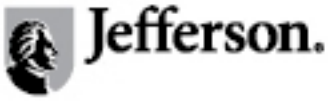

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Osteoarthritis associated with estrogen deficiency.

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Review

Osteoarthritis associated with estrogen deficiency

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Abstract

Osteoarthritis (OA) affects all articular tissues and finally leads to joint failure. Although articular tissues have long been considered unresponsive to estrogens or their deficiency, there is now increasing evidence that estrogens influence the activity of joint tissues through complex molecular pathways that act at multiple levels. Indeed, we are only just beginning to understand the effects of estrogen deficiency on articular tissues during OA development and progression, as well as on the association between OA and osteoporosis. Estrogen replacement therapy and current selective estrogen receptor modulators have mixed effectiveness in preserving and/or restoring joint tissue in OA. Thus, a better understanding of how estrogen acts on joints and other tissues in OA will aid the development of specific and safe estrogen ligands as novel therapeutic agents targeting the OA joint as a whole organ.

Introduction

Osteoarthritis (OA) is a very common chronic disease that affects all joint tissues, causing progressive irreversible damage and, finally, the failure of the joint as an organ [1]. Characteristic pathological changes in OA not only include joint cartilage degeneration but also subchondral bone thickening, osteophyte formation and synovial inflammation, all of which are associated with capsule laxitude and decreased muscle strength [1,2]. The pathological changes that occur in OA are the result of the action of biomechanical forces coupled with multiple autocrine, paracrine and endocrine cellular events that lead to a breakdown of the normal balance in tissue turnover within the joint [3,4].

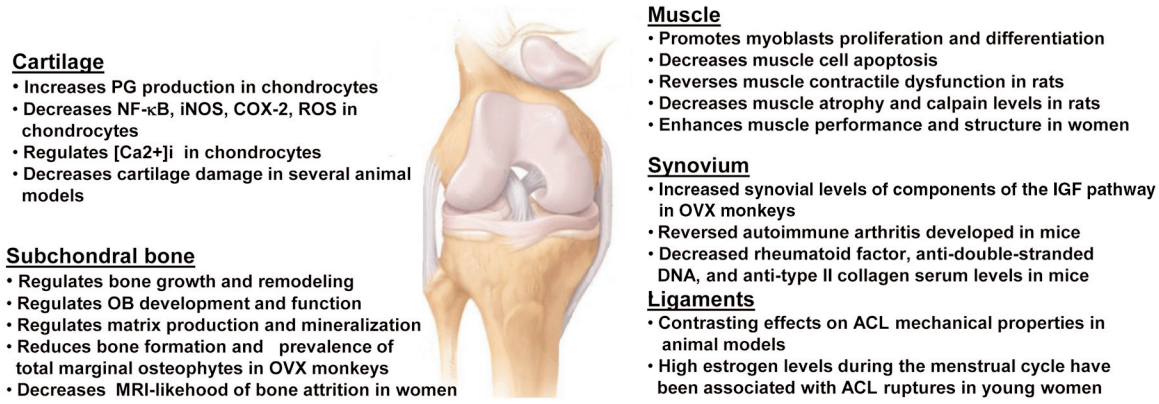
Among the multiple physiopathological mechanisms involved in OA, those related to sex hormone control have been attracting much attention, in particular those involving estrogens [5]. In contrast to other tissues such as the endometrium, breast, brain and non-joint bone, it was traditionally thought that joint tissues were non-responsive to estrogens and estrogen deficit. However, interest in estrogens was stimulated by the large proportion of postmenopausal women with OA and the complexity of their role in this disease. Indeed, considerable efforts have been made to understand the potential role of estrogens in the biology of joint tissues, as well as in the development and progression of OA, which has led to a better understanding of the effects of estrogen on joint tissues and on cartilage in particular [5-7].

There is increasing evidence that estrogens fulfill a relevant role in maintaining the homeostasis of articular tissues and, hence, of the joint itself. The dramatic rise in OA prevalence among postmenopausal women [8,9], which is associated with the presence of estrogen receptors (ERs) in joint tissues [10-14], suggests a link between OA and loss of ovarian function. This association indicates a potential protective role for estrogens against the development of OA. Indeed, recent *in vitro*, *in vivo*, genetic and clinical studies have shed further light on these issues.

This review is based on a literature search of peer-reviewed articles written in English in the Medline and PubMed databases from 1952 to April 2009 carried out using the keywords estrogen, menopause, estrogen replacement therapy (ERT) and selective estrogen receptor modulators (SERMs) alone or in various combinations with joint, cartilage, subchondral bone, synovium, ligaments, muscle, tendons, OA and osteoporosis (OP). Accordingly, it addresses the effect of estrogen deficit on all joint tissues and the dual action of

ACL = anterior cruciate ligament; AF = activation function; AP = activator protein; BMD = bone mineral density; E_2 = 17β-estradiol; ER = estrogen receptor; ERE = estrogen response element; ERK = extracellular signal regulated kinase; ERT = estrogen replacement therapy; IGF = insulin-like growth factor; IGFBP = IGF-binding protein; IL = interleukin; MAP = mitogen activated protein; MMP = matrix metalloproteinase; NCoR = nuclear receptor co-repressor; NF = nuclear factor; OA = osteoarthritis; OB = osteoblast; OP = osteoporosis; OVX = ovariectomized; PI3 = phosphatidylinositol-3; PKC = protein kinase C; SERM = selective estrogen receptor modulators; SMAD = mothers against decapentaplegic; SMRT = silencing mediator for the retinoic acid and thyroid hormone receptor; Sp = specificity protein; TGF = transforming growth factor; TNF = tumor necrosis factor.

Figure 1



Estrogen actions on target articular tissues. ACL, anterior cruciate ligament; [Ca2+]i, intracellular calcium concentration; COX-2, cyclooxygenase-2; IGF, insulin-like growth factor; iNOS, inducible nitric oxide synthase; MRI, magnetic resonance imaging; OB, osteoblast; OVX, ovariectomized; PG, proteoglycan.

estrogen deficit on the association of OP and cartilage damage. In addition, we emphasize the relevance of these effects in the onset and/or progression of OA as well as summarizing our current knowledge on how estrogen regulates the metabolism of joint tissues. Finally, we examine the effects of ERT and current SERMs in OA, as well as the development of new specific estrogen ligands as potential therapeutic strategies to treat this disease.

