Expression of Enhancer of Zeste Homolog 2 in B Lymphocyte Subsets in Hashimoto's Thyroiditis and the Therapeutic Mechanism and Efficacy of Its Inhibitors: A Postprint

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Abstract

Background: Thyroid autoantibodies are markers for the diagnosis of Hashimoto's thyroiditis (HT), and B lymphocytes play an important role in the pathogenesis of HT. Enhancer of Zeste homolog 2 (EZH2) is an epigenetic protein that plays an important role in the regulation of lymphocyte development and function.

Objective: This study investigates the expression of EZH2 in plasmablasts and plasma cells in HT thyroid tissue, and further explores the therapeutic effect of EZH2 inhibitors in an experimental autoimmune thyroiditis (EAT) model.

Methods: Six patients who underwent thyroid surgery at Peking University First Hospital between 2010 and 2020 were enrolled; thyroid tissue from the contralateral side of the tumor was obtained (3 cases of HT and 3 cases of normal thyroid tissue), and B lymphocyte-related gene expression was screened by RNA-seq. Thyroid tissues were collected from 16 HT patients and 8 healthy donor (HD) controls, and immunohistochemistry and immunofluorescence were used to verify EZH2 expression in B lymphocytes in HT thyroid tissue. Twenty-five HT thyroid fine-needle aspiration (FNA) samples, 19 HT peripheral blood samples, and 12 healthy donor PB samples were collected, and flow cytometry was performed to detect alterations in EZH2 expression in plasmablasts and plasma cells. Fifteen 7-week-old NOD.H-2h4 mice with EAT were divided into a control group (n=5), an EAT without injection group (n=5), and an EZH2 inhibitor GSK126 treatment group (10 mg/kg, 3 intraperitoneal injections per week, n=5). After 8 weeks, the degree of thyroid inflammation and TgAb levels were assessed.

Results: RNA-seq results showed that EZH2 levels were upregulated in HT thyroid tissue compared with normal thyroid tissue, with concomitant increases in several B lymphocyte phenotype-related genes such as CD19, CD27, CD38, and CD52. Immunohistochemistry revealed positive EZH2-stained cells in germinal centers (GC) in all 16 HT thyroid tissue specimens, with strong positivity; no positive cells were observed in the 8 normal thyroid tissue specimens. EZH2 staining was highly expressed in the GC area of HT thyroid tissue, and EZH2 was specifically expressed in CD19+ B lymphocytes. Flow cytometry results showed that the proportions of CD19+ B lymphocytes, plasmablasts, and plasma cells in HT FNA samples were higher than those in HD peripheral blood and HT peripheral blood samples ($P<0.01$), and the positive proportion of EZH2 in CD19+ B lymphocytes and plasmablasts in HT FNA samples was higher than that in HT peripheral blood ($P<0.005$). In mouse experiments, thyroid lymphocyte infiltration was increased in the EAT group compared with the control group. The inflammation score and TgAb level in the GSK126 treatment group were higher than those in the control group and lower than those in the EAT group, with statistically significant differences ($P<0.001$).

Conclusion: EZH2 expression is abnormally elevated in CD19+ B lymphocytes in HT thyroid tissue, which may promote the differentiation of B lymphocytes into plasma cells and thereby promote autoantibody production that destroys the thyroid. EZH2 inhibitors can alleviate thyroid inflammation in the EAT model. Increased EZH2 expression in plasmablasts may be involved in the pathogenesis of HT. EZH2 may represent a novel therapeutic target for HT, and the related mechanisms require further in-depth investigation.

Full Text

EZH2 Expression in B Lymphocyte Subsets of Hashimoto's Thyroiditis and the Therapeutic Mechanism and Effect of Its Inhibitors

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Abstract

Background: Thyroid autoantibodies are diagnostic markers for Hashimoto's thyroiditis (HT), and B lymphocytes play a crucial role in the pathogenesis of HT. Enhancer of zeste homolog 2 (EZH2) is an epigenetic protein that plays an important role in the regulation of lymphocyte development and function.

Objective: This study investigates EZH2 expression in plasmablasts and plasma cells in HT thyroid tissue and further explores the therapeutic effect of EZH2 inhibitors in an experimental autoimmune thyroiditis (EAT) model.

