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

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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 manifestated 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 ERα and ERβ 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 17β-estradiol elevated ERα expression but not ERβ 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 17β-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 17β-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-κB) 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 ERα gene, Khalkhali-Ellis *et al.* proved the presence of functional ERα 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 17β-estradiol, increases local levels of estrogen (Castagnetta *et al.* 2003). Induction of RA-FLS with b-estradiol leads to ectopic

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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 *et al.* 2005)
	OPG	Downstream mediators of estrogen on bone and decoy receptor for RANKL inhibiting osteoclastogenesis	(Mitani *et al.* 2005)
	Wnt-1 induced signaling /secreted protein-2 Wisp2	Connective tissue growth factor	(Tanaka *et al.* 2005)
	Matrix metalloproteinase (MMP)-3	Ability to degrade components of the extracellular matrix	(Yamaguchi *et al.* 2012)
	CCL13	Accumulation of leukocytes	(Yamaguchi *et al.* 2012)
	IL-1α	Leukocytic pyrogen, inducer of acute-phase response and lymphocyte-activating factor (LAF)	(Itoh *et al.* 2007)
	Sp1 and Sp3	Key regulators of leukotriene C(4) synthase gene	(Itoh *et al.* 2007)
	Inhibitory Fcγ receptor IIb	Critical role in the balance of tolerance and auto-immunity	(Engdahl *et al.* 2018)
	St6Gal1	Catalyzes the addition of α2,6 linked sialic acids to terminal Nglycans and can modify glycoproteins and/or glycolipids	(Engdahl *et al.* 2018)
	CCR6	Recruitment of proinflammatory IL17-producing T-cells to sites of inflammation.	(Andersson *et al.* 2015*b*)
	CDT	Alcohol biomarker	(Fleming *et al.* 2004)
	FAS-L	Involved in the regulation of cell death, leads to apoptosis of thymocytes	(Montagna *et al.* 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 *et al.* 2021)
	IFN-γ	Promote macrophage activation, mediate antiviral and antibacterial immunity, regulate Th1/Th2 balance, and control cellular proliferation and apoptosis	(Latham *et al.* 2003)
	IL-10	Limiting host immune response to pathogens, thereby preventing damage to the host and maintaining normal tissue homeostasis.	(Latham *et al.* 2003)
	GM-CSF	Promotes myeloid cell development and maturation, and dendritic cell differentiation and survival, inflammatory and autoimmune reactions	(Latham *et al.* 2003)
	IgG2a anti-CII Abs	Activating the complement cascade	(Latham *et al.* 2003)
	CR1	Removal of immune complexes and pathogens coated with C3b and C4b	(Latham *et al.* 2003)
	IL-1β	Activation of innate immunity facilitating the differentiation of IL-17-producing T cells and innate immune cells	(Plum *et al.* 2009)
	TNF-α	Responsible for a diverse range of signaling events within cells, leading to necrosis or apoptosis, resistance to infection and cancers	(Plum *et al.* 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 *et al.* 2009)
	IL-17	T cell activation to neutrophil mobilization and activation	(Plum *et al.* 2009)
	VEGF	Potent angiogenic factor	(Plum *et al.* 2009)
	FGF-2	Cellular proliferation, survival, migration, and differentiation	(Plum *et al.* 2009)

(*Continued*)

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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 *et al.* 2004)
	α1-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 *et al.* 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 *et al.* 2004)
	IL-2	Key growth and death factor for antigen-activated T lymphocytes, essential to maintain self-tolerance	(Engdahl *et al.* 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 *et al.* 2005)
	MCP-1	Regulate migration and infiltration of monocytes/macrophages	(Subramanian *et al.* 2005)
	RANKL	Regulates osteoclast formation, activation and survival in normal bone modeling and remodeling	(Ho *et al.* 2011)
	sRAGE	Monocyte- and neutrophil-mediated inflammation and mononuclear phagocyte survival and differentiation	(Pullerits *et al.* 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 *et al.* 2003)
	GAG	Cell signaling process, including regulation of cell growth, proliferation, promotion of cell adhesion, anticoagulation, and wound repair	(Ganesan *et al.* 2008*a*)
	MMP-2	Cleave protein called type IV collagen	(Ganesan *et al.* 2008*a*)

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 17β-estradiol were used as substrates in RA and OA synoviocytes, they got converted into 16α-, 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 16αOH-estrone (Schmidt *et al.* 2009). Estrogen instigated interleukin (IL)-1α 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, ERα but not ERβ intervened the effects of estrogen. It was reported that a GC-rich region in the IL-1α 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 ERα showed physical interaction irrespective of the presence of estrogen. ERα and histone deacetylase 2 (HDAC2) also interacted physically, and estrogen promoted the detachment of HDAC2 from ERα. These results indicated that estrogen induced the separation of corepressor

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HDAC2 from ERα, which escalated Sp1 transcriptional activity across the GC-rich region present in the IL-1α 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 Fcγ 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 17β-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 glycosoaminoglycan (GAG) loss in the articular tissues (Ganesan *et al.* 2008*a*). 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.* 2008*b*). 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-1β, 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). 17β-estradiol treatment in experimental arthritis RA models, CIA; antigen-induced arthritis (AIA); and CAIA, decreased IL-17+γδT cell number in joints, but elevated IL-17+ γδT cells (significant producers of IL-17) in draining LNs which suggested an estrogen-mediated interruption of IL-17+ γδT cell migration from LNs to joints obstructing progression of experimental arthritis (Andersson *et al.* 2015*a*) (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 17β-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.* 2015*b*). SAR development of a series of 4-(indazol-3-yl)phenols has directed toward the establishment of an orally active

