Estrogen receptor signaling and targets: Bones, breasts and brain (Review)

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Abstract. Estrogens are involved in a number of physiological functions, including in the development of the brain, growth, reproduction and metabolism. The biological actions of estrogens are achieved by binding to estrogen receptors (ERs) in numerous types of tissues. ERα and ERβ belong to the nuclear receptor superfamily and the G-protein coupled ER1 (GPER1) is a membrane receptor. The primary biologically active estrogen, 17β-estradiol demonstrates a high affinity for ERs. Mechanistically, estrogens bind to the ERs in the nucleus, and the complex then dimerize and bind to estrogen response elements (EREs) located in the promoter regions of the target genes. This is referred to as the genomic mechanism of ERs' function. Furthermore, ERs can also act through kinases and other molecular interactions leading to specific gene expression and functions, referred to as the non-genomic mechanism. While ERα and ERβ exert their functions via both genomic and non-genomic pathways, GPER1 exerts its function primarily via the non-genomic pathways. Any aberrations in ER signaling can lead to one of a number of diseases such as disorders of growth and puberty, fertility and reproduction abnormalities, cancer, metabolic diseases or osteoporosis. In the present review, a focus is placed on three target tissues of estrogens, namely the bones, the breasts and the brain, as paradigms of the multiple facets of the ERs. The increasing prevalence of breast cancer, particularly hormone receptor-positive breast cancer, is a challenge for the development of novel antihormonal therapies other than tamoxifen and aromatase inhibitors, to minimize toxicity from the long treatment regimens in patients with breast cancer. A complete understanding of the mechanism of action of ERs in bones may highlight options for novel targeted treatments for osteoporosis. Likewise, the aging of the brain and related diseases, such as dementia and depression, are associated with a lack of estrogen, particularly in women following menopause. Furthermore, gender dysphoria, a discordance between experienced gender and biological sex, is commonly hypothesized to emerge due to discrepancies in cerebral and genital sexual differentiation. The exact role of ERs in gender dysphoria requires further research.

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Contents

1. Introduction

Estrogens are hormones involved in a number of physiological functions both pre- and postnatally, including brain development, growth, reproduction and metabolism. The primary members of the estrogen family include estrone (E1), estriol and 17β-estradiol (17β-E2), which are primarily produced through the aromatization of testosterone in the granulosa cells in the ovaries and peripheral tissues such as the placenta, adipose tissue, osteoblasts, brain and smooth muscle cells (1,2). Steroidogenesis begins in the ovarian theca cells where cholesterol is converted to androgens, and is completed by the ovarian granulosa cells through the conversion of these androgens to estrogens. Estrogen synthesis begins with the conversion of cholesterol into pregnenolone by the P450 side chain cleavage enzyme. Pregnenolone is then converted into progesterone by 3-β-hydroxysteroid dehydrogenase (3β-HSD), or into 17-hydroxypregnenolone by 17-α-hydroxylase/17,20 lyase (CYP17). Progesterone is then converted into 17-hydroxyprogesterone by the CYP17 enzyme, whereas 17-hydroxypregnenolone is converted into 17-hydroxyprogesterone by 3β-HSD. Next, CYP17 converts

17-hydroxypregnenolone and 17-hydroxyprogesterone into dehydroepiandrosterone (DHEA) and androstenedione, respectively (1,2). Next, different 17β-HSD enzymes catalyze the synthesis of androstenediol from DHEA and testosterone from androstenedione. Finally, in the granulosa cells of the ovaries, the enzyme aromatase converts androstenedione and testosterone into E1 and E2, respectively (1,2). Another enzyme, 5α-reductase, converts testosterone into the potent androgen 5α-dihydrotestosterone. In men, estrogens are primarily produced in the peripheral tissues and, to a lesser extent in the testes by the aromatization of testosterone (1,2). The primary biologically active estrogen is 17β-E2, which demonstrates a high affinity for estrogen nuclear receptors and regulates vital physiological processes both pre- and postnatally. Estrone is a less active estrogen given its low affinity to estrogen receptors (ERs) and predominates in peri- and post-menopausal women. Estriol is primarily secreted by the placenta during pregnancy and has a weak affinity to ERs (1).

ERα, ERβ and G-protein coupled ER1 (GPER1) are the primary ERs and are expressed in numerous target tissues. ERα is primarily present in breast tissues, bones, the uterus, thecal ovarian cells, testes, epididymis, the prostate and the liver. ERβ is expressed in the bladder, granulosa ovarian cells, prostate epithelium, the colon and immune system cells. In adipose tissue and to a greater extent, in the brain and cardiovascular tissues, both ERα and ERβ are expressed (3,4) (Fig. 1). Expression of GPER1 has been detected in multiple tissues, including in the endometrium, breast, ovaries, brain, adrenal glands, kidneys, vasculature and heart endothelium (5). More recent studies on mice and *in vitro* have reported the homeostatic role of GPER1 in physiological actions such as energy homeostasis, cardiovascular function, bone and cartilage development, immune system response and neurodevelopment and neurotransmission (6). Any defects in ER signaling lead to a number of diseases, including those affecting growth and puberty, fertility and reproduction abnormalities, cancer, metabolic diseases, osteoporosis and neurodegeneration (4).

Estrogens and ERs exert their effects via genomic and non-genomic processes. Estrogens bind to ERs to cause ER dimerization and translocation to the nucleus, where they function with specific auxiliary elements known as estrogen response elements (EREs) located in the promoter regions of target genes. Alternatively, ERs also act via kinases and other molecular interactions leading to gene expression and functions through a non-genomic mechanism (7-10). The actions of ERα and ERβ antagonize each other in specific tissues such as the bones, breasts and brain, and in prostate cancer cells (8-12). ERβ has isoforms that can act separately and by different actions on specific tissues (11). For instance, the ERβ isoforms ERβ2 and ERβ5 inhibit ERα in breast cancer cells, exerting a protective role (8,10,12). Likewise, in breast cancer T47D cells, ERβ expression is upregulated compared with ERα (10). In addition, in the bone, ERα is primarily expressed in the cortical bone, whereas ERβ is expressed mostly in the trabecular bone (11).

