Am J Transl Res 2018;10(12):4331-4337
www.ajtr.org /ISSN:1943-8141/AJTR0037315

Original Article

Circadian protein CLK suppresses transforming growth factor-β expression in peripheral B cells of nurses with day-night shift rotation

Hui Yang[1*], Li-Tao Yang[2,3*], Jun Liu[4*], Shan Tang[1], Xiulan Zhao[1], Qiaohong Wang[1], Shaoguo Zhang[1], Meixia Shi[1], Wei Pan[1], Ping-Chang Yang[2]

[1]Department of Nursing, The First Hospital, Shanxi Medical University, Taiyuan 030001, Shanxi, China; [2]The Research Center of Allergy & Immunology, School of Medicine, Shenzhen University, Shenzhen 518060, Shanxi, China; [3]Longgang ENT Hospital, Shenzhen 518116, Shanxi, China; [4]Shenzhen Maternity & Child Health Hospital, Shenzhen 518052, Shanxi, China. [*]Equal contributors.

Received July 28, 2016; Accepted November 24, 2016; Epub December 15, 2018; Published December 30, 2018

Abstract: Background and aims: The mechanism of dysfunction of regulatory B cells is unclear. The circadian locomotor output cycles kaput (CLK) regulates immune responses. CLK expression can be increased by alteration of the circadian rhythm. This study tests a hypothesis that alteration of the circadian rhythm, such as engaging the day-night shift rotation (DNSR), interferes with the expression of transforming growth factor (TGF)-β in B cells (TGFbB cell). Methods: Peripheral blood samples were collected from DNSR nurses and persons with the regular circadian clock life style (RC). The frequency of TGFbB cells in the blood samples was assessed by flow cytometry. The expression of TGF-beta in B cells was assessed with real time RT-PCR. Results: We observed that the frequency of peripheral TGFbB cells was less in DNSR nurses as compared to RC subjects. The expression of CLK and histone deacetylase 11 in peripheral B cells was higher, the TGF-β expression was lower, in peripheral B cells of DNSR nurses. Over-expression of CLK repressed the expression of TGF-β in B cells, which was mediated by HDAC11. Conclusions: The CLK expression in peripheral B cells is higher in DNSR nurses, which suppresses the expression of TGF-β in B cells. To regulate the expression of CLK during the circadian clock alteration needs to be further investigated.

Keywords: B lymphocyte, interleukin-10, circadian CLK, nuclear factor-interleukin-3, nurse

Introduction

The transforming growth factor (TGF)-β-producing regulatory T cells (Treg) and B cells (TGFbB cells) or interleukin (IL)-10-producing B cells play an important role in the maintenance of the immune homeostasis in the body [1, 2]. By releasing the anti-inflammatory cytokine TGF-β, Tregs and TGFbB cells suppress other immune cells' activities and inhibit inflammation in the body [3]. The immune regulatory functions of TGFbB cells have been described in allergic diseases [4]. Yet, the mechanism underlying the dysfunction of TGFbB cells has not been fully understood.

Recent reports indicate that the circadian protein, Circadian Locomotor Output Cycles Kaput (CLK), plays a role in the suppression of TGF-β expression [5]. CLK can be up regulated by the alternation of circadian rhythm [6], such as in jet lag or engaging day-night work shift rotation (DNSR). CLK is involved in the development of natural killer cells and CD8α$^+$ dendritic cells, macrophage activation and aberrant CD4$^+$ T cell polarization [7]. Several lines indicate that CLK regulates the expression of immunoglobulin (Ig)E [8, 9]. IgE is produced by B cells and is a key mediator in allergic diseases. The regulation of IgE expression has not been fully understood yet. Our previous work indicates that the frequency of TGFbB cells is decreased in mice with allergic inflammation [4]. Yet, the mechanism underlying the suppression of TGF-β in B cells remains to be further investigated. Based on the information above, we hypothesize that circadian protein CLK may be one of the factors interferes with the TGF-β

expression in B cells. In this study, we observed that the CLK levels in peripheral B cells were higher in DNSR nurses than the subjects with regular circadian life style (RC subjects). In vitro study showed that overexpression of CLK inhibited the expression of TGF-β in B cells.