The effects of estrogen deficiency on components of the osteoarthritis joint

Different studies have provided compelling information on the relevant effects of estrogen deficiency on joint components in cell culture, animal models or humans. Although much of the attention has focused on the effects of estrogen on articular cartilage, estrogen deficiency also affects other joint tissues during the course of OA, such as the periarticular bone, synovial lining, muscles, ligaments and the capsule (Figure 1).

In vitro studies

Several experimental studies have shown that estrogens are implicated in the regulation of cartilage metabolism. Indeed, 17β-estradiol (E_2) enhances glycosaminoglycan synthesis in cultures of rabbit joint chondrocytes through the up-regulation of the uridine diphosphate glucose dehydrogenase gene [15]. Furthermore, estrogen (1 to 100 M) significantly impairs the release of C-telopeptide of type II collagen from TNF-α and oncostatin M-stimulated bovine cartilage explants *ex vivo* in a dose-dependent manner [16]. In addition, E_2 inhibits cyclooxygenase-2 mRNA expression in bovine articular chondrocytes and protects them from reactive oxygen species-induced damage [17,18]. However, the effects of high doses of estrogen on chondrocytes are contradictory. High concentrations of E_2 lead to deleterious effects such as suppression of DNA synthesis in human chondrocytes [19], as well as the inhibition of proteoglycan synthesis and cell division in both bovine chondrocytes and cartilage explants [20,21]. A significant difference in ER affinity for its ligand as a function of age was observed. Human chondrocytes from early pubertal individuals display a maximal response to estrogens, while chondrocytes from neonatal children do not respond at all [22]. Similarly, ERs from pubertal rabbit chondrocytes exhibit higher affinity for estrogens than pre-pubertal chondrocytes [23]. Thus, estrogen dose and donor age are the main factors that influence chondrocyte response to estrogen.

These and many other relevant findings *in vitro* (discussed below) clearly show that estrogen influences the activity of all joint tissues through complex molecular mechanisms acting at multiple levels.

In vivo studies

The effects of estrogen on joint tissues have primarily been studied in ovariectomized (OVX) animal models. Despite these studies, the influence of estrogen deficiency on cartilage remains unclear, even though there is significant evidence of the detrimental effect of estrogen loss in mature female animals [7]. An increase in cartilage turnover and surface erosion was observed in OVX Sprague-Dawley rats [24], as well as in cynomolgus macaques subjected to bilateral OVX [25]. Significantly, intact females had less severe OA than OVX females and although intact male mice showed more severe OA than intact females, orchiectomized mice develop less OA than intact males [26]. By contrast, such associations could not be shown in other earlier studies [7].

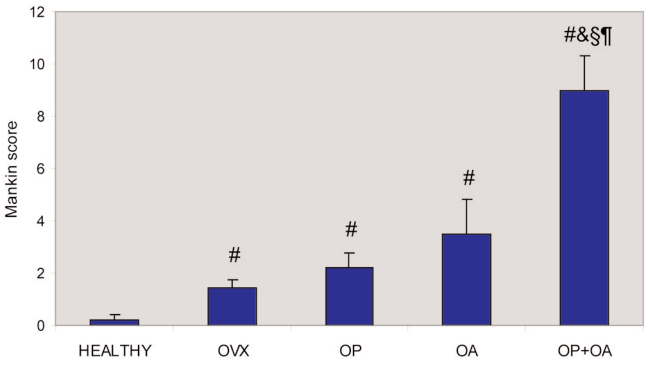
Relevant changes have also been described in the subchondral bone of OVX animals. Indeed, OVX cynomolgus monkeys have higher indices of bone turnover in

subchondral bone compared to epiphyseal/metaphyseal cancellous bone of the proximal tibia [27]. Moreover, the marginal osteophyte area is positively correlated with subchondral bone thickness in the medial tibial plateau of these animals [28]. Significantly, subchondral bone remodeling has also been described in conjunction with changes in joint cartilage in a guinea pig model of spontaneous OA [29]. We found that rabbit subchondral bone has mixed densitometric characteristics with a marked predominance of cortical bone [30]. In fact, subchondral knee bone mineral density (BMD) is significantly correlated with the BMD of the spine, and trabecular and cortical knee bone in healthy, OA, OP and OP/OA rabbits [31].

Our rabbit model is a valuable tool to study OP because rabbits have much faster bone turnover than rodents or primates, and in contrast to rodents, they reach skeletal maturity soon after their sexual development is complete [32]. Moreover, since OVX itself only causes mild osteopenia, which may be insufficient to provoke OP in these animals, moderate doses of methylprednisolone were administrated to ensure OP development [33]. We evaluated whether estrogen deficiency alone can induce OA alterations in healthy cartilage or, by contrast, whether OP subchondral bone is the origin of the cartilage changes in these animals. Estrogen deficiency leads to mild OA changes 22 weeks after isolated OVX in healthy articular cartilage, while OVX and methylprednisolone-induced OP play an additional role in these osteoarthritic changes (Figure 2). Thus, estrogen deprivation might produce a dual effect: a main direct action upon joint cartilage and a minor indirect effect on subchondral bone.

The influence of estrogen on the remaining joint tissues has not been studied directly in OA animal models. However, the involvement of these tissues in OA and the changes produced by estrogen in related animal models suggest a potential role of estrogen in OA changes. Indeed, the remodeling of the cruciate ligament is thought to occur early during knee OA in guinea pigs [29] and the potential role of endogenous estrogens in the disproportionate number of anterior cruciate ligament (ACL) injuries seen in female athletes has been studied in different animal models, although to date with negative results [34]. Besides, significant attenuation of histochemical and biochemical indices of muscle damage and inflammatory response were found in female rats after downhill running when compared with their male counterparts. Such an effect may possibly be explained by the higher circulating estrogen levels in these rats [35]. In addition, estrogen deficiency following OVX is often accompanied by an increase in fat mass, which in turn leads to increased adipokine levels, the role of which in OA is also now being investigated.

