Reproductive Sciences
2017, Vol. 24(9) 1235-1244

DOI: 10.1177/1933719116678686
journals.sagepub.com/home/rsx
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Estrogen Receptors and Signaling in Fibroids: Role in Pathobiology and Therapeutic Implications

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Abstract

Uterine fibroids are the most common gynecologic tumors with a significant medical and financial burden. Several genetic, hormonal, and biological factors have been shown to contribute to the development and growth of fibroid tumors. Of these factors, estrogen is particularly critical since fibroids are considered estrogen dependent because no prepubertal cases have been described in the literature and tumors tend to regress after menopause. Understanding the role of estrogen in fibroids is not only important for understanding the pathobiology of fibroids but also for the development of successful therapeutics. In this review, we discuss the types and structure of estrogen receptors (nuclear and membrane bound, including α and β receptors and G protein-coupled estrogen receptor 1 GPER1). Estrogen-signaling pathways in fibroids include genomic (direct and indirect) and nongenomic including Ras-Raf-MEK (MAPK/Erk Kinase)-mitogen-activated protein kinase (MAPK) and phosphatidylinositide 3-kinase (PI3K)-phosphatidylinositol-3,4,5-trisphosphate (PIP3)-Akt (Protein kinase B)-mammalian target of rapamycin (mTOR) pathways; shortly Ras-Raf-MEK-MAPK and PI3K-PIP3-Akt-mTOR pathways. Several aberrations in estrogen receptors and signaling pathways are implicated in fibroid pathobiology. Current therapeutic and research agents targeting ERs/signaling include gonadotropin-releasing hormone (GnRH) agonists, GnRH antagonists, aromatase inhibitors, selective ER modulators, gene therapy, and others. Future research can identify potential targets for the development of novel treatments. In particular, epigenomics of estrogen activity and individualized (precision) medicine appear to be attractive areas for future research.

Keywords

uterine leiomyoma, fibroid, estrogen, estrogen receptor, signaling, treatment

Introduction

Uterine fibroids, also called leiomyomas, are the most common gynecologic tumors. A fibroid tumor starts as a monoclonal proliferation of a single uterine smooth muscle cell.[1,2] The underlying processes driving the transformation of a myocyte into a uterine fibroid are not completely understood. However, several genetic, hormonal, and biologic factors have been shown to contribute to the development, growth, and maintenance of uterine fibroids. These factors include steroid hormones, growth factors, cytokines, chromosomal, genetic, and epigenetic aberrations.[3-6]

Estrogens, cholesterol-derived steroid hormones, play a vital role in the female reproductive physiology in addition to their pleiotropic effects on other body systems. Fibroids are considered estrogen dependent since no prepubertal cases have been described, and tumors tend to regress after menopause and on gonadotropin-releasing hormone agonists (GnRHa) treatment, which decreases ovarian estrogen production.[5-8] The role of estrogen in fibroid biology is complex and involves several other factors, including progesterone, growth factors, and genetic and epigenetic factors. In addition, the necessity of estrogen for leiomyoma growth represents a unique therapeutic opportunity through targeting estrogen receptors, signaling pathways, and estrogen response genes. This review discusses the types and structure of estrogen receptors and signaling in fibroids, the current therapeutic options targeting estrogen receptors/signaling, and future research directions.

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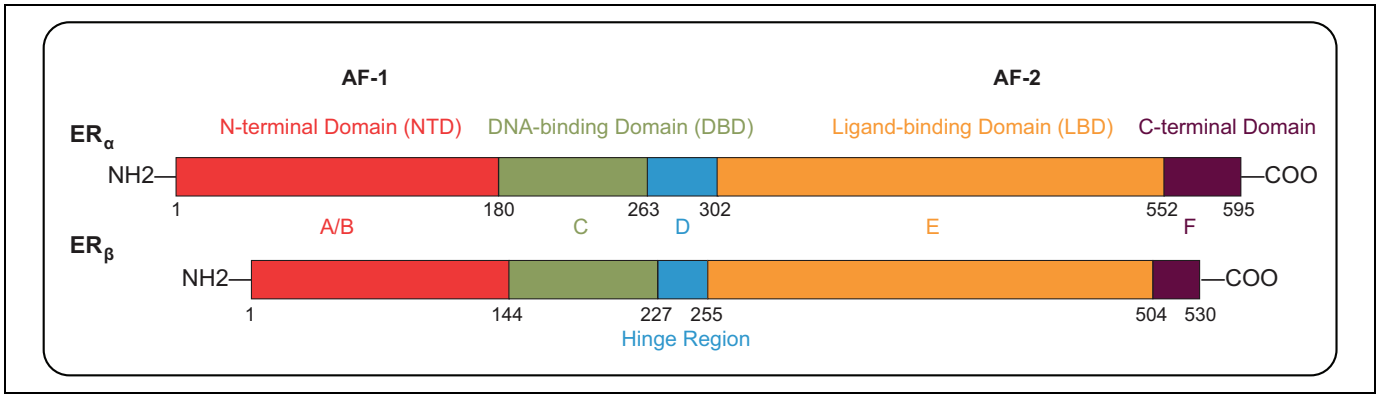

