

REVIEW

Estrogen-mediated differential protein regulation and signal transduction in rheumatoid arthritis

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Abstract

Exploration of the dual and opposing facets of estrogen necessitates a clear understanding to diminish the controversy of estrogen regulation in averting the systemic, autoimmune, joint degrading disorder, and rheumatoid arthritis (RA). Experimental evidences consider estrogen as a pivotal enzyme to modulate the disease progression via managing several cellular mechanisms targeting inflammatory markers such as TNF, ILs, nuclear factor kappa B, and other regulatory proteins like matrix metalloproteinases impeding joint erosion and cartilage degradation. Estrogen modulates cellular signaling associated with inflammation, oxidative stress, related cardiovascular risk, and miRNA regulation during RA progression. Studies determining estrogen regulation in RA complicate the resemblance of the outcome as they represent both hyper and hypo level of estrogen is linked to the disease. Although some reports deliver estrogen as malign, there is now increasing evidence of rendering protection dose dependently. Variation in estrogen level causes differential expression of certain proteins and their related signaling which is directly or indirectly linked to RA pathogenesis. This review summarizes the variations in protein expression levels by focusing on the *in vitro*, *in vivo*, and clinical studies of estrogen deficiency and treatment. Construction of protein–protein interaction network, GO, and KEGG pathway enrichment analysis of the differentially expressed proteins assist in hypothesizing a potential molecular mechanism of estrogen in RA via *in silico* studies. Targeting these differential proteins can emerge a new path for developing advanced therapeutic strategies.

Key Words

- ▶ controversy
- ▶ estrogen
- ▶ differential proteins
- ▶ rheumatoid arthritis
- ▶ pathway analysis

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Introduction

Female prevalence in specific diseases higher than male emerge various questions which are still ambiguous and need to be explored. Women are known to have a stronger immune system which sometimes pays a steep cost of becoming more prone to autoimmune diseases.

Rheumatoid arthritis (RA) is one such autoimmune disease (Smolen *et al.* 2018) which leads to joint inflammation causing cartilage degradation and functional loss of joint (Kaloni *et al.* 2020). RA also targets multiple organs including lungs, skin, heart, and kidney. The treatment

for men and women differs as sex hormones play a vital role in the causation of the disease. Women are affected more than men in RA, and the incidence of disease in patients (age<50) is in the ratio of 4–5:1 (female to male ratio) and 2–3:1 in older ages. An asymptomatic arousal of RA occurs in women during middle age or menopause. Disease flares are most common during postpartum period, and nulliparity increases the risk of the disease, whereas pregnancy often causes remission of the disease. The role of sex hormones has been manifested during different stages of RA, but still the role of hormonal factors remains controversial. Some studies showed that there is no relation between female sex hormones and RA. Pregnancy loss, parity, hormone replacement therapy (HRT), or oral contraceptives (OCs) have been reported as both protective and risk factors for RA (Warren & Halpert 2004). These controversies raise an interest to look through the pathways governed by the sex hormones during RA conditions.

In females, estrogen delivers a very important role not only in reproduction, behavior, cognition, and bone integrity but also in imparting various therapeutic interventions in several diseases. Estrogen controls cellular processes mainly through ‘classical’ mechanism where it disseminates into the cell and binds to estrogen receptor (ER) located in the nucleus. This complex then activates estrogen responsive elements which lead to recruitment of co-activators or co-repressors regulating the expression of associated proteins. Estrogen can also act more rapidly by ‘nongenomic’ pathway where it binds to ER situated in or vicinal to the plasma membrane. ER has two main forms ER α and ER β encoded by ESR1 and ESR2, respectively, different in chromosomal locations and major splice variants which regulate their activity in different diseases (Deroo & Korach 2006).

Reports on estrogen, the most studied female sex hormone regulating RA pathogenesis, revealed that its deficiency leads to inflammation in joints causing bone erosion. Various protein expression levels are found to be differentially regulated by estrogen treatment or during its deficiency. Whether these differentially regulated proteins lead to RA pathogenesis or whether RA conditions cause variations in protein expression levels needs to be verified. Estrogen mechanism regulating the dysregulated proteins has been clarified in various experiments, however, still uncertainty lures on estrogen regulation in RA, as many contradictory reports have been found. This review summarizes all the relevant published data including *in vitro*, *in vivo*, and clinical studies to shed light on the therapeutic implications of estrogen and estrogen-

related compounds to provide clarity toward controversial estrogen regulation in RA.

Experimental evidences of estrogen regulation in RA

In vitro studies

Estrogen regulates the differential expression of various proteins involved in this disease. Mitani *et al.* (2005) reported that RA fibroblast-like synoviocytes (RA-FLS) stimulation by 10–6M 17 β -estradiol elevated ER α expression but not ER β and significantly increased osteoprotegerin (OPG) mRNA expression but no change in receptor activator of nuclear factor kappa-B ligand (RANKL). Stimulation of RA-FLS by estrogen antagonist ICI 182780 indicated that estrogen inhibited bone erosion by stimulating the secretion of OPG (Mitani *et al.* 2005). Treatment of macrophages from RA synovial tissues with 17 β -estradiol (10⁻⁹ M) for 24 h increased Fas ligand (Fas-L) expression which modulates apoptosis (Montagna *et al.* 2009, Volpe *et al.* 2016). A recent study demonstrated that 17 β -estradiol treatment in RA synoviocytes reduces transforming growth factor β -activated kinase-1 (TAK1) significantly which is highly expressed in RA-FLS (Li & Li 2020). In 2012, Ganesan *et al.* cultured synovial fibroblasts from arthritic rats and treated them with pharmacological and physiological estrogen concentrations that exerted anti-inflammatory effects after stimulation by TNF- α (Ganesan *et al.* 2012). Recent study in 2021 established that estradiol treatment in primary articular chondrocytes subjected to acidosis-mediated injury reduced the expression of acid sensing ion channel 1a (ASIC1a) and autophagy level leading to protection against acidosis-mediated damage and autophagy (Hang *et al.* 2021). WAY-169916 (a selective ligand for ER) treatment in RA-FLS reduced TNF- α -mediated inflammatory gene expression via selective blockade of nuclear factor kappa B (NF- κ B) transcriptional activity (Steffan *et al.* 2004).¹

On contrary to the protective role of estrogen, there are some *in vitro* analyses concluding its inverse role in disease pathogenesis. Earlier in 2000, by utilizing *in vitro* cartilage invasion model and transfection of FLS with ER α gene, Khalkhali-Ellis *et al.* proved the presence of functional ER α in FLS, exerting a stimulatory effect on matrix metalloproteinase (MMP) expression, degradation and invasion of cartilage (Khalkhali-Ellis *et al.* 2000). In RA synoviocytes, the conversion of dehydroepiandrosterone (DHEA), an available steroid precursor to 17 β -estradiol, increases local levels of estrogen (Castagnetta *et al.* 2003). Induction of RA-FLS with b-estradiol leads to ectopic

Table 1 Differentially regulated proteins upon estrogen exposure with their respective functions.