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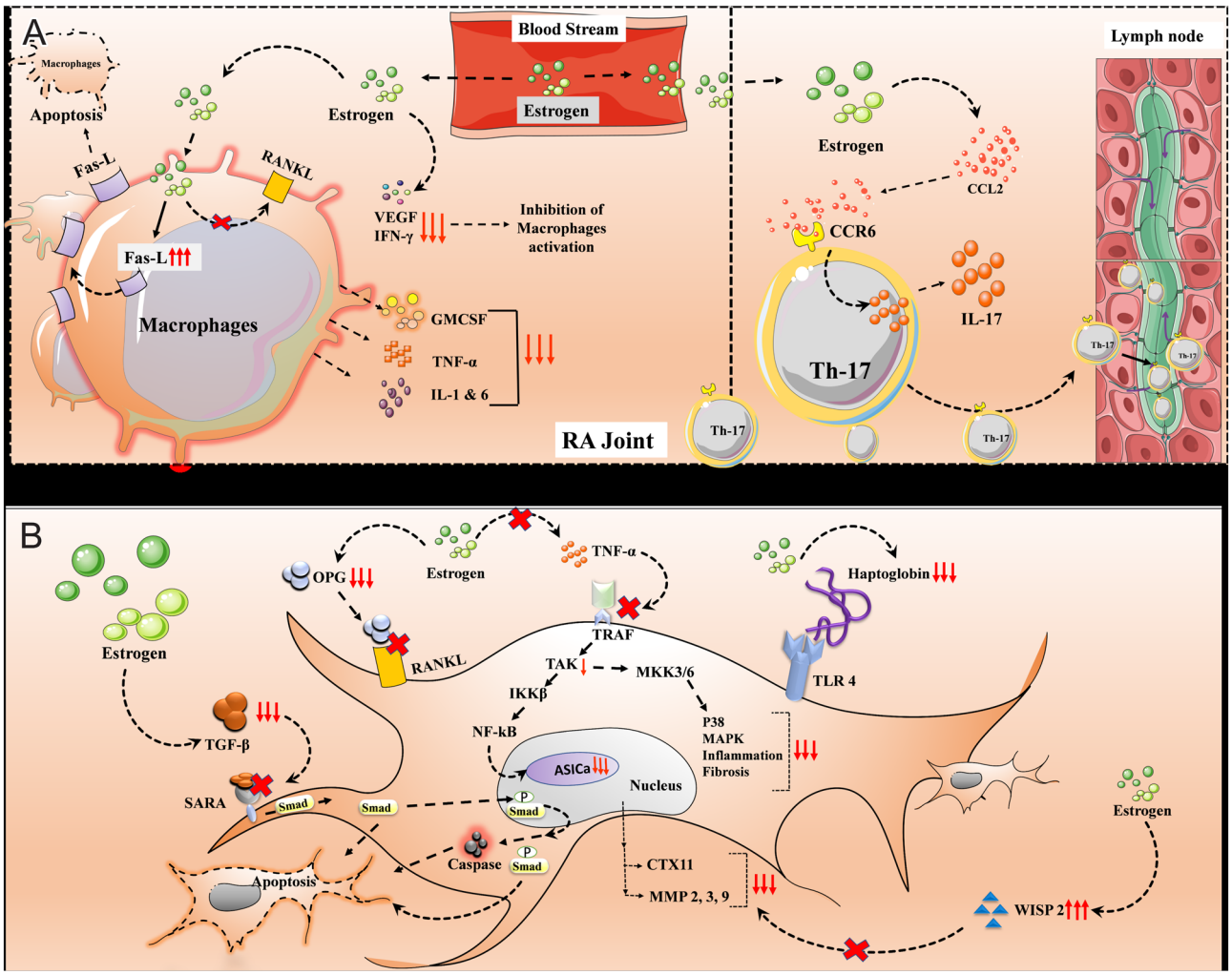

Figure 1

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 synoviocytes regulation in RA. Estrogen attenuating several inflammatory pathways like NFkB, TLR4, PI3-AKT by its action on specific proteins including RANKL,TRAF, TAK, TNF, OPG, and MMPs. Apoptosis of synoviocytes during RA progression can also be regulated by estrogen by targeting several proteins like smad, caspase, and TGFβ. 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, α1-acid glycoprotein (α1-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-kB 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 ERα, but not ERβ, or GPR-30 signaling is responsible for prevention of the disease symptoms as treatment with specific agonists propylpyrazoletriol (PPT; for ERα), diarylpropionitrile (DPN; for ERβ), 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 ERα-inactivated mice model and concluded that ERα 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 ERα−/− mice, whereas in Col2α1-ERα−/− (cartilage-specific) mice, estrogen reduced joint destruction but did not significantly decrease synovitis proving that ERα is responsible for the protective effect of estrogen for both joint destruction and synovitis but Col2α1-ERα−/− 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 ERα, but reduction in frequency of neutrophils in joints was mediated by ERα 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 ERα (Engdahl *et al.* 2014). A small-molecule ERβ 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 ERα (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 16α-LE2. Likewise in ArKO mice, antigen-specific T cell proliferation increased after immunization with type II collagen (CII) which got reversed after administration of 16α-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 17β-estradiol (estrogen 100 μM) 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.

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

Cells/cell lines			Treatment	Dosage	Mechanism	Reference
In vivo studies						
RA-FLS			17β-estradiol	10^{-6} M	↑ ERα ↑ (OPG)	(Mitani *et al.* 2005)
Macrophages			17β-estradiol	10^{-9}M	↓ FAS-L	(Montagna *et al.* 2009)
RA-FLS			17β-estradiol	100 nM	↓ TAK1	(Li & Li 2020)
Primary articular chondrocytes			Estradiol	500 nmol/mL	↓ ASIC1a ↓ autophagy level	(Hang *et al.* 2021)
RA-FLS			WAY-169916	-	↓ TNF-α ↓ NF-κB	(Steffan *et al.* 2004)
RA-FLS			b- estradiol	10^{-9} M	↑AID	(Igarashi *et al.* 2010)
RA-FLS			17-b-estradiol	10^{-6} M	↑ Wisp2	(Tanaka *et al.* 2005)
RA-FLS			Estrogen	10 nM	↓H_2O_2-induced apoptosis ↑TNF-α-induced MMP-3 ↑ CCL13	(Yamaguchi *et al.* 2012)
RA-FLS cell line MH7A			Estrogen	10 nM	↑ Sp1	(Itoh *et al.* 2007)
Animal (strain)	**Age/weight**	**Number**	**Treatment**	**Dosage**	**Measurement**	**Reference**
In vivo studies						
Female C57BL/6 mice	10 weeks	-	Estrogen	0.83 μg/day	↑Fc sialylation ↑Fcγ receptor IIb	(Engdahl *et al.* 2018)
DBA/1LacJ mice	-	-	17β-estradiol	60-day release estrogen pellets containing a total of either 2.5 or 0.36 mg of 17β-estradiol	Paw volume Severity of arthritis Serum estrogen levels ↓ T-cell production ↓ IL-10 ↓ GM-CSF ↓ IgG2a anti-CII Abs IgG1 anti-CII Abs	(Latham *et al.* 2003)
Male and female Wistar rats	80–100 g	(n = 6) per group	Estradiol benzoate	2.5, 5, and 10 μg	Histopathology Paw volume Lipid peroxide level Antioxidant assays Collagen level Total and sulfated glycosaminoglycans ↓ TNF-a ↓MMP-2	(Ganesan *et al.* 2008*a*)
Male and female Wistar rats	80–100 g	(n = 6) per group	Estradiol benzoate	2.5, 5, and 10 μg	Histopathology Paw volume Radiology Calcium and phosphorus Type II collagen antibodies in the serum Serum IL-1β, IL-6 and IL-10 ,TNF Bone turnover markers; Alkaline phosphatase Tartarate resistant acid phosphatase (TRAP) assayPGE2 analysis	(Ganesan *et al.* 2008*b*)

(*Continued*)