Figure 1. Structure of estrogen receptor α (top) and β (bottom). The sequence of the 5 domains is illustrated in different colors with amino acid numbers. DBD indicates DNA-binding domain; LBD, ligand-binding domain; NTD, *N*-terminal transactivation domain.

Methods

To prepare for this study, we searched PubMed and Google Scholar for relevant publications through 2016. We used key words, including fibroid, leiomyoma, estrogen, estrogen receptors, estrogen signaling, treatment, and therapeutics. We included cellular, molecular, animal, epidemiologic studies in addition to clinical trials and review articles. Because of the nature of many studies (particularly basic research), this is not considered a systematic review as defined by the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement, which primarily focuses on randomized trials and other clinical interventional studies.[9] We included about 100 references. Key information was obtained from these publications and appropriately incorporated and cited in this work.

Types and Structure of Estrogen Receptors

Estrogen exerts its effects on target cells by binding to its receptors, currently classified as nuclear and membrane bound.[10]

Nuclear Estrogen Receptors

Nuclear ERs are members of the nuclear hormone receptor superfamily; they serve as ligand-activated transcription factors (TFs) regulating gene expression. They are modular proteins composed of 5 domains (Figure 1). The *N*-terminal transactivation domain, also called the A/B domain, includes the activation function 1 domain, which is thought to bind to the transcription complexes. It is also responsible for several protein–protein interactions. The C domain is the DNA-binding domain (DBD) that binds to specific DNA sites called estrogen response elements (EREs). It is also involved in receptor dimerization and is the most conserved region of the receptor. The D domain, also known as the hinge region, is the flexible connection between the DBD and ligand-binding domain (LBD). It contains a nuclear localization sequence that gets activated upon ligand binding. It is also the site of certain posttranslational modifications. The E domain encompasses several regions, including the LBD, a dimerization domain, and a part of the nuclear localization region. The E-domain also contains the activation function 2 and is the site of binding to coactivators and corepressors. The carboxyl terminal domain is called the F domain, and it protects the receptor against improper ligand activation.[11-14]

Nuclear ERs are subdivided into ERα and ERβ, which are encoded by 2 distinct genes located on chromosome 6 (*ESR1*) and 14 (*ESR2*), respectively.[8,15,16] Furthermore, they have different transcriptional activation domains, diverse tissue distribution, and multiple splice variants.[17,18] Even though they exhibit different expression patterns and posttranslational modifications, depending on physiologic and pathologic conditions, they possess significant homology in the DBD and LBD. The shared sequence homology is approximately 97% in the DNA-binding regions and 59% in the ligand-binding region.[12,19] Both receptors are coexpressed at certain levels in a variety of tissues, but uterine tissue mainly expresses ERα.[20] These differences explain why estrogen exerts pleiotropic effects on tissues. 17β-Estradiol (E_2) is the fundamental ligand that activates ERα and ERβ. 17β-Estradiol is thought to induce proliferation of uterine tissue through ERα.[11] Therefore, antiestrogen drugs, such as GnRHa, aromatase inhibitors, and selective ER modulators (SERMs), may prevent estrogenic effects on estrogen-sensitive tissues.

