Tissue-Specific Regulation of Genes by Estrogen Receptors

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Semin Reprod Med 2012;30:14–22.

Abstract

Estrogens are frequently used in reproductive medicine. The Women's Health Initiative trial found that the risks of menopausal hormone therapy (MHT) exceed the benefits. The estrogens in MHT, however, were introduced prior to our understanding of the mechanism of action of estrogens. Estrogen signaling is highly complex, involving various DNA regulatory elements to which estrogen receptors bind. Numerous transcription factors and co-regulatory proteins modify chromatin structure to further regulate gene transcription. With a greater understanding of estrogen action, the major problem with the current estrogens in MHT appears to be that they are nonselective. This produces beneficial effects in bone, brain, and adipose tissue but increases the risk of breast and endometrial cancer and thromboembolism. Resurrecting MHT for long-term therapy will require the development of more selective estrogens, such as estrogen receptor (ER)β-selective estrogens and tissue-selective ERα agonists. These compounds will offer the best prospects to expand the indications of MHT and thus prevent the chronic conditions associated with menopause.

Keywords
- estrogen
- estradiol
- estrogen receptor
- gene regulation
- transcription

Drugs that interact with estrogen receptors (ERs) are commonly prescribed in reproductive medicine. Estrogens are used in contraceptives, fertility regimens, and menopausal hormone therapy (MHT). Whereas the therapeutic benefits of estrogens in contraceptives and fertility treatment are clear, the use of estrogens in MHT has become increasingly controversial with the findings of the Women's Health Initiative (WHI) trial.[1–3] Prior to the WHI, MHT was the second most frequently prescribed medication in the United States. Since the publication of the results of the WHI in 2002, the number of women using MHT has declined dramatically.[4] Discovering safer estrogens could resurrect MHT for its classic indications and offer a new therapeutic strategy for other conditions associated with menopause.

Postmenopausal women have been treated with estrogens for symptoms such as hot flashes and urogenital atrophy for ~70 years. More recently, MHT was used to prevent osteoporosis, fractures, and cardiovascular disease. The WHI was the first large randomized clinical trial to assess the risks and benefits of MHT.[1,3] The conclusion from the WHI was that the risks exceed the benefits, with an increase in breast cancer that was the most troublesome finding, particularly because there were no protective effects on heart disease. Whereas the results of the WHI remain controversial due to the design of the trial that enrolled subjects several years after onset of menopause (average age was 63 years), there were several clear outcomes. First, the combination of estrogens and progestins is more detrimental than estrogen treatment alone. Progestins were initially added to block the

Issue Theme The Important Role of Estrogen Receptor-β in Women's Health; Guest Editor, Emily J. Su, M.D., M.S.

DOI http://dx.doi.org/10.1055/s-0031-1299593.
ISSN 1526-8004.

proliferative effect of estrogens in the uterus because unopposed estrogen increases the risk of endometrial cancer. The WHI made it clear that progestins abolish the negative effects in the endometrium, but this comes at the expense of increasing the risk of breast cancer and heart disease.[1,5] Second, estrogens in current MHT regimens should be used mainly as a short-term treatment of menopausal symptoms such as hot flashes, night sweats, and urogenital atrophy in patients for whom the benefits outweigh the risks.

Although the restricted use of MHT minimizes the risk of adverse effects, it might be possible to expand the clinical indications to more chronic conditions if estrogens can be developed that can be used safely for longer periods. Most MHT regimens contain estradiol (E2), estrone, or equilin. The major problem with these estrogens is they act nonselectively in all tissues containing ERs. The nonselective action of these estrogens results from at least two major properties. First, these estrogens bind to the two estrogen receptors, ERα and ERβ, with relatively similar affinity.[6,7] Second, they act as nonselective agonists by triggering ER signaling pathways in all tissues containing ERs. The nonselective action produces beneficial effects in some tissue, such as bone, brain, and adipose tissue, and adverse effects in others, such as the mammary gland, uterus, and liver. To overcome the adverse effects of current estrogens, it will be necessary to develop selective ER agonists. A necessary step to develop these is to acquire a better understanding of the mechanisms whereby estrogens regulate gene expression through ERα and ERβ in different tissues. Here we review potential mechanisms whereby ERα and ERβ regulate genes to produce tissue-specific effects and discuss some potential new clinical indications for selective ER agonists for MHT.