Materials and methods

Human subjects

Twenty nurses (age: 25-27 years old) engaging with regular day-night shift rotation (DNSR) for 1-2 years were randomly recruited into this study in the First Hospital of Shanxi Medical University from January of 2015 to May of 2016. DNSR nurses with one of the following conditions were excluded from this study: Allergic diseases; autoimmune diseases; severe organ diseases; using immune suppressors and cancer. In addition, 20 healthy, non-DNSR subjects (age: 25-27 years old) were also recruited into this study to be a control group. The using human tissue in the present study was approved by the Human Ethic Committee at Shanxi Medical University.

Isolation of peripheral blood mononuclear cells (PBMC)

The blood samples (30 ml per subject) were collected from each human subject at 9 am by ulnar vein puncture. The PBMCs were isolated from the blood samples by gradient density centrifugation.

Isolation of B cells

$CD19^+$ B cells were isolated from the PBMCs by magnetic cell sorting (MACS) with a purchased B cell isolation reagent kit (Miltenyi Biotech) according to the producer's instructions. The purity of the isolated B cells was greater than 98% as checked by flow cytometry.

Cell culture

The B cells were cultured in RPMI1640 medium. The medium was supplemented with 2 mM L-glutamine, 0.1 mg/ml streptomycin, 100 U/ml penicillin and 10% fetal bovine serum. The medium was changed in 2 to 3 days. Before using for further experiments, the viability of the B cells was assessed by Trypan blue exclusion assay; it was greater than 99%.

Flow cytometry

The B cells were stained with FITC-labeled anti-CD19 mAb or isotype IgG (BD Bioscience) for 30 min at 4°C, washed with phosphate-buffered saline (PBS), fixed with 1% paraformaldehyde for 30 min, incubated with 0.5% saponin (Sigma Aldrich), washed with PBS, incubated with APC-labeled anti-TGF-β mAb or isotype IgG (BD Bioscience) for 30 min at 4°C, washed with PBS and analyzed with a flow cytometer (FACSCanto II, BD Bioscience). The frequency of $TGF\text{-}\beta^+$ B cells (TGFbB cells) in the PBMCs was calculated with Flowjo (TreeStar) with the data of isotype IgG staining as a gating reference.

Real time quantitative RT-PCR (RT-qPCR)

Total RNA was extracted from the harvested B cells using TRIzol (Invitrogen) and reversely transcribed into cDNA using a reverse transcription reagent kit (Invitrogen) following the manufacturer's instructions. The cDNA was then used for PCR assay of CLK and TGF-β mRNA expression. PCR assay was carried out on the CFX96 Real-time PCR Detection System (Bio-Rad) with the SYBR Green Master Mix (Invitrogen). The results were calculated with the $2^{-\Delta\Delta Ct}$ method and presented as fold change against the control group. The primers used in the present study include: GTGTTACATCGCGCCATCAT and CATTAGGAGGGCTGAGAGGG (CLK); GCAGCTGTCCAACATGATCG and GAGATCCGCAGTCCTCTCTC (TGF-β); GTCTTGCCTGTTCAGTGCAA and TGCATCCCTGATTTCCACCT (HDAC11); CGCAAAGACCTGTATGCCAA and CACACAGAGTACTTGCGCTC (β-actin).

Western blotting

The total proteins were extracted from the B cells used in the present study. The proteins were fractioned by SDS-PAGE (sodium dodecyl sulfate polyacrylamide gel electrophoresis) and transferred onto a PVDF (Polyvinylidene difluoride) membrane. The membrane was treated with 5% skim milk for 30 min at room temperature, followed by incubation with the anti-CLK mAb or anti-HDAC11 mAb or isotype IgG (Santa Cruz Biotech) overnight at 4°C, washed with Tris-buffered saline-Tween 20 (TBST) for 3 times, incubated with peroxidase-labeled second antibodies for 1 h at room temperature, developed by ECL (enhanced chemiluminescence). The results were recorded by photo-