2. ERs: Location, structure and function

Location and structure. Estrogen nuclear receptors belong to the nuclear receptor superfamily and thus have similar structural properties to thyroid and steroid hormone receptors. ERα consists of 595 amino acids and is encoded by the *ESR1* gene located on chromosome 6 (6q25.1) (12). ERβ is 530 amino acids long and is encoded by the *ESR2* gene on chromosome 14 (14q23.2) (13). ER, similar to all the other members of the nuclear receptor family of proteins is domain-structured. Specifically, ER consists of six domains: A, B, C, D, E and F (Fig. 2). The A and B domains, also referred to as the N-terminal domain, connects and activates the DNA transactivation function domain [activation function 1 (AF-1)]. The A and B domains are associated with receptor specificity and contribute to the transcriptional activation of target genes; they are also required for ligand-independent transcriptional regulation (12-15). Domain C, also known as the DNA-binding domain, is a highly preserved domain that enhances the DNA-binding ability of ER (12-15). Two zinc fingers are localized on the C domain, which interact with specific hormonal response elements, EREs, leading to receptor dimerization. Domain D is the hinge region between domains C and E, which binds to heat shock proteins. Domain D also acts to stabilize the DNA-binding function of the C domain and provides the receptor with flexibility in binding to EREs (14,15). Finally, the E and F domains are the ligand-binding domains (LBD), which are located at the C-terminus. The E and F domains exhibit a complex monitoring ligand-dependent action through the transcriptional activation function domain (AF-2) and coactivators in Helix 12. Helix 12 serves a key role in the LBD, modifying its structure when bound to a ligand; this results in the active form when ER is bound to an agonist, or the inactive form when bound to an antagonist, and this structural change modulates the transcriptional regulation (14,15). Finally, GPER1, previously known as GPR30, consists of 375 amino acids and is located on chromosome 7 (7p22.3). GPER1 consists of seven transmembrane α-helices, which form four extracellular segments and four cytosolic segments. The binding affinity of GPER1 to estrogen is weaker than that of the nuclear ERs. GPER1 was discovered 20 years ago and the details of its exact interactions with estrogens and other steroid hormones remain to be determined (16).

Different isoforms of ERα arise due to gene splicing (Fig. 2). ERα isoforms include ERα-46 and ERα-36, which are the primary reactive estrogen isoforms (17) ERα-36 is formed due to the presence of an alternative transcription site at the first intron and lacks AF1 and AF2 domains. ERα-36 contains exons 2-6 of the wild-type EPα and exon 9 (27 amino acids at the C-terminal) (18,19). ERα-46 is another ERα variant lacking exon 1; it contains all exons of the wild-type receptor (18). Numerous variants of ERβ have also been identified, including ERβ2, ERβ4 and ERβ5, which lack a different C-terminal sequence in order to bind to different EREs (20,21).

ERβ1 consists of 530 amino acids and is the full-length version of wild-type ERβ, whereas ERβ2, 3, 4 and 5 have a single LBD sequence. These differences result in a shorter LBD and a lack of AF2 domain function. ERβ1 is the only isoform that can bind to an estrogen-ligand, the remaining truncated ERβ isoforms have no estrogen-binding ability (20).

Function. Estrogens exert their effects upon binding to their receptors via complex genomic and non-genomic mechanisms (Fig. 3) (22). The mechanisms of ER expression and