Membrane-Bound Estrogen Receptors

Membrane-bound ERs (mERs) are localized at the plasma membrane and exhibit considerable similarities to the nuclear ERs. In fact, there is evidence that both nuclear and membrane ERs derive from the same transcript.[10] The cellular content of mER is only a fraction (3%-10%) of the nuclear receptors.[21,22] The mERs are located in small invaginations of the plasma membrane called caveolae that act like signaling hubs. Importantly, mERs require posttranslational modifications to be anchored to the plasma membrane including the attachment of fatty acid residues catalyzed by enzymes such as palmitoylacyltransferases.[23,24]

G protein-coupled estrogen receptor 1 (GPER1), also known as G protein-coupled receptor 30 (GPR30), is a 7-transmembrane

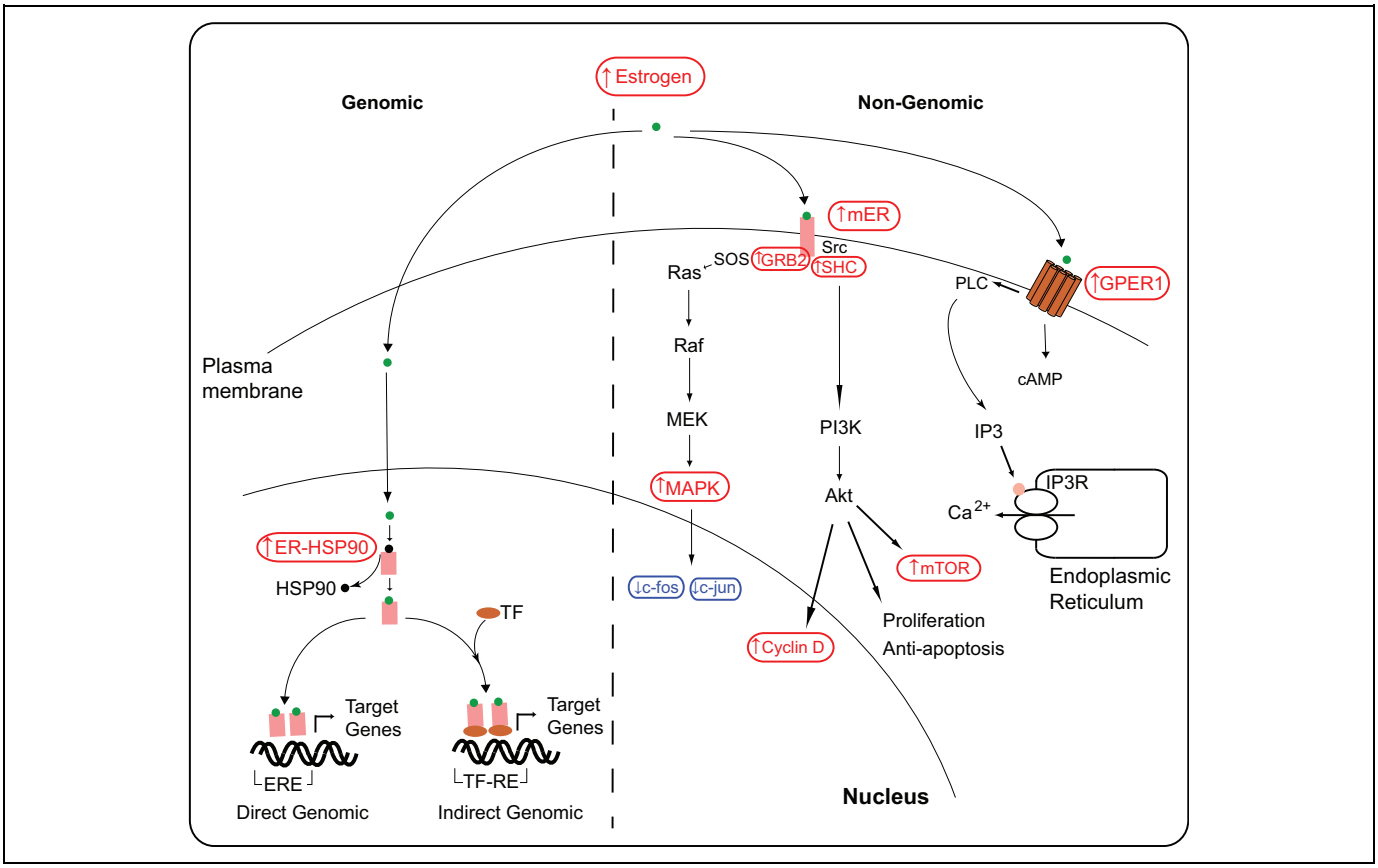

Figure 2. Estrogen pathways in uterine leiomyoma cells, including genomic and nongenomic pathways. ↑ and ↓ denote increased (red) or decreased (blue) levels and/or function, respectively. ER indicates estrogen receptor; ERE, estrogen response element; GPER1, G protein-coupled ER 1; HSP90, heat shock protein 90; IP3, inositol triphosphate; IP3R, inositol triphosphate receptor; mER, membrane-bound ER; PLC, phospholipase C; TF, transcription factor; TF-RE, transcription factor response element. (The color version of this figure is available online.)