Figure 2



Osteoarthritic cartilage damage is aggravated by ovariectomy plus glucocorticoid-induced osteoporosis in a rabbit model. Ovariectomy itself induces small disturbances in the cartilage, while no differences were found between articular cartilage from ovariectomized (OVX), osteoporosis (OP) and osteoarthritis (OA) rabbits. Bar graphs showing the total Mankin score from the histological evaluation of joint cartilage at the weight bearing area of the medial femoral chondyle in the different experimental groups. Healthy, controls; OVX, ovariectomized rabbits; OP, osteoporotic rabbits induced by OVX followed by parenteral methyprednisolone injections for 4 weeks; OA, osteoarthritic rabbits induced by partial medial meniscectomy and anterior cruciate ligament section of the knee; OP+OA, rabbits with experimentally induced OP followed by OA induction. Data are expressed as the mean ± standard deviation. $^{\#}P < 0.05$ versus healthy; $^{\&}P < 0.05$ versus OVX; $^{\S}P < 0.05$ versus OP; $^{\P}P < 0.05$ versus OA.

Human studies

Associations between polymorphisms in the human ERα gene (*ESR1*) and OA have been studied in different populations with mixed results. Haplotypes of the *Pvu*II and *Xba*I polymorphisms in the ERα gene have been associated with an increased prevalence of clinical and radiographic knee OA [36-38]. In addition, the exon 8 G/A *Btg*I polymorphism was also associated with knee OA in Asian populations [38]. However, other studies showed either no or only a modest inverse relationship between ERα gene polymorphisms and OA in Caucasian populations [39,40].

Numerous clinical studies have also shown that OA is related to estrogen levels [8,9,41-47]. Thus, the prevalence of OA is greater in women than men and a clear increase in OA prevalence is associated with the peak age of menopause [8,9,41]. Indeed, a nationwide population survey showed that radiographic generalized OA is three times more common in women aged 45 to 64 years compared to their male counterparts [9], and a hospital-based study found a high female to male ratio of 10:1 for OA, with a peak at 50 years of age [42]. In addition, 64% of females with knee OA suffered the onset of symptoms either perimenopausally or within 5 years of natural menopause or hysterectomy. In fact, the onset of symptoms of knee OA occurred before 50 years of age in 58% of females as opposed to only 20% of males [43].

Since the earliest studies of OA, generalized involvement of joints was described in postmenopausal females, and

predominant node formation with early signs of inflammation was observed in the proximal and distal interphalangeal joints of the hands [44]. Nodular hand OA is often associated with a polyarticular and symmetric involvement of major joints such as knees and hips [45]. Erosions may occur in the interphalangeal joints and are characteristic of erosive OA. This disorder tends to occur in middle-aged women, and it is often an acute condition with features of inflammation that subside over a period of months to years, leaving deformed joints and occasional ankylosis [46]. Lower levels of serum E_2 and its metabolite 2-hydroxyestrone in urine were recently reported in postmenopausal women who developed radiographically defined knee OA [47].

Failure of estrogen production at menopause is associated with a relevant loss of muscle mass and, therefore, significant impairment of muscle performance and functional capacity [48]. Diminished strength of the quadriceps in women but not men predict knee OA [49], and peri- and postmenopausal women also seem to have less lean body mass when compared with pre-menopausal women [50]. In addition, varus-valgus laxity has more frequently been described in women than in men [51].

The effect of estrogen deficiency on the association between osteoarthritis and osteoporosis

At this time, a complex and paradoxical relationship seems to exist between OA and OP, although there is increasing evidence supporting a close biomolecular and mechanical association between subchondral bone and cartilage [52]. Indeed, microarray profiles have identified a number of genes differentially expressed in OA bone that are key players in the structure and function of both bone and cartilage, including genes that participate in the Wingless-type mouse mammary tumor virus/β-catenin (Wnt/β-catenin) and transforming growth factor-β/mothers against decapentaplegic (TGF-β/SMAD) signaling pathways and their targets [53]. Wnt5b and other genes involved in osteoclast function are differentially expressed between male and female OA bone [53]. Furthermore, aggrecan production, as well as SOX9, type II collagen and parathyroid hormone-related protein mRNA expression was inhibited in sclerotic but not non-sclerotic osteoblasts (OBs), while expression of matrix metalloproteinases MMP-3 and MMP-13 and osteoblast-specific factor 1 by human OA chondrocytes was augmented in a co-culture system. Thus, sclerotic osteoarthritic subchondral OBs may contribute to cartilage degradation and chondrocyte hypertrophy [54].

Current methodological difficulties in detecting and closely following incipient OA lesions at early stages in humans are a major obstacle to better understanding the relationship between OA and OP. Therefore, animal models provide an alternative to study this relationship. However, some species may not be suitable for such studies since OVX provokes strong subchondral bone remodeling and loss in these animals (for example, rodents), and possibly ensuing indirect cartilage damage. Conversely, there are certain advantages to studying OP in rabbits [32] and, in this context, our group has developed an experimental model in mature rabbits where OP markedly aggravates the severity of OA estimated using the Mankin score (Figure 2). Moreover, the increased cartilage damage is correlated with loss of bone mass, suggesting a direct relationship between OA and OP [31].

Several cross-sectional studies have demonstrated an inverse relationship between OP and OA [55,56], while others produced opposite results [57]. However, some confounding variables such as race, obesity and physical activity could explain the mutually exclusive relationship between OA and OP. Thus, overweight individuals and/or those that undertake excessive physical activity could have a higher risk of developing OA and of having a higher bone mass. This controversial relationship is also witnessed at the regional level. Indeed, severe hip OA has a protective role against the age-related decrease in structural and mechanical properties of cancellous bone in the principal compressive region of the ipsilateral femoral head [58]. In turn, subchondral tibial BMD was correlated with future joint space narrowing and it has been proposed as a predictor of knee OA progression [59]. However, other studies have shown a decrease in subchondral BMD associated with knee OA. Indeed, in female patients with relatively mild OA of the knee, a significant decrease in periarticular subchondral BMD was evident, whether or not they had a low spine BMD [60].