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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	(Nilsson *et al.* 2009)
MRL/lpr mice	4–24 weeks	6-10	estrogen	60 mg/kg	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	(Yoneda *et al.* 2004)
Balb/c female mice	5–7 week	-	2-methoxyestradiol (2ME2)	100, 75, 50, 25, 10, 1 mg/kg, p.o., daily	Cell infiltration Pannus severity Cartilage lesion Bone resorption Quantitative histomorphometric analysis ↓IL-1β, TNF-α, IL-6 and IL-17 ↓Angiogenic cytokines (VEGF and FGF-2)	(Plum *et al.* 2009)
Female DBA/1 mice	8–10 weeks	5–10	17β-estradiol-3-benzoate	1 μg/mouse/day	Arthritis severity ↓IL-17+γδT cell number ↑IL-17+ γδT cells	(Andersson *et al.* 2015*a*)
Female Lewis rats	9 weeks	151–175 g *N* = 10	OE2-pellet	0·18 mg/60 days release	Paw volume Serum markers of cartilage degradation ↓Type II collagen degradation marker (CTX-II) ↓MMP-2 ↓MMP-9	(Nielsen *et al.* 2008)
Female Sprague–Dawley rats	6-month	10–11	17-estradiol	0.25-mg pellet	Histology Serum estradiol level Body weight ↓CTX-II	(Oestergaard *et al.* 2006)
DBA/1 mice	8–10	-	17β-estradiol-3-benzoate	1 μg/mouse/day	Histology ↓Severity of arthritis ↓Th17 cells number in joints ↑Th17 cells in lymph nodes ↑CCR6 ↑CCL20 Chemotaxis assay	(Andersson *et al.* 2015*b*)
Lewis rats	7 week	*n* = 8	Estradiol valerate (EV)	EV; 5 μg/0.1 mL sesame oil, weekly starting day—10	↓TNF-α and joint inflammation ↑systemic OPG ↓ RANKL/OPG protein RLX family peptide receptor 1 (RXFP1) gene expression)	(Ho *et al.* 2011)

(*Continued*)

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Table 2 Continued.

Cells/cell lines			Treatment	Dosage	Mechanism	Reference
Male and female Lewis rats	-	-	Idoxifene + estrogen	5 mg/kg	↓IL-6 levels ↓paw volume BMD Histology Bone mineral content Absorptiometry	(Badger *et al.* 1999)
B10.Q-ncf1*/*mice	7-19	5–10	17b-estradiol-3-benzoate	1.0 μg/mouse/day	Histology BMD Serum levels of COMP (cartilage oligomeric matrix protein) ↓Frequency of arthritis ↓IL-6	(Jochems *et al.* 2010)
CIA model	4 months 80–100 g	n = 10	estrogen	100 μg E2 for 3 days	↓TAK1 joint swelling	(Li & Li 2020)
DBA/1LacJ mice	-	-	Ethinyl estradiol	50 Ag EE or 200 Ag EE in 100 Al olive oil.	Histology ↓ TNF-a IFN-g, MCP-1 and IL-6 bCII-specific IgG2a antibodies ↓mRNA for cytokines, chemokines and chemokine receptors in joint tissue	(Subramanian *et al.* 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	(Yoshioka *et al.* 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)	Histology; synovitis, soft tissue inflammation, exudates, cartilage degradation ↑IL-6, TNF, MCP 1, and IFNγ levels Antigen-specific T cell proliferation	(Yang *et al.* 2010)
Wistar rats	-	-	17 β-estradiol	100 μM for 24 h	Rate of respiratory burst, phagocytosis and nitric oxide production Diameter of the wrists and the foot	(Jahan Tigh *et al.* 2018)
Sprague-Dawley (SD) rats	7–8 weeks 180–220 g	n = 7	Estradiol	0.1, 0.2, and 0.3 mg/kg	Severity of joint Cartilage damage IL-1β and TNF-α ASIC1a, GPER1, LC3, and Beclin1	(Hang *et al.* 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 administrating 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 administrating 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 16a-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 ERβ, 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,

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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 ERα 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 ERα 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 *Daniorerio* (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-1β, 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-κB, 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

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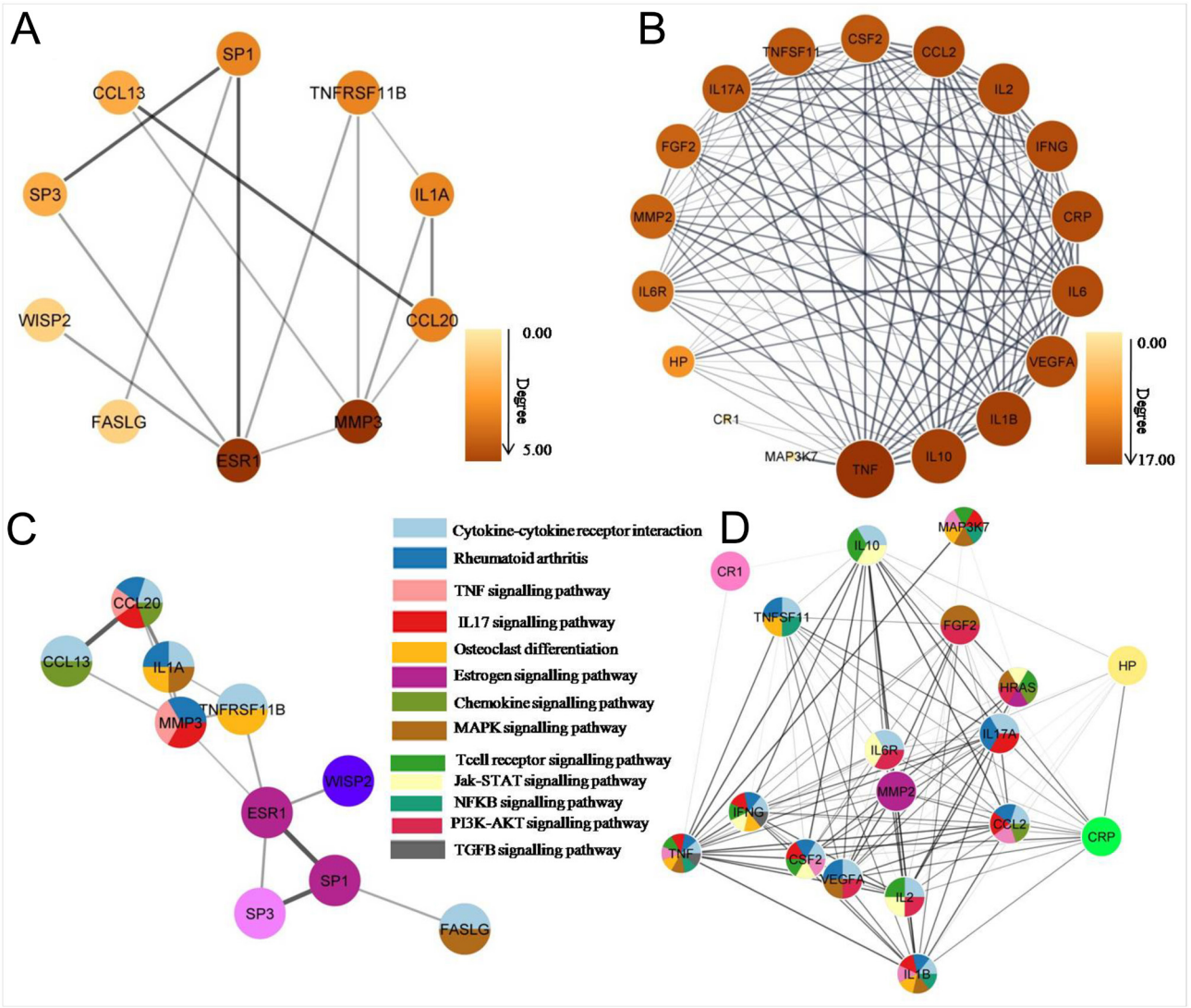