Differential Expression and Roles of Estrogen Receptor-α and Estrogen Receptor-β in Tissues

Estrogen effects are mediated by ERα and ERβ.[8] One way estrogens can exert tissue-specific effects is through the differential expression of ERα and ERβ in cell types. The tissue distribution of ERα and ERβ is different,[6,9,10] and therefore it is expected that estrogens will have different effects in tissues that express predominantly ERα or ERβ. ERα is the predominant receptor in the bone, uterus, liver, and adipose tissue, whereas ERβ is the predominant receptor in the ovary and intestinal tract. The brain, mammary gland, and cardiovascular system express both ERα and ERβ, indicating that both receptors have roles in these tissues. Estrogen receptor knockout mice, as well as gene regulation and functional studies in cell lines, demonstrated that ERα and ERβ have different biological roles even if present in the same tissue. The ERα knockout (ERKO) mice have rudimentary development of the uterus and mammary gland, whereas these tissues are normal in the ERβ knockout (BERKO) mice.[11] These findings indicate that the proliferative effects of estrogens in MHT that lead to an increased risk of breast and endometrial cancer are mediated by ERα and not ERβ. Further support for different roles of ERα and ERβ in cell proliferation has been generated by studying human cancer cells lines. For example, E2 stimulates proliferation of MCF-7 breast cancer cells that contain ERα but not ERβ, demonstrating that ERα mediates cell proliferation. When ERβ is expressed along with ERα in MCF-7 or T47D breast cancer cells, the proliferative response to estrogens is lost, demonstrating that ERβ has an antiproliferative role and blocks the effects of ERα.[12,13] Antiproliferative effects of ERβ are also observed in colon cancer cells.[14] The genes regulated by ERα in MCF-7 cells are different than when ERβ is present.[12,15] In ERα expressing MCF-7 cells, E2 stimulates the production of multiple proliferative genes, including c-myc, cyclin D1, cyclin B1, and cyclin A.[12] In contrast, when MCF-7 cells express ERβ along with ERα, these cell proliferative genes are not activated. Instead, growth inhibitory genes such as p21, p27, BTG2, and GADD45A are activated, and these proteins cause MCF-7 cells to arrest in the G2 phase of the cell cycle.[12,15] These findings demonstrated that ERα mediates the proliferative effects of estrogens in breast cancer cells, and that ERβ acts as a tumor suppressor by blocking ERα-mediated effects.

Estrogen Receptor-β-Selective Agonists for Cancer Chemoprevention

The antiproliferative effects of ERβ in human cancer cell lines suggest that ERβ-selective agonists could be used for cancer chemoprevention. Multiple synthetic ERβ-selective agonists have been developed, with diarylpropionitrile (DPN) and ERB-041 the most studied.[16–18] Several plant-derived ERβ-selective agonists have also been discovered, including MF101 and liquiritigenin (LIQ).[19,20] Some ERβ-selective agonists have been tested in animal models, which demonstrated a lack of proliferative effects in the mammary gland and uterus. In rats treated with ERB-041, no proliferative effects were observed in the mammary gland.[18] When MCF-7 cells are grown in mouse xenograft models, E2 produces large tumors, but no tumor formation occurred in mice treated with MF101 or LIQ.[19,20] These studies demonstrated that ERβ agonists do not promote proliferation of normal mouse mammary epithelial and human breast cancer cells. None of the ERβ-selective agonists that were tested produced proliferative effects in mouse or rat uterus as demonstrated by no increase in uterine weight or size after treatment. The ERβ-selective drug MF101 did not cause any endometrial abnormalities in postmenopausal women treated for 12 weeks.[21] These studies indicate that ERβ agonists will not increase the risk of endometrial cancer like current estrogens in MHT. ERβ also exerts antiproliferative effects in colon cancer cells in xenograft models.[14] *Apc*$^{Min/+}$ mice have a mutation in the adenomatous polyposis coli (Apc) gene, which leads to the formation of numerous adenomatous polyps throughout the intestinal tract.[22] The ERβ agonist DPN reduces intestinal tumorigenesis in *Apc*$^{Min/+}$ mice,[23] indicating that the antitumor effect is mediated by ERβ. Several findings in humans provide evidence for an antiproliferative role for ERβ. A significant reduction in the amount of ERβ is found in breast[24] and colonic[25] cancer tumors compared with normal tissue. The WHI found that MHT decreased the risk of

colon cancer,[1] which is consistent with the observation that the colon expresses mainly ERβ. Together, these findings indicate that ERβ-selective estrogens might be useful drugs to prevent breast and colon cancer in postmenopausal women.