Mechanisms underlying the effects of estrogen on joint tissues

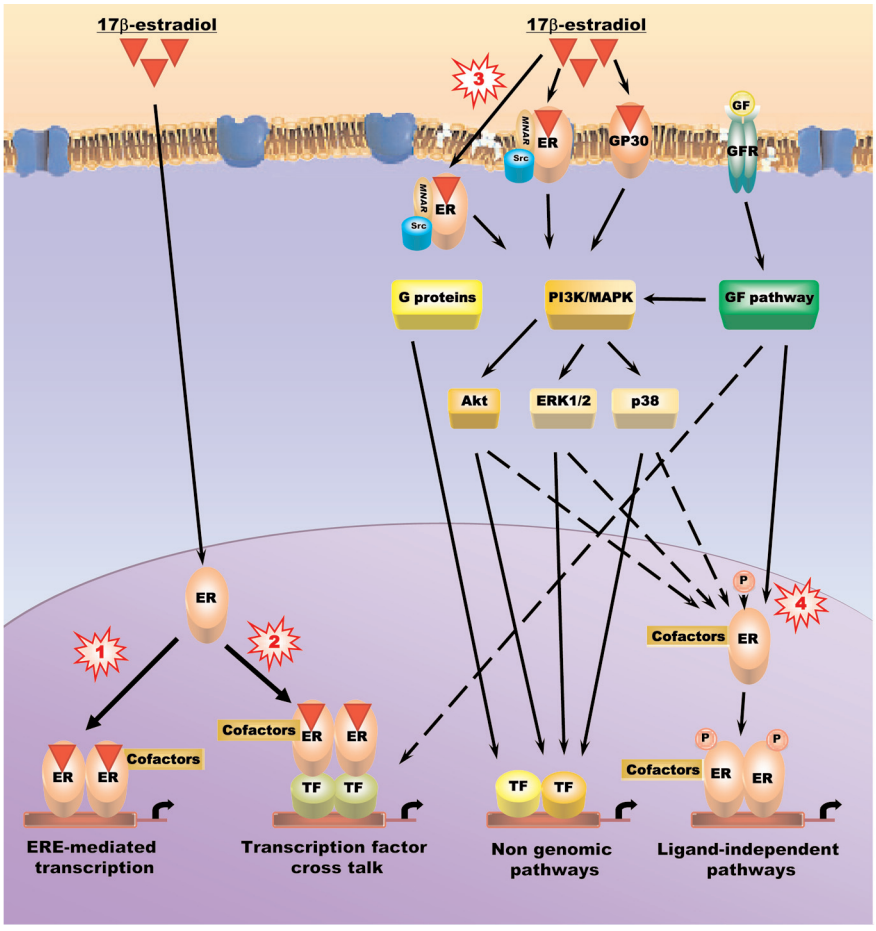
Estrogen influences the biology of joint tissues by regulating the activity and expression of key signaling molecules in several distinct pathways (Figure 3).

Canonical estrogen receptor signaling pathway (estrogen response element-dependent)

Estrogen primarily exerts its effects on target tissues by binding to and activating ERs. ERs act as ligand-activated transcription factors in the nucleus that specifically bind to estrogen response elements (EREs) in the promoters of target genes such as the human oxytocin, prolactin, cathepsin D, progesterone receptor, vascular endothelial growth factor, insulin-like growth factor (IGF)-1, or c-fos genes [61], as diagrammatically shown in Figure 3 (pathway 1). The ERE is a 13 base-pair inverted sequence that binds ERs as dimers. Because imperfect palindromic EREs, or even half EREs, are often seen in the regulatory region of estrogen target genes, transcriptional synergism might occur that could include the co-operative recruitment of co-activators, direct interaction between ER dimers, or allosteric modulation of the DNA-ER complexes [62].

ERs contain four functional domains. The variable amino-terminal A/B domain harbors the constitutive activation

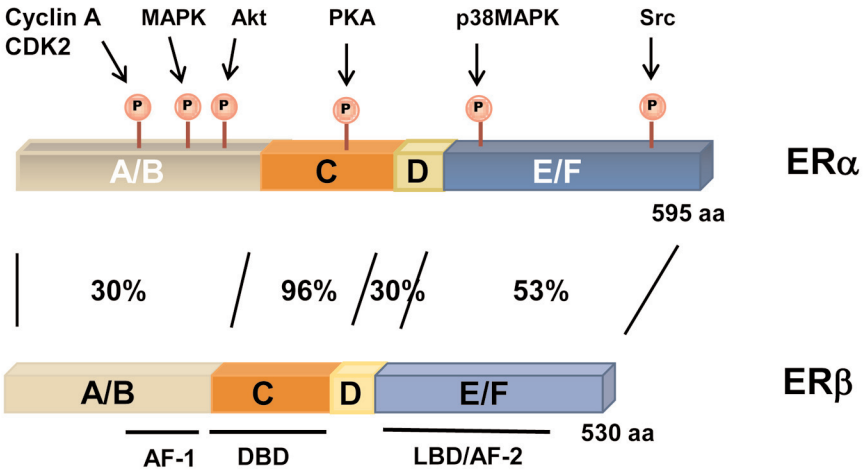
Figure 3



Intracellular signaling pathways used to regulate the activity of estrogens, estrogen receptors, and selective estrogen receptor modulators on articular tissues. Pathway 1: canonical estrogen signaling pathway (estrogen response element (ERE)-dependent) - ligand-activated estrogen receptors (ERs) bind specifically to EREs in the promoter of target genes. Pathway 2: non-ERE estrogen signaling pathway - ligand-bound ERs interact with other transcription factors, such as activator protein (AP)-1, NF-κB and Sp1, forming complexes that mediate the transcription of genes whose promoters do not harbor EREs. Co-regulator molecules regulate the activity of the transcriptional complexes. Pathway 3: non-genomic estrogen signaling pathways - ERs and GP30 localized at or near the cell membrane might elicit the rapid response by activating the phosphatidylinositol-3/Akt (PI3K/Akt) and/or protein kinase C/mitogen activated protein kinase (PKC/MAPK) signal transduction pathways. Pathway 4: ligand-independent pathways - ERs can be stimulated by growth factors such as insulin-like growth factor (IGF)-1, transforming growth factor-β/mothers against decapentaplegic (TGF-β/SMAD), epidermal growth factor (EGF) and the Wnt/β-catenin signaling pathway in the absence of ligands, either by direct interaction or by MAP and PI3/Akt kinase-mediated phosphorylation. Since members of these signaling pathways are transcription factors, some of them, such as SMADs 3/4, can elicit estrogen responses by interacting with ER in the non-ERE-dependent genomic pathway. ERK, extracellular signal regulated kinase; GF, growth factor; GFR, growth factor receptor; MNAR, Modulator of nongenomic action of estrogen receptors; TF, transcription factor.