Methods: We collected thyroid tissues from 6 patients who underwent thyroid surgery at Peking University First Hospital between 2010 and 2020, obtaining tissue from the contralateral lobe (3 HT cases and 3 normal thyroid tissues), and screened for B lymphocyte-related gene expression via RNA-seq. We collected thyroid tissues from 16 HT patients and 8 healthy donor (HD) thyroid tissues, and verified EZH2 expression in B lymphocytes in HT thyroid tissues using immunohistochemistry and immunofluorescence. We collected fine-needle aspiration (FNA) samples from 25 HT patients, peripheral blood from 19 HT patients, and peripheral blood from 12 healthy individuals, and used flow cytometry to detect altered EZH2 expression in plasmablasts and plasma cells. Fifteen 7-week-old NOD.H-2h4 mice with EAT were divided into control (n=5), EAT without injection (n=5), and EZH2 inhibitor GSK126 treatment (10 mg/kg, 3 intraperitoneal injections/week, n=5) groups. After 8 weeks, thyroid inflammation severity and TgAb levels were assessed.

Results: RNA-seq results showed that EZH2 levels were upregulated in HT thyroid tissues compared with normal thyroid tissues, with corresponding increases in B lymphocyte phenotype-related genes such as CD19, CD27, CD38, and CD52. Immunohistochemical results revealed strongly positive EZH2 staining in germinal center (GC) regions in all 16 HT thyroid tissue specimens, while no positive cells were observed in 8 normal thyroid tissues. EZH2 staining was highly expressed in GC regions of HT thyroid tissues and specifically expressed in CD19+ B lymphocytes. Flow cytometry analysis showed that the proportions of CD19+ B lymphocytes, plasmablasts, and plasma cells in HT FNA samples were higher than those in HD peripheral blood and HT peripheral blood samples ($P<0.01$). The positive rate of EZH2 in CD19+ B lymphocytes and plasmablasts was higher in HT FNA samples than in HT peripheral blood ($P<0.005$). In mouse experiments, lymphocytic infiltration in the thyroid was increased in the EAT group compared with the control group. The GSK126 treatment group showed inflammatory scores and TgAb levels higher than the control group but lower than the EAT group, with statistically significant differences ($P<0.001$).

Conclusion: EZH2 expression is abnormally elevated in CD19+ B lymphocytes in HT thyroid tissue, potentially promoting B lymphocyte differentiation into plasma cells and thereby enhancing autoantibody production that destroys thyroid tissue. EZH2 inhibitors can alleviate thyroid inflammation in the EAT

model. Increased EZH2 expression in plasmablasts may be involved in the pathogenesis of HT. EZH2 may represent a novel therapeutic target for HT, though further mechanistic studies are needed.

Key words: Hashimoto's thyroiditis; B-lymphocyte subsets; Enhancer of Zeste homolog 2 protein; Hypothyroidism; Targeted therapy

Introduction

Hashimoto's thyroiditis (HT) is an organ-specific autoimmune thyroid disease and the leading cause of hypothyroidism, which can significantly impair patients' quality of life and even be life-threatening. Current clinical management of HT primarily focuses on symptomatic treatment and hormone replacement therapy for hypothyroidism. Therefore, further investigation into the pathogenesis of HT is needed to develop novel therapeutic strategies and reduce the incidence of hypothyroidism.

The pathogenesis of HT is directly related to the infiltration of numerous lymphocytes, including T and B lymphocytes in germinal centers (GC) visible on histopathological sections, and the production of autoantibodies. Recent studies have shown that, in addition to genetic and environmental factors, epigenetic regulation also contributes to the development of HT. Enhancer of zeste homolog 2 (EZH2) is an important epigenetic protein that plays a crucial role in regulating the development and function of immune cells such as lymphocytes. Research has demonstrated that EZH2 participates in the pathogenesis of autoimmune diseases such as lupus nephritis in systemic lupus erythematosus and inflammatory bowel disease through epigenetic mechanisms. Abnormally elevated EZH2 can promote CD4+ T lymphocyte adhesion and migration, activate B lymphocytes, regulate plasma cell differentiation, and enhance antibody production, while EZH2 inhibitors can suppress autoantibody formation. Thus, EZH2 may represent a novel therapeutic target for autoimmune diseases. This study aims to investigate EZH2 expression in B lymphocytes in HT thyroid tissue and explore the effect of EZH2 inhibitors in alleviating thyroid inflammation in an experimental autoimmune thyroiditis (EAT) mouse model, providing a theoretical basis for the future use of EZH2 small-molecule inhibitors in HT treatment.