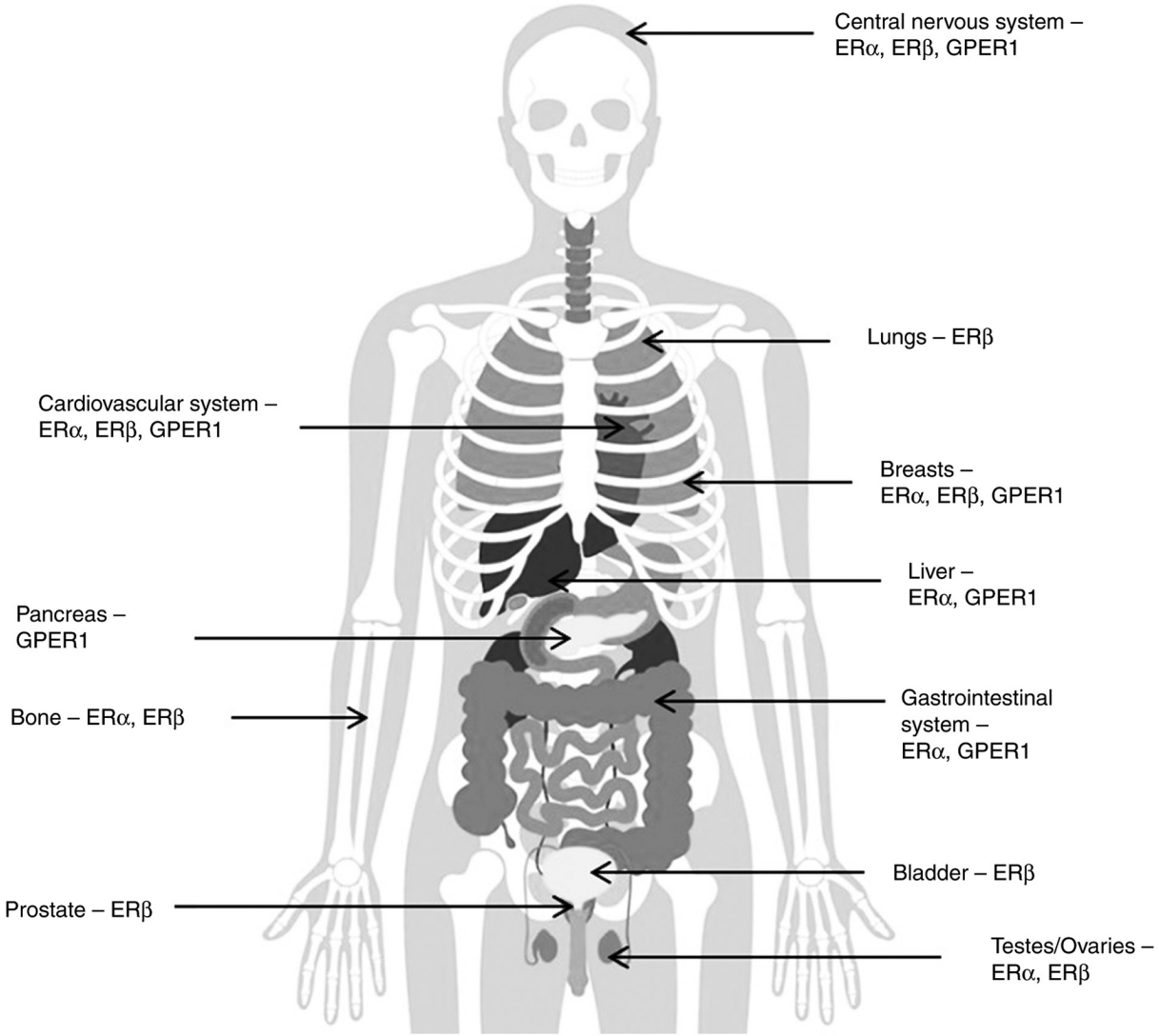

Figure 1. Locations of the normal expressions of ERα, ERβ and GPER1 in human tissues. ER, estrogen receptor; GPER1, G-protein coupled ER1.

action are complex. Both ERα and ERβ are activated via AF-1 and AF-2 domains, which are located in the N-terminal domain and LBD, respectively, and mediate synergistic transcriptional regulation (14,23). The genomic action of ERs regulates the transcription of numerous target genes involved in the growth and differentiation of the cell. When estrogen hormones bind to ERα, the receptor is activated, dimerizes and translocates to the nucleus where it interacts with transcriptional coactivators. The activated form of ERα targets the promoters of the target genes binding to EREs. Moreover, ERα is able to bind to serum-responsive elements forming a complex, which then interacts with transcription factors, including activator protein 1 and specific protein 1, to modulate the expression of genes lacking EREs in their promoter regions (9,10,15,24).

ERα also exerts non-genomic activity (25,26). Estrogen binds to membrane ERs and regulates ion channel opening, or activates related enzymes and Ca^{2+} mobilization that triggers the activation of nuclear transcription factors. This involves the fast activation of intracellular signaling pathways including the cyclic adenosine monophosphate and the growth factor receptor PI3K/Akt or Ras/MAPK pathways. The ERα-E2 complex binds with a number of different proteins leading to the generation of a complex molecule within the cytoplasm. Such proteins include the Src protein kinase and the p85 subunit of PI3K. Such complexes can rapidly trigger the activation of the MAPK and Akt pathways (27). Furthermore, ERs can also act via a ligand-independent mechanism through interaction with growth factors such as insulin-like growth factor (IGF) and epidermal growth factor. These growth factors trigger the phosphorylation of the ERs via the growth factor membrane receptor to regulate intracellular signaling pathways. Through this mechanism, ERs can regulate gene expression without the need for ligand binding (22,23).

The non-genomic mechanisms of ERα involving the plasma membrane are based on post-translational alterations. The two mechanisms, genomic and non-genomic, are not completely independent. In fact, there is an interchange between the two mechanisms of action, particularly via the phosphorylation of ERα or its cofactors. An example of the overlap between the two types of mechanisms of ER signaling is the estrogenic activation of cyclin D1 via the AP-1 binding site, which requires MAPK activity and formation of an ER/Src/PI3K complex (7,28).