Regulation of estrogen	Differentially regulated proteins	Function of proteins	Reference
Upregulation by estrogen exposure	ER- α	Nuclear transcription factors of estrogen	(Mitani <i>et al.</i> 2005)
	OPG	Downstream mediators of estrogen on bone and decoy receptor for RANKL inhibiting osteoclastogenesis	(Mitani <i>et al.</i> 2005)
	Wnt-1 induced signaling /secreted protein-2 Wisp2	Connective tissue growth factor	(Tanaka <i>et al.</i> 2005)
	Matrix metalloproteinase (MMP)-3	Ability to degrade components of the extracellular matrix	(Yamaguchi <i>et al.</i> 2012)
	CCL13	Accumulation of leukocytes	(Yamaguchi <i>et al.</i> 2012)
	IL-1 α	Leukocytic pyrogen, inducer of acute-phase response and lymphocyte-activating factor (LAF)	(Itoh <i>et al.</i> 2007)
	Sp1 and Sp3	Key regulators of leukotriene C(4) synthase gene	(Itoh <i>et al.</i> 2007)
	Inhibitory Fc γ receptor IIb	Critical role in the balance of tolerance and auto-immunity	(Engdahl <i>et al.</i> 2018)
	St6Gal1	Catalyzes the addition of α 2,6 linked sialic acids to terminal Nglycans and can modify glycoproteins and/or glycolipids	(Engdahl <i>et al.</i> 2018)
	CCR6	Recruitment of proinflammatory IL17-producing T-cells to sites of inflammation.	(Andersson <i>et al.</i> 2015b)
	CDT	Alcohol biomarker	(Fleming <i>et al.</i> 2004)
	FAS-L	Involved in the regulation of cell death, leads to apoptosis of thymocytes	(Montagna <i>et al.</i> 2009)
Downregulation by E2 exposure	TAK1	Key mediator of toll-like receptors and pro-inflammatory cytokine	(Li & Li 2020)
	ASIC1a	Potent proton sensors to detect extracellular acidification in the periphery and brain.	(Hang <i>et al.</i> 2021)
	IFN- γ	Promote macrophage activation, mediate antiviral and antibacterial immunity, regulate Th1/Th2 balance, and control cellular proliferation and apoptosis	(Latham <i>et al.</i> 2003)
	IL-10	Limiting host immune response to pathogens, thereby preventing damage to the host and maintaining normal tissue homeostasis.	(Latham <i>et al.</i> 2003)
	GM-CSF	Promotes myeloid cell development and maturation, and dendritic cell differentiation and survival, inflammatory and autoimmune reactions	(Latham <i>et al.</i> 2003)
	IgG2a anti-CII Abs	Activating the complement cascade	(Latham <i>et al.</i> 2003)
	CR1	Removal of immune complexes and pathogens coated with C3b and C4b	(Latham <i>et al.</i> 2003)
	IL-1 β	Activation of innate immunity facilitating the differentiation of IL-17-producing T cells and innate immune cells	(Plum <i>et al.</i> 2009)
	TNF- α	Responsible for a diverse range of signaling events within cells, leading to necrosis or apoptosis, resistance to infection and cancers	(Plum <i>et al.</i> 2009)
	IL-6	Host defense due to its wide range of immune and hematopoietic activities and its potent ability to induce the acute phase response	(Plum <i>et al.</i> 2009)
	IL-17	T cell activation to neutrophil mobilization and activation	(Plum <i>et al.</i> 2009)
	VEGF	Potent angiogenic factor	(Plum <i>et al.</i> 2009)
	FGF-2	Cellular proliferation, survival, migration, and differentiation	(Plum <i>et al.</i> 2009)

(Continued)

Table 1 (Continued).

Regulation of estrogen	Differentially regulated proteins	Function of proteins	Reference
	Haptoglobin	Binding hemoglobin, thus preventing iron loss and renal damage, antioxidant, antibacterial activity and involved in acute phase response	(Steffan <i>et al.</i> 2004)
	α 1-AGP	Immunomodulatory protein, important binding proteins in plasma, modulating pharmacokinetics and pharmacodynamics of many drugs, bind and transport several endogen ligands related to inflammation	(Steffan <i>et al.</i> 2004)
	CRP	Interaction with Fc receptors leads to the generation of proinflammatory cytokines, innate immune system surveillance molecule for altered self and certain pathogens	(Steffan <i>et al.</i> 2004)
	IL-2	Key growth and death factor for antigen-activated T lymphocytes, essential to maintain self-tolerance	(Engdahl <i>et al.</i> 2014)
	IFN-g	Promote macrophage activation, mediate antiviral and antibacterial immunity, enhance antigen presentation, orchestrate activation of the innate immune system, coordinate lymphocyte–endothelium interaction, regulate Th1/Th2 balance, and control cellular proliferation and apoptosis	(Subramanian <i>et al.</i> 2005)
	MCP-1	Regulate migration and infiltration of monocytes/macrophages	(Subramanian <i>et al.</i> 2005)
	RANKL	Regulates osteoclast formation, activation and survival in normal bone modeling and remodeling	(Ho <i>et al.</i> 2011)
	sRAGE	Monocyte- and neutrophil-mediated inflammation and mononuclear phagocyte survival and differentiation	(Pullerits <i>et al.</i> 2009)
	sIL-6R	Ligand–receptor complex with IL-6 stimulates a variety of cellular responses including proliferation, differentiation, and activation of inflammatory processes	(D'Elia <i>et al.</i> 2003)
	GAG	Cell signaling process, including regulation of cell growth, proliferation, promotion of cell adhesion, anticoagulation, and wound repair	(Ganesan <i>et al.</i> 2008a)
	MMP-2	Cleave protein called type IV collagen	(Ganesan <i>et al.</i> 2008a)

expression of activation-induced cytidine deaminase (AID) generating somatic mutations of *TP53* causing its dysfunction and tumor-like properties as well as elevation in *Wisp2* expression (Tanaka *et al.* 2005, Igarashi *et al.* 2010). Similarly, estrogen induction also suppressed apoptosis induced by H_2O_2 , promoted TNF- α -induced MMP-3 productions, and escalated CC motif chemokine ligand 13 (*CCL13*) gene expressions promoting inflammatory cells infiltration through ERK-1/2 signaling on the synovial fibroblasts leading to RA progression (Yamaguchi *et al.* 2012). Schmidt *et al.* reported that when estrone and 17 β -estradiol were used as substrates in RA and OA synoviocytes, they got converted into 16 α -, 4-, and 2-hydroxylated estrogens and their 4- and 2-methylation products. The precursor estrogens get transformed to proinflammatory metabolites in RA synoviocytes. RA

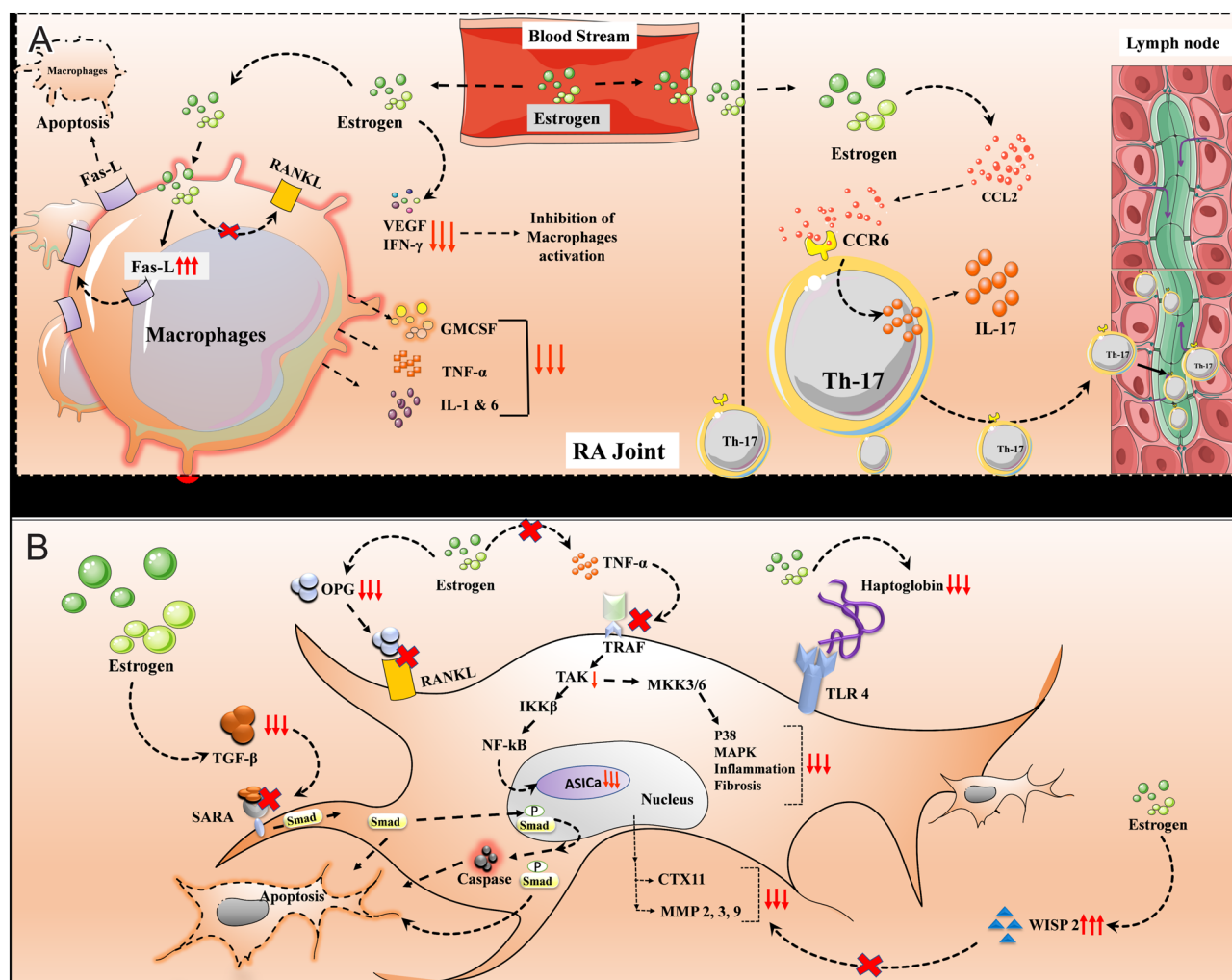
synovial cells majorly generate proliferative 16 α OH-estrone (Schmidt *et al.* 2009). Estrogen instigated interleukin (IL)-1 α mRNA expression in both types of cells: RA patient's primary synoviocytes as well as rheumatoid fibroblast-like cell line MH7A, depending upon expression of estrogen receptor. In MH7A cells, ER α but not ER β intervened the effects of estrogen. It was reported that a GC-rich region in the IL-1 α gene promoter was accountable for the response to estrogen and the estrogen treatment upregulated specificity protein-1 (Sp1) and specificity protein-3 (Sp3) which were cohered to the GC-rich region. Sp1 and ER α showed physical interaction irrespective of the presence of estrogen. ER α and histone deacetylase 2 (HDAC2) also interacted physically, and estrogen promoted the detachment of HDAC2 from ER α . These results indicated that estrogen induced the separation of corepressor