Figure 2
Protein–protein interaction network constructed by Cytoscape 3.8.2. (A) PPI of upregulated targets on the basis of degree; the color of the node is positively related to the degree of contribution of the node in the network. (B) PPI of downregulated targets on the basis of degree. (C) PPI network 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

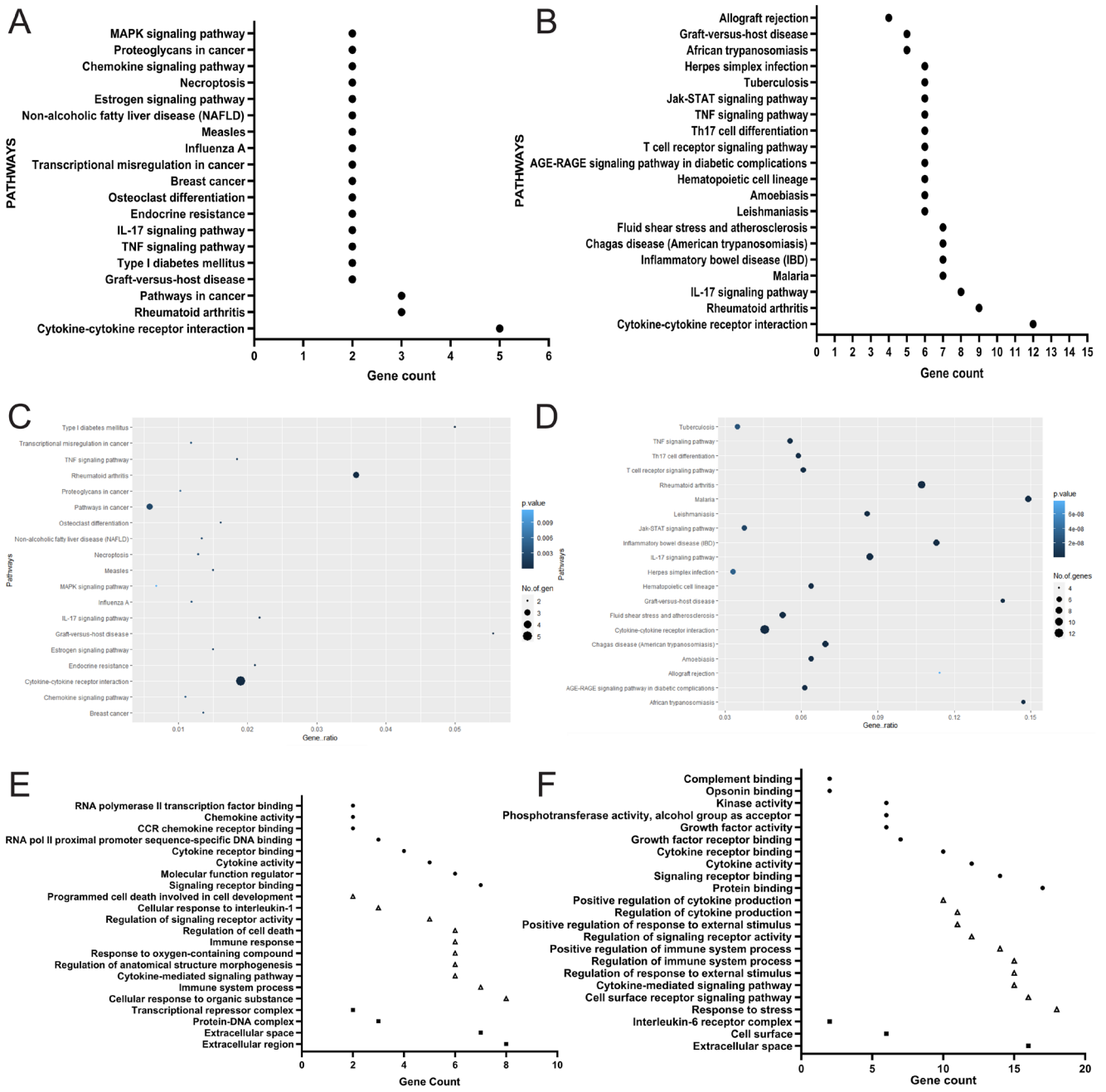

Figure 3

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-κB, 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 haves 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

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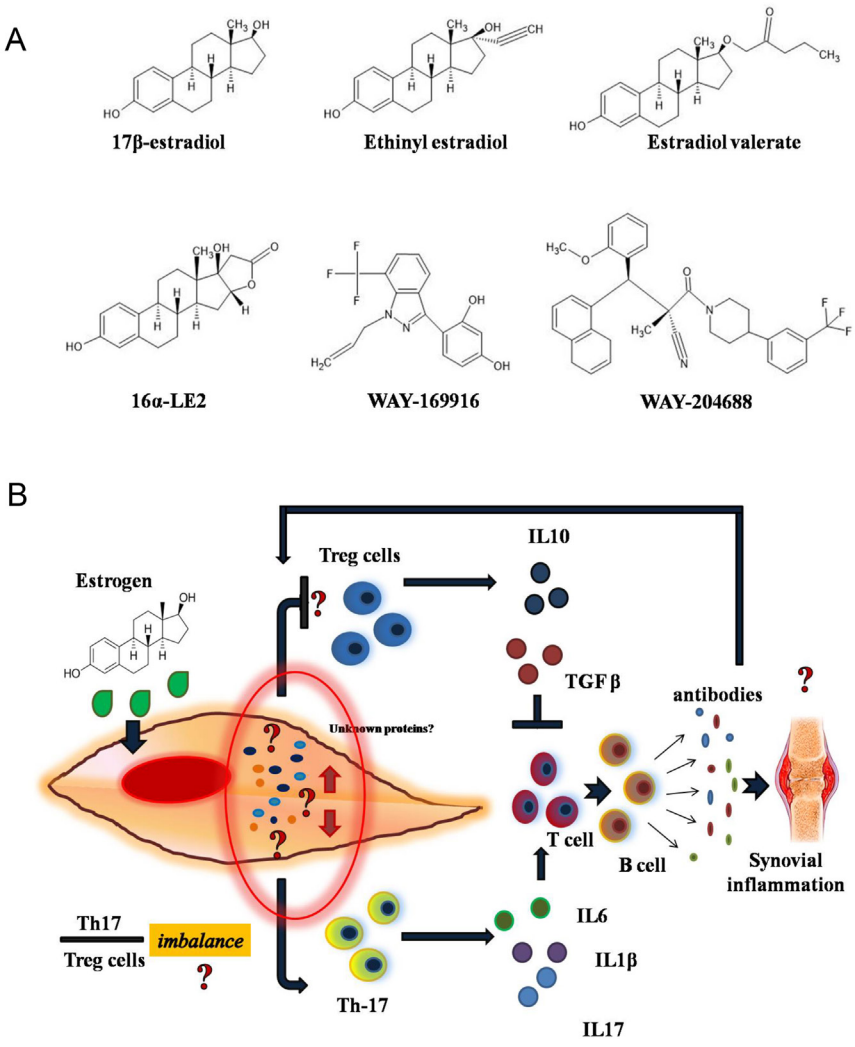