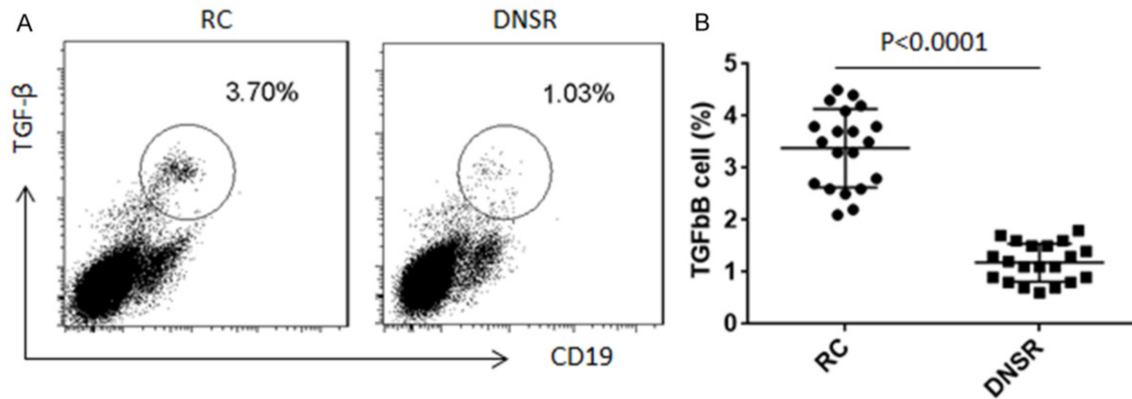

Figure 1. Frequency of TGFbB cells in DNSR nurses and RC subjects. A: The representative flow cytometry dot plots show the frequency of TGFbB cells in the peripheral blood of RC subjects and DNSR nurses. B: The scatter dot plots show the individual datum of TGFbB cell frequency of RC subjects and DNSR nurses.

graphing with an image station (UVI, Cambridge, UK).

Chromatin immunoprecipitation assay (ChIP)

ChIP assay was performed on B cells with a purchased ChIP reagent kit (Sigma Aldrich) following the manufacturer's instructions. Briefly, B cells were harvested from the culture and fixed with 1% formalin for 15 min to cross link the chromatin and the around proteins. After sonication to shear the DNA into small pieces (around 200-500 bps), the samples were precleared by incubation with protein G-agarose beads for 2 h at 4°C. The samples were centrifuged at 13,000 rpm for 10 min at 4°C to collect the supernatant to be incubated with anti-HDAC11 mAb, or anti-Foxp3 mAb, or isotype IgG, overnight at 4°C. The immune complexes in the samples were precipitated by incubation with protein G-agarose beads for 2 h at 4°C. The agarose beads were collected by centrifugation, and then washed; the immune complexes on the beads were eluted in elution buffer. DNA in the immune complex was recovered by reverse crosslinking at 65°C for 4 h. The samples were digested with proteinase K for 1 h at 45°C to remove proteins. The DNA was recovered by phenol/chloroform extraction and ethanol precipitation. The DNA or input (10%, collected before antibody precipitation) was analyzed by qPCR with primers of the TGF-β promoter: CGAAGGAGGTCACTGTGAGT and TGTTCACACAAGCGTCAGTG. The results were presented as fold change against the input.

Over expression of CLK in B cells

$CD19^+$ B cells were prepared and transfected with the CLK-plasmids or control plasmids (Sangon Biotech, Taiyuan, China) and lipotecfemine following the manufacturer's instructions. The results of the transfection were checked by Western blotting.

RNA interference (RNAi)

$CD19^+$ B cells were collected from healthy subjects and transduced with HDAC11 shRNA or control shRNA (Santa Cruz Biotech) following the manufacturer's instructions. The effects of RNAi were checked by Western blotting.

Induction of TGF-β expression in B cells

$CD19^+$ B cells were isolated from PBMCs by MACS with commercial reagent kits (Miltenyi Biotech) following the manufacturer's instructions. The purity of the isolated B cells was greater than 98% as checked by flow cytometry. Following published procedures [10], B cells were cultured in the presence of LPS (20 μg/ml) for 48 h. The B cells were harvested at the end of culture and analyzed by RT-qPCR as described above.