HDAC2 from ER α , which escalated Sp1 transcriptional activity across the GC-rich region present in the IL-1 α gene promoter (Itoh *et al.* 2007). These *in vitro* findings distinctly revealed that estrogen impacts the activity of joint tissues entailed in RA via intricating molecular mechanisms engaged at various levels (Fig. 1A).

In vivo studies

The role of estrogen on RA progression has majorly been studied in ovariectomized (OVX) animal models. It has been observed that decrease in estrogen level in the body during menopause reduces IgG sialylation which develops a pro-inflammatory pattern leading to onset of RA. Galactosylation is a necessary requirement for sialylation at the Asn297 site of IgG which along with the increase of glycosylation pattern of human IgG mediates anti-inflammatory effects. Accordingly, Engdahl *et al.*, in 2018, demonstrated that induced estrogen in postmenopausal (OVX) mice immunized with ovalbumin caused significant increase in Fc sialylation of total and ovalbumin-specific IgG along with inhibitory Fc γ receptor IIB expression on bone marrow leukocytes. Estrogen instigated anti-inflammatory activity of IgG by increasing Fc sialylation and triggering ST6GAL1 expression in antibody-producing cells (Engdahl *et al.* 2018). In 2003, three individual strains of collagen induced arthritis (CIA)-susceptible mice were treated with time-release pellets of 17 β -estradiol which caused a significant inhibition of arthritis along with decrease in T cell production of interferon (IFN)- γ , IL-10, and granulocyte-macrophage colony-stimulating factor (GM-CSF) levels generated by lymph nodes (LNs) cells (Latham *et al.* 2003). Study by Ganesan *et al.* demonstrated treatment with physiological doses of estrogen in arthritis-induced OVX rats significantly restored the antioxidant levels, attenuated the TNF- α and MMP-2 levels, and damage to reticulin organization along with collagen and glycosaminoglycan (GAG) loss in the articular tissues (Ganesan *et al.* 2008a). Ganesan *et al.* proved the attenuation of inflammation in CIA rats after estrogen treatment by measuring the disease severity including paw volume, radiology, joint histopathology, cytokine levels, bone turnover markers, pain mediator (prostaglandin E2) level, and immune response to type II collagen (Ganesan *et al.* 2008b). The development of CIA got enhanced in CR1/2-deficient DBA/1 mice compared to WT mice (low dose CIA), and it was reported that estrogen is a major factor that controls CR1 expression. CR1+B220+B-cell numbers and expression of CR1 got reduced compared to sham-operated WT mice proposing that estrogen promoted CR1 expression

in B cells (Nilsson *et al.* 2009). Effects of estrogen deficiency on RA were also studied in OVX MRL/lpr mice model where significant elevation in serum rheumatoid factor (RF), anti dsDNA was observed, and anti type II collagen autoimmune arthritis developed in OVX rats more severely than sham model and got entirely recovered on estrogen administration. Beside this, an elevation in RANKL, decrease in OPG mRNA expression, high amount of CD4+T cells bearing RANKL, and expansion of osteoclast formation and bone resorption pits were observed in estrogen deficiency conditions in RA model (Yoneda *et al.* 2004). Plum *et al.* showed that upon treating collagen antibody-induced arthritis (CAIA) mouse model with 2-methoxyestradiol (2ME2), reduction in TNF- α , IL-1 β , IL-6 and IL-17, angiogenic cytokines like VEGF and FGF-2, synovial inflammation, bone resorption, degradation of articular cartilage, pannus production, osteoclast activity along with prevention of neovascularization into the joint were observed (Plum *et al.* 2009). 17 β -estradiol treatment in experimental arthritis RA models, CIA; antigen-induced arthritis (AIA); and CAIA, decreased IL-17+ γ δ T cell number in joints, but elevated IL-17+ γ δ T cells (significant producers of IL-17) in draining LNs which suggested an estrogen-mediated interruption of IL-17+ γ δ T cell migration from LNs to joints obstructing progression of experimental arthritis (Andersson *et al.* 2015a) (Fig. 1B). Chondroprotective effects were evaluated by examining type II collagen degradation biomarkers after estrogen therapy (ET) on CIA rats. It demonstrated the detainment of time of onset of the disease symptoms along with reduction in paw volume and hind paw inflammation, significant decrease in type II collagen degradation marker (CTX-II) expression in serum or tissue, and reduction in MMP-2 and MMP-9 in paw extracts of the rats (Nielsen *et al.* 2008). Oestergaard *et al.* illustrated the attenuation of catabolic function of chondrocytes by showing a significant decrease in serum CTX-II after ET in ovariectomized Sprague-Dawley rats (Oestergaard *et al.* 2006). In a study by Andersson *et al.*, reduction in severity of arthritis was observed in 17 β -estradiol-treated OVX DBA/1 mice with established CIA. Estrogen altered the IL-17 producing T helper cells (Th17) phenotype, and lesser Th17 cells were noticed in joints compared to controls, whereas there were more Th17 cells present in LNs during the early phase of RA. It was also observed that estrogen elevated CCR6 on Th17 cells and also escalated corresponding ligand (CCL20) within LNs which leads to withholding of Th17 cells in LNs in the course of CIA induction phase (Andersson *et al.* 2015b). SAR development of a series of 4-(indazol-3-yl)phenols has directed toward the establishment of an orally active

**Figure 1**

Mechanistic regulation of estrogen during RA. (A) Estrogen regulation on the inflammatory and apoptosis-mediated molecules in macrophages during RA. Estrogen helps in transporting Th17 cells from RA joints back to lymph nodes thus attenuating the release of IL17 in RA joints which causes inflammation. (B) Estrogen mediating synovocytes regulation in RA. Estrogen attenuating several inflammatory pathways like NF κ B, TLR4, PI3-AKT by its action on specific proteins including RANKL, TRAF, TAK, TNF, OPG, and MMPs. Apoptosis of synovocytes during RA progression can also be regulated by estrogen by targeting several proteins like smad, caspase, and TGF β . A full color version of this figure is available at <https://doi.org/10.1530/JME-22-0010>.