function (AF)-1, which modulates transcription in a gene- and cell-specific manner. The central and most conserved C domain contains the DNA binding domain, and it also mediates receptor dimerization. The D domain is a less well understood region. Finally, the carboxy-terminal multifunctional E/F domain holds the ligand-binding domain as well as sites for cofactors, transcriptional activation (AF-2) and nuclear localization (Figure 4) [63]. There are two receptor subtypes, ERα and ERβ, which are different proteins encoded by distinct genes located on chromosomes 6 (q24-q27) and 14 (q21-q22), respectively [64]. These two receptor subtypes have 96% amino acid homology in the DNA binding domain but only 53% identity in the ligand-binding domain. As a result, similar ERE binding properties have been associated with a partially distinct spectrum of ligands for each receptor, although with similar affinities for estrogen. Even weaker amino acid identity is found in the A/B domain of ERα and ERβ (Figure 4). Both receptors also show little conservation in AF-2 and, therefore, several proteins may direct ERα and ERβ to different targets as observed in their contrasting effects at the activator protein (AP)-1 site of the collagenase promoter. Thus, ERα and ERβ have different

Figure 4



Structural composition of estrogen receptor (ER)α and ERβ. Both receptors have four functional domains that harbor a DNA-binding domain (DBD), a ligand-binding domain (LBD) and two transcriptional activation functions (AF-1 and AF-2), as indicated for ERβ. The percent of homology in these domains between ERα and ERβ is indicated, as well as the location of several phosphorylation sites in ERα whereby this receptor is activated by important kinases that modulate a wide variety of cellular events. aa, amino acids; Akt, serine/threonine specific-protein kinase family encoded by the Akt genes; CDK2,cyclin-dependent kinase 2; MAPK, mitogen activated protein kinase; PKA, protein kinase A; Src: steroid receptor coactivator.

transcriptional activities that may contribute to their distinct tissue-specific actions [63,65].

Both ERs are distributed widely throughout the body, displaying distinct but overlapping expression in a variety of tissues. ERα is highly expressed in classical estrogen target tissues such as the uterus, placenta, pituitary and cardiovascular system, whereas ERβ is more abundant in the ventral prostate, urogenital tract, ovarian follicles, lung, and immune system. However, the two ERs are co-expressed in tissues such as the mammary gland, bone, and certain regions of the brain [66]. Although both ER subtypes can be expressed in the same tissue, they may not be expressed in the same cell type. Nonetheless, in cells where the two ER subtypes are co-expressed, ERβ can antagonize ERα-dependent transcription [64]. The generation of human ERα and ERβ mRNA transcripts is a complex process that is controlled by sophisticated regulatory mechanisms leading to the generation of several isoforms/variants for each receptor subtype. Most ERα variants only differ at the 5' untranslated region and they are involved in tissue-specific regulation of ERα gene expression. Several species-specific and common ERβ isoforms have been described, many of which are expressed as proteins in tissues [67].

In articular tissues, both ER types are expressed by the chondrocytes [10], subchondral bone cells [11], synoviocytes [12], ligament fibroblasts [13] and myoblasts [14] in humans and other species. However, ERα is predominant in cortical bone and ERβ predominates in cartilage, cancellous bone and synovium [10,12,68]. More mRNA transcripts for both subtypes of ERs were found in male than in female human cartilage, but there were no differences between different joints, or between cartilage from OA patients and the normal population [10]. In bone, ERα and ERβ are expressed by OBs and they are differentially expressed during rat OB maturation [69]. Pre-osteoclasts express ERα, while osteoclast maturation and bone resorption is associated with the loss of ERα expression [70]. ERβ mRNA and protein are predominantly found in the stroma and lining cells of normal human synovium, independent of sex or menopausal status of the tissue donor [12]. Fibroblasts from human ACL, medial cruciate ligament and patellar tendon express functional ER transcripts. Indeed, 4 to 10% of ACL cells express ERs in patients with acute ACL injuries, approximately twice the proportion found in control subjects [13,71]. In human skeletal muscle, ERα mRNA expression was 180-fold higher than that of ERβ [72]. Remarkably, individuals that undergo high endurance training have more ERα and ERβ mRNA transcripts in skeletal muscles than moderately active individuals [73].

Characterizing the phenotypes of knockout models has advanced our understanding of the role of ER in biological processes. Indeed, ERβ plays a significant role in bone remodeling in female ER knockout mice, whereas ERα does so in both sexes. Thus, male and female $ER\alpha^{-/-}$ mice show decreased bone turnover and greater cancellous bone volume, even though the cortical thickness and BMD was reduced. Female $ER\beta^{-/-}$ mice have slightly increased trabecular bone volume, while male animals do not show any change in their bones. Male and female double $ER^{-/-}$ mice

showed significant defects in cortical bone and BMD, while female mice alone displayed a profound decrease in trabecular bone volume [74]. A recent study has shown that $ER\alpha^{-/-}\beta^{-/-}$ double knockout increased osteophytosis and thinning of the lateral subchondral plate, both osteoarthritic characteristics, in the knee of transgenic mice [75]. These results confirm the relevant changes described in subchondral bone of OVX animal models [27-29]. However, no difference in cartilage damage was observed between the $ER\alpha^{-/-}$, $ER\beta^{-/-}$ and $ER\alpha^{-/-}\beta^{-/-}$ double knockout and wild-type mice at 6 months of age, although the cartilage damage was very mild in all mice [75]. Whether the absence of significant cartilage damage in all ER knockout mice groups reflects some important differences between ER knockout mice, which lack ER expression since birth, and OVX models that show significant OA cartilage changes associated with estrogen depletion at a later age [7,24-26] remains to be established.