Statistics

All the data are presented as mean ± SD. The differences between two groups were determined by Student t test or two-way analysis of variance (ANOVA) along with the Bonferroni cor-

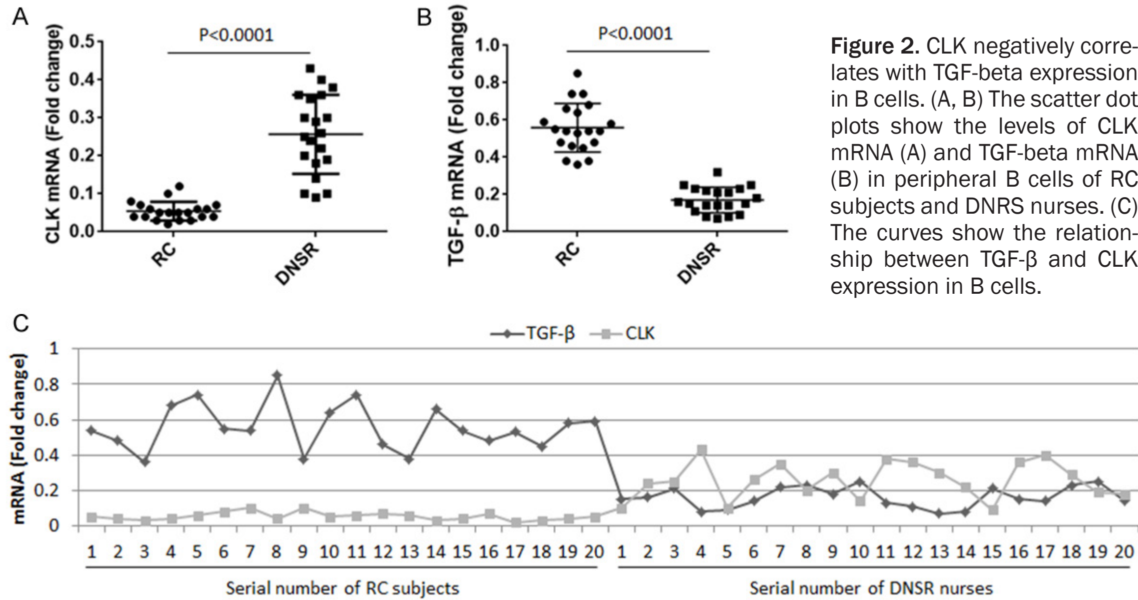

Figure 2. CLK negatively correlates with TGF-beta expression in B cells. (A, B) The scatter dot plots show the levels of CLK mRNA (A) and TGF-beta mRNA (B) in peripheral B cells of RC subjects and DNRS nurses. (C) The curves show the relationship between TGF-β and CLK expression in B cells.

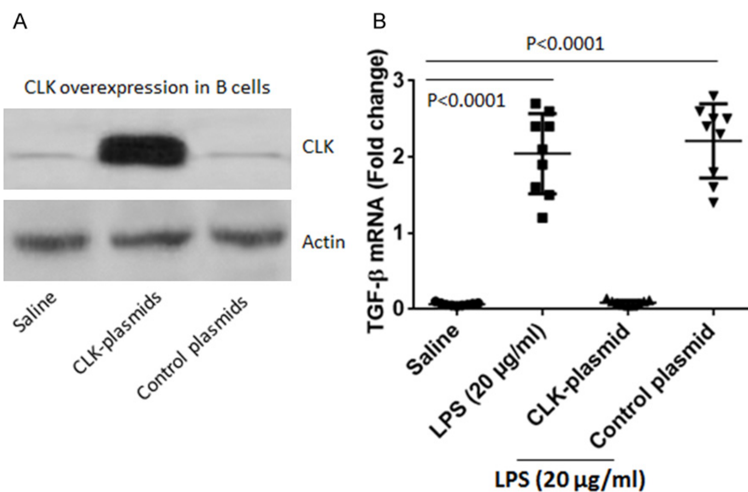

Figure 3. CLK suppresses TGF-β expression in B cells. A: The immune blots show the CLK levels in B cells after treating with the agents denoted on the X axis. B: The scatter dot plots show the TGF-beta mRNA levels in B cells after treating with the agents on the X axis. Samples were analyzed in triplicate in each experiment. The experiments were repeated 3 times. Each dot presents an individual datum.

rection. P<0.05 was set as the criterion of significance.