Tissue Selective Estrogen Receptor-α Agonists for the Prevention of Obesity, Diabetes, and Metabolic Syndrome

One important action of estrogens is its anti-inflammatory property. Estrogens repress the transcription of numerous proinflammatory cytokine genes, suggesting that safer estrogens could be used to prevent and treat inflammatory conditions associated with menopause.[26,27] The WHI reported data on four inflammatory conditions that increased during menopause: cardiovascular disease, Alzheimer's disease, osteoporosis, and type 2 diabetes. These conditions are associated with increased production of proinflammatory cytokines that contribute to the pathogenesis of these diseases. The initial WHI findings indicated that MHT increased the risk of cardiovascular disease in the total subject population that averaged 63 years of age.[1] However, in a subset of women between 50 and 59 years, there was a decrease in the incidence of cardiovascular events that was nonsignificant, possibly due to the small number of subjects in this age group.[5] MHT also increased the risk of Alzheimer's disease.[28] These studies indicate that these inflammatory conditions might not benefit from estrogens, although it remains possible that estrogens might be protective of cardiovascular disease and Alzheimer's disease if started near the onset of menopause. The WHI showed a clear benefit of MHT on preventing osteoporosis and fractures.[29,30] The main problem with using MHT for this indication is that the beneficial effects are quickly lost after stopping MHT, thus requiring long-term use that increases the risk of breast cancer and thromboembolism. The WHI found that MHT decreases the risk for type 2 diabetes.[31,32] Similarly, a reduction in type 2 diabetes by MHT was also observed in the Heart and Estrogen/Progestin Replacement Study[33] and Nurses' Health Study.[34]

The drop in estrogen levels during menopause leads to weight gain and metabolic complications.[35,36] Clinical studies found that estrogens in MHT prevent weight gain and fat redistribution to the abdomen, known as visceral fat.[37–40] The increase in visceral fat during menopause is particularly ominous because it produces proinflammatory cytokines that can lead to hypertension, lipid and cholesterol abnormalities, insulin resistance, and atherosclerosis, which together are the clinical manifestations that define the metabolic syndrome.[41,42] The incidence of metabolic syndrome in women in the United States is 21%, and a large proportion of women with metabolic syndrome are postmenopausal.[43] In fact, postmenopausal status alone is a risk factor for metabolic syndrome.[44] In the Study of Women's Health across the Nation, the incidence of metabolic syndrome increased progressively from 6 years before to 6 years after the final menstrual period.[44] The metabolic syndrome increases the risk of type 2 diabetes, heart attacks, and strokes, which are among the leading causes of death in postmenopausal women.[42] Based on these observations, if safer estrogens could be developed for long-term therapy, they could represent a new class of estrogens to prevent osteoporosis, fractures, obesity, type 2 diabetes, and metabolic syndrome in postmenopausal women.

Most evidence suggests that ERα mediates the beneficial effects of estrogens in the bone and adipose tissue. A male with a rare mutation that results in a nonfunctional ERα presented with severe osteopenia, and with hyperinsulinemia, impaired glucose tolerance, and acanthosis nigricans,[45] which are clinical manifestations of insulin resistance. Studies in rodents also indicate that ERα mediates the effects of estrogens in bone and fat tissue. Female ERKO mice have shorter and thinner bones compared with wild-type mice,[46] whereas the bones in BERKO mice are thicker with increased cortical density.[47] Henie et al showed that ERKO mice exhibit increased weight gain, visceral adiposity, and insulin levels, and impaired glucose tolerance.[48] The increase in body weight and fat content were not observed in BERKO mice, indicating that ERβ does not mediate these effects.[49] The ERα-selective agonist 4,4',4''-(4-Propyl-[1*H*]-pyrazole-1,3,5-triyl) *tris*phenol (PPT) reduces weight gain and fat accumulation in mice.[50,51] In an obese mouse model (ob/ob), E2 treatment improved glucose tolerance and insulin sensitivity, and this effect was mimicked by PPT.[52] In contrast, the ERβ agonist DPN does not alter total body weight gain in rats.[53]