As regards muscle, $ER\alpha^{-/-}$ mice have lower tetanic tension per calculated anatomical cross-sectional and fiber areas in tibialis anterior and gastrocnemius than in wild-type mice. In contrast, $ER\beta^{-/-}$ and wild-type mice were comparable in all measures. These results suggest that the effects of estrogen on skeletal muscle are mainly mediated by ERα [76]. With respect to ligaments, no changes in medial cruciate ligament or ACL viscoelastic or tensile mechanical properties were observed in $ER\beta^{-/-}$ mice [77].

Non-estrogen response element-mediated genomic ER signaling

The second genomic mechanism involves the interaction of ligand-bound ERs with other transcription factors like Fos/Jun (AP-1-responsive elements), c-Jun/NF-κB and specificity protein 1 (Sp1) recruiting co-regulators to form initiation complexes that regulate the transcription of genes whose promoters do not harbor EREs [64,78]. In this tethering mechanism, ERs do not bind directly to DNA (Figure 3, mechanism 2) and, thus, ERs can up-regulate the expression of promoters containing AP-1 sites, such as the collagenase and IGF-1 genes. Interestingly, E_2 exerts distinct transcriptional activation on the AP-1 site of the collagenase promoter depending on whether ERα or ERβ is involved: it elicits transcriptional activation with ERα, while it represses transcription with ERβ [65,78]. The interaction of ERs with Sp1 activates uteroglobin, retinoic acid receptor alpha, IGF-binding protein 4 (IGFBP4), TGF-α, bcl2 and the low-density lipoprotein receptor genes [61,78]. Similarly, suppression of IL-6 expression by E_2 occurs through interactions of the ligand bound ER with the NF-κB complex [64].

Ligand-dependent activation of ERs, both ERE and non-ERE-mediated, attracts co-regulator molecules that modify the chromatin state, thereby recruiting or hindering the transcriptional complex and representing another level of control in ER gene regulation [61,63,79]. Co-activators stimulate transcription by interacting with helix 12 (H12) of the AF-2 region through their short 'nuclear receptor boxes', transducing ligand signals to the basal transcriptional machinery. The best characterized co-activators include the steroid receptor co-activator (SRC) family (SRC1, SRC2 and SRC3) and members of the mammalian mediator complex (thyroid receptor associated proteins, vitamin-D receptor interacting proteins, activator-recruited cofactor) [63,79]. Alternatively, co-repressors that impede transcription include the nuclear receptor co-repressor (NCoR) and the silencing mediator for the retinoic acid and thyroid hormone receptor (SMRT), which interact with ligand-free ER through an elongated amino acid sequence called the CoRNR-box. By contrast, if H12 assumes a 'charge clamp' configuration in response to agonist binding, then it could not hold the long NCoR/SMRT helices. Thus, agonist binding reduces the affinity of ERs for co-repressors and increases their affinity for co-activators [63,79]. In addition, both SMRT and NCoR recruit the protein SIN3 and histone deacetylases to form a large co-repressor complex, implicating histone deacetylation in transcriptional repression [79].

In rabbit articular chondrocytes, ERα activation inhibits NF-κB p65 activity and, subsequently, decreases IL-1β-stimulated inducible nitric oxide synthase expression and nitric oxide production [80]. Moreover, ERα and, particularly, ERβ transfection significantly enhances MMP-13 promoter activity through an AP-1 site, which may be modulated through the sites of the Runt-related (Runx) and PEA-3 Ets transcription factors in a rabbit synovial cell line lacking endogenous ER [81]. A normal balance between classic ERE-mediated and non-ERE-mediated ERα, genomic and non-genomic, pathways in cortical bone have also been described in $ER\alpha^{-/NERKI}$ mice and its disruption can lead to an aberrant response to estrogen [82].

Non-genomic ER signaling pathways

Estrogens may also exert their ligand-dependent effects through non-genomic mechanisms that are responsible for more rapid effects, occurring within seconds or minutes of stimulating cell signal transduction pathways, such as the mitogen activated protein (MAP) kinases, in particular the extracellular signal regulated kinase 1/2 (ERK 1/2), p38 and phosphatidylinositol-3 (PI3) kinase/Akt pathways [64]. A small ER population and/or a G-protein-coupled receptor termed GP30, localized at or close to the cell membrane, may elicit these responses [83,84]. ER translocation to the cell membrane is nourished by its interaction with membrane proteins such as caveolin 1/2, striatin and the adaptor proteins Shc and p130 Cas [64]. S-palmitoylation and myristoylation of ERα also promote ERα association with the plasma membrane and its interaction with caveolin-1 [64]. Furthermore, interaction between ER, the tyrosine kinase cSrc and an adaptor protein called modulator of nongenomic action of estrogen receptors (MNAR) generates a signaling complex that may be crucial for the important cSrc activation

and further kinase phosphorylation [85]. Thus, several molecular processes have been shown to mediate the non-genomic effects of ER (Figure 3, pathway 3). However, the precise mechanisms involved in ER localization in the cell membrane, as well as the interaction between ERs and signaling pathways, are yet to be fully established.

There appears to be sexual dimorphism in the non-genomic pathways described in human articular and rat growth plate chondrocytes. Thus, only female cells respond to estrogens by promoting a rapid protein kinase C (PKC)-α-mediated increase in proteoglycan production and alkaline phosphatase activity (PKC increase occurred within 9 minutes and was maximal at 90 minutes). Treatment with the PKC inhibitor chelerythrine blocked these effects [86,87]. PKC activation initiated a signaling cascade involving the ERK1/2 and p38 MAP kinase pathways, which in turn mediate the downstream biological effects of estrogen on alkaline phosphatase activity and [(35)S]-sulfate incorporation in rat growth plate chondrocytes. A membrane receptor has been proposed to elicit this response, although its precise nature remains to be established [88].

Estrogen also regulates intracellular calcium concentrations ($[Ca^{2+}]_i$) in a sex-specific and cell maturation state-dependent manner in rat growth plate chondrocytes. Indeed, E_2 more rapidly increased $[Ca^{2+}]_i$ in resting zone chondrocytes than in growth-zone chondrocytes from female rats, while no effect was observed in chondrocytes from male rats. This effect is mediated by membrane-associated events, phospholipase C-dependent inositol triphosphate-3 production and Ca^{2+} release from the endoplasmic reticulum [89]. In the light of the higher prevalence of OA in postmenopausal females, it has been proposed that these intrinsic sex-specific differences may contribute to OA development [86]. In addition, inclusion of the gender variable when interpreting experimental data and the functional adaptation of donor cells in transplants between organisms of different sexes should be considered [86].