Results

Lower frequency of TGFbB cell is detected in DNSR nurses

Based on published data that circadian disruption disturbs the immune homeostasis [11] and associates with autoimmune diseases [12], we hypothesize that circadian disruption may affect the immune regulatory system. Thus, we assessed the frequency of peripheral TGFbB cells, one of the major cell populations of immune regulation [13], in DNSR nurses. The results showed that the frequency of TGFbB cell was lower in the peripheral blood samples of DNSR nurses than that in RC subjects (**Figure 1**). The results implicate that the generation of TGFbB cells in DNSR nurses is compromised.

High levels of the CLK in peripheral B cells of DNSR nurses

The CLK is one of the circadian proteins [14], which can regulate the expression of TGF-β [5]. Thus, we checked the levels of the CLK in peripheral B cells in DNSR nurses. The results showed that the levels of CLK mRNA were higher in peripheral B cells of DNSR nurses than that in RC subjects (**Figure 2A**). Considering that CLK might modulate the expression of TGF-β in B cells, we also assessed the expression of TGF-β in the B cells. The results showed

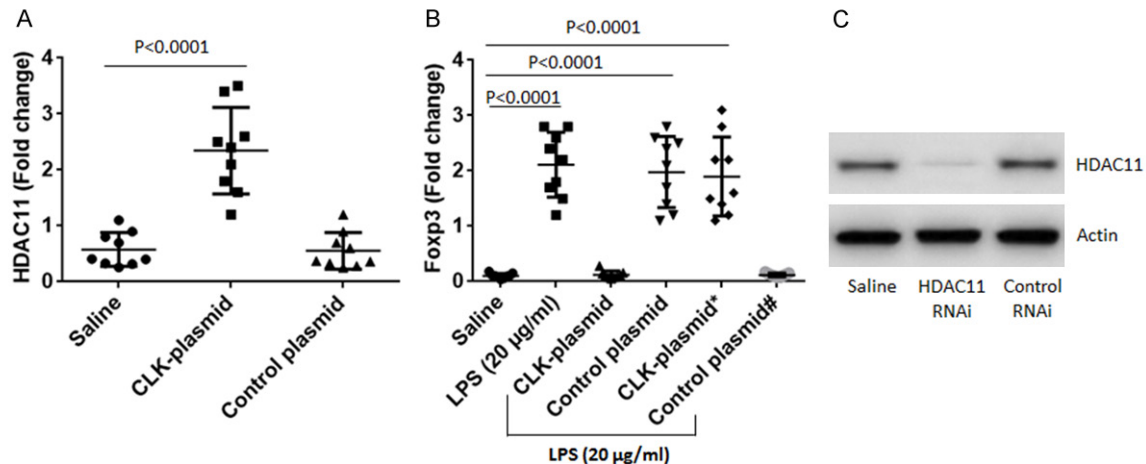

Figure 4. HDAC11 mediates the effects of CLK on suppression of TGF-beta gene transcription in B cells. A: The scatter dot plots show the levels of HDAC11 at the TGF-β promoter locus of B cells after the treatment denoted on the X axis. CLK-plasmid: B cells were transfected with CLK-carrying plasmids to over express CLK. Control plasmid: B cells were transfected with empty plasmids used as controls. B: The scatter dot plots show the levels of Foxp3 (TGF-beta gene transcription factor) at the TGF-beta promoter locus in B cells after treatment in the culture as denoted on the X axis. *, HDAC11-deficient B cells. #, B cells were transduced with control shRNA used as controls. C: The immune blots show the results of HDAC11 RNAi. LPS: The presence of LPS in the culture.

that the TGF-β levels in peripheral B cells were much lower in DNSR nurses than that in RC subjects (**Figure 2B**). We then performed a correlation assay with the data of CLK and TGF-β of B cells; the results showed a negative correlation (r = -0.7665, P<0.0001) between CLK and TGF-β in the B cells (**Figure 2C**). The results suggest that CLK may compromise the generation of TGFbB cells in DNSR nurses.