receptor that is structurally unrelated to the nuclear ERs. It is controversial whether it is localized at the plasma membrane[25] or at the endoplasmic reticulum.[26] It is encoded by the *GPER* gene located on chromosome 7,[27] and its expression is genetically independent of other ERs. Finally, it displays more rapid estrogen response when compared with nuclear ERs.[27-29]

Estrogen Signaling Pathways

Estrogen-dependent signaling pathways can be classified as genomic and nongenomic. While genomic pathways depend on modulation of transcriptional activities through gene expression, nongenomic pathways are typically mediated through rapid activation of signaling cascades.[14,30] Figure 2 illustrates different estrogen-signaling pathways and their effects in fibroids.

In the direct genomic pathway, estrogen–ER complexes directly bind to regulatory regions of target genes to modulate gene expression.[31] Unbound receptors are attached to a molecular chaperone known as heat shock protein 90 (HSP90) that protects these receptors from degradation. It also helps maintain high-affinity hormone-binding conformation.[32,33] After estrogen binds to ER, HSP90 dissociates. Then, receptor dimerization and conformational changes allow ER to bind to EREs located within the regulatory region of target genes.[31] Afterward, several coregulator proteins, such as steroid receptor coactivator 1, are attached to the complex to facilitate transcriptional processes.[34]

In the indirect genomic pathway, ligand–ER complexes do not directly bind to DNA. Instead, they bind to certain DNA-binding TF through protein–protein interaction. Therefore, in this situation, DNA response elements' consensus sequences of estrogen-responsive genes are TF response elements rather than EREs.[30,35] Thus, estrogen can change the expression of genes that do not have an ERE-like region in their promoter region. The net result may be the activation or repression of target gene expression in estrogen-sensitive tissue. These TF include specificity protein 1, nuclear factor–κB, CCAAT/enhancer-binding protein β, GATA binding protein 1, and signal transducer and activator of transcription 5.[36,37]

In the nongenomic pathway, estrogen binds to ER (mER, GPER1, and some subtypes of nuclear ERα and ERβ) to rapidly modulate signaling pathways.[27] Ligand–ER complexes mostly activate protein kinase pathways, including mitogen-activated protein kinase (MAPK) through the Ras–Raf–MEK–MAPK pathway and phosphatidylinositide 3-kinases