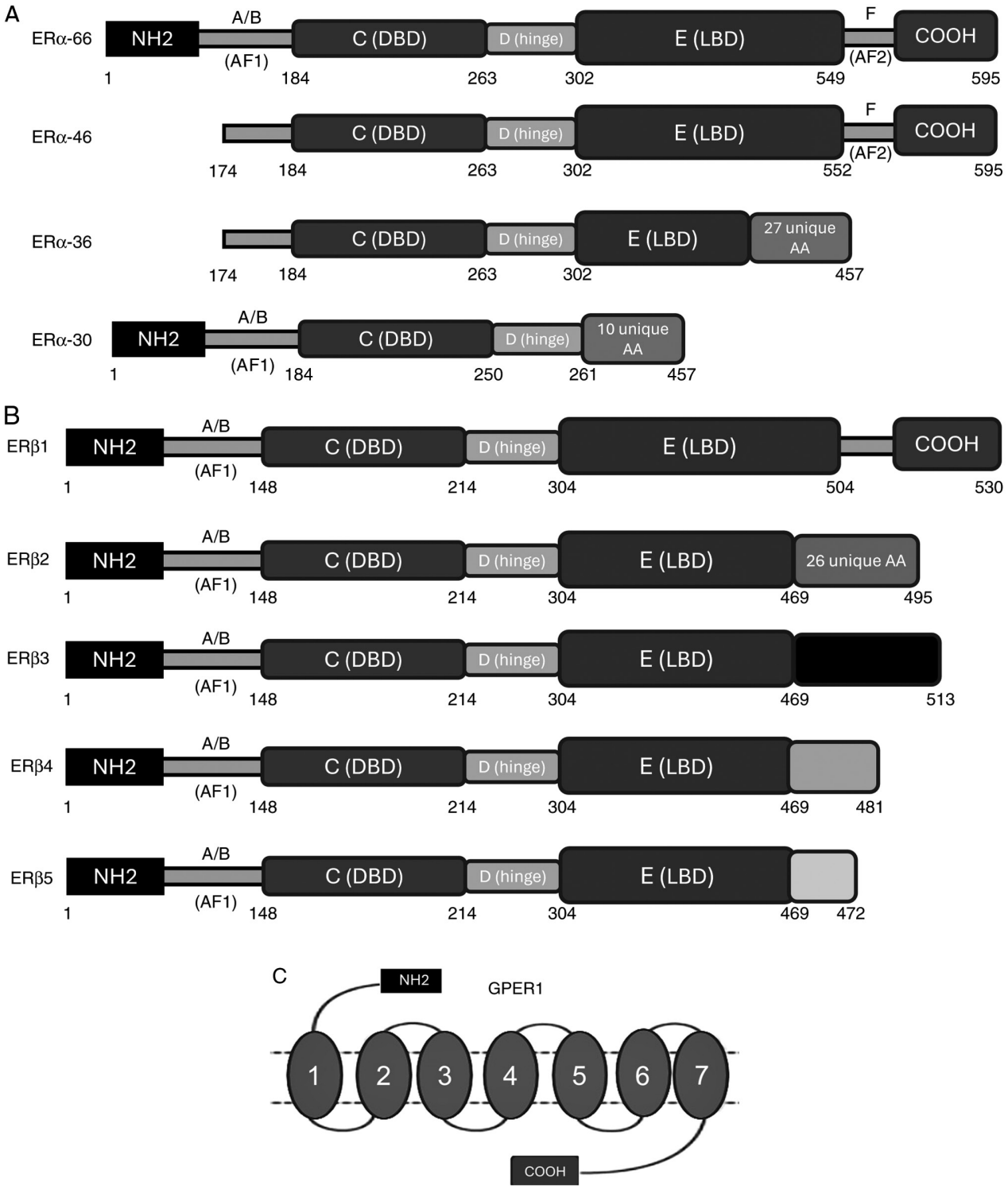

Figure 2. ER structure. (A) ERα and (B) ERβ isoforms. (C) GPER1 with 7 helices. ER, estrogen receptor; AA, amino acid; DBD, DNA binding domain; LBD, ligand binding domain; GPER1, G-protein coupled ER1; AF, activation factor.

In the present review, ERs' effects in three target tissues as paradigms of the multiple facets of the ER, including bone and growth disorders, cancer, gender dysphoria and ageing, are discussed. The antagonistic action of ERs in the aforementioned tissues complicates the understanding of their mechanisms of action (4). The increasing prevalence of breast cancer, particularly hormone receptor-positive breast cancer, remains a challenge for the development of novel anti-hormonal therapies other than tamoxifen and aromatase inhibitors, to minimize the toxicity of long treatment regimens in patients with breast cancer. A complete understanding of the mechanisms of action of ERs in the bones may result in the identification of novel pathways for the treatment of osteoporosis. Aging of the brain and related diseases such as dementia and depression are associated with the lack of estrogens, particularly in women after menopause. ERs serve

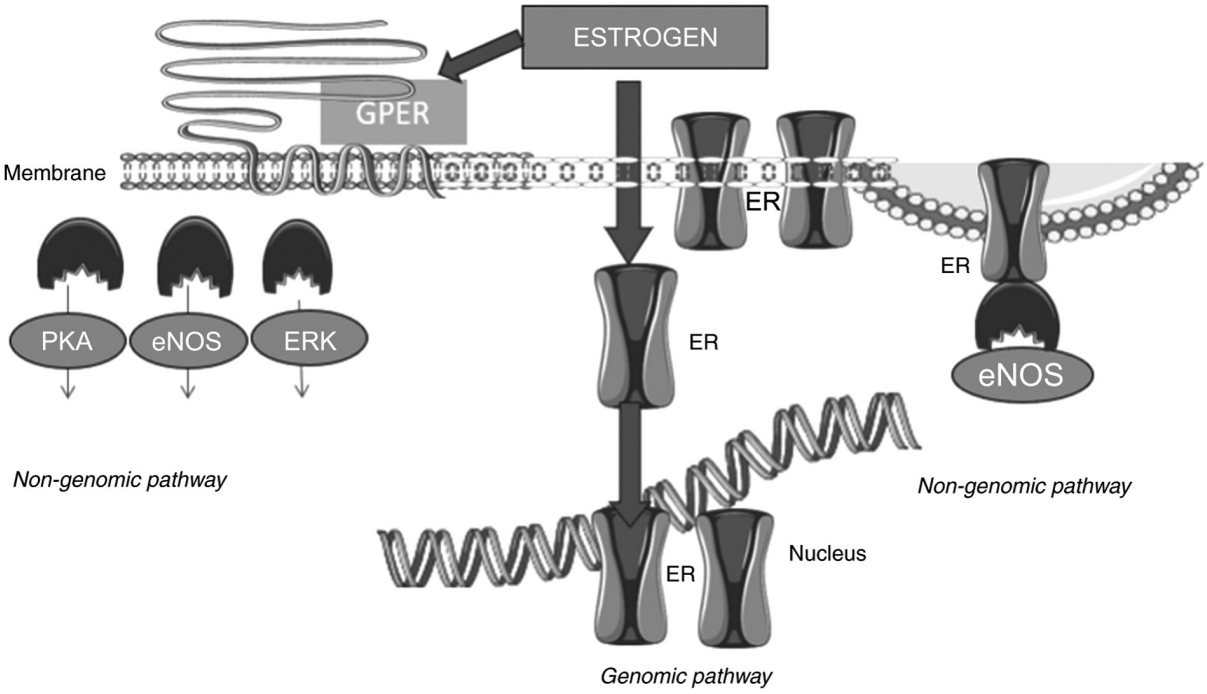

Figure 3. Estrogen receptor signaling pathway. By the genomic mechanism, ER after ligand binding and dimerization, translocates to the nucleus, where it directly binds to the responsive elements of target genes involved in cell growth, inflammation, proliferation, survival and protein synthesis. By the non-genomic mechanism, estrogens bind to ERs modulating eNOS activity. Estrogens also bind to the GPER located on the membrane to regulate signaling through eNOS, PKA and extracellular signal-regulated kinases ERK or MAPK. ER, estrogen receptor; eNOS, endothelial nitric oxide synthase; GPER, G-protein coupled ER; PKA, protein kinase A.

a key role in aging. Estrogens are known to exert a neuroprotective role on the brain via ERs. Decreases of estrogens with age is followed by brain cell degeneration and decreased of memory and cognition. Gender dysphoria, a marked incongruence between experienced gender and biological sex, is commonly hypothesized to arise from discrepant cerebral and genital sexual differentiation. The exact role of ERs in gender dysphoria is a challenge that requires further research.