Both ERK phosphorylation kinetics and the duration of phospho-ERK nuclear retention determine the pro- or anti-apoptotic effects of estrogen in bone cells. In fact, E_2-induced transient ERK phosphorylation (lasting 30 minutes) leads to anti-apoptotic effects in OBs and osteocytes, whereas it produces pro-apoptotic signals in osteoclasts through sustained ERK phosphorylation (for at least 24 hours) [90]. Also, the ERK 1/2 and PI3K/Akt/Bad pathways mediate the anti-apoptotic effect of estrogens in C2C12 muscle cells following activation of ERα and ERβ located in diverse cellular compartments such as the mitochondria and perinucleus [91]. Divergent ER-induced gene expression has been found depending on whether the genomic or non-genomic signaling pathways are activated in different cell types. In osteoblastic OB-6 cells, E_2 stimulated complement 3 (C3) and IGF-1 expression after 24 hours, which did not occur following estren administration. This discrepancy is explained by the ERE present in the promoter of the C3 gene and by ER regulating IGF-1 through a protein-protein interaction that influences the AP-1 enhancer. Since estren is a non-genotropic ER activator, it did not activate these ERE- or AP-1-containing genes [92].

Ligand-independent signaling pathways

The stimulation of growth factors such as those of the IGF-1, epidermal growth factor, TGF-β/SMAD and Wnt/β-catenin signaling pathways can activate ERs or associated co-regulators via kinase phosphorylation in the absence of ER ligands [64,93-95]. In turn, ERα may also regulate growth factor signaling [64,93-95]. Crosstalk between growth factors and ERs occurs in both the nuclear and cytoplasmic compartments, promoting highly active interactions [64,93-95] (Figure 3, pathway 4).

In OBs, estrogen and TGF-β/SMAD signaling pathways may interact at several levels: activation of the TGF-β pathway by estrogens via TGF-β mRNA induction; increase of estrogen and TGF-β/SMAD signaling due to cytoplasmic MAP kinase activity; direct interaction between ERs and the SMAD proteins in the cytoplasm or nucleus; and interaction between ERs and the TGF-β-inducible early-response gene (*TIEG*) and Runx-2 transcription factors in the nucleus. Both TIEG and Runx-2 expression are induced by E_2 and TGF-β and, furthermore, TIEG appears to be required for the E_2 and TGF-β-induced regulation of Runx2 expression [95]. Thus, a relevant inhibition of osteoclastic bone resorption by osteocytes occurs as a result of TGF-β enhancement by estrogen [96].

ERs can interact with members of the Wnt/β-catenin signaling system in both the presence and absence of the ligand [97]. Bone response to mechanical forces can be influenced by interactions between the β-catenin and T-cell factor nuclear complex, and ERα in OBs. Indeed, ER modulators suppressed the accumulation of active β-catenin in the nucleus of OBs *in vitro* within 3 hours following a single period of dynamic strain of magnitude similar to the estimated strain that OBs regularly experience *in vivo*. Accordingly, microarray analysis performed with RNA extracted from the tibia of $ER\alpha^{-/-}$ mice demonstrated the abrogation of dynamic axial loading-induced expression of Wnt-responsive genes (compared with RNA from the tibia of wild-type mice) [98]. These results suggest that ERα is required for early Wnt/β-catenin-induced bone cell responses to mechanical strain. Indeed, the reduced effectiveness of the bone cell responses to mechanical load associated with estrogen deficiency may alter the bone mass in postmenopausal OP women.

In cynomolgus monkey joint cartilage, IGFBP2-mediated activation of the IGF system induces IGF-1 production, which in turn leads to increased sulfate incorporation into proteoglycans following estrogen administration [99]. In addition,

ERs might interact with the TGF-β and Wnt/β-catenin signaling cascades in articular chondrocytes. Both the Wnt/β-catenin and TGF-β/SMAD signaling pathways play a prominent role in bone and cartilage biology. The TGF-β/SMAD pathway fulfils a beneficial role in bone and cartilage maintenance/repair, although it is also an important protagonist of osteophyte formation [95,100]. In turn, the Wnt/β-catenin system is essential in many biological aspects of bone, from differentiation, proliferation and cellular apoptosis to bone mass regulation and its ability to respond to mechanical load [101]. Activation of the Wnt/β-catenin pathway has also been implicated in OA cartilage damage, and Wnt inhibitors such as the secreted frizzled related protein 3 and Dickkopf-1 might modulate the susceptibility to, and the progress of, hip OA [102].

Although our understanding of the different molecular mechanisms by which estrogen deficits could act on articular tissues and their contribution to OA development has advanced significantly in recent years, it is still limited and more research will be necessary to identify therapeutic targets for this very prevalent disease.

The effects of estrogen replacement therapy and selective estrogen receptor modulators on articular tissues

ERT has displayed mixed effects on joint tissues in various animal and human studies while SERMS conversely have demonstrated a homogeneous response in these tissues (a general description of the effects of SERMs on different tissues is presented in Table 1).

In vivo studies

Estrogen administration in OVX animals has paradoxical effects on joint cartilage, in contrast to the clear benefits of SERM administration [24]. While intra-articular E_2 injections [103] and high supraphysiological estrogen concentrations [104] caused deleterious effects on joint cartilage in a dose- and time-dependent fashion, the beneficial effects of long-term estrogen treatment have been seen in different models [24,25,99]. Early estrogen administration maximizes its positive effects on cartilage [16] and, in turn, tamoxifen decreases cartilage damage in a rabbit model of OA, even in males [105]. Furthermore, tamoxifen antagonized the chondrodestructive effects of high dose intra-articular E_2 during early knee OA in rabbits [106]. Also, NNC 45-0781 and levormeloxifen both inhibited the OVX-induced acceleration of cartilage and bone turnover, and they significantly suppressed cartilage damage in female Sprague-Dawley rats [24,107].