Figure 4
(A) Structures of estrogen-related compounds modulating RA compounds. These compounds are induced either *in vitro* or *in vivo* for determining estrogen effect on RA conditions. (B) Hypothesis of estrogen regulation in RA: It is known that estrogen regulates cytokines and other inflammatory 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 phytestrogen 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.* 2018*a*,*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.

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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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References

Adams BD, Furneaux H & White BA 2007 The micro-ribonucleic acid (miRNA) miR-206 targets the human estrogen receptor-α (ERα) and represses ERα messenger RNA and protein expression in breast cancer cell lines. *Molecular Endocrinology* **21** 1132–1147. (https://doi.org/10.1210/me.2007-0022)

Ammari M, Jorgensen C & Apparailly F 2013 Impact of microRNAs on the understanding and treatment of rheumatoid arthritis. *Current Opinion in Rheumatology* **25** 225–233. (https://doi.org/10.1097/BOR.0b013e32835d8385)

Andersson A, Grahnemo L, Engdahl C, Stubelius A, Lagerquist MK, Carlsten H & Islander U 2015*a* IL-17-producing γδT cells are regulated by estrogen during development of experimental arthritis. *Clinical Immunology* **161** 324–332. (https://doi.org/10.1016/j.clim.2015.09.014)

Andersson A, Stubelius A, Karlsson MN, Engdahl C, Erlandsson M, Grahnemo L, Lagerquist MK & Islander U 2015*b* Estrogen regulates T helper 17 phenotype and localization in experimental autoimmune arthritis. *Arthritis Research and Therapy* **17** 32. (https://doi.org/10.1186/s13075-015-0548-y)

Andersson K, Pullerits R, Forsblad-d'Elia H, Erlandsson M, Silfverswärd ST & Bokarewa M 2018 THU0015 oestrogen dependent regulation of micro-RNA in rheumatoid arthritis. *Annals of the Rheumatic Diseases* **77** 236.3–237. (https://doi.org/10.1136/annrheumdis-2018-eular.6662)

Badger AM, Blake SM, Dodds RA, Griswold DE, Swift BA, Rieman DJ, Stroup GB, Hoffman SJ & Gowen M 1999 Idoxifene, a novel selective estrogen receptor modulator, is effective in a rat model of adjuvant-induced arthritis. *Journal of Pharmacology and Experimental Therapeutics* **291** 1380–1386.

Baker L, Meldrum KK, Wang M, Sankula R, Vanam R, Raiesdana A, Tsai B, Hile K, Brown JW & Meldrum DR 2003 The role of estrogen in cardiovascular disease. *Journal of Surgical Research* **115** 325–344. (https://doi.org/10.1016/S0022-4804(03)00215-4)

Caggiano TJ, Brazzale A, Ho DM, Kraml CM, Trybulski E, Chadwick CC, Chippari S, Borges-marcucci L, Eckert A, Keith JC, *et al.* 2007 Estrogen receptor dependent inhibitors of NF-kappaB transcriptional activation-1 synthesis and biological evaluation of substituted 2-cyanopropanoic acid derivatives: pathway selective inhibitors of NF-kappaB, a potential treatment for rheumatoid arthritis. *Journal of Medicinal Chemistry* **50** 50–53. (https://doi.org/10.1021/jm701013k)

Canet LM, Sánchez-Maldonado JM, Cáliz R, Ramos AR, Lupiañez CB, Canhão H, Martínez-Bueno M, Escudero A, Segura-Catena J, Sorensen SB, *et al.* 2019 Polymorphisms at phase I-metabolizing enzyme and hormone receptor loci influence the response to anti-TNF therapy in rheumatoid arthritis patients. *Pharmacogenomics Journal* **19** 83–96. (https://doi.org/10.1038/s41397-018-0057-x)

Capobianco G, De Muro P, Cherchi GM, Formato M, Lepedda AJ, Cigliano A, Zinellu E, Dessole F, Gordini L & Dessole S 2010 Plasma levels of C-reactive protein, leptin and glycosaminoglycans during spontaneous menstrual cycle: differences between ovulatory and anovulatory cycles. *Archives of Gynecology and Obstetrics* **282** 207–213. (https://doi.org/10.1007/s00404-010-1432-2)

Castagnetta LA, Carruba G, Granata OM, Stefano R, Miele M, Schmidt M, Cutolo M & Straub RH 2003 Increased estrogen formation and estrogen to androgen ratio in the synovial fluid of patients with rheumatoid arthritis. *Journal of Rheumatology* **30** 2597–2605.

Chadjichristos C, Ghayor C, Kypriotou M, Martin G, Renard E, Ala-Kokko L, Suske G, De Crombrugghe B, Pujol JP & Galéra P 2003 Sp1 and Sp3 transcription factors mediate interleukin-1β down-regulation of human type II collagen gene expression in articular chondrocytes. *Journal of Biological Chemistry* **278** 39762–39772. (https://doi.org/10.1074/jbc.M303541200)

Chakraborty D, Gupta K & Biswas S 2021 A mechanistic insight of phytoestrogens used for rheumatoid arthritis: an evidence-based review. *Biomedicine and Pharmacotherapy* **133** 111039. (https://doi.org/10.1016/j.biopha.2020.111039)

Chen ZZ, Zhang XD, Chen Y & Wu YB 2017 The role of circulating miR-146a in patients with rheumatoid arthritis treated by Tripterygium wilfordii Hook F. *Medicine* **96** e6775. (https://doi.org/10.1097/MD.0000000000006775)

Cohen A, Shmoish M, Levi L, Cheruti U, Levavi-Sivan B & Lubzens E 2008 Alterations in micro-ribonucleic acid expression profiles reveal a novel pathway for estrogen regulation. *Endocrinology* **149** 1687–1696. (https://doi.org/10.1210/en.2007-0969)