Materials and Methods

1.1 Study Subjects and Specimen Collection

The study involved three parts: (1) Thyroid tissues from 6 patients who underwent thyroidectomy between 2010-2020 were obtained from the contralateral lobe to the tumor (3 HT cases and 3 healthy controls, aged 35-51 and 22-59

respectively) for RNA extraction and RNA-seq analysis; (2) Thyroid tissue sections from 16 HT patients and 8 healthy donor thyroid tissues (distant from papillary thyroid carcinoma) collected between 2010-2020 were used for immunohistochemistry and immunofluorescence to verify EZH2 expression patterns; (3) Peripheral blood from 12 healthy donors (aged 22-62) and 19 HT patients (aged 25-67), and FNA samples from 25 HT patients (aged 27-74) were collected for flow cytometry analysis of B lymphocyte subsets and EZH2 expression (collected between 2017-2020). The study was approved by the Ethics Committee (Approval No. 2020 科研 062).

1.2 Diagnostic Criteria for HT Patients

HT was diagnosed based on thyroid autoantibodies combined with thyroid ultrasound, postoperative pathology, or fine-needle aspiration pathology. Exclusion criteria included: (1) Pathological evidence of other thyroid diseases; (2) Other malignancies, particularly EZH2-related tumors such as melanoma, lung cancer, or bladder cancer; (3) Pregnancy or conditions affecting thyroid hormone-binding globulin; (4) Hematological malignancies such as leukemia or lymphoma; (5) Other autoimmune diseases beyond thyroid autoimmunity such as systemic lupus erythematosus or psoriasis. Healthy controls had normal thyroid function, negative thyroid autoantibodies, and normal thyroid ultrasound, with exclusion of thyroid disease history/family history and other autoimmune diseases or malignancies.

1.3 Experimental Animals

Female NOD.H-2h4 mice (7 weeks old, 20-25 g) were purchased from Cyagen (Suzhou) Biotechnology Co., Ltd. The experimental protocol was approved by the Animal Management Committee of Peking University First Hospital (Approval No. J2023021) and complied with Chinese Ministry of Health animal management regulations.

1.4 Reagents and Instruments

EZH2 antibody (Cell Signaling Technology, USA), CD19 antibody (Biolegend, USA), immunohistochemistry kit (PV9000, Zhongshan Jinqiao), FITC-CD19, BV510-CD27, PerCp-Cy5.5-CD38, APC-CD138, PE-Cy7-IgD, BV421-EZH2 (Biolegend, USA), goat anti-mouse/rabbit fluorescent secondary antibodies (Invitrogen, Alexa Fluor 488/595, USA), GSK126 (Selleck, USA), lysis solution (BD, USA), bovine thyroglobulin (bTg, Sigma-Aldrich, USA), laser confocal microscope (Leica, Germany), flow cytometer (BD FACS Canto II, USA).

1.5 Experimental Methods

1.5.1 RNA-seq Detection: Commercial kits were used to extract RNA from fresh thyroid tissues (Part 1). Shanghai Biotechnology Corporation performed the sample testing. Data analysis used fold-change and t-test statistical methods

to screen differentially expressed genes with criteria: Fold Change (linear) <0.5 or >1.5; t-test, P<0.05.

1.5.2 Immunohistochemistry: Thyroid specimens (Part 2) were processed as paraffin sections, baked, dewaxed, rehydrated, subjected to antigen retrieval, endogenous peroxidase inactivation, and blocking. EZH2 (1:100) or CD19 (1:100) antibodies were incubated for immunohistochemical staining. Secondary antibodies (PV9000, Zhongshan Jinqiao) were incubated at room temperature for 30 minutes, followed by washing, counterstaining, dehydration, clearing, and mounting.

1.5.3 Immunofluorescence and Laser Confocal Microscopy: Paraffin sections were baked, dewaxed, rehydrated, and repaired with citrate buffer at 95°C for 25 minutes, then incubated with 3% hydrogen peroxide at room temperature for 15 minutes. EZH2 (1:400) and CD19 (1:500) were co-incubated for immunofluorescence. Goat anti-mouse/rabbit fluorescent secondary antibodies (Alexa Fluor 488/595) were incubated at room temperature for 2 hours, followed by mounting with DAPI-containing medium. A Leica laser confocal microscope with 405/488/543 lasers was used to observe target cell staining.