Although selective ERα agonists have exciting potential to prevent postmenopausal osteoporosis, weight gain, visceral fat accumulation, obesity, diabetes, and metabolic syndrome, there is very little impetus to develop them for these indications because they will likely require long-term use, which could increase the risk of breast cancer and thromboembolic events, as well as endometrial cancer in the absence of progestins. PPT increases uterine size, similar to E2 in rats,[54] which supports the pessimistic perception of using ERα-selective agonists for long-term MHT. One strategy to exploit the beneficial metabolic effects of estrogens is to improve the safety of estrogens by developing tissue-selective ERα drugs that act as agonists in adipose tissue and bone but lack action in the mammary gland and uterus. To date, no tissue-selective ERα agonists have been identified. We found that extracts from two plants, Radix *Glycyrrhiza uralensis* (RG) and Radix *Pueraria Montana var. lobata* (RP), act as tissue-selective ERα agonists (submitted). We demonstrated that E2 functioned as a non–selective-tissue ERα agonist because it decreased fat accumulation, stimulated mammary gland ductal formation, increased uterine size in mice, and stimulated MCF-7 cell tumor formation in a mouse xenograft model. RG and RP mimicked the beneficial ERα agonist effect of E2 in fat by reversing weight gain and fat accumulation. However, in contrast to E2, RG and RP did not elicit any proliferative effects in the mammary gland, uterus, or MCF-7 cell tumor xenograft model. Furthermore, gene expression profiling demonstrated that RG and RP regulated genes in abdominal fat similar to E2 but not in mammary gland and uterus. These observations suggest that tissue-selective ERα agonists might be a new therapeutic approach to prevent

chronic conditions in postmenopausal women such as obesity, diabetes, osteoporosis, and metabolic syndrome.

Basic Mechanism of Estrogen Receptor Regulation of Gene Transcription

Because the major problem with current estrogens in MHT is that they behave as nonselective agonists, it is important to understand the mechanism in which different classes of estrogens such as nonselective estrogens, ER subtype-selective estrogens, and tissue-selective ERα agonists regulate different genes in various tissues. A greater understanding of how estrogens regulate genes in a tissue-specific manner could facilitate the development of more selective ER agonists for MHT. Although E2 acts as an agonist in all tissues by regulating gene transcription, the set of genes it regulates is different in each tissue. The mechanism whereby E2 regulates distinct genes in different tissues is not entirely clear. Several basic steps are involved in estrogen-mediated transcriptional regulation.[8] The first step involves E2 binding to the ER, which causes a conformational change that allows the E2-ER complex to bind to specific regulatory elements in target genes. The best characterized ER regulatory element is a 13 nucleotide inverted palindrome known as the estrogen response element (ERE). The E2-ER complex binds directly to the ERE as a dimer. In addition to binding directly to DNA, the E2-ER complex can bind indirectly to chromatin via transcription factors such as AP-1 and NFκB.[55–57] Finally, the E2-ER complex can bind to DNA adjacent to transcription factors such as Sp1 and FoxA1, which stabilizes ER binding and promotes the assembly of transcription complexes.[58–60] Once the E2-ER complex is tethered to the regulatory element, it can recruit co-regulatory proteins such as coactivator proteins involved in activating target genes.[61] The next step for transcriptional activation of ER target genes involves the interaction of the E2-ER complex and coactivators with mediator proteins and basal transcription factors.[62] The structure of chromatin is ultimately changed through histone acetylation and other modifications, and then RNA polymerase II initiates gene transcription.[63] Much less is known about the mechanisms and regulatory elements involved in transcriptional repression, but corepressor proteins are involved in repression by recruiting histone deacetylases.[64]

Tissue-Specific Regulation of Genes

Whereas the estrogens used in MHT act as agonists in all tissues containing ERs, they regulate genes in a tissue-specific manner. We examined the effects of E2 on gene regulation in four tissues in mice: the mammary gland, uterus, gonadal fat, and liver. E2 acted as an agonist in these tissues as demonstrated by the regulation of numerous genes (►**Table 1**). The greatest number of genes regulated was in the uterus, followed by the gonadal fat, liver, and mammary gland. There

Table 1 Estradiol-Regulated Genes in Mouse

Tissue Type	No. of Genes Regulated by E2
A. Estradiol-regulated genes in different mouse tissues	
Fat	986
Uterus	1896
Mammary gland	419
Liver	462
Fat and uterus	379
Fat and mammary gland	157
Fat and liver	44
Mammary gland, liver, and fat	16
Mammary gland, liver, and uterus	5
Mammary gland, liver, uterus, and fat	5
B. Estradiol-regulated genes in different fat tissues in mice	
Fat Type	**No. of Genes Regulated by E2**
Subcutaneous	631
Brown	187
Gonadal	497
Subcutaneous and brown	27
Subcutaneous and gonadal	91
Brown and gonadal	25
Brown, gonadal, and subcutaneous	10