non-steroidal ligand WAY-169916 which can be inherently used in the treatment of RA with bare minimal classical proliferative effects linked with estrogen. Its daily oral treatment in the AIA disease model for arthritis resulted in improved modifications in hind paw scores and development in cartilage lesions in the tarsal joints and histological scores of synovitis along with reducing three serum acute phase proteins haptoglobin, α 1-acid glycoprotein (α 1-AGP), and c-reactive protein (CRP) majorly in spleen, liver, and popliteal LNs (Steffan *et al.* 2004). Similarly, development of substituted 2-cyanopropanoic acid derivatives escorted toward the production of WAY-204688, an orally active, pathway-selective, ER-dependent anti-inflammatory agent, which impeded proinflammatory

genes transcriptional activation mediated by NF- κ B which was proved to be effective orally in preclinical models of inflammatory diseases like RA (Caggiano *et al.* 2007). In 2011, Yuan Ho *et al.* demonstrated the effect of estradiol valerate (EV) in rat AIA model of RA. Treatment with EV reduced circulating TNF- α and inflammation in joints, increased systemic OPG, decreased RANKL/OPG protein and mRNA ratio in joints of AIA rats along with augmenting RLX family peptide receptor 1 (RXFP1) gene expression (Ho *et al.* 2011). In another study, male and female rats with AIA were treated with idoxifene, a selective ER modulator, and estrogen, prophylactically (days 0–21) or therapeutically (days 10–21) and a significant inhibition of paw inflammation, improvement of integrity in joints, and

significant reduction of IL-6 levels were observed in both rats. Moreover, valuation of the tibiotarsal joints of female rats showed protection of bone, cartilage, and soft tissue. It was reported that prophylactic dosing was more effective than therapeutic treatment in reducing paw inflammation in these animals (Badger *et al.* 1999). In 2010, it was demonstrated in a well-established model of postmenopausal RA, that ER α , but not ER β , or GPR-30 signaling is responsible for prevention of the disease symptoms as treatment with specific agonists propylpyrazoletriol (PPT; for ER α), diarylpropionitrile (DPN; for ER β), G1 (for GPR-30), or physiologic dose of estradiol caused elevated bone mineral density (BMD) and reduced bone resorption markers in serum and cartilage degradation markers, while no effective result was observed after DPN or G1 treatment (Yoneda *et al.* 2004). Engdahl *et al.* used ER α -inactivated mice model and concluded that ER α is the main receptor which is responsible for the protective effect of estrogen treatment to amend arthritis in AIA model as estrogen diminished synovitis and joint destruction in WT mice (OVX, treated with estrogen, AIA mice) but no effect was observed in synovitis or joint destruction in total ER α -/- mice, whereas in Col2 α 1-ER α -/- (cartilage-specific) mice, estrogen reduced joint destruction but did not significantly decrease synovitis proving that ER α is responsible for the protective effect of estrogen for both joint destruction and synovitis but Col2 α 1-ER α -/- is necessary for preventing joint destruction and not for synovitis protection by estrogen. After estrogen treatment, monocytes and macrophages frequency got reduced in the joints mediated by ER α , but reduction in frequency of neutrophils in joints was mediated by ER α expression in chondrocytes. Furthermore, after estrogen treatment, depletion of cytokines like IFN- γ , IL-2, IL-6, and IL-17 developed by splenocytes treated with the T-cell mitogen conA was observed which was also mediated by ER α (Engdahl *et al.* 2014). A small-molecule ER β selective agonist, ERB-041, had shown potent anti-inflammatory activity in Lewis rat model of AIA by partially or fully altering a large number of genes and protein expressions in the disease model (Follettie *et al.* 2006). Selective ER modulator (SERM) raloxifene, estradiol, and also endogenous estrogen treatment, diminished the frequency of arthritis, attenuated joint degradation, and resisted generalized osteoporosis along with reducing the serum levels of IL-6 in B10.Q-ncf1^{*/*}mice. B10.Q-ncf1^{*/*}mice are B10.Q mice with a mutated Ncf1 gene in which CIA evolve as a chronic relapsing disease, mimicking human RA (Jochems *et al.* 2010). Recently in a study, administration of estrogen in CIA model significantly reduced TAK1

expression and attenuated the arthritis development (Li & Li 2020). Butyrate treatment in autoimmune arthritis animal model resulted in reduction in arthritis score compared to the control group along with inhibition of HDAC2 in osteoclasts and HDAC8 in T cells, causing acetylation of glucocorticoid receptors and ER α (Kim *et al.* 2018). Treatment of DBA/1LacJ mice with bovine type II collagen (bCII) and orally active estrogen (ethinyl estradiol) leads to reduction in histological and clinical signs of CIA along with decrease in proliferation and secretion of MCP-1, TNF- α , IFN- γ , and IL-6 by bCII peptide-specific T cells, bCII-specific IgG2a antibodies production, and mRNA of receptors for cytokines and chemokines in joint tissue (Subramanian *et al.* 2005). An estrogen-replete CIA rat model was used by Yoshioka *et al.* in 2008 to analyze the impact of ET in arthritis and BMD. Estrogen treatment in CIA rats resulted in milder development of arthritis, suppression of deterioration in BMD along with improvements in histomorphometrical parameters of bone resorption (Yoshioka *et al.* 2008). Yang *et al.* in 2010 reported that estrogen deficiency in aromatase-deficient (ArKO) mice cognate with significant elevation in LPS-induced serum IL-6, TNF, MCP-1, and IFN- γ levels and increase of AIA disease symptoms and antigen-specific T cell proliferation which got significantly diminished by administration of 16 α -LE2. Likewise in ArKO mice, antigen-specific T cell proliferation increased after immunization with type II collagen (CII) which got reversed after administration of 16 α -LE2, which significantly decreased the severity of CIA, which was linked with repression of anti-CII-specific IgG (Yang *et al.* 2010). In 2018, mesenchymal stem cells amalgamated with 17 β -estradiol (estrogen 100 μ M) were administered in CIA-induced wistar rats and a inhibition in amount of edema and swelling of the palms of the hands and feet, decrease in inflation along with repressive effect on inflammatory mediators of spleen-inherent immune cells in RA model were observed (Jahan Tigh *et al.* 2018). Recently in 2021, Hang *et al.* demonstrated that estradiol treatment in rats with AA lead to decrease in cartilage damage and reduction in expression of inflammatory cytokines in the serum. The protection of the rat cartilage with AA against acidosis-mediated damage occurred by attenuating ASIC1a expression through the PI3K-AKT-mTOR pathway, majorly by G-protein coupled estradiol receptor 1 (GPER1), and also prevented autophagy by the selective ASIC1a blocker psalmotoxin-1 (PCTX-1), respectively (Hang *et al.* 2021). Illustration of these therapeutic endeavors of estrogen treatment in *in vivo* animal models summarized in Table 2 infers it as extremely potential for targeting RA.

Table 2 *In vitro* and *in vivo* studies of estrogen treatment with their mechanism.

Cells/cell lines	Treatment	Dosage	Mechanism	Reference
<i>In vivo</i> studies				
RA-FLS	17 β -estradiol	10 ⁻⁶ M	↑ ER α ↑ (OPG)	(Mitani <i>et al.</i> 2005)
Macrophages	17 β -estradiol	10 ⁻⁹ M	↓ FAS-L	(Montagna <i>et al.</i> 2009)
RA-FLS	17 β -estradiol	100 nM	↓ TAK1	(Li & Li 2020)
Primary articular chondrocytes	Estradiol	500 nmol/mL	↓ ASIC1a	(Hang <i>et al.</i> 2021)
RA-FLS	WAY-169916	-	↓ autophagy level	(Steffan <i>et al.</i> 2004)
RA-FLS	b- estradiol	10 ⁻⁹ M	↓ TNF- α	(Igarashi <i>et al.</i> 2010)
RA-FLS	17-b-estradiol	10 ⁻⁶ M	↓ NF- κ B	(Tanaka <i>et al.</i> 2005)
RA-FLS	Estrogen	10 nM	↑ AID	(Yamaguchi <i>et al.</i> 2012)
			↑ Wisp2	
			↓ H ₂ O ₂ -induced apoptosis	
			↑ TNF- α -induced MMP-3	
			↑ CCL13	
			↑ Sp1	(Itoh <i>et al.</i> 2007)
RA-FLS cell line MH7A	Estrogen	10 nM	Measurement	Reference
<i>In vivo</i> studies				
Female	Estrogen	0.83 μ g/day	↑ Fc sialylation	(Engdahl <i>et al.</i> 2018)
C57BL/6 mice			↑ Fc γ receptor IIb	
DBA/1Lad mice	17 β -estradiol	60-day release estrogen pellets containing a total of either 2.5 or 0.36 mg of 17 β -estradiol	Paw volume Severity of arthritis Serum estrogen levels ↓ T-cell production ↓ IL-10 ↓ GM-CSF ↓ IgG2a anti-CII Abs IgG1 anti-CII Abs Histopathology Paw volume Lipid peroxide level Antioxidant assays Collagen level Total and sulfated glycosaminoglycans ↓ TNF- α ↓ MMP-2	(Latham <i>et al.</i> 2003)
Male and female Wistar rats	Estradiol benzoate	2.5, 5, and 10 μ g	Histopathology Paw volume Radiology Calcium and phosphorus Type II collagen antibodies in the serum Serum IL-1 β , IL-6 and IL-10, TNF Bone turnover markers; Alkaline phosphatase Tartarate resistant acid phosphatase (TRAP) assay PGE2 analysis	(Ganesan <i>et al.</i> 2008a)
Male and female Wistar rats	Estradiol benzoate	2.5, 5, and 10 μ g	Histopathology Paw volume Radiology Calcium and phosphorus Type II collagen antibodies in the serum Serum IL-1 β , IL-6 and IL-10, TNF Bone turnover markers; Alkaline phosphatase Tartarate resistant acid phosphatase (TRAP) assay PGE2 analysis	(Ganesan <i>et al.</i> 2008b)

(Continued)

Table 2 Continued.