Over-expression of CLK suppresses TGF-β expression in B cells

Data reported above implicate that CLK suppresses TGF-β expression in B cells. To test this, we over-expressed CLK in B cells by transfecting B cells with CLK gene carrying plasmids (**Figure 3A**). The CLK-over-expressing B cells were stimulated with LPS in the culture. As analyzed by RT-qPCR, the over-expression of CLK markedly inhibited the expression of TGF-β in B cells (**Figure 3B**). The results support our hypothesis that CLK is one of the factors down regulation of TGF-β in peripheral B cells.

HDAC11 mediates the effects of CLK on the suppression of TGF-beta in B cells

Published data indicate that HDAC11 suppresses the immune regulatory molecule, IL-10, in B cells [15]. We were wondering if HDAC11 was also involved in the suppression of TGF-β, another important immune regulatory molecule, in B cells. To test this, we also assessed the levels of HDAC11 at the TGF-β promoter locus of wild B cells and B cells with CLK-over-expression after exposing to LPS in the culture. As shown by the ChIP results, over-expression of CLK markedly increased the HDAC11 levels (**Figure 4A**) as well as decreased the Foxp3 (the transcription factor of TGF-β) levels (**Figure 4B**) at the TGF-β promoter locus. To corroborate the results, in separate experiments, we knocked down the gene of HDAC11 in B cells by RNAi, which resulted in about 10 folds down of the expression of HDAC11 in the B cells (**Figure 4C**). The HDAC11-deficient B cells were then transfected with CLK plasmids and cultured in the presence or absence of LPS for 48 h. The results showed that the deficiency of HDAC11 abolished the CLK-suppressed TGF-β expression in B cells (**Figure 4B**). The data suggest that HDAC11 is a critical factor in the CLK-suppressed TGF-β expression in peripheral B cells.

Discussion

A large number of people work at DNRS, such as doctors, nurses, emergency medical staff, pilots and police, which represent a significant part of the population. The present data

showed that CLK levels were higher in peripheral B cells of DNRS nurses. The CLK was negatively correlated with the expression of TGF-β in the B cells. Over expression of the CLK in B cells markedly suppressed the expression of TGF-β. Since TGF-β is an important molecule in the regulatory T cells and TGFbB cells, which are the major immune regulatory cells in the body and are the major components of the immune regulatory system, the data suggest that CLK may be one of the causative factors of the immune tolerance dysfunction.

The most symptoms of DNRS are fatigue and the related safety issues. In addition, increased risk of breast cancer has been noted in those engaging works of shift rotation [16], which has a potential link to clock gene polymorphisms [17] or melatonin expression suppression caused by exposing to light at night [18]. It is also reported that shift work and long-term exposure to light at night are associated with cardiovascular disorders [19] and metabolic syndrome and obesity [20]. The present data show a novel aspect of the study about circadian rhythm. The CLK levels were found higher in peripheral B cells of DNSR nurses, which suppressed the expression of TGF-β in B cells.

TGFbB cells are a fraction of immune regulatory cells and play important roles in the maintenance of the immune homeostasis in the body. Our previous work showed that the TGFbB cells had an immune suppressor function by showing inhibitory effects on $CD4^+$ T cell proliferation [21]. Others also found that TGFbB cells inhibited Th1 cells [10]. Yet, how TGFbB cells get dysfunction is less clear. The present study has revealed a previously unknown phenomenon that CLK plays a role in compromising the TGF-β expression in B cells. We observed this phenomenon in DNSR nurses, in which the frequency of peripheral TGFbB cell was decreased and the expression of TGF-β was impaired.

The CLK is an essential activator of elements in the pathway of circadian rhythms [22]. Besides, the CLK is also an immune regulator, such as increases 3T3-L1 cell (a fibrocyte-like cell line) proliferation via up regulating the Wnt signaling [23]; regulates muscle insulin sensitivity [24] and regulates bone reabsorption [25]. The present data have added novel information to the understanding of the CLK by showing that the CLK can suppress the expression of TGF-β in B cells.

