in T cells, we analyzed the quantitative mRNA expression of gene of HIF-1α (HIF1A) and the metabolic or Th17-associated genes: glucose transporter 1 or GLUT1 (SLC2A1), IL-17 (IL17A), IFN-γ (IFNG), STAT3(signal transducer and activator of transcription 3), RORC(RORγt), SOCS1(suppressor of cytokine signaling 1), and SOSC3(suppressor of cytokine signaling 3) in CD4 T cells from SLE patients by quantitative PCR. The results in Fig. 5 demonstrated that among the metabolic or Th17-associated genes, GLUT1 (SLC2A1) gene expression was significantly increased in patients with SLE (Fig. 5a). Moreover, HIF1A gene expression was positively correlated with the expression of GLUT1 andIL-17 but not IFN-γ (Fig. 5b). These results indicate HIF-1α gene expression is positively correlated with glycolysis- and Th17-associated genes in SLE patients. We further analyzed the possible association of HIF1A gene expression and clinical manifestations in SLE patients. However, there is no significant differences between both groups of high or low expression levels of HIF1A in most of the clinical manifestations except alopecia in SLE patients (p=0.04 by Chi-square test) (Table 1).

Discussion

In this study, we demonstrated increased HIF-1α expression in T cells from SLE patients in both quantitative real-time PCR analysis and protein expression. In addition, HIF-1α gene expression was positively correlated with glycolysis- and Th17-associated pathways in SLE patients. Our results suggest that HIF-1α may contribute to the activation of pro-inflammatory Th17 cells via metabolic reprogramming

in SLE. Recent accumulating evidences reveal that HIF-1-α, the transcription factor mediates the metabolic switch from oxidative phosphorylation to glycolysis [6], regulates T cell metabolism and modulates immune responses within localized inflammatory lesions [10, 20]. Elevated HIF-1α level is strongly associated with increased disease activity in a variety of inflammatory and autoimmune diseases, including multiple sclerosis [21], rheumatoid arthritis [22], inflammatory bowel disease [23]. and type 1 diabetes mellitus [24]. In addition, previous study demonstrated that HIF-1α enhanced Th17 development, and HIF-1α-deficient mice are resistant to induction of experimental autoimmune encephalitis [10]. A recent report showed that silencing of HIF-1α reduced development of autoimmune manifestation in MRL/lpr mice [15]. Taken together, all these studies suggest that increased HIF-1α expression contributes to the development of immunopathology in autoimmune diseases.

SLE is a systemic autoimmune disease characterized by abnormal production of autoantibodies and immune dysfunctions. Numerous aberrations of the T cell responses were reported and were implicated in the pathogenesis of SLE [25]. T cells from both human lupus patients and lupus-prone mice present a hyperactive metabolic profile in T cell metabolism [26]. Inhibition of GLUT1 in lupus-prone mice MRL/lpr T cells ameliorates disease activity [27]. Recent studies also reported HIF-1α is overexpressed in human lupus CD4 T cells [28]. Moreover, in a recent study, Kono et al. reported treatment of MRL/lpr mice with BPTES, a selective Glutaminase 1 (Gls1) inhibitor, improved autoimmune pathology in a Th17-dependent manner via reducing HIF-1α protein in Th17 cells. In addition, T cells from patients with SLE treated with BPTES displayed decreased Th17 differentiation [29]. HIF-1α-dependent glycolytic pathway and metabolic reprogramming is considered to promote Th17 development, and HIF-1α–deficient mice have reduced Th17 cell differentiation [11]. All these results support our finding that HIF-1α expression is increased in T cells from SLE patients, and is positively correlated with Th17 associated pathway, suggesting that HIF-1α may play an important role in immune pathogenesis of lupus. Recent study reported that Th17 cells and IL-17 levels are elevated in several autoimmune diseases including SLE [30]; nevertheless, a positive correlation between Th17 and SLE disease activity has not been firmly established. This suggests it is quite diverse and heterogeneous in the pathogenesis of SLE. The correlation between HIF1-α and SLE disease activity still awaits further investigation.