Cells/cell lines		Treatment	Dosage	Mechanism	Reference
DBA/1 mice	6 week	-	-	Severity of arthritis serum C5 levels ↓CR1+B220 ⁺ B-cell ↓CR1 expression Histology Immunohistology Bone resorption ↑Serum RF ↑Anti dsDNA ↑Anti type II collagen autoimmune arthritis IL-2, IL-4, IFN- γ ↑RANKL ↓OPG mRNA ↑CD4 ⁺ T cells bearing RANKL Cell infiltration Pannus severity Cartilage lesion Bone resorption Quantitative histomorphometric analysis ↓IL-1 β , TNF- α , IL-6 and IL-17 ↓Angiogenic cytokines (VEGF and FGF-2)	(Nilsson <i>et al.</i> 2009)
MRL/lpr mice	4–24 weeks	6–10	60 mg/kg	estrogen	(Yoneda <i>et al.</i> 2004)
Balb/c female mice	5–7 week	-	100, 75, 50, 25, 10, 1 mg/kg, p.o., daily	2-methoxyestradiol (2ME2)	(Plum <i>et al.</i> 2009)
Female DBA/1 mice	8–10 weeks	5–10	1 μ g/mouse/day	17 β -estradiol-3- benzoate	(Andersson <i>et al.</i> 2015a)
Female Lewis rats	9 weeks	151–175 g N = 10	0.18 mg/60 days release	OE2-pellet	(Nielsen <i>et al.</i> 2008)
Female Sprague– Dawley rats	6-month	10–11	0.25-mg pellet	17-estradiol	(Oestergaard <i>et al.</i> 2006)
DBA/1 mice	8–10	-	1 μ g/mouse/day	17 β -estradiol-3- benzoate	(Andersson <i>et al.</i> 2015b)
Lewis rats	7 week	n = 8	EV; 5 μ g/0.1 mL sesame oil, weekly starting day—10	Chemotaxis assay ↓TNF- α and joint inflammation ↑systemic OPG ↓RANKL/OPG protein RLX family peptide receptor 1 (RXFP1) gene expression	(Ho <i>et al.</i> 2011)

(Continued)

Table 2 Continued.

Cells/cell lines	Treatment		Dosage	Mechanism	Reference
Male and female Lewis rats	-	-	Idoxifene + estrogen	↓IL-6 levels ↓paw volume BMD Histology Bone mineral content Absorptiometry Histology BMD Serum levels of COMP (cartilage oligomeric matrix protein) ↓Frequency of arthritis ↓IL-6 ↓TAK1 joint swelling	(Badger <i>et al.</i> 1999)
B10.Q-nf1*/mice	7-19	5-10	17b-estradiol-3-benzoate	1.0 µg/mouse/day	(Jochems <i>et al.</i> 2010)
CIA model	4 months 80-100 g	n = 10	estrogen	100 µg E2 for 3 days	(Li & Li 2020)
DBA/1LacJ mice	-	-	Ethinyl estradiol	50 Ag EE or 200 Ag EE in 100 Al olive oil.	(Subramanian <i>et al.</i> 2005)
Female Sprague-Dawley rats	Seven month 260-330 g	n = 10	17b-estradiol	20 µg/kg 3 times per week Body weight severity of inflammation ↓BMD Bone histomorphometry Histology; synovitis, soft tissue inflammation, exudates, cartilage degradation ↑IL-6, TNF, MCP 1, and IFNγ levels Antigen-specific T cell proliferation Rate of respiratory burst, phagocytosis and nitric oxide production Diameter of the wrists and the foot Severity of joint Cartilage damage IL-1β and TNF-α ASIC1a, GPER1, LC3, and Beclin1	(Yoshioka <i>et al.</i> 2008)
ArKO mice Male DBA/1J mice	8 and 10 weeks	-	16-LE2 8-VE2 17-estradiol	16-LE2 (3 g/kg), the selective ER agonist 8-VE2 (100 g/kg), 17-estradiol (3 g/kg)	(Yang <i>et al.</i> 2010)
Wistar rats	-	-	17β-estradiol	100 µM for 24 h	(Jahan Tigh <i>et al.</i> 2018)
Sprague-Dawley (SD) rats	7-8 weeks 180-220 g	n = 7	Estradiol	0.1, 0.2, and 0.3 mg/kg	(Hang <i>et al.</i> 2021)

Clinical studies

In postmenopausal women with RA, estrogen treatment significantly elevated the Fc sialylation of IgG and expression of ST6GAL1 in human antibody-producing cells delivering an idea for high risk of RA with low estrogen level condition such as menopause (Engdahl *et al.* 2018). Clinical observations of patients demonstrated significant correlation of plasma glutamate level and radiographic bone erosions in the presence of low levels of CRP and estradiol. The patients with low levels of CRP and estradiol have the highest levels of glutamate. In RA patients with estradiol levels >65 pmol/L combined with low systemic inflammatory activity, no such erosions were observed. Glutamate and bone resorption relationship was found to be affected by estradiol as well as systemic inflammatory activity (Hajati *et al.* 2009). Study on 88 postmenopausal women with active RA after 2 years of HRT treatment demonstrated improvement in BMD in the hip and lumbar spine, significant upregulation of the bone anabolic factor, insulin-like growth factor 1 (IGF-1), and reduction of serum levels of soluble IL-6 receptor (sIL-6R) (D'Elia *et al.* 2003). In a study by Pullerits *et al.* in 2009, consequences of HRT on serum soluble receptor for advanced glycation end product (sRAGE) levels in RA patients were analyzed through a long range before and after treatment initiation. Patients encountering HRT showed significantly reduced levels of sRAGE in serum at 1 and 2 years in comparison to the levels at initiation of the study which is correlated with increase in serum estradiol and decreased bone/cartilage turnover markers such as C-terminal propeptide of type I procollagen, carboxyterminal telopeptide of type I collagen, and cartilage oligomeric matrix protein and increase in total BMD in hip and femoral neck (Pullerits *et al.* 2009). Study in three European populations with 2936 RA patients and 2197 healthy controls revealed that after the administration of anti-TNF drugs, a significant correlation was observed between CYP3A4rs11773597 and CYP2C9rs1799853 variants and alterations in DAS28. It was also reported that the CYP3A4 and ESR2 single-nucleotide polymorphisms (SNPs) correlated with TRIM4 and ESR2 mRNAs expression in peripheral blood mononuclear cells (PBMCs) and that the CYP2C9rs1799853 SNP regulates the efficacy of multiple drugs. ESR2GGG haplotype is significantly associated with a reduced chance of having poor response to anti-TNF drugs (Canet *et al.* 2019). Stauber *et al.* reported a significant increase in carbohydrate-deficient transferring (CDT) levels in 11 postmenopausal women receiving estrogen replacement in comparison with 34 postmenopausal women not administering estrogen. CDT is an alcohol biomarker

approved by the U.S. Food and Drug Administration recently (Fleming *et al.* 2004). Studies conducted on postmenopausal RA patients receiving treatment of transdermal oestradiol (50 mg daily) over 6 months demonstrated that patients who had acquired greater enhancements in serum estrogen while administering HRT manifested improvements in a number of parameters of disease activity such as early morning stiffness, erythrocyte sedimentation rate (ESR), articular index (AI), and visual analog pain scale (VPS) (Hall *et al.* 1994).

On the contrary, ERB-041 is considered as well tolerated and safe thrive to exemplify anti-inflammatory activities in RA patients, inspite of showing strong evidence of its activity in preclinical arthritis models (Roman-Blas *et al.* 2010). In a clinical study in 2003, it was observed that the estrogen levels in synovial fluids of RA patients were significantly elevated compared to controls, and 16 α -hydroxyestrone and 4-hydroxyestradiol, biologically active estrogen derivatives, were also found to be higher in RA patients (Castagnetta *et al.* 2003). Although the reports from clinical studies confer some interesting results, it is still confined, and more research is necessary to specifically target this prevalent disease.