Nakamura et al reported that another circadian protein PER2 was higher in mast cells of mice with allergic disorders; using an antagonist of PER2 could stabilize the sensitized mast cells to attenuate allergic inflammation [26]. Our data show that the CLK, another circadian protein, suppresses TGF-β expression in B cells. Whether the suppression of TGF-β in B cells has the causal relationship with the decrease in TGFbB cell in the peripheral blood system of DNSR nurses is to be further investigated. Our recent study also found that the circadian protein NFIL3 was associated with the initiation of food allergy (un-published data). These data suggest that circadian alteration may be a facilitating factor in the initiation of some immune diseases, such as allergy.

In summary, the present data show that the CLK levels were higher in DNSR nurses that were negatively correlated with the frequency of peripheral TGFbB cells. In vitro study showed that over expression of CLK suppressed the expression of TGF-β in B cells. Therefore, the regulation of CLK expression during DNSR period needs to be further investigated.

Acknowledgements

This study was supported by grants from the innovation of science and Technology Commission of Shenzhen Municipality (JCYJ20150402-090413008, JCYJ20160422101725667, JCYJ-20140418095735611, JCYJ201604290919-35720 and ZDSYS201506050935272), the Natural Science Foundation of China (8137-3176, 31570932, 31400856, 81571790 and 81501573).

Disclosure of conflict of interest

None.

Authors' contribution

LTY, JL, ST, XZ, QW, SZ, MS and WP performed experiments, analyzed data and reviewed the manuscript. HY and PCY designed the project, supervised experiments and wrote the paper.

Address correspondence to: Hui Yang, Department of Nursing, The First Hospital, Shanxi Medical University, 85 Jiefang Road South, Taiyuan 030001, Shanxi, China. E-mail: huiryang@Mailnesia.com; Dr. Ping-Chang Yang, The Research Center of Allergy & Immunology, School of Medicine, Shenzhen University, 3688 Nanhai Blvd, Room 722A of Medical

School Building, Shenzhen 518060, Shanxi, China. E-mail: pcy2356@szu.edu.cn

References

[1] Liston A, Gray DH. Homeostatic control of regulatory T cell diversity. Nat Rev Immunol 2014; 14: 154-65.

[2] Rosser EC, Mauri C. Regulatory B cells: origin, phenotype, and function. Immunity 2015; 42: 607-12.

[3] Lykken JM, Candando KM, Tedder TF. Regulatory B10 cell development and function. Int Immunol 2015; 27: 471-7.

[4] Liu ZQ, Yang G, Geng XR, Liu JQ, Mo LH, Liu ZG, Yang PC. Micro RNA-17-92 cluster mediates interleukin-4-suppressed IL-10 expression in B cells. Am J Transl Res 2016; 8: 2317-24.

[5] Chen WD, Yeh JK, Peng MT, Shie SS, Lin SL, Yang CH, Chen TH, Hung KC, Wang CC, Hsieh IC, Wen MS, Wang CY. Circadian CLOCK mediates activation of transforming growth factor-beta signaling and renal fibrosis through cyclooxygenase 2. Am J Pathol 2015; 185: 3152-63.

[6] Tong X, Muchnik M, Chen Z, Patel M, Wu N, Joshi S, Rui L, Lazar MA, Yin L. Transcriptional repressor E4-binding protein 4 (E4BP4) regulates metabolic hormone fibroblast growth factor 21 (FGF21) during circadian cycles and feeding. J Biol Chem 2010; 285: 36401-9.

[7] Male V, Nisoli I, Gascoyne DM, Brady HJ. E4BP4: an unexpected player in the immune response. Trends Immunol 2012; 33: 98-102.

[8] Rothman PB. The transcriptional regulator NFIL3 controls IgE production. Trans Am Clin Climatol Assoc 2010; 121: 156-71.

[9] Kashiwada M, Levy DM, McKeag L, Murray K, Schröder AJ, Canfield SM, Traver G, Rothman PB. IL-4-induced transcription factor NFIL3/E4BP4 controls IgE class switching. Proc Natl Acad Sci U S A 2010; 107: 821-6.

[10] Tian J, Zekzer D, Hanssen L, Lu Y, Olcott A, Kaufman DL. Lipopolysaccharide-activated B cells down-regulate Th1 immunity and prevent autoimmune diabetes in nonobese diabetic mice. J Immunol 2001; 167: 1081-9.