1.5.4 Flow Cytometry Staining: Based on references [12,13], flow cytometry (Part 3) was used to detect B lymphocyte proportions and EZH2 expression. Single-cell suspensions were prepared by lysing red blood cells with lysis solution (BD, San Diego, CA, USA). Cells were stained with FITC-CD19, BV510-CD27, PerCp-Cy5.5-CD38, APC-CD138, BV421-EZH2 (all 1:100), and PE-Cy7-IgD (1:200) at room temperature for 30 minutes. Isotype-matched antibodies served as negative controls. Samples were washed, resuspended, and analyzed on a BD FACS Canto II flow cytometer using FACS Diva software (BD, San Diego, CA, USA).

1.5.5 Experimental Animal Grouping: Fifteen 7-week-old female NOD.H-2h4 mice were randomly divided into three groups (n=5 each): Group A (wild-type control) received no treatment; Group B (EAT group) received 0.9% sodium chloride solution (equal volume) intraperitoneally 3 times/week starting with iodized water feeding; Group C (EZH2 inhibitor group) received GSK126 (10 mg/kg) intraperitoneally 3 times/week starting with iodized water feeding. Groups B and C were fed 0.05% sodium iodide (NaI) high-iodine water for 8 weeks to establish the EAT mouse model [14]. After 8 weeks, mice were sacrificed to assess thyroid inflammation severity and TgAb levels.

1.5.6 Assessment of Thyroid Inflammation Severity: Mouse thyroid tissues were fixed in 4% formalin, routinely paraffin-embedded, and sectioned (5 m). HE staining was performed and observed under light microscopy. Lymphocytic infiltration was expressed as a percentage. Thyroid inflammation was scored as: 0 = no mononuclear cell infiltration; 1 = 2-3 follicular spaces with infiltration; 2 = 2-3 infiltration foci within one follicle; 3 = 10-40% area infiltrated; 4 = 41-80% area infiltrated; 5 = >80% area infiltrated [14].

1.5.7 TgAb Measurement: Mouse serum was collected via retro-orbital bleed-

ing. TgAb levels were detected by ELISA. Commercial bTg was diluted to 4 g/mL in 0.05M carbonate buffer for coating. After washing with PBST and blocking with 3% BSA, mouse serum (diluted 1:50 in PBST) was added to 96-well plates. After washing, HRP-labeled goat anti-mouse IgG secondary antibody was incubated, followed by color development. OD values were measured at 490 nm using a microplate reader, and absolute values (test OD - blank OD) were compared.

1.6 Statistical Methods

GraphPad Prism 7 (GraphPad Software, USA) was used for data processing, statistical analysis, and graphing. Normally distributed data were expressed as mean ± standard deviation ($\bar{x}\pm s$) and compared using independent samples t-test between two groups or one-way ANOVA among multiple groups. Non-normally distributed data were expressed as median (P25, P75) and compared using non-parametric tests. $P<0.05$ was considered statistically significant.

Results

2.1 RNA-seq Results

RNA-seq analysis of HT and normal thyroid tissues revealed upregulated EZH2 levels in HT thyroid tissues, with corresponding increases in B lymphocyte phenotype-related genes including CD19, CD27, CD38, and CD52 [Figure 1: see original paper].

2.2 Immunohistochemistry and Immunofluorescence Results

EZH2 immunohistochemical staining was strongly positive in GC regions in all 16 HT thyroid tissue specimens, while no positive cells were observed in 8 normal thyroid tissues. EZH2 staining was highly expressed in GC regions of HT thyroid tissues and specifically expressed in CD19+ B lymphocytes [Figure 2: see original paper]. Immunofluorescence double staining for EZH2 and CD19 confirmed specific high expression of EZH2 in CD19+ B lymphocytes [Figure 3: see original paper].