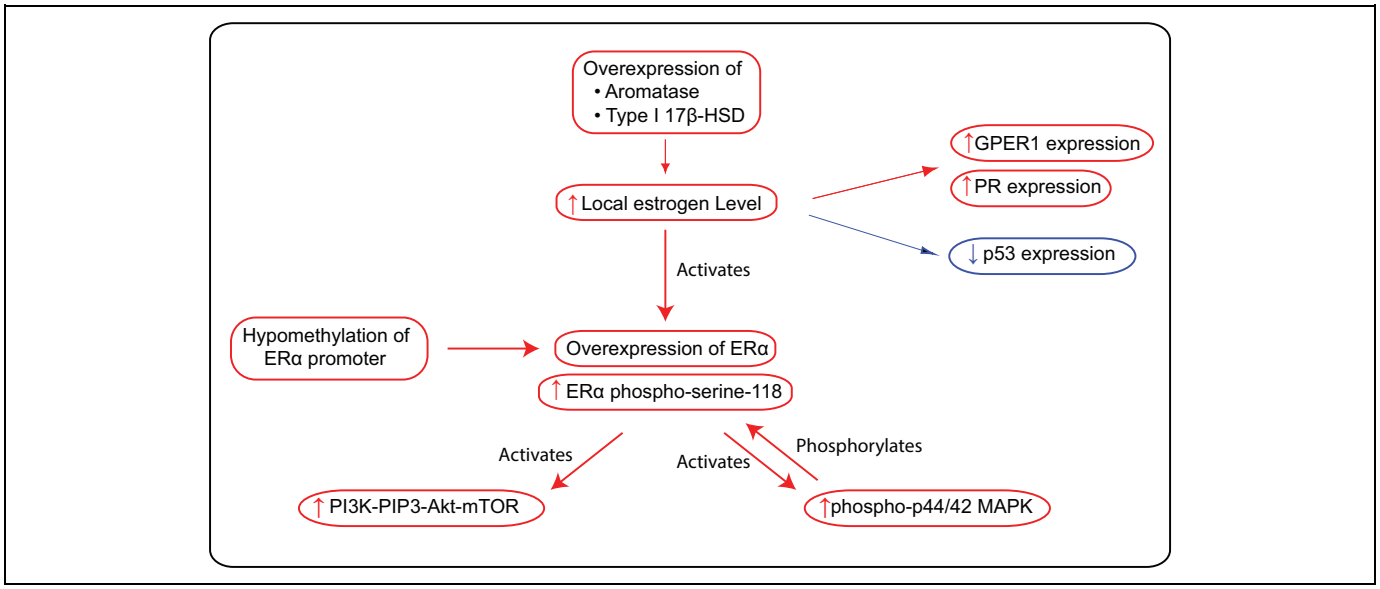

Figure 3. Aberrations in estrogen pathways in uterine leiomyoma cells. ↑ and ↓ denote increased (red) or decreased (blue) levels and/or function, respectively. ER indicates estrogen receptor; GPER1, G protein-coupled ER 1; 17β-HSD, 17β-hydroxysteroid dehydrogenase; PR, progesterone receptor. (The color version of this figure is available online.)

(PI3K)–Akt through the PI3K–phosphatidylinositol-3,4,5-trisphosphate (PIP3)–Akt–mammalian target of rapamycin (mTOR) pathway. Subsequently, these pathways can indirectly modulate the expression of certain genes.[27,30]

In the Ras–Raf–MEK–MAPK pathway, the binding of estrogen to receptors initiates a cascade of molecular events, which include the activation of the small guanine nucleotide-binding protein (G protein) Ras through substitution of guanosine diphosphate by guanosine-5′-triphosphate. Ras activation is followed by Raf activation, which subsequently phosphorylates (and activates) MEK protein. In turn, MAPK is phosphorylated (and activated), which then leads to the activation of several TFs of the activating protein 1 family, including c-Fos and c-Jun. This process regulates transcription of target genes. The Ras–Raf–MEK–MAPK pathway regulates several cellular processes, including proliferation, survival, and apoptosis.[14,38,39]

The PI3K–PIP3–Akt–mTOR pathway can be activated by both mERs and GPER1. In this pathway, estrogen binding to receptors leads to the activation of PI3K, which in turn phosphorylates the plasma membrane lipid phosphatidylinositol-4,5-bisphosphate to PIP3. In turn, this process leads to the recruitment and activation of Akt proteins, which regulate the mTOR, glycogen synthase kinase 3, and other proteins and TFs. Of note, the tumor suppressor phosphatase and tensin homolog (PTEN) inactivates PIP3 by dephosphorylation at carbon 3. This pathway regulates important processes, including cell cycle, proliferation, and survival.[14,40] From the above discussion, it is evident that a rapid nongenomic signaling pathway works in a similar manner to growth factor signaling. Interestingly, there is evidence of cross talk between rapid estrogen signaling and growth factor signaling through receptor tyrosine kinases.[27,30]

G protein-coupled estrogen receptor 1 (GPER1, also known as GPR30), similar to other G protein-coupled receptors, works as a guanine nucleotide exchange factor. G protein-coupled ER 1 activates 2 main signal transduction pathways. First, it activates adenylate cyclase enzyme, which generates cyclic adenosine monophosphate. Second, it activates the phospholipase C (PLC) enzyme, which generates inositol trisphosphate (IP3), and IP3 releases calcium from the endoplasmic reticulum to the cytosol.[28,41,42] In addition, activation of the G protein of GPER1 leads to Src phosphorylation, which induces matrix metalloproteinase (MMP) activation. Matrix metalloproteinase cleaves pro-heparin-binding epidermal growth factor (pro-HB-EGF) releasing free heparin-binding epidermal growth factor-like growth factor (HB-EGF) which, in turn, binds to and activate epidermal growth factor receptors (EGFRs).[28,43] This transactivation represents an important integrator of estrogen and growth factor cellular signaling.