E2, estradiol.

was very little overlap of regulated genes between tissues. The greatest overlap occurred between the uterus and fat. E2 regulated 1896 genes in the uterus and 986 genes in the gonadal fat, of which 379 genes were commonly regulated. Only 5 or 16 genes were commonly regulated in three tissues, and 5 genes were commonly regulated in all four tissues. These findings demonstrate that E2 regulates mostly different genes in the four tissue types. We also compared the genes regulated by E2 in three fat tissues that are more closely related (►**Table 1**). E2 acted as an agonist in gonadal, subcutaneous, and brown fat by regulating between 187 and 631 genes. E2 regulated 497 genes in gonadal fat and 631 genes in subcutaneous fat. Despite the fact that adipocytes in these two fat tissues are both derived from multipotent mesenchymal stem cells, only 91 genes were commonly regulated.[65] Surprisingly, only 10 genes were commonly regulated by E2 in all three fat depots. These findings demonstrate that E2 regulation of genes is highly tissue specific, even among closely related tissues. Now that the factors involved in ER signaling are more completely defined, it is likely that some of them are responsible for tissue-specific regulation of gene transcription by estrogens. Studies indicate that differential expression of ER subtypes, co-regulatory proteins, and transcription factors in various tissues, as well as the presence of different regulatory elements for ERα and ERβ and epigenetic modifications, are involved in tissue-specific gene regulation in response to estrogens.

Tissue-Specific Recruitment of Estrogen Receptors

A possible mechanism whereby E2 regulates distinct genes in different tissues is by binding to different regulatory elements in various tissues. One way to test this possibility is by using the ChIP-sequencing technique that identifies protein binding sites in the genome. After cells are treated with E2, the cells are fixed in formaldehyde that covalently cross-links the ER to its regulatory element. ERs attached to regulatory elements throughout the genome are immunoprecipitated using an antibody to ERα or ERβ. After immunoprecipitation the protein-DNA cross-links are reversed, and the DNA is collected, processed, and then sequenced using an automated DNA sequencing system. After sequences are determined, they are mapped to the human genome library to identify the nearest gene using bioinformatics tools.

We performed ChIP sequencing in U2OS cells that express a stably transfected ERα or ERβ to identify genome-wide ER binding sites. In response to E2 treatment, 11,975 binding sites were identified for ERβ[66] and 15,947 binding sites for ERα (unpublished data). Approximately 30% of the ERα binding sites overlapped with ERβ binding sites. ERα and ERβ binding sites were also different in MCF-7 cells.[67,68] In these cells there were 4405 ERα and 1897 ERβ binding sites, of which 1386 binding sites were common to both receptors. These results demonstrate that many ERα and ERβ binding sites are different in U2OS and MCF-7 cells. Because the binding sites are different, it is expected that the genes regulated by ERα and ERβ in response to E2 will be different. In fact, when we examined the genes regulated by E2 in U2OS-ERα or U2OS-ERβ cells, we found that E2 regulated 725 genes in U2OS-ERα cells and 436 genes in U2OS-ERβ cells.[69] Of the 725 genes regulated in the U2OS-ERα cells, only 236 were regulated in the U2OS-ERβ cells. Monroe et al showed that when ERα and ERβ were both expressed in U2OS cells, E2 regulated 102 genes exclusively by the ERα/ERβ heterodimer.[70] These findings demonstrate that ERα, ERβ, and the ERα/ERβ heterodimer regulate a distinct set of genes, and they indicate that one mechanism whereby different estrogens cause tissue-specific gene regulation is due to the differential expression of ERα and ERβ in cells. To investigate if ERβ-selective compounds regulate different genes than E2, U2OS-ERβ cells were treated with different ERβ-selective compounds and E2.[69] We found that many genes regulated by ERβ-selective compounds were not regulated by E2.[69] For example, LIQ regulated 430 genes, whereas E2 regulated 200 genes in the U2OS-ERβ cells. Furthermore, E2 regulated 489 genes in U2OS-ERα cells, whereas LIQ only regulated 3 genes. These findings demonstrated that ERβ-selective compounds produce major differences in gene regulation with both ERα and ERβ compared with E2, suggesting that they will elicit distinct tissue-specific effects. Importantly, these results suggest that ER subtype–specific drugs might be valuable alternatives to estrogens in MHT to regulate a different set of genes than nonselective estrogens such as E2 that activate both ERα and ERβ.