2.3 Flow Cytometry Results

Flow cytometry gating strategies for B lymphocyte subsets in HT FNA and peripheral blood samples are shown in [Figure 4: see original paper]. The proportions of CD19+ B lymphocytes, plasmablasts (CD27+CD38+ B lymphocytes), and plasma cells (CD27+CD138+ B lymphocytes) in HT FNA samples were significantly higher than those in HD peripheral blood and HT peripheral blood samples ($P<0.05$) . The positive rate of EZH2 in CD19+ B lymphocytes and plasmablasts was higher in HT FNA samples than in HT peripheral blood

(P<0.005), while no significant difference was observed in EZH2 positivity in plasma cells between HT peripheral blood and HT FNA samples (P>0.05) .

2.4 EAT Mouse Model Results

Lymphocytic infiltration in thyroid tissues was increased in the EAT group compared with the control group [Figure 5: see original paper]. The GSK126 treatment group showed inflammatory scores and TgAb levels higher than the control group but lower than the EAT group, with statistically significant differences (P<0.001) .

Discussion

B lymphocytes and thyroid autoantibodies play important roles in HT pathogenesis. Thyroglobulin antibody (TgAb) and thyroid peroxidase antibody (TPOAb), hallmark antibodies of HT, can damage thyroid follicular cells through antibody-dependent cell-mediated cytotoxicity (ADCC) and/or complement-dependent cytotoxicity. Targeting B lymphocytes to explore the mechanisms of thyroid autoantibody production and prevent thyroid tissue destruction may be a direction to prevent irreversible hypothyroidism. Studies have shown that selective B lymphocyte depletion immunotherapy in multiple sclerosis can effectively reduce disease recurrence and new inflammation [15-16], suggesting B lymphocytes as a potential therapeutic target for autoimmune diseases.

This study found abnormal distribution of B lymphocyte subsets such as plasmablasts and plasma cells in HT patient thyroids, confirming the important role of B lymphocytes in HT pathogenesis. Further results demonstrated that EZH2 was specifically overexpressed in CD19+ B lymphocytes in GCs of HT thyroid tissues, with elevated EZH2 levels in plasmablasts. EZH2, a member of the polycomb protein family and a catalytic subunit of polycomb repressive complex 2 (PRC2) [17], is an important epigenetic regulator. EZH2 can suppress gene expression through histone methyltransferase-mediated chromatin remodeling [18], and can also activate downstream genes through PRC2-independent mechanisms by methylating non-histone targets or directly interacting with other proteins [19]. Studies have reported that EZH2 can suppress B lymphocyte differentiation gene expression, regulate B lymphocyte differentiation, maintain antibody gene random mutation, and promote antibody diversification and affinity maturation in the immune system [7]. In autoimmune diseases such as systemic lupus erythematosus and Sjögren's syndrome, EZH2 has been shown to promote B lymphocyte proliferation and antibody secretion [8-9]. Therefore, we hypothesize that abnormal elevation of EZH2 in CD19+ B lymphocytes, particularly plasmablasts, in HT thyroid tissues may promote B lymphocyte differentiation into plasma cells, thereby enhancing thyroid autoantibody production and accelerating the destruction of thyroid follicular cells, leading to

irreversible hypothyroidism. Thus, EZH2 may play an important role in B lymphocyte differentiation in HT thyroid tissue and represent a novel therapeutic target.

EZH2 plays different pathogenic roles in different autoimmune diseases. Most studies demonstrate that EZH2 promotes B lymphocyte differentiation, function, and antibody production through epigenetic suppression/activation of downstream genes or signaling pathways. However, other studies have found that in colitis and experimental autoimmune encephalomyelitis, EZH2 promotes autoimmune inflammatory responses by mediating Toll-like receptor (TLR)-induced pro-inflammatory gene expression and activating macrophages and microglia [11,20]. In inflammatory bowel disease, EZH2 promotes inflammation by inhibiting myeloid-derived suppressor cell function [11,20]. Therefore, EZH2 plays different roles in autoimmune diseases, and its mechanisms require further investigation.

In this study, 8-week treatment with the EZH2 inhibitor GSK126 in the EAT model significantly alleviated thyroid inflammation as assessed by HE staining and serum TgAb levels. Previous studies have shown that elevated EZH2 promotes autoantibody production in multiple sclerosis and lupus patients [9,21], and EZH2 inhibition can ameliorate autoimmune diseases such as systemic lupus erythematosus and inflammatory bowel disease [11,22]. This study observed similar immunosuppressive effects of EZH2 inhibitors in the EAT model. More specific drugs targeting EZH2 epigenetic activity have entered clinical trials, and GSK126 is a potent EZH2 enzymatic inhibitor that has been shown in preclinical and clinical studies to broadly reduce EZH2 methyltransferase activity and effectively inhibit its pathogenic effects [23]. This study suggests that EZH2 inhibitors may have significant development prospects and translational significance for HT treatment.