Although nuclear receptors are generally involved in transcriptional activity, membrane receptors are more commonly involved in rapid signaling. However, there is evidence that both receptor categories are capable of rapid signaling as well as transcriptional activity modulation.[28]

Estrogen Receptors and Signaling in Fibroid Pathobiology

Clinical and laboratory studies support the essential role of estrogen in the development and growth of fibroids.[44-46] The complexity of estrogen-responsive pathways in fibroids and their interactions with several other etiologic factors is mirrored by a constellation of estrogen-related aberrations in fibroids. Figure 3 shows a simplified diagrammatic presentation of estrogen-signaling aberrations. Although plasma

estrogen levels are similar in women with and without fibroids, tissue levels are higher in women with fibroids.[47] This correlation illustrates the role of local fibroid aromatase activity in converting androstenedione to estrone, which is then converted to estradiol by 17β-hydroxysteroid dehydrogenase (17β-HSD). Fibroid tissue was found to overexpress both aromatase and 17β-HSD type 1 compared to normal myometrium.[48-50] Therefore, it is not surprising that aromatase inhibitors were shown to inhibit proliferation of leiomyoma cells in experiments and to reduce tumor size in clinical trials.[45,46,51]

One of the major roles of estrogen in fibroid development appears to be the induction of progesterone receptor expression through ERα, rendering tumorigenic tissue more responsive to progesterone signals.[52] Using a xenograft fibroid animal model, Ishikawa and colleagues showed that estrogen provides a microenvironment in which progesterone can induce fibroid growth.[53]

Several studies demonstrated that the levels of ERα and ERβ messenger RNA (mRNA) transcripts are higher in fibroid cells compared to normal myometrium.[54,55] More recently, Tian and colleagues found that GPER1 expression is higher in fibroids compared to matched endometrium.[56] They also found that E_2 increases the levels of GPER1 mRNA in fibroid cells but decreased it in myometrial cells. This discrepancy represents an example of perturbed responsiveness of leiomyoma cells and how it can contribute to leiomyomatogenesis.

In addition to abnormal expression of ERs, there is evidence of aberrant receptor phosphorylation in fibroids. Hermon and colleagues demonstrated that the ERα phospho-serine-118 level is higher in fibroids compared to normal myometrium. They also suggested that ERα is phosphorylated by phospho-p44/42 MAPK.[57] This proposed feedback by MAPK on ERs represents an example of the complexity and interconnectedness of signaling pathways in fibroids. Estrogens also regulate the expression of several genes, including c-Fos and c-Jun, connexin 43, progesterone receptor, insulin-like growth factor 1, and insulin-like growth factor receptors.[58-61] Although estrogen upregulates the expression of platelet-derived growth factor and EGFR, it downregulates the expression of EGF.[62-64] Estrogen was also shown to inhibit tumor suppressor p53 expression, which can partly contribute to leiomyoma growth.[65]

There is also evidence of aberrant rapid estrogen signaling in leiomyoma. Nierth-Simpson and colleagues found that estrogen stimulates protein kinase Cα within a few minutes in both fibroid and myometrial cells. Remarkably, they found a divergence in the effect on MAPK phosphorylation in which estrogen increases phosphorylated MAPK in fibroid cells but not in myometrial cells. This differential effect can be a contributing factor in fibroid pathobiology.[66] In addition, Yu and colleagues found that several molecules of the Ras–Raf–MEK–MAPK pathway are overexpressed in fibroids compared to myometrium.[67] Also, 2 independent groups (Lessl and colleagues and Gustavsson and colleagues) found that mRNA transcript levels of c-Fos and c-Jun (members of the downstream effectors of the MAPK pathway) are lower in fibroids than in myometriums.[60,68] In addition, there is evidence suggesting that the aberrant PI3K–PIP3–Akt–mTOR pathway can be implicated in fibroid pathogenesis. For example, Crabtree and colleagues described the upregulation of mTOR signaling in fibroids in humans and animal models.[69] In addition, another group discovered a higher expression of glycogen synthesis kinase-3 and cyclin D_2 in fibroids than in myometriums.[70]

Finally, there is evidence describing epigenomic aberrations affecting ERs and signaling in fibroids. Asada and colleagues described promoter hypomethylation of ERα, typically overexpressed in fibroids.[71] Using sodium bisulfite sequencing and bisulfite restriction mapping, they identified 7 CpG sites in the promoter of ERα gene that were hypomethylated in fibroids compared to myometriums. In addition, they described a correspondingly higher level of ERα mRNA in fibroid tissue. Another study by Maekawa and colleagues described an additional epigenetic aberration in fibroids. Using genome-wide DNA methylation analysis, they identified aberrant DNA methylation and aberrant mRNA levels of 22 target genes of ERα in fibroid tissues compared to myometrium. These genes have the consensus sequence of EREs. Importantly, some of the identified genes control cellular processes, including apoptosis and cellular senescence, while others are tumor suppressors. These findings can provide insight into tumor transformation mechanisms in fibroids and how they are closely linked to estrogen signaling.[72]

Therapeutic Targeting of Estrogen Receptors/Signaling in Fibroids

The critical role of estrogen in fibroid pathobiology makes it a logical therapeutic target through modulating estrogen levels, ERs, and estrogen-signaling pathways.