Estrogen modulating microRNA expression in RA

miRNAs are short non-coding nucleotide sequence involved in early development, cell proliferation, differentiation, and various biological activities by regulating the expression at mRNA level. Sex hormones regulate various miRNA, and their aberrant pattern can alter its expression profile and functions. Recently, reports revealed that miRNAs play a major role in pathogenesis and progression of RA (Ammari *et al.* 2013).

Recently, studies are in progress to investigate the role of estrogen in modulating miRNA regulation in RA. miR-155 expression is high in RA patients which increases TNF- α , IL- β , and various inflammatory factors involved in pathogenesis of RA (Su *et al.* 2017). *Silybummarianum* (milk thistle) plant contains active component silibinin, an agonist of ER β , helps in the regulation of inflammation by decreasing miR-155 (Dupuis *et al.* 2018). miR-22 negatively regulates Cyr61 by binding with its 3' UTR of mRNA (Lin *et al.* 2014). Deregulation in miR-146a was present only in females having RA, proposing the connection of estrogen with miR-146a in immune cells (Zhou *et al.* 2015). Positively related expression of miR-146 is associated with RA severity (Chen *et al.* 2017), and *in vivo* studies suggested that estrogen reduced miR-146a, miR-143, miR-126, miR-125b,

let-7e, miR-145, and miR-125a and increased miR-451, miR-148a, miR-223, miR-18a, miR-486, and miR-708 in mice splenic lymphocyte (Dai *et al.* 2008). Estrogen treatment activates ER α resulting in miRNA profile alteration in RA which increases the production of essential proteins linked to miRNA biogenesis such as Drosha, Dicer, and DGRC8 (Andersson *et al.* 2018). Studies conducted in human cell lines suggested that ER α decreased miR-206 expression (Meyers *et al.* 2001) while ER- β agonist increased miR-206 expression in breast cancer cell line (Adams *et al.* 2007). *In vivo* studies on estrogen treatment at several time points showed alteration of miRNA expression according to their respective tissue in *Danio rerio* (Zebrafish) (Cohen *et al.* 2008). Exposure of estrogen treatment on ACI female rats depicted alteration of miRNA profile and related protein expression (Kovalchuk *et al.* 2007). These recent studies emerged a new field of miRNA-mediated regulation of estrogen which can contribute largely in miRNA related targeted therapy to prevent RA.

Estrogen role in modulating cardiovascular effects in RA

Numerous clinical studies accounted that patients with RA are twice at higher risk of encountering cardiovascular complications as compared to general population (Hansildaar *et al.* 2021). The greater susceptibility of postmenopausal women in encountering cardiovascular disease (CVD) events highlights the influence of sex hormones in the progression of CVD. Estrogen level has the potential to regulate blood vessel metabolism enhancing antioxidant activity (Duan *et al.* 2021). The main circulating female sex hormone estrogen has been reported to act as an immunomodulator as well as a cardioprotective agent (Baker *et al.* 2003, Iorga *et al.* 2017). The cardioprotective influence of estrogen could be attributed to plethora of mechanisms such as:

1. Differential expression of estrogen receptors on mitochondrial membrane regulating mitochondrial function in cardiac tissue.
2. Antioxidant effects of estrogen in rat model have been highlighted via upregulation of hydrogen (Iorga *et al.* 2017).
3. Presence of ERs on human monocytes indicative of their influence on the release of cytokines (Baker *et al.* 2003).

However, the HRT clinical trials involving estrogen and progesterone treatment showed opposite effect on the

progression of CVD wherein it establishes the CV risk in healthy postmenopausal women population and have no influence in ameliorating the CV risk in postmenopausal women with pre-existing CVD. This failure of HRT trials suggests the influence of progesterone in interfering with the mechanism of estrogen interaction or some other risk factors (Baker *et al.* 2003, Iorga *et al.* 2017). Certain research studies have examined that risk of CVD in women with RA increases with early menopause which evident the role of female sex hormones in contemplating the CV risks among RA patients. However, independent HRT trials involving the administration of only estrogen need to be further conducted to highlight the role of estrogen in influencing the CV risk among RA patients (Pfeifer *et al.* 2014).

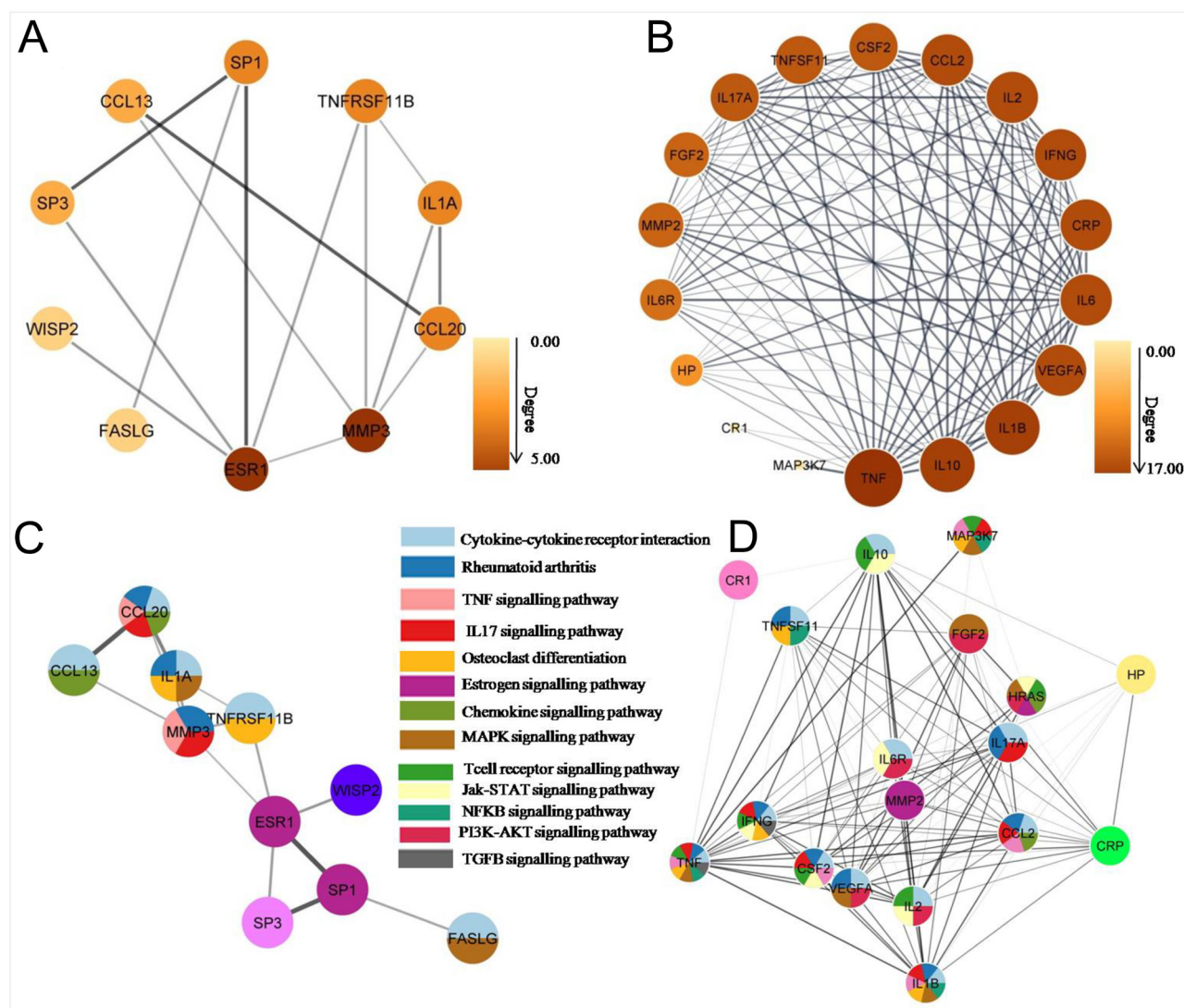
In silico studies

PPI network construction

A PPI network was established with estrogen-regulated differentially expressed proteins in RA. The network of upregulated and downregulated proteins was constructed by Cytoscape 3.8.2. Upregulated proteins network (Fig. 2A) was constructed on the basis of degree of contribution of the proteins in the network and is denoted by color of the nodes from darker to lighter. ESR1, MMP3, CCL20, and IL1A have consecutively darker shades depicting their importance with degree values of 5, 5, 3, and 3 respectively. Downregulated proteins TNF, IL-10, IL-1 β , VEGFA, and IL-6 represent the darker nodes with degree of 17, 16, 16, 15, and 15, respectively (Fig. 2B). These proteins play a major role in RA progression. KEGG pathway enrichment of upregulated proteins showed involvement of MAPK, estrogen, TNF, IL-17, chemokine, cytokine-cytokine receptor signalling pathways, thus regulating the progression of RA (Fig. 2C). Downregulated proteins are majorly involved in T cell receptor, JAK-STAT, Th17 cell differentiation, NF- κ B, PI3K-AKT, TLR, TGF- β pathways (Fig. 2D).