In the present review, a comprehensive literature search was performed using PubMed and the search terms 'ER', 'structure', 'function', 'bones', 'cancer', 'breast cancer', 'brain' and 'gender dysphoria' along with the appropriate Boolean modifiers.

3. ERs in the bones

Estrogens are vital for skeletal growth and for the maintenance of bone mass in both males and females. Estrogen action is mediated via ERα and ERβ in both the growth plate and mineralized bone from early in human development to adulthood (29). During puberty, estrogens begin exhibiting their roles in the pubertal growth spurt by binding to ERs on osteoblasts and chondrocytes. Estrogens promote osteoblastic activity and inhibit osteoclastic activity via advancing of the growth plate, bone proliferation and epiphyseal fusion (29,30). The fact that estrogen deficiency leads to osteopenia and presumably osteoporosis in postmenopausal women is well established (29). Both estrogens and androgens also act directly and/or via the *ERα* gene in osteoblasts by altering the physiological balance of the osteoprotegerin/receptor activator of NF-κB ligand system (30). Another mechanism involved in the osteoprotective actions of estrogens is achieved via the upregulation of the Fas ligand (*FasL*) gene. *FasL* action is mediated by ERα, resulting in increased apoptosis in differentiated osteoclasts (31). Moreover, androgens also contribute to skeletal growth and bone mineral density either via a direct action on the growth plate or indirectly through their aromatization to estrogens (30).

Estrogen deficiency leads to an increase in osteoblast and osteocyte apoptosis in both humans and rodents. There are different patterns of expression of ERs on specific bone cells, including osteoblasts and osteoclasts, and on cartilage cells known as chondrocytes, and these patterns of expression are influenced by age and sex. *ERα* chondrocyte knock-out mice have been reported to exhibit increased longitudinal bone growth and wider growth plates (32). However, *ERα* deletion from the osteoclast lineage does not affect the cortical bone mass (33). *ERα* knock-out mice demonstrated trabecular bone loss and high bone turnover, which may be related to increased osteoclast counts in females compared with males. However, deletion of the *ERα* gene in osteoblast progenitors in female mice demonstrated no effect on trabecular bone mass (11,34,35). Furthermore, ERβ is also highly expressed in osteoclasts (23). Studies in humans reported similar findings (11,34,35). Expression of ERα and ERβ also differs in bone compartments in humans, where ERβ is expressed in higher levels on the trabecular bone whereas ERα is primarily expressed in the cortical bone (11,34,35).

The clinical implications of ER function were first described approximately four decades ago. The first case of estrogen resistance in a male patient of tall stature, with markedly delayed skeletal maturation and osteoporosis, was

Table I. ERα, ERβ and GPER actions in cancer tissues.

	Role of receptors		
Cancer type	ERα	ERβ	GPER
Ovarian	Tumor promoter (41)	Tumor suppressor (41); ERβ and ERβ2 suppress ERα (42); ERβ5 tumor promoter (42)	GPER1 tumor suppressor (43); GPER30 tumor promoter (44)
Prostatic	Tumor promoter (42)	Tumor suppressor ERβ5 (45); Tumor promoter (45)	Tumor suppressor (46)
Gastric/intestinal	Tumor suppressor (47); ERα36 tumor promoter (48)	ERβ5 tumor promoter (49)	Tumor promoter (50); Tumor suppressor (50)
Liver	Tumor suppressor (51); Tumor promoter (51)	Tumor promoter (51)	Tumor suppressor (52)
Pancreatic	Tumor promoter (53)	Tumor promoter (53)	Tumor suppressor (53)
Melanoma	Tumor promoter (54)	Tumor suppressor (54)	Tumor suppressor (54)
Lung	Tumor promoter (55)	Tumor promoter (56)	Tumor suppressor (57)

GPER, G-protein coupled estrogen receptor; ER, estrogen receptor.

described in 1986. This patient was reported to possess a disruptive mutation in the *ERα* gene (36) and the administration of estrogen did not improve the condition of the patient (36). A similar clinical presentation was observed in male patients with an aromatase deficiency. The aromatase gene *CYP19*, is located on chromosome 15q21.1 and is expressed in a number of tissues, including the placenta, ovaries, testes, adipose tissues and the brain. Specific promoters in each tissue type lead to different splice variants and different expression profiles of the *CYP19* gene in the respective tissues (37). The aromatase enzyme serves an essential role in sex steroid physiology by converting C19 androgenic steroids (androstenedione and testosterone) to C18 estrogens (E2 and E1), which promote growth acceleration, skeletal maturation and epiphyseal fusion. Aromatase gene inactivating mutations in males cause delayed bone maturation and decreased bone mineralization. In contrast with estrogen resistance, administration of estrogen in patients with aromatase deficiency, promotes the closure of growth plates (38). Conversely, females with an aromatase deficiency demonstrate different degrees of virilization, from an infant with ambiguous genitalia to a woman with polycystic ovarian syndrome (39).

4. ERs and cancer

Estrogen nuclear receptors are involved in tumor development and progression in numerous types of cancer (10). Gynecological, endocrine gland, gastrointestinal and lung cancer may exhibit either up- or downregulation of ERs. Both ERα and ERβ are expressed in numerous types of tumors but usually in different cancer types (40). ERα expression appears to predominate in breast, ovarian, endometrial and endocrine gland cancer, whereas ERβ is upregulated in gastrointestinal and lung cancer (10). Conversely, ERβ inhibits the proliferation of colon and lung cancer, whereas ERα exerts an onco-protective role in breast cancer, with decreased ERα levels associated with a poorer prognosis (40). The polymorphic expression of ERs in different types of cancer supports the notion that estrogenic function is tissue-dependent. However, the exact mechanisms of action in the different types of tissues remain incompletely understood. The present review focuses on breast cancer, with a summary of ER expression in other types of cancer provided in Table I (41-57).