Gonadotropin-Releasing Hormone Agonists (GnRHa)

Gonadotropin-releasing hormone agonists are synthetic peptides that are structurally similar to the natural GnRH molecule. Binding of GnRHa to receptors is followed by an initial increase in gonadotropin secretion. However, continuous treatment is followed by receptor desensitization and a decrease in the gonadotropin output. As a result, the pituitary–ovarian axis is suppressed and a hypoestrogenic state is created with estrogen and progesterone, as low as menopausal levels.[73] In addition, GnRHa were demonstrated to have direct effects on fibroid cells, including inhibition of the expression of the vascular endothelial growth factor, fibroblastic growth factor, and platelet-derived growth factor.[74]

A study conducted by Wang and colleagues demonstrated that GnRHa directly inhibit proliferation and induce apoptosis of human leiomyoma cells.[75] In addition, Chegini and Kornberg showed that GnRHa therapy led to a decrease in Extracellular Signal-Regulated Kinase (ERK), focal adhesion kinase (FAK), and pERK1/2 expression in both leiomyoma and myometrium.[76]

Clinical studies have shown that GnRHa substantially reduce tumor volume, bleeding, and volume-related symptoms.[8,77]

However, due to significant side effects, such as loss of bone mineral density, they can be used only for a short period. Typically, they are used to transiently improve symptoms and reduce tumor size preoperatively.

Gonadotropin-Releasing Hormone Antagonists

Unlike GnRHa, GnRH antagonists directly inhibit GnRH receptors without the initial stimulatory phase. Therefore, the end result is similar to that of long-term GnRHa administration. Even though some observational studies showed that GnRH antagonists reduce tumor volume,[78,79] no randomized controlled studies have been reported.

Aromatase Inhibitors

Estrogen is locally produced by leiomyoma cells through aromatase activity. However, mechanisms underlying the gonadotropin-independent expression of aromatase in fibroid tissue are not completely understood.[3,80] It is reported that fibroid tissue contains high levels of aromatase, which results in higher levels of estrogen compared to adjacent myometrium.[46] Moreover, it has been demonstrated that aromatase inhibitors are able to shrink fibroid volume, thus suggesting that the aromatase activity and the in situ estrogen production in these benign tumors are key mechanisms in hormone-dependent fibroid growth.[3] However, this mechanism of estrogen deprivation has negative systemic side effects, such as hot flushes, amnesia, and osteoporosis, which restrict long-term regimens.[81]

As described before, because fibroid cells have intrinsic aromatase enzyme activity independent of the ovaries, aromatase enzyme has been proposed as a logical target in the treatment of fibroids. Several clinical trials have demonstrated that aromatase inhibitors reduce both leiomyoma volume and related symptoms.[51,82,83,84] A study conducted by Duhan and colleagues showed that a 3-month treatment with letrozole, an aromatase activity inhibitor, was as effective as GnRH agonists. Moreover, letrozole was associated with fewer side effects, such as the initial flare-up and menopausal symptoms.[51] It is thought that aromatase inhibitors lead to complete cessation of estrogen synthesis in the fibroid tissue, while it is only partially inhibited in the ovaries.[85] Therefore, aromatase inhibitors represent a promising therapeutic option, but further studies are needed.

Selective Estrogen Receptor Modulators

Selective ER modulators are nonsteroidal ER ligands with mixed agonist and antagonist activity. Their effects on different estrogen-sensitive tissues, such as breast[86,87] and uterus,[73] are organ specific, which allows selective stimulation or inhibition of estrogen-like action in specific organs.

The mixed agonism/antagonism of SERMs may be explained by 3 interrelated mechanisms: ERα/ERβ expression ratio of a particular tissue, the ratio of coactivator to corepressor proteins, and the type of ER conformational changes induced by drug binding, which in turn determines how strongly the ligand–receptor complex recruits coactivators (resulting in an agonist response) relative to corepressors (resulting in antagonism).[88]

The main agents in this group are tamoxifen, commonly used in the treatment of breast cancer, and raloxifene, used as an antiresorptive drug in the treatment of osteoporosis.[89] Other members include afimoxifene,[90] arzoxifene,[91] and bazedoxifene.[92]