Tissue-Specific Estrogen Receptor-α and Estrogen Receptor-β Cistromes

Another way to achieve tissue-specific gene regulation is through the existence of tissue-specific regulatory elements in ER target genes. Brown and coworkers coined the term *cistrome* to define all cis-regulatory elements that ER interacts with throughout the genome.[71,72] Based on studies using tiling arrays[59,73,74] and ChIP sequencing,[66,75] it has become clear that the ER cistrome is much more complex then initially anticipated and is tissue specific. ER binding sites are more diverse and complex than the classical ERE. Most of the ER regulatory elements require transcription factors for activity, including AP1, FoxA1, and Sp1.[59,66,73–76] One of the most complex regulatory elements defined comes from the NKG2E gene, which requires a collaboration between the transcription factors c-jun, heat-shock factor 2, and CCAAT/enhancer-binding protein β and a unique variant ERE for E2 to fully activate this gene.[77] Another possibility for the tissue-specific effects of estrogens is that the ERα and ERβ cistromes are tissue specific. This was examined for ERα when the binding sites for ERα on chromosomes 1 and 6 were compared in MCF-7 and U2OS cells.[71] There were 1090 ERα binding sites in MCF-7 cells and 1137 binding sites in U2OS cells. Only 172 ERα binding sites were common to both cell types. These findings demonstrate that the ERα cistrome is tissue specific. Different ERα and ERβ cistromes in various tissues likely account for some differences in gene expression profiles observed after treatment with E2 (►**Table 1**).

Tissue-Specific Expression and Recruitment of Coregulators

Coregulator proteins interact directly with nuclear receptors to form a bridge between the receptor and basal transcriptional machinery to regulate gene transcription.[78] There are two major classes of coregulators: coactivators that activate and corepressors that repress gene transcription.[79] Coregulators contain enzymatic activity that cause posttranslational modifications of chromosomal proteins involved in gene regulation. Some possess acetyltransferase or methyltransferase activity, which leads to the acetylation and methylation of histones.[79] These modifications are important for activating and repressing genes. Because of the central role of coregulators in ER-mediated transcriptional regulation, the differential expression of coregulators in various tissues could lead to tissue-specific effects. Northern blot analysis showed distinct tissue-specific expression patterns of co-regulatory genes.[80] There is also tissue-specific expression of co-regulators in reproductive organs.[81] These observations suggest that tissues will respond differently to estrogenic drugs dependent on the expression of specific co-regulator proteins. Tamoxifen is known to act as an agonist in the endometrium, whereas it acts as an antagonistic in the mammary gland. Shang and Brown demonstrated that the agonist effect of tamoxifen in the uterus is due to the expression of high levels of the coactivator NCOA1.[82] In contrast, it functions as an antagonist in the breast cancer cells because of low expression of NCOA1 and high expression of NCOA3. Different coactivators participate in E2-mediated proliferation of MCF-7 breast cancer cells. Using small interfering RNA to knock down coactivators, it was shown that proliferation of MCF-7 cells in response to E2 treatment was abolished in cells where there was a knockdown of NCOA3, but not NCOA1 or NCOA2, demonstrating that only NCOA3 is required for E2-mediated proliferation.[83] Coactivator-associated arginine methyltransferase 1 (CARM1) is a unique coactivator in that it inhibits E2-mediated proliferation and gene regulation when overexpressed in MCF-7 cells.[84] About 16% of genes upregulated by E2, some of them proliferative genes, were downregulated in the presence of CARM1. These findings demonstrate that the response to E2 is determined by expression of specific coregulator proteins in cells.