Breast cancer is the most common cancer among women, accounting for 30% of all new female cancer cases, with a rising global incidence in both pre- and postmenopausal women (58). Breast tumors are categorized based on histopathological analysis as either lobular or ductal, *in situ* or invasive carcinoma (59). According to the molecular profile, breast cancer can be classified as positive or negative for ERα, progesterone receptor (PR) and human epidermal growth factor receptor 2 (HER2). The latter classification of breast cancers relies on three primary subtypes as follows: Luminal, which is $ER^{+}PR^{+}HER2^{-}$, HER2-positive, which is $ER^{-}PR^{-}HER2^{+}$, or triple-negative, which is $ER^{-}PR^{-}HER2^{-}$. The majority of breast cancer cases (~80%) are ERα-positive. The luminal type is separated into two subclasses, A and B. Both subclasses express ERα but vary in terms of aggressiveness. Luminal A cases are typically low-grade, whereas luminal B can be of higher grade and with worse prognosis. Luminal B cases demonstrate upregulated expression of HER2, reduced ERα expression and increased cancer cell proliferation (60). Knowing the molecular profile of breast cancer allows for the selection of the most appropriate treatment, management and follow-up for patients.

Physiologically, ERα is expressed in the ducts and lobules of the breast gland, while ERβ is expressed in the luminal, myoepithelial and stromal cells. ERα serves a major role in breast development, while ERβ depletion has no effect on mammary gland growth and development (60). The etiology of breast cancer is multifactorial. Mutations in tumor suppressor genes such as *BRCA1*, *BRCA2*, *CHEK2*, *TP53* or *PTEN*, or gain of function mutations in oncogenes such as *EGFR*, *IGF1R* and *HER2* only account for a small

percentage of breast cancer cases (58). Given that ERα is found only in 10% of breast tissue cells, mutations in the *ERα* gene are not the primary cause of breast cancer formation. However, ERα dysregulation is involved in breast cancer development and progression. ERα is found in ~80% of breast cancer cells (61), which shows the importance of the role of estrogens in hormone-dependent breast cancer. Numerous studies have linked the occurrence of breast cancer with exposure to estrogen replacement therapy in menopausal women, and the involvement of ERs has been hypothesized (62,63).

The pathophysiological mechanisms of the carcinogenic effects of estrogen in the breast are still under evaluation. Estrogens, through ER-dependent and independent mechanisms, can promote the upregulation or downregulation of numerous molecules involved in cell proliferation, differentiation and apoptosis (40). ERα functions as a transcription factor for several tumor-associated genes, including IGF1 receptor (IGF1R), cyclin D1, the anti-apoptotic protein BCL-2 and vascular endothelial growth factor (62-64). Moreover, estrogens, primarily 17β-E2, can stimulate ERα-mediated genes such as the *LRP16* gene, which interferes with and downregulates ERα-mediated transcription of E-cadherin (64). The reduction of E-cadherin enhances the invasiveness of breast cancer (64). Furthermore, estrogens may upregulate the expression of *Wnt11* via an ER-dependent mechanism in breast cancer (65). Likewise, studies have reported that phosphorylation of ERα at serine residues 118 and 167 in breast cancer cells induces expression of genes that impact tumor progression and treatment responsiveness (65-67). Conversely, estrogens can promote cell proliferation via non-ER related cell membrane phosphorylation of target molecules such as Akt (67). Such knowledge may allow the development of innovative cancer treatments based on non-ER-related mechanisms of estrogen action.

ERα is expressed in ~80% of breast cancer cells, meaning that these breast cancer cases respond to endocrine therapy. Conversely, patients with *ESRα* mutant-positive metastases demonstrate resistance to hormonal therapy. Missense mutations in *ESRα* have been identified in >51 residues most of which are within the ER-LBD. Such mutations are either hormone-dependent, hormone-independent or neutral. Y537S and D538G are the most common mutations and are characterized as hormone-independent mutations (68). Other mutations such as K303R and E380Q result in estrogen hypersensitivity and are characterized as hormone-dependent. Finally, neutral mutations such as S432L and V534E have also been reported (69). ERα splice variants are also involved in breast cancer. ERα-46 is expressed in ~70% of breast cancer tissues and inhibits cell proliferation and ERα-66-regulated target gene transcription. ERα-46 appears to enhance endocrine responses by inhibiting selected ERa-66 endocrine responses. Via this mechanism, ERα-46 appears to increase the response to endocrine therapy (70). ERα-36 regulates non-genomic signaling. It has been reported that patients with breast cancer with tumors expressing high levels of ERα-36 benefit less from tamoxifen therapy compared with those with low levels of ERα-36 expression. Thus, ERα-36 is suggested to act as an antagonist of tamoxifen contributing to hormone therapy resistance and cancer metastases (71).

Genomic studies of breast cancer have reported the role of epigenetics in ERα dysregulation. The loss of ERα expression can be the result of hypermethylation of CpG islands within the ERα promoter region. Deacetylation and histone methylation of the ER promoter are also epigenetic events involved in ERα deregulation. Demethylating agents and methylation inhibitors may thus serve as potential treatments for ERα-negative or endocrine therapy-resistant tumors (72).

ERβ is expressed in breast cancer cells, but to a lesser degree than ERα. There are conflicting studies regarding the role of ERβ in breast cancer. Some studies have identified a protective effect of ERβ expression in breast cancer cells by antagonizing the action of Erα (73). Moreover, the expression of ERβ is associated with a better prognosis and with an increased response to endocrine therapy (74). Other studies have reported opposing results, demonstrating that ERβ expression is associated with adverse outcomes in breast cancer and is not predictive of resistance to tamoxifen (75,76). More recently, studies have focused on the protective effects of ERβ-5 in patients with breast cancer and the positive association with longer relapse-free survival times. ERβ-5 has also been reported to induce the sensitivity of breast cancer cell lines to chemotherapy-induced apoptosis (77,78).

Finally, GPER1 expression is detected in 60% of breast cancer cases, primarily in Luminal A cases. GPER1 downregulation in the tumor cells is associated with poorer outcome (66). Likewise, studies have reported that expression of GPER1 decreases during tamoxifen treatment. Thus, breast cancers with low expression of GPER1 show endocrine treatment resistance (79).