Additionally, our results showed no change in B lymphocyte subset distribution in PB samples from HT patients compared with healthy donors, but a significant increase in CD19+ cell percentage in HT FNA samples, particularly plasmablasts and plasma cells, supporting HT as an organ-specific autoimmune disorder. FNA samples may be more representative than PB samples for reflecting HT autoimmune characteristics. Previous literature also suggests that HT is characterized by thyroid lymphocyte infiltration including B and T lymphocytes, and that abnormal B lymphocyte function and autoantibody formation are the main immune responses in autoimmune thyroid disease [6].

This study has limitations. First, we only investigated EZH2 expression in HT patients without exploring the function and pathogenic mechanisms of EZH2+ B lymphocyte subsets. Future functional studies are needed to confirm the role of EZH2 in regulating B lymphocyte differentiation and stimulating plasmablast differentiation into plasma cells at the cellular and animal levels. Second, the upstream regulatory mechanisms of abnormal EZH2 elevation remain unclear and require further exploration.

In conclusion, this study found that abnormal EZH2 overexpression in HT may promote B lymphocyte differentiation into plasma cells, thereby enhancing thyroid autoantibody production, and that EZH2 inhibitors can alleviate thyroid inflammation in mouse models. Increased EZH2 expression in plasmablasts may be involved in HT pathogenesis. EZH2 may serve as a future therapeutic target for HT, though further mechanistic studies are needed.

Author Contributions

YI Shengguo conceived the study, designed the research protocol, proposed the research question, implemented part of the experimental protocol, and drafted the manuscript. CAO Yedi and ZHAO Xue were responsible for sample collection, conducted experiments, and implemented the research process. LU Guizhi and ZHANG Yang collected, acquired, and cleaned data. CONG Tiechuan and ZHANG Lanbo screened patients and collected thyroid samples. ZHANG Jixin performed thyroid pathological diagnosis. LIANG Zhenwei was responsible for thyroid ultrasound and imaging selection and data retention. QU Chenxue conducted clinical laboratory tests and measurements. ZHANG Junqing was responsible for quality control and review of the article and supervision. GAO Ying designed the research question, revised the protocol, revised the final version, reviewed the article, and took overall responsibility.

Conflict of Interest

This article has no conflict of interest.

References

[1] RALLI M, ANGELETTI D, FIORE M, et al. Hashimoto's thyroiditis: an update on pathogenic mechanisms, diagnostic protocols, therapeutic strategies, and potential malignant transformation[J]. Autoimmun Rev, 2020, 19(10): 102649. DOI: 10.1016/j.autrev.2020.102649.

[2] MCLACHLAN S M, RAPOPORT B. Breaking tolerance to thyroid antigens: changing concepts in thyroid autoimmunity[J]. Endocr Rev, 2014, 35(1): 59-105. DOI: 10.1210/er.2013-1055.

[3] DE LEO S, LEE S Y, BRAVERMAN L E. Hyperthyroidism[J]. Lancet, 2016, 388(10047): 906-918. DOI: 10.1016/S0140-6736(16)00278-6.

[4] BRIX T H, HEGEDÜS L. Twin studies as a model for exploring the aetiology of autoimmune thyroid disease[J]. Clin Endocrinol, 2012, 76(4): 457-464. DOI: 10.1111/j.1365-2265.2011.04318.x.

[5] CAÑAS C A, CAÑAS F, BONILLA-ABADÍA F, et al. Epigenetics changes associated to environmental triggers in autoimmunity[J]. Autoimmunity, 2016, 49(1): 1-11. DOI: 10.3109/08916934.2015.1086996.

[6] WANG B, SHAO X Q, SONG R H, et al. The emerging role of epigenetics in autoimmune thyroid diseases[J]. Front Immunol, 2017, 8: 396. DOI: 10.3389/fimmu.2017.00396.

[7] NUTT S L, KEENAN C, CHOPIN M, et al. EZH2 function in immune cell development[J]. Biol Chem, 2020, 401(8): 933-943. DOI: 10.1515/hsz-2019-0436.