Tissue-Specific Epigenetic Modifications

Another likely possible mechanism whereby E2 regulates different genes in tissues is through the differentiation state of the cells. During differentiation there is different expression of transcription factors and co-regulatory proteins as well as epigenetic modifications that can determine which genes are regulated by ERs. To explore this issue, we examined Caco-2, a human colon carcinoma cell line, HeLa, and Ishikawa cells for responses to two ERβ-selective agonists, MF101 and LIQ. We chose these three cell lines because they did not express ERα or ERβ. All three cell lines were infected with an adenovirus that expressed ERβ and then were treated for 6 hours with MF101 or LIQ, and the gene expression profiles were determined with microarrays.[69] Although the cells received the same amount of ERβ and concentration of drugs, and they were exposed to uniform treatment times, there was very little overlap in the regulated genes in the three cell lines. Only three genes were commonly regulated by MF101, and no genes were commonly regulated by LIQ in the three cell types. We found that three major nuclear receptor coactivators, NCOA1, NCOA2, and NCOA3, were expressed similarly in the three cell types, suggesting that the difference in gene regulation was not due to the differential expression of coactivators.[69] Another explanation for the different regulation of genes in the three cell types is that differences in differentiation state led to the differential expression of some transcription factors involved in ER-mediated transcription. FoxA1 is considered to be a pioneer factor because it enhances ERα recruitment to cis-regulatory elements in the genome. Many ERα binding sites overlap with FoxA1 binding sites, indicating that FoxA1 cooperates with ERα to regulate target genes.[74] Krum et al compared the genes regulated in MCF-7 and U2OS cells and found that $<10\%$ were common to both cell types,[71] demonstrating the existence of cell-type specific gene expression in these cells. The different gene expression profile was shown to be related to the expression of FoxA1, which was expressed in MCF-7 cells but not in U2OS cells.[71] These findings suggest that some tissue-specific effects of estrogens are due to the different expression of transcription factors such as FoxA1 that results from differentiation state of the cell.

Another possibility for the different gene regulation in the various cell types is that certain ER target genes are turned off by epigenetic changes. During cell differentiation, several epigenetic modifications occur in the chromosomes without altering the DNA sequence. Several major epigenetic modifications have been discovered that have been classified into three main groups: DNA methylation, histone modifications, and nucleosome positioning.[85] FoxA1 recognizes monomethylated and demethylated histone H3 near an ER binding site.[71] Once FoxA1 recognizes these methylated histones, it interacts with ER to open up chromatin structure and facilitate the recruitment of transcription factors leading to increased transcription.[71] These findings demonstrate that epigenetic changes are important to mark the sites where transcription factors bind and interact with ERs at cis-regulatory elements. Therefore, epigenetic modifications in different target genes that occur during differentiation can determine if the gene will bind transcription factors at ER regulatory elements.

Conclusion

Estrogens have been used for >70 years in MHT to prevent and treat menopausal symptoms such as hot flashes, night sweats, and vaginal dryness. Later they were used to prevent osteoporosis, fractures, and heart disease. The publication of the results from the WHI dramatically changed the outlook for MHT when it was reported that the risks exceed the benefits. The number of postmenopausal women taking MHT plummeted, mainly due to the increased risk of breast cancer. The major estrogens used currently in MHT were

discovered many years prior to the discovery of ERα and ERβ, regulatory elements, co-regulator proteins, and basic transcriptional mechanisms. It is now known that the major problem with current estrogens in MHT is that they are nonselective. They bind to ERα and ERβ nonselectively. They act as nonselective ER agonists that trigger ER signaling in all tissues containing ERs. These nonselective actions produce beneficial effects in the brain, bone, and adipose tissue but cause proliferation of cells in the mammary gland and uterus, increasing the risk of breast and endometrial cancer. One potential means to resurrect MHT for long-term therapy will be to develop more selective estrogens that act in a tissue-selective manner. The design of tissue-selective estrogens will require a better understanding of how estrogens regulate different genes in target tissues. Some mechanisms for tissue-specific gene regulation have been elucidated, including the differential expression of ERα and ERβ, co-regulator proteins and transcription factors, and the presence of different ERα and ERβ regulatory elements and epigenetic modifications in various tissues. Designing drugs that act more selectively such as ERβ subtype selective estrogens and tissue-selective ERα agonists are potential future prospects to prevent previous indications for MHT, such as osteoporosis, as well as new indications, such as obesity, type 2 diabetes, metabolic syndrome, and breast and colon cancer.

Disclosures

Sreenivasan Paruthiyil, Isaac Cohen, and Mary Tagliaferri are employees of Bionovo, Inc. Terence P. Speed is on the scientific advisory board of Bionovo, Inc. Dale C. Leitman has received financial support for research from Bionovo, Inc. Chaoshen Yuan, Candice B. Herber, and Moshe Olshansky have nothing to disclose.

Acknowledgment

We thank the National Center for Complementary and Alternative Medicine for funding our research.

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