The exact mechanisms of action and antagonism of ERs in different types of cancer is an ongoing area of research, which may highlight novel strategies for the development of therapeutics for the management of specific types of cancer. Research on novel treatments for combating endocrine-resistant tumors is also ongoing.

5. ERs in gender dysphoria

ERα and ERβ expression are upregulated during the development of the central nervous system (CNS) (80). ERα is primarily located in the hypothalamic nuclei, the periaqueductal grey, the parabrachial nucleus, the medial amygdala, the preoptic area and on the bed nucleus of the stria terminalis. ERα is also expressed to a lesser degree in the locus coeruleus. ERβ is also expressed in the aforementioned areas, but is primarily expressed in the lateral septum, the basolateral amygdala and the trigeminal nuclei. Lower levels of ERβ are also observed in the hippocampus, the locus coeruleus, the cerebral cortex and the cerebellum. The most recently discovered ER, GPER1, is also expressed in the brain, with high levels in the olfactory bulbs, the hypothalamus, cortex, the hippocampus, and the Purkinje and granule cells of the cerebellum (80). ERs function in the brain primarily through non-genomic pathways and to a lesser extent via the genomic pathways, where ERs regulate brain development, sexual behavior, cognitive ability, temperature homeostasis and appetite (81-83).

The masculinization of the brain in humans is primarily mediated by the androgen receptor (AR) and partly by

Table II. Studies on the contribution of ERs in gender dysphoria.

First author, year	Gender	ERs	Findings	(Refs.)
Henningsson *et al*, 2005	MTF	ERβ	Significant association with a dinucleotide CA polymorphism in the ERβ gene. The higher number of CA repeats implies greater transcription activation and therefore lower feminization or greater defeminization	(85)
Hare *et al*, 2009	MTF	ERβ	Unable to replicate the significant association between longer CA repeat lengths in the ERβ gene	(86)
Ujike *et al*, 2009	MTF and FTM	ERβ and ERα	No difference in allelic or genotypic distribution of the genes, thus no evidence that genetic variants of sex hormone-related genes confer individual susceptibility to transsexualism	(87)
Fernández *et al*, 2014	MTF and FTM	ERβ	No difference in allelic or genotypic distribution of the sex hormone-related genes between transgender patients and controls	(88)
Cortés-Cortés *et al*, 2017	MTF and FTM	ERα	The polymorphism XbaI-rs9340799 and the haplotypes L-C-G and L-C-A are associated with FTM in adults	(89)
Fernández *et al*, 2018	MTF and FTM	ERα and ERβ	The key receptors implicated in sexual differentiation of the brain have a specific allele combination for ERβ and ERα in MTFs. The FTM gender is associated with specific polymorphisms of ERβ and ERα. Thus, ERα and ERβ serve a key role in the sexual differentiation of the brain	(90)
Foreman *et al*, 2019	MTF	ERα and ERβ	Overrepresented alleles and genotypes are proposed to under-masculinize/feminize on the basis of their reported effects in other disease contexts	(91)
Ramírez *et al*, 2021	MTF and FTM	ER coactivators	The coactivators SRC-1 and SRC-2 could be considered as candidates for increasing the list of potential genes for gender incongruence. The role of SRCs in ER-mediated gene expression is known	(92)

MTF, male to female transgender; FTM, female to male transgender; ER, estrogen receptor; SRC, steroid receptor coactivator.

ERs (81). In humans, the expression of aromatase is highest in the thalamus (82). During embryogenesis, fetal testosterone passes into the brain and is metabolized by the enzyme aromatase to E2, which exerts its action by binding to ERs, which masculinizes specific brain regions. It has been previously reported in mouse knockout studies that ERβ has a defeminization role in male brains and in male behavior (83). The effects of sex steroids on the brain during development determine sexual differentiation. A suggested pathway for brain masculinization is the direct initiation of gene expression via the activation of AR and ERs. Gender dysphoria, a marked discordance between experienced gender and biological sex, is commonly hypothesized to be attributed to discrepant of typical male or female brain and genital sexual differentiation (84). The contribution of ERs to the development of gender incongruence has been widely studied with contradicting and only partially convincing results (85,86). Interactions of genes and polymorphisms may contribute to the polygenic bases of gender dysphoria. However, genetics is not the only determining factor to change sexual orientation. The majority of studies that investigated the implication of the genetic component focused on polymorphisms related to ERα and ERβ (Table II) (85-92).

Based on the limited available published data, the primary receptors involved in sexual differentiation of the brain have a specific allele combination for ERβ, ERα and AR in trans females, whose gender incongruence is associated with a specific genotypic combination of ERs and AR variants. Specific polymorphisms of ERβ and ERα have also been reported in trans males. Both ERα and ERβ serve a key role in the typical sexual differentiation of the human brain. Estrogens, primarily E2, regulate the growth, development and function of the reproductive system and the CNS. The mechanisms of ERs and their interactions with estrogens in gender differentiation are not completely understood. The two receptors ERα and ERβ bind with the E2 ligand, resulting

in the dimerization of the receptor, resulting in the necessary fundamental changes in the LBD and thus allowing for coactivators to be engaged. This step is required for the transcriptional regulation of gender-determination genes dependent on E2 (93,94). Moreover, the E2-ER complex interacts with steroid receptor coactivators (SRCs) to activate the transcription of the target genes. SRCs can also influence the ability of the transcription factors to activate or inhibit the expression of numerous genes (95). There are three SRC members: SRC-1, SRC-2 and SRC-3. It has been suggested that variations at the DNA level in SRCs can affect the function of the E2-ER complex and therefore modify the transcription of the genes regulated by E2. The coactivators SRC-1 and SRC-2 are hypothesized to serve a key role in the potential expression of the gene for gender incongruence (92). Overall, it is hypothesized that gender incongruence has a complex underlying pathophysiology that involves interactions between sex steroids, sex steroid receptors, coactivators and genes (92). Furthermore, reduced androgen levels and androgen signaling may serve a role in the acquisition of a female gender identity of male-to-female transsexuals, whereas a higher number of CA repeats in the ERβ gene lead to reduced ERβ signaling and inhibit the ERβ dimerization on the male brain, which results in increased transcriptional activation and thus lower feminization, may explain the acquisition of a male gender identity in female-to-male transexuals.