[8] HE C M, YANG Y L, CHEN Z L, et al. EZH2 promotes T follicular helper cell differentiation through enhancing STAT3 phosphorylation in patients with primary sjögren's syndrome[J]. Front Immunol, 2022, 13: 922871. DOI: 10.3389/fimmu.2022.922871.

[9] ZHANG M Z, IWATA S, HAJIME M, et al. Methionine commits cells to differentiate into plasmablasts through epigenetic regulation of BTB and CNC homolog 2 by the methyltransferase EZH2[J]. Arthritis Rheumatol, 2020, 72(7): 1143-1153. DOI: 10.1002/art.41208.

[10] ZHEN Y X, SMITH R D, FINKELMAN F D, et al. Ezh2-mediated epigenetic modification is required for allogeneic T cell-induced lupus disease[J]. Arthritis Res Ther, 2020, 22(1): 133. DOI: 10.1186/s13075-020-02225-9.

[11] ZHOU J, HUANG S, WANG Z Y, et al. Targeting EZH2 histone methyltransferase activity alleviates experimental intestinal inflammation[J]. Nat Commun, 2019, 10(1): 2427. DOI: 10.1038/s41467-019-10176-2.

[12] DISANO K D, GILLI F, PACHNER A R. Memory B cells in multiple sclerosis: emerging players in disease pathogenesis[J]. Front Immunol, 2021, 12: 676686. DOI: 10.3389/fimmu.2021.676686.

[13] SANZ I, WEI C, JENKS S A, et al. Challenges and opportunities for consistent classification of human B cell and plasma cell populations[J]. Front Immunol, 2019, 10: 2458. DOI: 10.3389/fimmu.2019.02458.

[14] ZHAO N, WANG Z Z, CUI X J, et al. In vivo inhibition of microRNA-326 in a NOD.H-2h4 mouse model of autoimmune thyroiditis[J]. Front Immunol, 2021, 12: 620916. DOI: 10.3389/fimmu.2021.620916.

[15] HAUSER S L, BAR-OR A, COHEN J A, et al. Ofatumumab versus teriflunomide in multiple sclerosis[J]. N Engl J Med, 2020, 383(6): 546-557. DOI: 10.1056/NEJMoa1917246.

[16] HAUSER S L, BAR-OR A, COMI G, et al. Ocrelizumab versus interferon beta-1a in relapsing multiple sclerosis[J]. N Engl J Med, 2017, 376(3): 221-234. DOI: 10.1056/NEJMoa1601277.

[17] RINGROSE L, PARO R. Epigenetic regulation of cellular memory by the Polycomb and Trithorax group proteins[J]. Annu Rev Genet, 2004, 38: 413-443.

DOI: 10.1146/annurev.genet.38.072902.091907.

[18] HERVIOU L, CAVALLI G, CARTRON G, et al. EZH2 in normal hematopoiesis and hematological malignancies[J]. Oncotarget, 2016, 7(3): 2284-2296. DOI: 10.18632/oncotarget.6198.

[19] KIM J, LEE Y, LU X D, et al. Polycomb- and methylation-independent roles of EZH2 as a transcription activator[J]. Cell Rep, 2018, 25(10): 2808-2820.e4. DOI: 10.1016/j.celrep.2018.11.035.

[20] ZHANG X L, WANG Y, YUAN J, et al. Macrophage/microglial Ezh2 facilitates autoimmune inflammation through inhibition of Socs3[J]. J Exp Med, 2018, 215(5): 1365-1382. DOI: 10.1084/jem.20171417.

[21] TSOU P S, COIT P, KILIAN N C, et al. EZH2 modulates the DNA methylome and controls T cell adhesion through junctional adhesion molecule A in lupus patients[J]. Arthritis Rheumatol, 2018, 70(1): 98-108. DOI: 10.1002/art.40338.

[22] WU L L, JIANG X Y, QI C J, et al. EZH2 inhibition interferes with the activation of type I interferon signaling pathway and ameliorates lupus nephritis in NZB/NZW F1 mice[J]. Front Immunol, 2021, 12: 653989. DOI: 10.3389/fimmu.2021.653989.

[23] DUAN R, DU W F, GUO W J. EZH2: a novel target for cancer treatment[J]. J Hematol Oncol, 2020, 13(1): 104. DOI: 10.1186/s13045-020-00937-8.

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