Due to their efficacy as antiestrogenic agents in breast cancer, SERMs have been evaluated as potential therapeutics in fibroids. However, tamoxifen failed to reduce tumor size. Even worse, due to its agonistic effects on endometrial ERs, it carries the risk of developing endometrial pathology, especially in premenopausal women.[89,93,94] In addition, tamoxifen has stimulatory effects on the ovaries because it increases the level of gonadotropins, which may result in ovarian cysts.[81,95] On the other hand, raloxifene has a more favorable profile by reducing in vitro cell proliferation and by upregulating apoptosis.[96-98] Moreover, it is a more pure uterine antagonist since it has no endometrial agonist activity.[87] However, raloxifene exhibits a suboptimum reduction in leiomyoma size in postmenopausal women[99,100] and possesses poor pharmacokinetic properties.[81] Randomized controlled trials in premenopausal women with fibroids were inconclusive.[101] For these reasons, clinical efficacy of raloxifene in fibroids is also considered to be limited.

To date, the few available clinical trials show insufficient evidence for the efficacy of SERM on fibroid size reduction or symptom improvement. Moreover, due to the adverse reactions, the safety of SERM is uncertain, and it should be used with caution in women with symptomatic uterine fibroids.

Gene Therapy

Gene therapy targeting ERs represents an intriguing strategy for fibroid therapy.[102,103] Al-Hendy and colleagues used adenovirus with a dominant-negative ER to inhibit downstream estrogen signaling and found that this inhibits proliferation and induces apoptosis in a cell line; and induces tumor growth arrest in a nude mouse animal model.[103]

Others

2-Methoxyestradiol (2ME) is an endogenous metabolite of E_2. It is formed by methylation of 2-hydroxyestradiol by the catechol-O-methyltransferase (COMT) enzyme. Salama and colleagues demonstrated that it induces apoptosis and inhibits cell proliferation and collagen biosynthesis in human and rat leiomyoma cells.[104] Another study by the same group showed that 2ME treatment as well as COMT enzyme overexpression inhibit proliferation of human leiomyoma cells. They showed the likelihood of this effect by modifying microtubule dynamics and estrogen signaling. Specifically, they proposed

that it inhibits nuclear shuttling of ERs. They concluded that 2ME is a promising therapeutic agent in the treatment of fibroids.[104,105]

Conclusions and Future Directions

Estrogen-related pathways in fibroids demonstrate 2 basic characteristics: complexity and connectedness. The previous brief discussion demonstrates the high degree of complexity of the involvement of different types of receptors (nuclear vs membrane bound and α vs β), different signaling effects (genomic vs nongenomic), and activation of several signaling pathways (Ras/Raf/MEK/ERK, PI3K/Akt/mTOR, and PLC/IP3/calcium). This complexity presents challenges in which several aberrations at different times can be operational but can also be seen as opportunities for the development of several therapeutic targets.

Connectedness, the second characteristic, is also evident in the cross talk between estrogen and growth factor-induced pathway, the interaction between estrogen stimulation and progesterone-responsiveness, and the impact of epigenomic aberrations on ERs and signaling. Therefore, a primary abnormality can lead to derangement in several pathways, with potentially amplifying cascades.

The combination of complexity and connectedness of estrogen-related pathways in fibroids presents an opportunity to improve the efficacy of current therapeutics through dual- or even multitargeting. These approaches are currently being used in cancer therapy, and it seems reasonable to assume that they can be beneficial in fibroids, given their complex pathobiologic processes.

To better understand the underlying processes driving the development and growth of fibroids, genomics and epigenomics are potential areas for further research. The recently available high-throughput studies, including whole genome sequencing, provide powerful tools to further enrich our understanding of fibroids. As demonstrated in our discussion, certain epigenetic aberrations affect ERs and signaling in fibroids. Given that these tools have been available for a short period of time, it is expected that more aberrations can be discovered, which can be useful in developing new therapeutic modalities.

Finally, as evident from the previous discussion, different patients may have varying aberrations and, therefore, may respond differently to therapeutics with different targets. Therefore, it is conceivable that treatment needs to be individualized to patients, according to their specific etiologic aberrations. Thus, individualized medicine (or precision medicine) can be tailored to patient-specific disease processes and driven by specific markers.

Authors' Note

The sponsor had no involvement in the study design; collection, analysis, and interpretation of the data; writing of the report; or decision to submit the article for publication.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: This work was supported by an award to Mostafa Borahay from the Institute for Translational Sciences at the University of Texas Medical Branch supported in part by NCATS [NIH Grant CTSA UL1TR000071].

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