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17β-Estradiol enhances the activation of IFN-α signaling in B cells by down-regulating the expression of let-7e-5p, miR-98-5p and miR-145a-5p that target IKKε

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ABSTRACT

The activation of IFN-α signaling in B cells contributes to the pathogenesis of systemic lupus erythematosus (SLE). Many studies suggest that estrogens are closely related to the gender difference in the prevalence of SLE. However, the underlying mechanism of the interaction between estrogens and the activation of IFN-α signaling in SLE B cells remains incompletely understood. In the present study, we first found that healthy female mice showed an up-regulated type I IFN-induced gene signature in B cells compared with age-matched male mice, and an *in vivo* study revealed that the gender difference was related to 17β-estradiol. Moreover, we found that 17β-estradiol could enhance the activation of IFN-α signaling in an ERα-dependent manner by down-regulating the expression of three microRNAs, including let-7e-5p, miR-98-5p and miR-145a-5p. These microRNAs could target the 3′UTR of the IKKε-encoding gene IKBKE directly and regulate the expression of IKKε, which can promote the activation of IFN-α signaling. In addition, compared with age-matched male mice, female mice showed a higher level of IKKε and lower levels of let-7e-5p, miR-98-5p and miR-145a-5p in B cells. Moreover, peripheral blood mononuclear cells from women showed a higher level of IKKε and lower levels of let-7e-5p, miR-98-5p and miR-145a-5p compared with those from age-matched men. These data suggest that 17β-estradiol amplifies the activation of IFN-α signaling in B cells *via* IKKε by down-regulating the expression of let-7e-5p, miR-98-5p and miR-145a-5p. Our findings may provide a new perspective for understanding the mechanism underlying the gender difference in the prevalence of SLE.

1. Introduction

Females are more susceptible to systemic lupus erythematosus (SLE) than males [1–3]. It is generally accepted that this gender difference is related to sex chromosomes, sex hormones and other sex-biased factors. For instance, the course of SLE is accompanied by fluctuations in the sex hormonal state in women [4–6]. Estrogens can trigger disease onset and exacerbate the SLE course [7,8], and in particular, induce a lupus-type phenotype in non-autoimmune disease-prone mice [9,10].

Multiple studies have shown that estrogens can affect the activation and function of B cells, T cells, dendritic cells, monocytes and other immune cells [11–13]. Of note, B cells play a central role in the pathogenesis of SLE through the production of pathogenic autoantibodies, the activation of autoreactive T cells, the production of pro-inflammatory cytokines and the organization of ectopic lymphoid tissue [14–19]. Toll-like receptors (TLRs), particularly TLR7 and TLR9, are also critically involved in these processes [20,21]. Previous studies have shown that estrogens can enhance TLR expressions in peritoneal leukocytes and resident macrophages [22]. Moreover, estrogens can affect the maturation and function of B cells [23,24]. All of these data indicate that estrogens may regulate TLR expressions and the activation of TLR-mediated signaling pathways in B cells.

As is well known, interferon-α (IFN-α) has been identified as a critical cytokine in the pathogenesis of SLE [25,26]. IFN-α can induce and accelerate the SLE symptoms in patients and mice [27,28]. Importantly, IFN-α can impact the function of B cells through a variety of mechanisms, including TLR7 expression, survival and differentiation [29–34]. What's more, immune cells, including B cells, display an up-regulated IFN-I-induced gene signature in SLE patients [35–42]. Several recent studies have demonstrated that estrogens can promote the TLR7- and TLR9-ligand-induced production of IFN-α in pDCs [43,44]. However, it remains unknown whether estrogens can regulate the activation of IFN-α signaling in B cells.

Abbreviations: SLE, systemic lupus erythematosus; IFN-α, interferon-α; TLR, Toll like receptor; ER, estrogen receptor; IKKε, inhibitor of kappa B kinase epsilon; UTR, untranslated region; si-ERα, small interfering RNA specific to ERα; si-ERβ, small interfering RNA specific to ERβ; JAK1, just another kinase 1; STAT1, signal transducer and activator of transcription 1.

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The inhibitor of kappa B kinase ε (IKKε; encoded by IKBKE), a noncanonical IκB kinase, can promote IFN-I signaling by promoting the phosphorylation of STAT1 [45]. IKKε was recently found to be associated with some autoimmune diseases [46,47]. Although new SLE-associated sequence variants in IKBKE have been identified as risk loci for SLE [46,47], the biological function of IKKε in SLE remains poorly understood. It is also unknown whether and how estrogens regulate IKKε expression and the activation of IFN-α-induced JAK1-STAT1 signaling in B cells.

Numerous studies have shown that microRNAs (miRNAs) are involved in the modulation function of estrogens on the immune response and inflammatory processes [48,49]. The dysregulation of miRNAs participates in the prevalence of autoimmune diseases, including SLE [50,51]. In particular, there are sex-based differences in the expression of miRNAs in mouse and rat [52,53]. Interestingly, several studies have shown that estrogens can regulate the expression of various miRNAs, such as miR-146a-5p, miR-125a-5p, miR-125b-5p, miR-143-3p, miR-145a-5p, let-7e-5p, miR-126 and miR-181a-2, in many cell types [48,54,55].