D'Elia HF, Mattsson LA, Ohlsson C, Nordborg E & Carlsten H 2003 Hormone replacement therapy in rheumatoid arthritis is associated with lower serum levels of soluble IL-6 receptor and higher insulin-like growth factor 1. *Arthritis Research and Therapy* **5** R202–R209. (https://doi.org/10.1186/ar761)

Dai R, Phillips RA, Zhang Y, Khan D, Crasta O & Ahmed SA 2008 Suppression of LPS-induced interferon-{gamma} and nitric oxide in splenic lymphocytes by select estrogen-regulated microRNAs: a novel mechanism of immune modulation. *Blood* **112** 4591–4597. (https://doi.org/10.1182/blood-2008-04-152488)

Deroo BJ & Korach KS 2006 Estrogen receptors and human disease. *Journal of Clinical Investigation* **116** 561–570. (https://doi.org/10.1172/JCI27987)

Duan H, Khan GJ, Shang LJ Jr & Peng H, Hu WC, Zhang JY, Hua J, Cassandra A, Rashed MMA & Zhai KF 2021 Computational pharmacology and bioinformatics to explore the potential mechanism of Schisandra against atherosclerosis. *Food and Chemical Toxicology* **150** 112058. (https://doi.org/10.1016/j.fct.2021.112058)

Dupuis ML, Conti F, Maselli A, Pagano MT, Ruggieri A, Anticoli S, Fragale A, Gabriele L, Gagliardi MC, Sanchez M, *et al.* 2018 The natural agonist of estrogen receptor β silibinin plays an immunosuppressive role representing a potential therapeutic tool in rheumatoid arthritis. *Frontiers in Immunology* **9** 1903. (https://doi.org/10.3389/fimmu.2018.01903)

Engdahl C, Börjesson AE, Forsman HF, Andersson A, Stubelius A, Krust A, Chambon P, Islander U, Ohlsson C, Carlsten H, *et al.* 2014 The role of total and cartilage-specific estrogen receptor alpha expression for the ameliorating effect of estrogen treatment on arthritis. *Arthritis Research and Therapy* **16** R150. (https://doi.org/10.1186/ar4612)

Engdahl C, Bondt A, Harre U, Raufer J, Pfeifle R, Camponeschi A, Wuhrer M, Seeling M, Mårtensson IL, Nimmerjahn F, *et al.* 2018 Estrogen induces St6gal1 expression and increases IgG sialylation in mice and patients with rheumatoid arthritis: a potential explanation for the increased risk of rheumatoid arthritis in postmenopausal women. *Arthritis Research and Therapy* **20** 84. (https://doi.org/10.1186/s13075-018-1586-z)

Fleming MF, Anton RF & Spies CD 2004 A review of genetic, biological, pharmacological, and clinical factors that affect carbohydrate-deficient transferrin levels. *Alcoholism, Clinical and Experimental Research* **28** 1347–1355. (https://doi.org/10.1097/01.ALC.0000139815.89794.BE)

Follettie MT, Pinard M, Keith JC, Wang L, Chelsky D, Hayward C, Kearney P, Thibault P, Paramithiotis E, Dorner AJ, *et al.* 2006 Organ messenger ribonucleic acid and plasma proteome changes in the adjuvant-induced arthritis model: responses to disease induction and therapy with the estrogen receptor-β selective agonist ERB-041. *Endocrinology* **147** 714–723. (https://doi.org/10.1210/en.2005-0600)

Ganesan K, Tiwari M, Balachandran C, Manohar BM & Puvanakrishnan R 2008*a* Estrogen and testosterone attenuate extracellular matrix loss in collagen-induced arthritis in rats. *Calcified Tissue International* **83** 354–364. (https://doi.org/10.1007/s00223-008-9183-9)

Ganesan K, Balachandran C, Manohar BM & Puvanakrishnan R 2008*b* Comparative studies on the interplay of testosterone, estrogen and progesterone in collagen induced arthritis in rats. *Bone* **43** 758–765. (https://doi.org/10.1016/j.bone.2008.05.025)

Ganesan K, Balachandran C, Manohar BM & Puvanakrishnan R 2012 Effects of testosterone, estrogen and progesterone on TNF-α mediated cellular damage in rat arthritic synovial fibroblasts. *Rheumatology International* **32** 3181–3188. (https://doi.org/10.1007/s00296-011-2146-x)

Hajati AK, Alstergren P, Näsström K, Bratt J & Kopp S 2009 Endogenous glutamate in association with inflammatory and hormonal factors modulates bone tissue resorption of the temporomandibular joint in patients with early rheumatoid arthritis. *Journal of Oral and Maxillofacial Surgery* **67** 1895–1903. (https://doi.org/10.1016/j.joms.2009.04.056)

Hall GM, Daniels M, Huskisson EC & Spector TD 1994 A randomised controlled trial of the effect of hormone replacement therapy on disease activity in postmenopausal rheumatoid arthritis. *Annals of the Rheumatic Diseases* **53** 112–116. (https://doi.org/10.1136/ard.53.2.112)

Hang X, Zhang Z, Niu R, Wang C, Yao J, Xu Y, Tao J, Li L & Chen F 2021 Estrogen protects articular cartilage by downregulating ASIC1a in rheumatoid arthritis. *Journal of Inflammation Research* **14** 843–858. (https://doi.org/10.2147/JIR.S295222)

Hansildaar R, Vedder D, Baniaamam M, Tausche AK, Gerritsen M & Nurmohamed MT 2021 Cardiovascular risk in inflammatory arthritis: rheumatoid arthritis and gout. *Lancet: Rheumatology* **3** e58–e70. (https://doi.org/10.1016/S2665-9913(20)30221-6)

Hill DA, Crider M & Hill SR 2016 Hormone therapy and other treatments for symptoms of menopause. *American Family Physician* **94** 884–889.

Ho TY, Santora K, Chen JC, Frankshun AL & Bagnell CA 2011 Effects of relaxin and estrogens on bone remodeling markers, receptor activator of NF-kB ligand (RANKL) and osteoprotegerin (OPG), in rat adjuvant-induced arthritis. *Bone* **48** 1346–1353. (https://doi.org/10.1016/j.bone.2011.03.684)

Igarashi H, Hashimoto J, Tomita T, Yoshikawa H & Ishihara K 2010 TP53 mutations coincide with the ectopic expression of activation-induced cytidine deaminase in the fibroblast-like synoviocytes derived from a fraction of patients with rheumatoid arthritis. *Clinical and Experimental Immunology* **161** 71–80. (https://doi.org/10.1111/j.1365-2249.2010.04163.x)

Ikuta K, Waguri-Nagaya Y, Kikuchi K, Yamagami T, Nozaki M, Aoyama M, Asai K & Otsuka T 2012 The Sp1 transcription factor is essential for the expression of gliostatin/thymidine phosphorylase in rheumatoid fibroblast-like synoviocytes. *Arthritis Research and Therapy* **14** R87. (https://doi.org/10.1186/ar3811)