GO enrichment and KEGG pathway analysis

To elucidate the properties of the differentially regulated estrogen targets linked with RA, the associated biological processes, cellular components, and molecular functions of the targets were interpreted by Cytoscape 3.8.2 and represented by R studio. Upregulated targets illustrated 84 GO entries, out of which 72 entries are associated with biological processes including cellular response to organic substance, immune system process, cytokine-mediated signaling pathway and eight entries are of

**Figure 2**

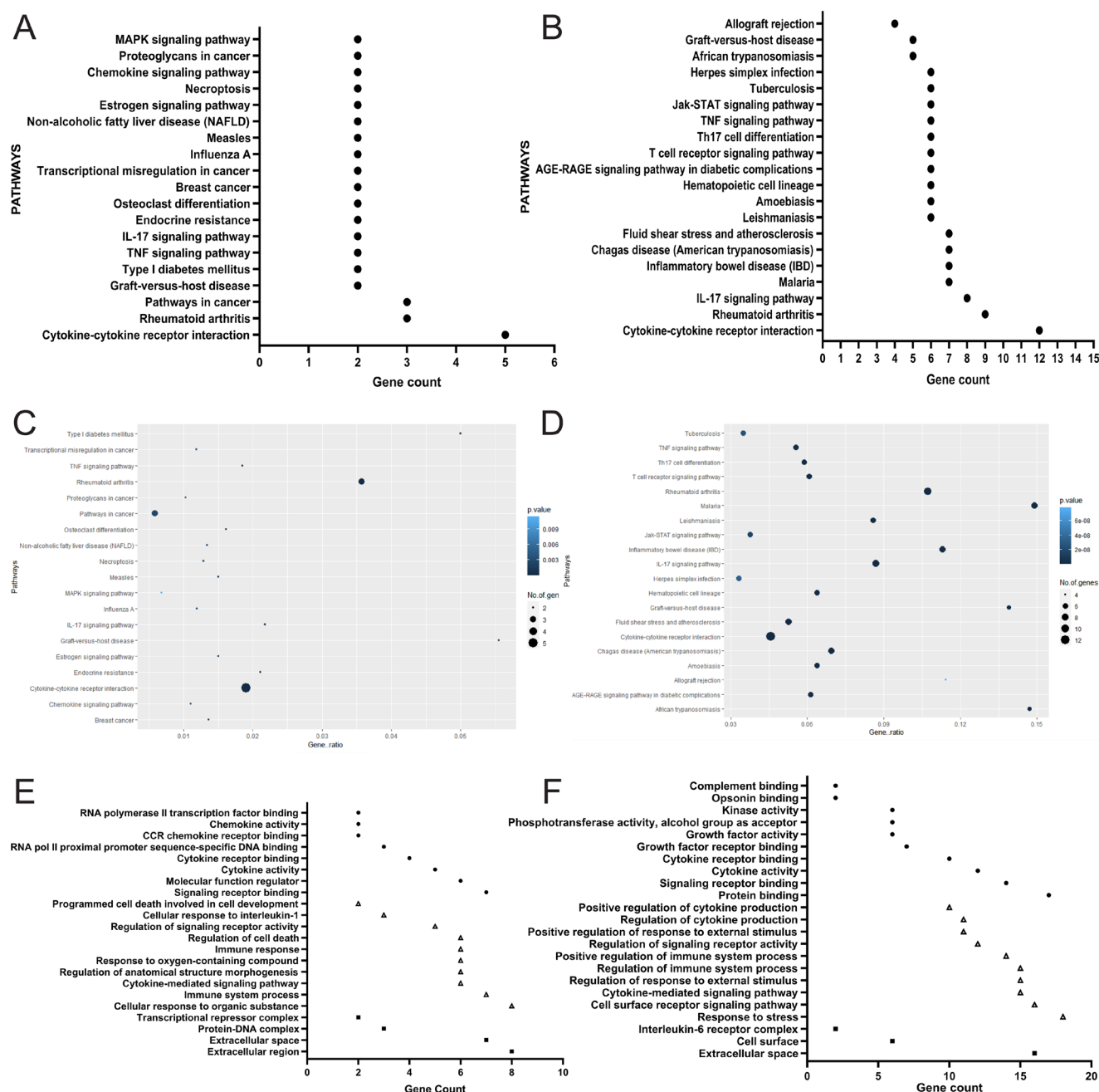
Protein-protein interaction network constructed by Cytoscape 3.8.2. (A) PPI of upregulated targets on the basis of degree; the color of the node is positively related to the degree of contribution of the node in the network. (B) PPI of downregulated targets on the basis of degree. (C) PPI network of upregulated proteins according to involved pathways; the color of the node is representative of the different signaling pathway as represented. (D) PPI network of downregulated proteins according to involved pathways. A full color version of this figure is available at <https://doi.org/10.1530/JME-22-0010>.

molecular functions like signaling receptor binding, molecular function regulator, cytokine activity, cytokine receptor binding, and four cell component entries include extracellular region, extracellular space, protein-DNA complex, and transcriptional repressor complex. Downregulated proteins illustrated total of 478 GO entries out of which 460 entries are associated with biological processes that include regulation of immune system process, positive regulation of cytokine production, response to stress and 15 entries are of molecular functions that include signaling receptor binding molecular function regulator, cytokine activity, cytokine receptor binding, and three cell component entries include extracellular

space, cell surface, and IL-6 receptor complex (Fig. 3E and F depicts the top 10 according to P value < 0.05). Illustration of the KEGG enrichment analysis sorted according to P value and gene count were represented in Fig. 3A and B, and KEGG pathway analysis according to gene ratio was illustrated using R studio (Fig. 3C and D).

Analysis of estrogen involvement in regulating proteins obtained via curative database

Analyzing the GO enrichment reports as well as the pathways involved, it was evident that the differentially

**Figure 3**

KEGG and GO analysis of potential targets related to occurrence and development of RA: (A) Histogram representation of pathways of upregulated proteins. (B) Histogram representation of pathways of downregulated proteins. (C) Representation in bubble chart of upregulated proteins involved pathways. (D) Representation in bubble chart of downregulated proteins involved pathways. (E) GO ontology of upregulated proteins. (F) GO ontology of downregulated proteins. A full color version of this figure is available at <https://doi.org/10.1530/JME-22-0010>.

regulated proteins directly or indirectly link with RA progression. The downregulated proteins direct more toward anti-inflammatory activities and RA protection. Few differentially regulated proteins indicate a proinflammatory function. Majorly, the evidence-based databases suggest

that estrogen is responsible for upregulation of SP1 and SP3. Contradictorily, the upregulation of SP1 that regulates the expression of gliostatin/thymidine phosphorylase is responsible for angiogenesis and arthritic activity (Ikuta *et al.* 2012). However, it is worth noting that targeting

Sp3/Sp1 ratio in cartilage can contribute to prevention of tissue degradation (Chadjichristos *et al.* 2003). Since mRNAs level not always lead to protein expression thus a proteomic validation is desirable for complete understanding.

In the protective part, the anti-rheumatic/anti-inflammatory nature of estrogen is well documented as we found from the curated databases that estrogen is found responsible for downregulation of CRP level and most of the cytokines and MMPs. However, few exceptional studies found that CRP and leptin level were elevated during menstruation cycle (Capobianco *et al.* 2010). Although the literature study inclined toward the protective effect of estrogen in RA, other related domains need to be evaluated further.