6. ERs and the aging brain

Estrogens have been recognized as potent neuroprotective agents. Estrogens, via their ERs, mediate gene transcription. The participation of ERs in regulating differentiation, proliferation and inflammation of neurons and synapses is fundamental to general physiology. The levels of sex hormones decrease with age, although this decrease is more pronounced during the menopause in women (96). The levels of ERs in the brain decrease just after birth and their actions in the CNS become slowly restricted to different regions of the brain. ERα is expressed in the amygdala and the hypothalamus, whereas ERβ is distributed in the cerebral cortex in the cerebellum, hippocampus, periventricular nuclei, bed nucleus of the stria terminalis, substantia nigra, ventral tegmental area and the dorsal raphe nucleus (80,97-99). Brain-derived neurotrophic factor (BDNF), found in the brain, regulates synaptic genesis and development (60,80). BDNF protein levels increase following ERβ activation in postmenopausal mice, which serves a crucial role in promoting the survival and differentiation of neurons (60). Both ERα and in particular ERβ, contribute to neuroprotection via the striatum in the basal ganglia of the brain, which regulates muscle tone and coordinates motor ability (99). Hippocampal GPER1 also contributes to estrogen-dependent conciliation memory via the regulation of hippocampal synaptic plasticity and neuroprotection. BDNF expression induced by selective GPER1 activation via the E2-PI3K/Akt and E2-MAPK signaling pathways promotes synaptic plasticity, exerting a neuroprotective effect (99).

Behavioral manifestations such as emotion and cognition are controlled by sex steroid hormones and have been studied by functional magnetic resonance imaging (fMRI). During the follicular and luteal phases in healthy women with a normal menstrual cycle, fMRI demonstrated differences in several areas of the brain, such as the amygdala, anterior hippocampus and several cortical regions (100). The role of ERs in neurodevelopment is vital in the adult brain, in addition to their more established roles in sexual behavior and reproduction.

During aging, several modifications and adjustments to tissues in the brain occur that result in adverse events. The levels of both ERα and ERβ, found in synapses of the cornu ammonis neurons in the hippocampus of female rats, decrease as the animal ages (101). As the expression of ERs is associated with neuroprotection, this level of neuroprotection may decrease as an individual ages, particularly during pre-menopause and menopause, increasing the risk of adverse neurological events in women, whereas men, who exhibit a gradual slow decrease in testosterone levels, may experience longer neuroprotective effects (102). Complete loss of estrogen during menopause is associated with neurological changes, since perimenopausal females have an increased risk of depression that is reduced when they receive treatment with estrogens (102,103).

Menopausal women demonstrate a decrease in cognitive function and in both verbal and working memory. According to the Women's Health Initiative Memory Study, the incidence of memory and cognitive impairment in menopausal women was 4.5% (104). Moreover, there is evidence of changes in the cognitive test performance of women that are consistently related to the reproductive period and menopausal transition. Population-based studies reported an increased risk of dementia by up to 23% in females with late menarche, early menopause and a short reproductive period (96,105). A recent study investigated the factors associated with a decrease in cognition and found a synergistic association with an early age of menopause and vascular risk impacting the cognitive function in menopausal women for >3 years of follow-up after menopause (106). The same study reported that hormone treatment with estrogens attenuated this association to preserve cognition (106). Conversely, an age-related shift in ERα or ERβ may underlie a decreased response to E2, suggesting that estrogen treatment may not always lead to cognitive preservation. The effects of ERα or ERβ on cognitive function are dose-dependent (107). Furthermore, hormone replacement will interact with locally produced E2, such that the effective dose of E2 to optimize hippocampal function will depend on local E2 and the relative expression of functional ERα and Erβ (96).

Neurodegenerative diseases, such as Alzheimer's disease (AD), are associated with estrogen depletion. A higher prevalence of AD and a more rapid cognitive decline were reported in women compared with men (106). Furthermore, a meta-analysis reported that estrogen replacement treatment could benefit postmenopausal women with neurodegenerative disease (108). Numerous studies have reported the neuroprotective function of estrogens, including acting as antioxidants, enabling DNA repair, inducing growth factor expression and regulating cerebral blood flow. Parkinson's disease (PD) affects motor skills, followed by cognitive impairment. Women with lower estrogen levels are more likely to develop PD compared with those with higher estrogen levels (60,108). The role of estrogen-based treatments in this group of women for the

prevention of dementia, loss of motor ability and depression is still under evaluation.

7. Conclusions and future perspectives

Undoubtedly, an understanding of the role of ERs in different tissues, the estrogen signaling mechanisms and the interactions between the three ERs (ERα, Erβ and GPER1) is required for the development of novel therapeutics. Bridging the gap between animal and human studies may open future research avenues for the development of novel treatments for ER-related diseases. Notably, ER signaling members in cancer may be targets for next-generation ER-targeted treatments. The rising incidence of breast cancer and also other types of cancer, related to ER pathology, is a hallmark for the need for more targeted treatments focused on ERs. Existing hormonal auxiliary treatments for osteoporosis are promising. However, the association between hormone replacement therapy and the development of breast cancer highlights the need for more targeted treatments focusing on ER signaling pathways. Although notable advancements have been made regarding sex hormone signaling in the brain, a deeper understanding of the spatiotemporal expression and ER signaling in the brain is still required. Further research on the physiological and pathophysiological aging of the brain and the differences between sexes observed in these processes is required. *In vivo* and integrated *in vitro* studies assessing the neuroprotective effects of estrogen highlight ERs as potential therapeutic targets in neurodegenerative diseases and aging.

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MT wrote, edited and reviewed the article. AK and KP wrote the structure, location, function of ERs sections of the article. NS wrote, edited, reviewed and supervised the writing of the manuscript. Data authentication is not applicable.

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Competing interests

The authors declare that they have no competing interests.

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