Iorga A, Cunningham CM, Moazeni S, Ruffenach G, Umar S & Eghbali M 2017 The protective role of estrogen and estrogen receptors in cardiovascular disease and the controversial use of estrogen therapy. *Biology of Sex Differences* **8** 33. (https://doi.org/10.1186/s13293-017-0152-8)

Itoh Y, Hayashi H, Miyazawa K, Kojima S, Akahoshi T & Onozaki K 2007 17β-Estradiol induces IL-1α gene expression in rheumatoid fibroblast-like synovial cells through estrogen receptor α (ERα) and augmentation of transcriptional activity of Sp1 by dissociating histone deacetylase 2 from ERα. *Journal of Immunology* **178** 3059–3066. (https://doi.org/10.4049/jimmunol.178.5.3059)

Jahan Tigh M, Abtahi Froshani S & Afzal Ahangaran N 2018 Effect of mesenchymal stem cells treated with 17β-estradiol on the pattern of intrinsic immunity responses in collagen-induced rheumatoid arthritis in Wistar rats. *Armaghane Danesh* **23** 42–56.

Jochems C, Islander U, Erlandsson M, Engdahl C, Lagerquist M, Gjertsson I, Ohlsson C, Holmdahl R & Carlsten H 2010 Role of endogenous and exogenous female sex hormones in arthritis and osteoporosis development in B10. Q-ncf1 */* mice with collagen-induced chronic arthritis. *BMC Musculoskeletal Disorders* **11** 284. (https://doi.org/10.1186/1471-2474-11-284)

Kaloni D, Chakraborty D, Tiwari A & Biswas S 2020 In silico studies on the phytochemical components of Murraya koenigii targeting TNF-α in rheumatoid arthritis. *Journal of Herbal Medicine* **24** 100396. (https://doi.org/10.1016/j.hermed.2020.100396)

Khalkhali-Ellis Z, Seftor EA, Nieva DRC, Handa RJ, Price RH, Kirschmann DA, Baragi VM, Sharma RV, Bhalla RC, Moore TL, *et al.* 2000 Estrogen and progesterone regulation of human fibroblast-like synoviocyte function in vitro: implications in rheumatoid arthritis. *Journal of Rheumatology* **27** 1622–1631.

Kim DS, Kwon J, Lee SH, Kim EK, Ryu J & Lu TT 2018 Attenuation of rheumatoid inflammation by sodium butyrate through reciprocal targeting of HDAC2 in osteoclasts and HDAC8 in T cells. *Frontiers in Immunology* **9** 1525. (https://doi.org/10.3389/fimmu.2018.01525)

Kovalchuk O, Tryndyak VP, Montgomery B, Boyko A, Kutanzi K, Zemp F, Warbritton AR, Latendresse JR, Kovalchuk I, Beland FA, *et al.* 2007 Estrogen-induced rat breast carcinogenesis is characterized by alterations in DNA methylation, histone modifications and aberrant microRNA expression. *Cell Cycle* **6** 2010–2018. (https://doi.org/10.4161/cc.6.16.4549)

Latham KA, Zamora A, Drought H, Subramanian S, Matejuk A, Offner H & Rosloniec EF 2003 Estradiol treatment redirects the isotype of the autoantibody response and prevents the development of autoimmune arthritis. *Journal of Immunology* **171** 5820–5827. (https://doi.org/10.4049/jimmunol.171.11.5820)

Li X & Li M 2020 Estrogen downregulates TAK1 expression in human fibroblast-like synoviocytes and in a rheumatoid arthritis model. *Experimental and Therapeutic Medicine* **20** 1764–1769. (https://doi.org/10.3892/etm.2020.8848)

Lin J, Huo R, Xiao L, Zhu X, Xie J, Sun S, He Y, Zhang J, Sun Y, Zhou Z, *et al.* 2014 A novel p53/microRNA-22/Cyr61 axis in synovial cells regulates inflammation in rheumatoid arthritis. *Arthritis and Rheumatology* **66** 49–59. (https://doi.org/10.1002/art.38142)

Mann S, Chakraborty D & Biswas S 2022 An alternate perspective of an underutilized fruit tree *Choerospondias axillaris* in health promotion and disease prevention: a review. *Food Bioscience* **47** 101609. (https://doi.org/10.1016/j.fbio.2022.101609)

Meyers MJ, Sun J, Carlson KE, Marriner GA, Katzenellenbogen BS & Katzenellenbogen JA 2001 Estrogen receptor-β potency-selective ligands: structure-activity relationship studies of diarylpropionitriles and their acetylene and polar analogues. *Journal of Medicinal Chemistry* **44** 4230–4251. (https://doi.org/10.1021/jm010254a)

Mitani M, Miura Y, Saura R, Kitagawa A, Fukuyama T, Hashiramoto A, Shiozawa S, Kurosaka M & Yoshiya S 2005 Estrogen specifically stimulates expression and production of osteoprotegerin from rheumatoid synovial fibroblasts. *International Journal of Molecular Medicine* **15** 827–832. (https://doi.org/10.3892/ijmm.15.5.827)

Montagna P, Brizzolara R, Soldano S, Pizzorni C, Sulli A & Cutolo M 2009 Sex hormones and leflunomide treatment of human macrophage cultures: effects on apoptosis. *International Journal of Clinical and Experimental Medicine* **2** 221–232.

Moran-Moguel MC, Petarra-Del Rio S, Mayorquin-Galvan EE & Zavala-Cerna MG 2018 Rheumatoid arthritis and miRNAs: a critical review through a functional view. *Journal of Immunology Research* **2018** 2474529. (https://doi.org/10.1155/2018/2474529)

Nielsen RH, Christiansen C, Stolina M & Karsdal MA 2008 Oestrogen exhibits type II collagen protective effects and attenuates collagen-induced arthritis in rats. *Clinical and Experimental Immunology* **152** 21–27. (https://doi.org/10.1111/j.1365-2249.2008.03594.x)

Nilsson KE, Andre M, Diaz de Ståhl T & Kleinau S 2009 Enhanced susceptibility to low-dose collagen-induced arthritis in CR1/2-deficient female mice – possible role of estrogen on CR1 expression. *FASEB Journal* **23** 2450–2458. (https://doi.org/10.1096/fj.08-125849)

Oestergaard S, Sondergaard BC, Hoegh-andersen P, Henriksen K, Qvist P, Christiansen C, Tankó LB & Karsdal MA 2006 Effects of ovariectomy and estrogen therapy on type II collagen degradation and structural integrity of articular cartilage in rats implications of the time of initiation. *Arthritis and Rheumatism* **54** 2441–2451. (https://doi.org/10.1002/art.22009)

Pfeifer EC, Crowson CS, Amin S, Gabriel SE & Matteson EL 2014 The influence of early menopause on cardiovascular risk in women with rheumatoid arthritis. *Journal of Rheumatology* **41** 1270–1275. (https://doi.org/10.3899/jrheum.131234)