Considering KEGG pathway analysis, the proteins that are involved in various signaling pathways including NF- κ B, PI3K-AKT, TLR, and TGF- β which drive toward RA are more found to be downregulated by estrogen. The contradiction arises with some of the proteins that have functions toward increasing RA but were found to be upregulated by estrogen exposure. After analyzing the PPI network and the respective GO of the proteins, it was found that most of the study related to estrogen exposure has been done with respect to inflammatory cytokines and their related molecules. But rarely studies can be found of cell- or tissue-specific proteins that might get differentially regulated upon estrogen exposure causing the attenuation of cytokine release during RA. This vast area of dealing with those proteins that link estrogen with inflammatory molecules needs to be explored to have a clear view of the estrogen pathways involved in RA management.

Future pharmacological perspectives

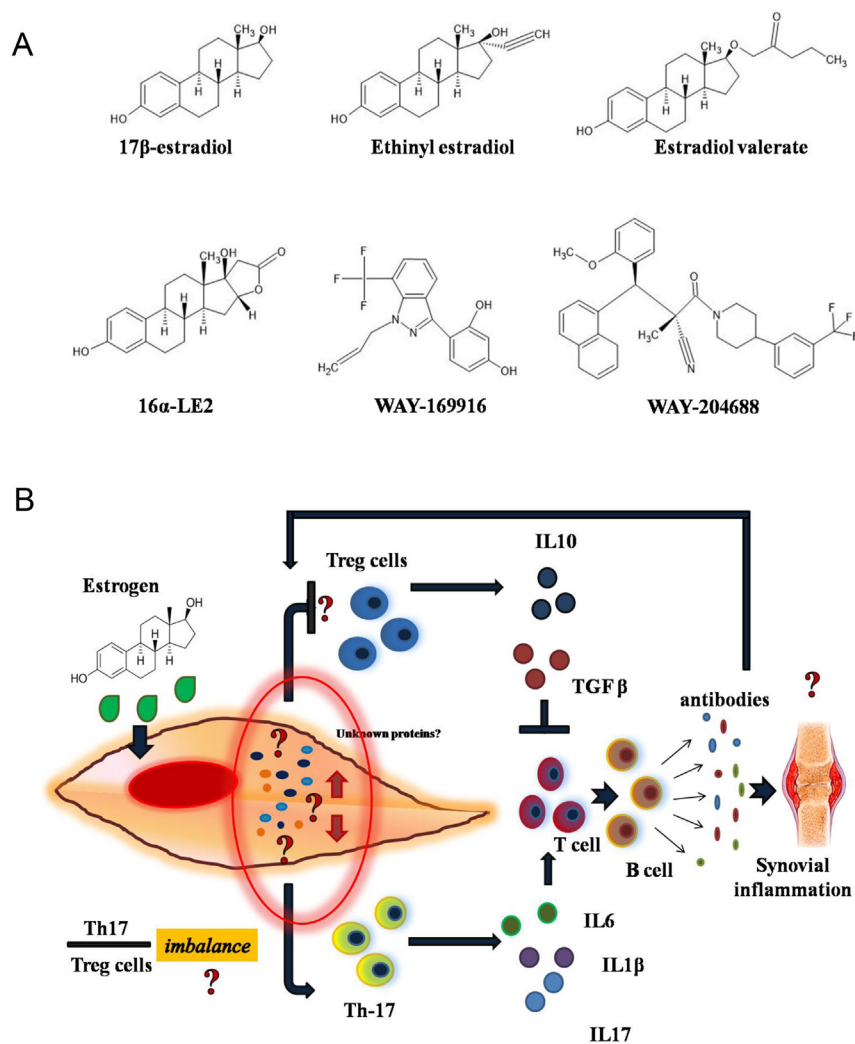
Substantial evidences proved that estrogen can be utilized as an adequate treatment for RA remission. Currently used synthetic drugs sometimes could not provide overall treatment since RA is not only a disease of joints. Estrogen induction can provide a treat-to-target therapeutic approach. Estrogen treatment has been used in different diseases including osteoporosis, heart diseases, stroke, and dementia (Hill *et al.* 2016). A normal level of estrogen should be present in the body to resist the development of RA, which can be executed by medications or through estrogen consisting diet like soyabeans, grapes. Thus, in future the utilization of phytestrogen as medication for RA patients can be upraised. Intake of phytestrogen as supplements or drugs can be beneficial for RA patients, which diminish the deficiency of estrogen in RA patients.

Future directions

By summarizing this review with all the experimental evidences and the *in silico* studies analyzed although we can conclude an important and protective role of estrogen in RA, still some questions remained unresolved: Whether estrogen plays a role in modulating the post-translational modification of the proteins in RA and its related autoimmunity? What role does estrogen play in combating oxidative stress which is an important factor to deal in RA? What is the role of estrogen in other types of arthritis? How much productive it would be if a combination of estrogen and phytestrogens is used for therapy. Other parameters concerning the effects of estrogen on RA conditions such as anti-oxidative, anti-proliferative, and immunomodulatory need to be superintended further. All these questions need to be answered in near future by more extensive researches and clinical trials to acquire a suitable remedy for RA.

Conclusion

A clear understanding of the role of estrogen and the molecular mechanisms in inducing changes in RA condition will contribute to improved therapeutic strategies to revamp damaged articular tissues and cartilages in RA. Various improved therapeutic approaches of estrogen and estrogen-related compounds (Fig. 4A) should be promoted for providing protection against disease progression because this disease condition is often triggered due to estrogen deficiency as it causes imbalance in the ratio of T regulatory (Treg) and Th17 cells by more activation of Th17 than Treg cells, leading to release of cytokines like IL-17, IL-6 which causes activation of T cell ultimately leading to the production of autoantibodies from B cells (Fig. 4B). *In vitro*, *in vivo*, and clinical manifestations of estrogen effect provided us the knowledge of the differentially expressed proteins and their related molecular mechanisms in RA pathogenesis. *In silico* studies also cleared some of the controversies of estrogen regulation in RA, as analysis of the differentially regulated proteins provided the idea of their linked pathways, functions, and their involvement in this disease progression. Targeting these differentially expressed proteins can lead to a path to retard the disease progression. New specific therapeutic implications can be innovated to maintain the homeostasis environment by controlling the deregulation of these protein expressions mediated by estrogen deficiency. Estrogen has been also found to be beneficial for protecting against CVD risk which arises mostly in RA patients either due to dysfunctioning of cellular functions internally or as an effect of the steroidal

**Figure 4**

(A) Structures of estrogen-related compounds modulating RA conditions. These compounds are induced either *in vitro* or *in vivo* for determining estrogen effect on RA conditions. (B) Hypothesis of estrogen regulation in RA: It is known that estrogen regulates cytokines and other inflammatory molecules but its action on cellular proteins that control the inflammatory regulations are yet to be revealed. This major gap needs to be revealed. A full color version of this figure is available at <https://doi.org/10.1530/JME-22-0010>.

medications which are administrated to RA patients frequently. Recently, the studies on miRNA and other small molecule regulation upon estrogen exposure elucidated the potential mechanism of estrogen further in dealing with RA (Moran-Moguel *et al.* 2018).

Besides the protective role of estrogen in RA, it also exerts others beneficial actions as well as some risks simultaneously. To compensate the harmful effects of estrogen, recently the use of phytomedicines for treating RA has come into trend and the field of phytosterogen has emerged as an effective treatment devoid of any side effects (Kaloni *et al.* 2020, Chakraborty *et al.* 2021). Many other plant compounds such as liquiritin, imperatorin, salicin, and quercetin have been shown to ameliorate the deteriorating effects of RA (Zhai *et al.* 2018a,b, 2019, Mann *et al.* 2022). Overall, our current study provides us with the knowledge of estrogen influence on RA pathogenesis and its linked differential expression of proteins and their mechanism which could explain hormonal cause

as a major link in RA progression apart from genetic or environmental factors. Still a huge gap exists in this field of estrogen regulation in RA pathophysiology and needs to be explored at a deeper level with more experimental and clinical trials to evolve a better remedy.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of this review.

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Author contribution statement

Debolina Chakraborty: Conceptualization; Data curation; Formal analysis; Methodology; Software, Validation; original draft, Ashish sarkar, Sonia Mann, Prachi Agnihotri, Mohd Saquib, Swati Malik: Writing, Monu:

Preparing illustrations, Rajkamal Kumavat, Anushka Mathur: review and editing, Sagarika Biswas: Investigation; Project administration; Resources; Supervision; Visualization, Funding acquisition.

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