Altered signaling and function contributes to aberrant immune cell function and autoimmunity in T cells in patients with SLE and lupus-prone mice [25, 31], and numerous abnormalities in the metabolism of these cells have been reviewed [32]. Emerging evidence suggests that metabolic abnormalities and mitochondrial clearance play important roles in SLE pathogenesis [33, 34]. Furthermore, activation of mTORC1 and immune metabolism dysfunction resulted in abnormal T cell activation in SLE patients and lupus mice [32, 35].

In summary, our current results have demonstrated that HIF-1α expression is increased in T cells from SLE patients, and is positively correlated with Th17 associated pathway, suggesting that HIF-1α may play an important role in immune pathogenesis of lupus. It also implicates HIF-1α is a potential novel therapeutic target for SLE.

Declarations

Author contribution: C-S W, H-J L, I-T C and P-N H designed the study; S-C W, H-Y L, and H-J L performed the experiments and analyzed the results; C-S W, H-J L, I-T C and P-N H drafted the figures and manuscript.

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Data availability The datasets generated during and/or analyzed during

the current study are available from the corresponding author on reasonable request.

Ethics approval: This study complies with the Declaration of Helsinki, that the research protocol was approved by the Research Ethics Committee of the Far Eastern Memorial Hospital and National Taiwan University Hospital, and that written informed consent has been obtained from the subjects.

All the experimental procedures and the use of the animals were approved by the Institutional Animal Care and Use Committee (IACUC) in National Taiwan University Medical Center.

Conflicts of interest: The authors declare no competing interests.

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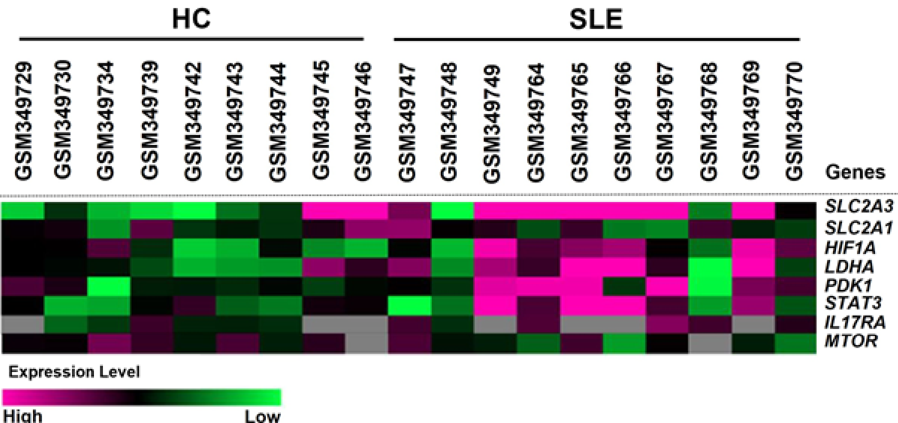
Tables

TABLE 1 Clinical manifestations of 43 lupus patients with high and low HIF-1α gene *(HIF1A)* expression (dichotomized at median)