In subchondral bone, the effects of long-term ERT have only recently begun to be studied. ERT limits bone formation in both subchondral bone and epiphyseal/metaphyseal cancellous bone of the proximal tibia in OVX cynomolgus monkeys [27]. ERT also reduces the prevalence of marginal osteophytes, particularly in the lateral tibial plateau, while the presence of axial osteophytes is not affected. However, neither the cross-sectional area in osteophytes nor its static and dynamic histomorphometric parameters are significantly influenced by ERT [28,108]. In addition, a significant effect of ERT has been described on several components of the IGF system in the synovial fluid of OVX female adult cynomolgus monkeys, suggesting a potential stimulatory effect of estrogen on joint tissues *in vivo* [109]. In turn, estrogen administration reversed OVX-induced contractile muscle and myosin dysfunction, as well as the OVX-induced increase of muscle wet mass in mature female mice caused by fluid accumulation [110].

Clinical studies

The effect of ERT on the risk of developing OA and on its progression in postmenopausal women remains unclear. Unlike observational clinical studies, some radiographic studies have suggested a protective effect of ERT on the radiographic detection of OA or its progression [111-115]. In a cross-sectional study, ERT significantly reduced the risk of radiographic hip OA, particularly in long-term users [111]. Similarly, an initial cross-sectional analysis of two of the largest studies found an inverse association between ERT use and radiological knee OA, suggesting that ERT may have a chondroprotective effect. However, a subsequent follow-up analysis failed to show significant ERT protection against either the development or progression of radiographic knee OA [112-115]. Additionally, contradictory results were described regarding the association between ERT and the requirement for arthroplasty [116]. Nevertheless, in the largest study, females that received estrogen alone had significantly fewer arthroplasties, particularly in the hip. Thus, unopposed estrogen administration might have a protective effect against the risk of joint replacement, an effect that may be particularly relevant in hip compared to knee OA [117].

Magnetic resonance imaging-estimated subchondral bone attrition and bone-marrow abnormalities associated with cartilage degradation in knee OA was delayed or prevented by ERT or alendronate in postmenopausal women [118]. In turn, ERT may preserve muscle performance. A 12-month trial showed that ERT protects against the detrimental effects of estrogen deficiency on skeletal muscle in early postmenopausal women, thereby positively influencing muscle performance and structure. Moreover, high-impact physical training provided additional benefits [119].

Development of novel estrogen ligands

Recently, novel ER ligands, both pathway-selective and ERβ-selective, have been developed due to the potent anti-inflammatory activity they have been attributed [120,121] (Table 1). Indeed, the pathway-selective ER ligands WAY-169916 and WAY-204688 inhibit NF-κB transcriptional activity in the absence of conventional estrogenic activity in different animal models of inflammatory diseases [122,123].

Table 1

Partial list of selective estrogen receptor modulators and selective estrogen receptor ligands in clinical development

Pharmacologic group	Compound name	ER action (main target tissues)	Indications and stage of development
Chloroethylene	Clomiphene	ER antagonist (brain)	Ovulation induction*
Triphenylethylenes	Tamoxifen	ER antagonist (breast) ER agonist (bone, uterus and serum cholesterol)	Breast cancer therapy and prevention* Beneficial effects on BMD Beneficial cartilage effect. Animal models
	Toremifene	Similar to tamoxifen	Breast cancer therapy and prevention*
	Ospemifene	Similar to tamoxifen	Vaginal atrophy. Phase III
Benzothiophenes	Raloxifene	ER antagonist (breast) ER agonist (bone and serum cholesterol)	OP therapy and prevention* Breast cancer therapy and prevention*
	Arzoxifene	ER antagonist (breast and uterus) ER agonist (bone and serum cholesterol)	OP therapy and prevention. Phase III Breast and uterine cancer therapy. Phase II
Naphthalenes	Lasofoxifene	ER agonist (bone and serum cholesterol) High bioavailability	OP treatment. Phase III Vaginal atrophy. Phase III
Indoles	Pipendoxifene	ER antagonist (breast)	Breast cancer therapy. Phase II
	Bazedoxifene	ER agonist (bone and blood lipids)	OP treatment and prevention. Phase III
Hydroxy-chromanes	NNC 45-0781	Tissue-selective partial ER agonists	Postmenopausal OP prevention. Preclinical Beneficial cartilage effect. Animal models
	NNC 45-0320 NNC 45-1506		
Steroidals	HMR-3339	ER agonist (bone and serum cholesterol)	Decrease serum cholesterol. Phase II Postmenopausal OP treatment. Preclinical
	Fulvestrant	Steroid ER antagonist (breast)	Refractory breast cancer
Selective ER ligands	Pinaberel (ERB-041)	ERβ-selective agonist	Chronic arthritis/endometriosis. Phase II
	WAY-169916	NF-κB activity inhibition. No classical ER action	Anti-inflammatory. Preclinical studies
	WAY-204688	Similar to WAY-169916	

*Products currently on the market. Levormeloxifen, a discontinued selective estrogen receptor modulator, also showed beneficial effects on cartilage in an animal model. BMD, bone mineral density; ER, estrogen receptor; OP, osteoporosis.

The suppressive effects of estrogen on inflammatory mediators, including NF-κB, inducible nitric oxide synthase, cyclooxygenase-2, and reactive oxygen species in articular chondrocytes [17,18,80], in association with other selective estrogenic benefits on joint tissues might reflect their potential utility in OA treatment.

Conclusion

Progressive structural and functional changes on articular structures commence at early menopause and persist post-menopause, leading to an increase in the prevalence of OA in the latter population and representing a big impact on health costs worldwide. Both experimental and observational evidence support a relevant role for estrogens in the homeostasis of joint tissues and, hence, in the health status of joints. Indeed, estrogens influence their metabolism at many crucial levels and through several complex molecular mechanisms. These effects of estrogens at joints are either significantly dampened or lost as a result of postmenopausal ovary insufficiency.

A better understanding of the role that estrogen and its deficiency plays in the molecular mechanisms of menopause-induced osteoarthritic changes that affect the different joint structures will help further development of new and precise therapeutic strategies to prevent and/or restore damaged articular tissues in OA. These improved therapeutic approaches must be devoid of the widely known undesirable effects of estrogens in other target tissues. Thus, in OA, which represents a particularly challenging disease due to its effects upon different joint structures, these therapeutic options should target the joint as a whole organ rather than focusing only on cartilage damage.

Competing interests

The authors declare that they have no competing interests.

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