Plum SM, Park EJ, Strawn SJ, Moore EG, Sidor CF & Fogler WE 2009 Disease modifying and antiangiogenic activity of 2-methoxyestradiol in a murine model of rheumatoid arthritis. *BMC Musculoskeletal Disorders* **10** 46. (https://doi.org/10.1186/1471-2474-10-46)

Pullerits R, D'Elia HF, Tarkowski A & Carlsten H 2009 The decrease of soluble RAGE levels in rheumatoid arthritis patients following hormone replacement therapy is associated with increased bone mineral density and diminished bone/cartilage turnover: a randomized controlled trial. *Rheumatology* **48** 785–790. (https://doi.org/10.1093/rheumatology/kep079)

Roman-Blas JA, Castañeda S, Cutolo M & Herrero-Beaumont G 2010 Efficacy and safety of a selective estrogen receptor β agonist, ERB-041, in patients with rheumatoid arthritis: a 12-week, randomized, placebo-controlled, phase II study. *Arthritis Care and Research* **62** 1588–1593. (https://doi.org/10.1002/acr.20275)

Schmidt M, Hartung R, Capellino S, Cutolo M, Pfeifer-Leeg A & Straub RH 2009 Estrone/17β-estradiol conversion to, and tumor necrosis factor inhibition by, estrogen metabolites in synovial cells of patients with rheumatoid arthritis and patients with osteoarthritis. *Arthritis and Rheumatism* **60** 2913–2922. (https://doi.org/10.1002/art.24859)

Smolen JS, Aletaha D, Barton A, Burmester GR, Emery P, Firestein GS, Kavanaugh A, McInnes IB, Solomon DH, Strand V, *et al.* 2018 Rheumatoid arthritis. *Nature Reviews: Disease Primers* **4** 18001. (https://doi.org/10.1038/nrdp.2018.1)

Steffan RJ, Matelan E, Ashwell MA, Moore XWJ, Solvibile WR, Trybulski E, Chadwick CC, Chippari S, Kenney T, Eckert A, *et al.* 2004 Synthesis and activity of substituted 4-(indazol-3-yl)phenols as pathway-selective estrogen receptor ligands useful in the treatment of rheumatoid arthritis. *Journal of Medicinal Chemistry* **47** 6435–6438. (https://doi.org/10.1021/jm049194)

Su LC, Huang AF, Jia H, Liu Y & Xu WD 2017 Role of microRNA-155 in rheumatoid arthritis. *International Journal of Rheumatic Diseases* **20** 1631–1637. (https://doi.org/10.1111/1756-185X.13202)

Subramanian S, Tovey M, Afentoulis M, Krogstad A, Vandenbark AA & Offner H 2005 Ethinyl estradiol treats collagen-induced arthritis in DBA/1LacJ mice by inhibiting the production of TNF-α and IL-1β. *Clinical Immunology* **115** 162–172. (https://doi.org/10.1016/j.clim.2005.01.006)

Tanaka I, Morikawa M, Okuse T, Shirakawa M & Imai K 2005 Expression and regulation of WISP2 in rheumatoid arthritic synovium. *Biochemical and Biophysical Research Communications* **334** 973–978. (https://doi.org/10.1016/j.bbrc.2005.06.196)

Volpe E, Sambucci M, Battistini L & Borsellino G 2016 Fas-Fas ligand: checkpoint of T cell functions in multiple sclerosis. *Frontiers in Immunology* **7** 382. (https://doi.org/10.3389/fimmu.2016.00382)

Warren MP & Halpert S 2004 Hormone replacement therapy: controversies, pros and cons. *Best Practice and Research: Clinical Endocrinology and Metabolism* **18** 317–332. (https://doi.org/10.1016/j.beem.2004.02.005)

Yamaguchi A, Nozawa K, Fujishiro M, Kawasaki M, Takamori K, Ogawa H, Sekigawa I & Takasaki Y 2012 Estrogen inhibits apoptosis and promotes CC motif chemokine ligand 13 expression on synovial fibroblasts in rheumatoid arthritis. *Immunopharmacology and Immunotoxicology* **34** 852–857. (https://doi.org/10.3109/08923973.2012.664149)

Yang YH, Ngo D, Jones M, Simpson E, Fritzemeier KH & Morand EF 2010 Endogenous estrogen regulation of inflammatory arthritis and cytokine expression in male mice, predominantly via estrogen receptor α. *Arthritis and Rheumatism* **62** 1017–1025. (https://doi.org/10.1002/art.27330)

Yoneda T, Ishimaru N, Arakaki R, Kobayashi M, Izawa T, Moriyama K & Hayashi Y 2004 Estrogen deficiency accelerates murine autoimmune arthritis associated with receptor activator of nuclear factor-κB ligand-mediated osteoclastogenesis. *Endocrinology* **145** 2384–2391. (https://doi.org/10.1210/en.2003-1536)

Yoshioka T, Hagino H, Yamasaki D, Okano T & Teshima R 2008 Effect of estrogen replacement therapy on arthritis and bone mineral density in estrogen-replete rats with collagen-induced arthritis. *Modern Rheumatology* **18** 23–28. (https://doi.org/10.1007/s10165-007-0011-2)

Zhai KF, Duan H, Khan GJ, Xu H, Han FK, Cao WG, Gao GZ, Shan LL & Wei ZJ 2018*a* Salicin from Alangium chinense ameliorates rheumatoid arthritis by modulating the Nrf2-HO-1-ROS pathways. *Journal of Agricultural and Food Chemistry* **66** 6073–6082. (https://doi.org/10.1021/acs.jafc.8b02241)

Zhai KF, Duan H, Chen Y, Khan GJ, Cao WG, Gao GZ, Shan LL & Wei ZJ 2018*b* Apoptosis effects of imperatorin on synoviocytes in rheumatoid arthritis through mitochondrial/caspase-mediated pathways. *Food and Function* **9** 2070–2079. (https://doi.org/10.1039/c7fo01748k)

Zhai KF, Duan H, Cui CY, Cao YY, Si JL, Yang HJ, Wang YC, Cao WG, Gao GZ & Wei ZJ 2019 Liquiritin from Glycyrrhiza uralensis attenuating rheumatoid arthritis via reducing inflammation, suppressing angiogenesis, and inhibiting MAPK signaling pathway. *Journal of Agricultural and Food Chemistry* **67** 2856–2864. (https://doi.org/10.1021/acs.jafc.9b00185)

Zhou X, Zhu J, Zhang H, Zhou G, Huang Y & Liu R 2015 Is the microRNA-146a (rs2910164) polymorphism associated with rheumatoid arthritis? Association of microRNA-146a (rs2910164) polymorphism and rheumatoid arthritis could depend on gender. *Joint Bone Spine* **82** 166–171. (https://doi.org/10.1016/j.jbspin.2014.12.009)

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