	HIF1A low ($2^{-\Delta CT} \leq 0.0248$, n=21)		*HIF1A* high ($2^{-\Delta CT} > 0.0248$, n=22)		*p*
Median age (IQR)	53.0	(46.0 to 65.0)	50.0	(40.0 to 58.0)	0.36
Male sex (%)	2	(9.5)	6	(27.3)	0.09
Visual disturbance n (%)	0	(0)	1	(4.5)	0.30
Vasculitis n (%)	1	(4.8)	1	(4.5)	0.97
Arthritis n (%)	8	(38.1)	4	(18.2)	0.21
Proteinuria n (%)	3	(14.3)	4	(18.2)	0.63
Rash n (%)	9	(42.9)	9	(40.9)	0.88
Alopecia n (%)	13	(61.9)	6	(27.3)	0.04
Mucosal ulcers n (%)	7	(33.3)	7	(31.8)	0.91
Median SLEDAI (IQR)	6.0	(5.5 to 10.0)	6.0	(4.0 to 10.0)	0.52
Median anti-ds-DNA IU/mL (IQR)	68.4	(38.4 to 228.1)	128.8	(45.9 to 284.4)	0.47
Median C3 mg/dL (IQR)	83.1	(72.2 to 102.5)	94.3	(72.2 to 111.0)	0.54
Median C4 mg/dL (IQR)	16.5	(11.7 to 24.1)	17.0	(13.4 to 21.1)	0.94
Median WBC (k/μL) (IQR)	5.4	(3.9 to 6.9)	5.9	(4.6 to 7.1)	0.51
Median Hb (g/dL) (IQR)	12.6	(11.3 to 13.5)	13.3	(12.1 to 14.7)	0.11
Median Platelet(k/μL) (IQR)	223.0	(194.0 to 278.5)	235.5	(206.0 to 261.0)	0.67
Median ESR 1hr(mm/hr) (IQR)	8.0	(4.3 to 13.0)	7.0	(5.5 to 21.0)	0.83

Figures

a



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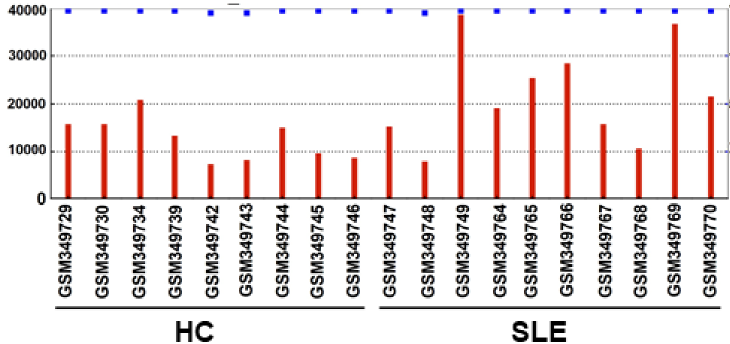


Figure 1

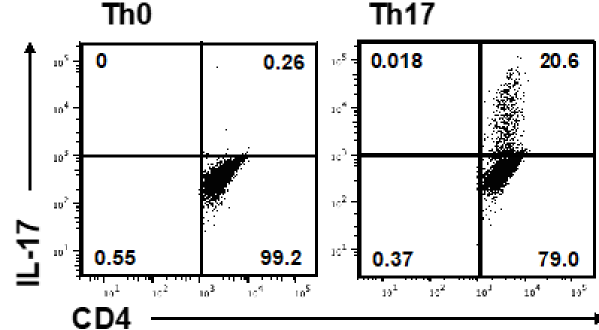
Increased HIF-1α gene expression in the transcriptome profiles of T cells from SLE patients. Gene Expression Omnibus (GEO) data of SLE patients (GDS4719) and healthy controls (HC) were analyzed by GEO tools.. This dataset included expression profiling of CD3+ T cells isolated from 10 SLE patients and 9 HC. (A) Selected gene expression of CD3+ T cell from SLE patients and HC are presented with heatmap. (B) HIF1A expression is increased in SLE patients compared to HC in GSE13887.