[11] Cuesta M, Boudreau P, Dubeau-Laramee G, Cermakian N, Boivin DB. Simulated night shift disrupts circadian rhythms of immune functions in humans. J Immunol 2016; 196: 2466-75.

[12] Damasceno A, Moraes AS, Farias A, Damasceno BP, dos Santos LM, Cendes F. Disruption of melatonin circadian rhythm production is related to multiple sclerosis severity: a preliminary study. J Neurol Sci 2015; 353: 166-8.

[13] Stolp J, Turka LA, Wood KJ. B cells with immune-regulating function in transplantation. Nat Rev Nephrol 2014; 10: 389-97.

[14] Geiger SS, Fagundes CT, Siegel RM. Chrono-immunology: progress and challenges in understanding links between the circadian and immune systems. Immunology 2015; 146: 349-58.

[15] Villagra A, Cheng F, Wang HW, Suarez I, Glozak M, Maurin M, Nguyen D, Wright KL, Atadja PW, Bhalla K, Pinilla-Ibarz J, Seto E, Sotomayor EM. The histone deacetylase HDAC11 regulates the expression of interleukin 10 and immune tolerance. Nat Immunol 2009; 10: 92-100.

[16] Davis S, Mirick DK, Stevens RG. Night shift work, light at night, and risk of breast cancer. J Natl Cancer Inst 2001; 93: 1557-62.

[17] Truong T, Liquet B, Menegaux F, Plancoulaine S, Laurent-Puig P, Mulot C, Cordina-Duverger E, Sanchez M, Arveux P, Kerbrat P, Richardson S, Guénel P. Breast cancer risk, nightwork, and circadian clock gene polymorphisms. Endocr Relat Cancer 2014; 21: 629-38.

[18] Hill SM, Blask DE, Xiang S, Yuan L, Mao L, Dauchy RT, Dauchy EM, Frasch T, Duplesis T. Melatonin and associated signaling pathways that control normal breast epithelium and breast cancer. J Mammary Gland Biol Neoplasia 2011; 16: 235-45.

[19] Scheer FA, Hilton MF, Mantzoros CS, Shea SA. Adverse metabolic and cardiovascular consequences of circadian misalignment. Proc Natl Acad Sci U S A 2009; 106: 4453-8.

[20] Karlsson B, Knutsson A, Lindahl B. Is there an association between shift work and having a metabolic syndrome? Results from a population based study of 27,485 people. Occup Environ Med 2001; 58: 747-52.

[21] Liu ZQ, Wu Y, Song JP, Liu X, Liu Z, Zheng PY, Yang PC. Tolerogenic CX3CR1+ B cells suppress food allergy-induced intestinal inflammation in mice. Allergy 2013; 68: 1241-8.

[22] Dunlap JC. Molecular bases for circadian clocks. Cell 1999; 96: 271-90.

[23] Zhu Z, Hua B, Xu L, Yuan G, Li E, Li X, Sun N, Yan Z, Lu C, Qian R. CLOCK promotes 3T3-L1 cell proliferation via Wnt signaling. IUBMB Life 2016; 68: 557-68.

[24] Liu J, Zhou B, Yan M, Huang R, Wang Y, He Z, Yang Y, Dai C, Wang Y, Zhang F, Zhai Q. CLOCK and BMAL1 regulate muscle insulin sensitivity via SIRT1 in male mice. Endocrinology 2016; 157: 2259-69.

[25] Xu C, Ochi H, Fukuda T, Sato S, Sunamura S, Takarada T, Hinoi E, Okawa A, Takeda S. Circadian clock regulates bone resorption in mice. J Bone Miner Res 2016; 31: 1344-55.

[26] Nakamura Y, Nakano N, Ishimaru K, Ando N, Katoh R, Suzuki-Inoue K, Koyanagki S, Ogawa H, Okumura K, Shibata S, Nakao A. Inhibition of IgE-mediated allergic reactions by pharmacologically targeting the circadian clock. J Allergy Clin Immunol 2016; 137: 1226-35.