In past studies, we have found significant gender differences in the global gene expression profiles in B cells and revealed that these gender differences are related to estrogens [56]. In the present study, we found an up-regulated IFN-I-induced gene signature in B cells from female mice compared with age-matched male mice. Moreover, the fundamental gender difference was related to 17β-estradiol. 17β-Estradiol amplified the IFN-α-induced activation of JAK1-STAT1 signaling in B cells *via* IKKε by down-regulating the expression of let-7e-5p, miR-98-5p and miR-145a-5p. Furthermore, compared with age-matched male mice, female mice showed a higher level of IKKε and lower levels of let-7e-5p, miR-98-5p and miR-145a-5p in B cells. Interestingly, a similar phenomenon was also existent in peripheral blood mononuclear cells (PBMCs) from women and age-matched men. Together, these data demonstrate that 17β-estradiol amplifies the activation of IFN-α signaling *via* miRNA-dependent pathways in B cells. Our findings provide a new perspective for understanding the mechanism of sex bias in the prevalence of SLE.

2. Materials and methods

2.1. Mice

Female and male C57BL/6 mice, 4–6 weeks old, were obtained from Model Animal Research Center at Nanjing University and maintained under specific pathogen-free conditions. Female C57BL/6 mice, ovariectomized at 4-week-old, were also obtained from Model Animal Research Center at Nanjing University. All experiments were conducted in accordance with institutional guidelines for animal care and used based on the Guide for the Animal Care Committee at Nanjing University.

2.2. Purification of murine splenic B cells and cell culture

Lymphocytes of spleen were isolated by Ficoll density centrifugation according to standard procedures. Murine splenic B cells were purified using the mouse B cell Isolation Kit (Miltenyi Biotec, Auburn, CA, USA) and the purity of B cells was always above 92%. For B cells culture, purified B cells were cultured in phenol red-free RPMI 1640 medium containing 10% charcoal-stripped FBS, 2 mM L-glutamine, 100 IU/mL penicillin, 100 μg/mL streptomycin, and 1% nonessential amino acids.

2.3. Isolation of human peripheral blood mononuclear cells

Blood samples were obtained from 15 healthy menopausal women and 10 healthy age-matched men. Human peripheral blood mononuclear cells (PBMCs) were separated from plasma by Ficoll centrifugation (Lymphoprep, Nycomed, Oslo, Norway) according to the standard procedures. The harvested PBMCs were stored in TRIZOL reagent (Invitrogen, Carlsbad, CA, USA) at −80 °C for using.

2.4. Cell viability assay

The effect of 17β-estradiol on viability of murine B cells was evaluated using the Cell Counting Kit 8 (CCK-8) according to the manufacturer's instructions. In brief, murine B cells were cultured in 96-well plates (5×10^3/100 μL/well) and incubated with various concentrations of 17β-estradiol for 24 h. Subsequently, the CCK-8 solution was added to each well (10 μL/well) and incubated for another 3 h. The absorbance at 450 nm was measured using microplate reader (Synergy HT, Bio-Tek).

2.5. Reverse transcription PCR (RT-PCR) and quantitative real-time PCR (qPCR) analysis

Total RNA was extracted using TRIZOL (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. RNA concentration was quantified by a SmartSpecTM Plus spectrophotometer (Bio-Rad, Hercules, CA) and RNA integrity was analyzed with formaldehyde denaturalization agarose gel electrophoresis. The method to quantify mRNA and miRNA was performed as described previously [57]. qPCR assays of mRNA and mature miRNAs were carried out on a StepOne Plus real-time polymerase chain reaction system or ABI Vii 7 detection system (Applied Biosystems, Foster City, CA) using SYBR Green PCR Master Mix. The $2^{-\Delta\Delta Ct}$ method was used for qPCR gene expression analysis. All quantification data were presented as a ratio to the GAPDH or U6 level.

2.6. Western blot analysis

Proteins were extracted using lysis buffer and the protein concentration of each sample was detected using the BCA protein assay kit (Pierce Chemical, Rockford, IL). Appropriate amounts of proteins (50 μg) were electrophoresed on SDS polyacrylamide gels with Tris-glycine running buffer and electrically transferred onto polyvinylidene difluoride membranes (MilliporeCorp, Bedford, MA). After blocking with 3% (w/v) bovine serum albumin (BSA) in Tris buffered saline (TBS)/Tween-20 (Bio-Rad Laboratories, Richmond, CA, USA) for 1 h, the membranes were washed and then incubated with primary antibodies against TLR7, phos-STAT1 (Ser 727), total STAT1 and IKKε which were purchased from Cell Signaling Technology (Danvers, MA) (all dilution at 1:1000) over night at 4 °C. After washing, the membranes were incubated at room temperature with the secondary antibody AffiniPure Goat Anti-Rabbit IgG (H + L) purchased from Beyotime Institute of Biotechnology (Haimen, China) (dilution at 1:3000) for 1 h. The ECL Plus western blotting detection reagents (Millipore, USA) were used to visualize protein expressions. The control protein GAPDH (dilution at 1:1000) was purchased from Cell Signaling Technology (Danvers, MA). Integrated density values were then calculated using an AlphaImager 3400 (Alpha Innotech).

2.7. Mouse ovariectomy and 17β-estradiol exposure

Twelve C57BL/6 mice, 4 weeks old, were surgically ovariectomized (OVX) after anesthesia using chloral hydrate and divided into two groups randomly. Mice were well owed to recover for two weeks and then implanted with Alzet mini-osmotic pumps (Model 1004; Durect Corp., Cupertino, CA, USA) delivering 0.2 μg/day 17β-estradiol or vehicle (0.5% ethanol and 99.5% polypropyleneglicol) for 4 weeks. Implants were placed in the subscapular region.