Figure 2

Increased HIF-1α expression in T cells from SLE patients. CD4 T cells were isolated from patients with SLE and healthy controls (HC) for HIF-1α expression. (a) The HIF-1α protein expression was analyzed with Western blotting and intracellular staining in flow cytometry. The expression level was presented with mean fluorescence intensity (MFI). The results are presented as median ± interquartile range (IQR) (*** $p < 0.001$ by Mann-Whitney U test). (b) Quantitative analysis of HIF-1α gene expression in T cells from

SLE and HC. HIF-1α gene (HIF1A) mRNA expression was normalized with β-actin and expressed as 2-ΔCT. The comparison of HIF1A mRNA expression in CD4+ T between SLE patients (n=43) and HC (n=20). SLE patients with disease activity scores (SLEDAI) > 6 (n=16) and ≤ 6 (n=27) are shown in bar graphs. Results are expressed as median ± IQR in bar graphs. (* p< 0.05 by Mann-Whitney U test)

a



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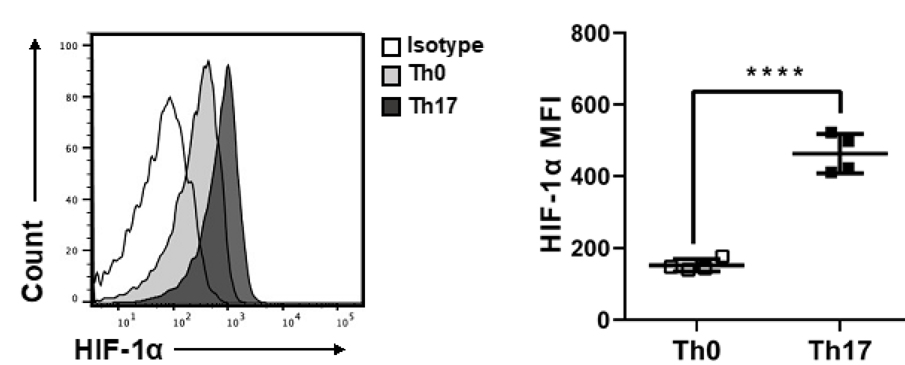

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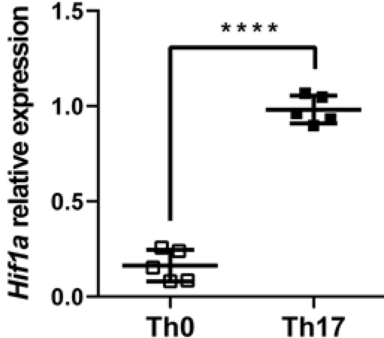
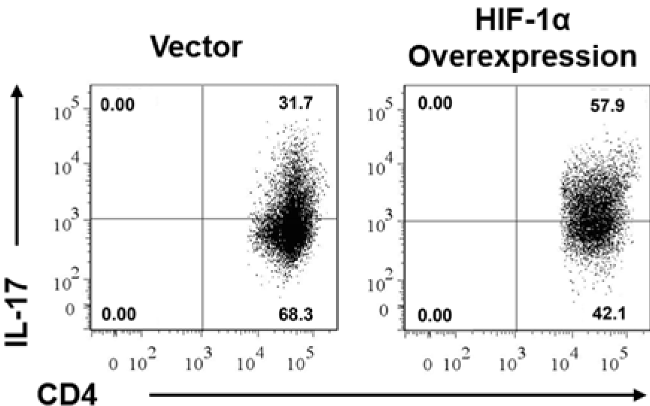


Figure 3

Enhanced HIF-1α expression in Th17-skewing murine T cells (a) Naïve murine CD4+ T cells were cultured under the Th17-skewing condition (with 50 ng/ml IL-6, 25 ng/ml IL-23, and 5 ng/ml TGF-β), and 10 ug/ml neutralizing antibodies against IFN-γ and IL-4 for 4 days, then re-stimulated with anti-CD3/CD28 Abs for 24 hours. The expression of IL-17 was analyzed by intracellular cytokine staining in flow cytometry. (b) The expression of HIF-1α in Th0 and Th17 cells was analyzed by intracellular staining in flow cytometry. The expression level was presented with MFI. The results are presented as mean ± SD. (c) Quantitative real-time PCR of HIF-1α gene (Hif1a) expression in Th0 and Th17 cells. The bar graph represents the mean ± SD of Hif1a relative expression (n=5) (**** $p<0.0001$ by two-tailed Student’s t test).



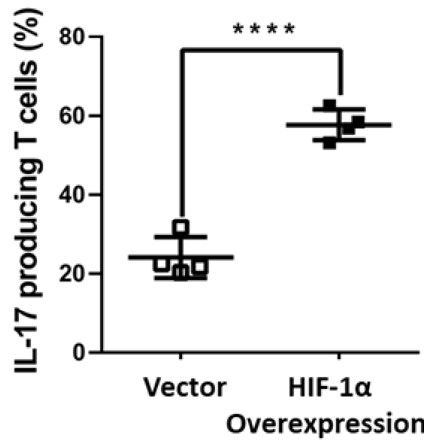
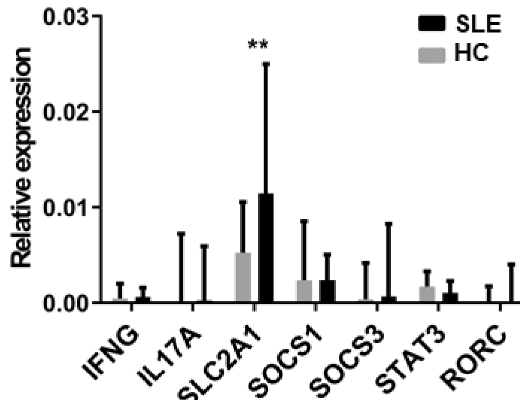
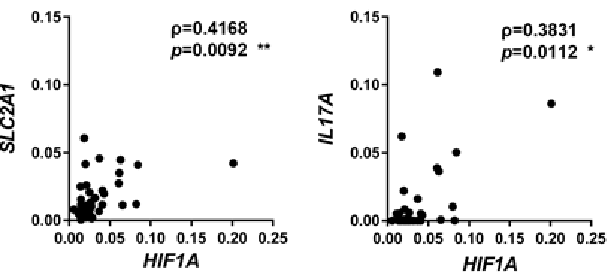


Figure 4

Overexpression of HIF-1α in murine T cells enhanced IL-17 production. Naïve CD4 T cells from mice were cultured under the Th17-skewing condition for 4 days, and re-stimulated with a combination of anti-CD3/anti-CD28 Abs for 24 hours. The Th17-skewed cells were then infected with HIF-1α-GFP-containing lentivirus at day 4. The expression of IL-17 in T cells with or without HIF-1α overexpression was analyzed by intracellular cytokine staining in flow cytometry. (**** $p < 0.0001$ by two-tailed Student’s t-test)





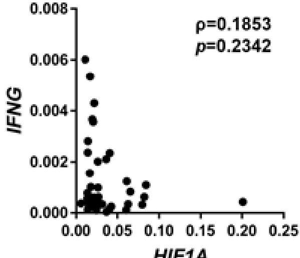


Figure 5

HIF-1α gene expression is positively correlated with glycolysis- and IL-17-associated genes in SLE patients. Quantitative mRNA expression of HIF-1α gene and selected metabloism- and Th17- associated genes in CD4 T cells from SLE patients and HC. Target gene expression is normalized with β-actin and expressed as 2-ΔCT. (a) Gene expression of glucose transporter 1 (SLC2A1), suppressor of cytokine signaling 1 (SOCS1), suppressor of cytokine signaling 3 (SOCS3), signal transducer and activator of

transcription 3 (STAT3), RORγt (RORC), IL-17 (IL17A) and IFN-γ (IFNG) in CD4 T cells from SLE patients and HC. Results are expressed as median ± IQR. (** $p < 0.01$, by Mann-Whitney U test) (b) Correlation between HIF1A, SLC2A1, IFNG, and IL17A mRNA expression in SLE patients. (* $p < 0.05$, ** $p < 0.01$, by Spearman's rank correlation test)

Supplementary Files

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- Sup1.tif
- Sup2.tif