Recent Advances in the Understanding of Nuclear Receptors- and Drug-Metabolizing Enzymes-Mediated Inter-Individual Differences

Review

Estrogen Receptor β as a Possible Double-Edged Sword Molecule in Breast Cancer: A Mechanism of Alteration of Its Role by Exposure to Endocrine-Disrupting Chemicals

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Estrogen is essential for the growth and development of mammary glands and its signaling is associated with breast cancer growth. Estrogen can exert physiological actions *via* estrogen receptors α/β (ER α/β). There is experimental evidence suggesting that in ER α/β -positive breast cancer, ER α promotes tumor cell proliferation and ER β inhibits ER α -mediated transcriptional activity, resulting in abrogation of cell growth. Therefore, ER β is attracting attention as a potential tumor suppressor, and as a biomarker and therapeutic target in the ER α/β -positive breast cancer. Based on this information, we have hypothesized that some endocrine-disrupting chemicals (EDCs) that can perturb the balance between ER α and ER β expression levels in breast cancer cells might have effects on the breast cancer proliferation (*i.e.*, down-regulation of the α -type of ER). We have recently reported that 4-methyl-2,4-bis(4-hydroxyphenyl)pent-1-ene (MBP), an active metabolite of bisphenol A, in ER α/β -positive human breast cancer significantly down-regulates ER α expression, yet stimulates cell proliferation through the activation of ER β -mediated transcription. These results support our hypothesis by demonstrating that exposure to MBP altered the functional role of ER β in breast cancer cells from suppressor to promoter. In contrast, some EDCs, such as Δ^9 -tetrahydrocannabinol and bisphenol AF, can exhibit anti-estrogenic effects through up-regulation of ER β expression without affecting the ER α expression levels. However, there is no consensus on the correlation between ER β expression levels and clinical prognosis, which might be due to differences in exposed chemicals. Therefore, elucidating the exposure effects of EDCs can reveal the reason for inconsistent functional role of ER β in ER α/β -positive breast cancer.

Key words estrogen receptor α ; estrogen receptor β ; breast cancer; 4-methyl-2,4-bis(4-hydroxyphenyl)pent-1-ene; endocrine-disrupting chemical

1. INTRODUCTION

Estrogen plays key role in the development and maintenance of reproductive functions. The steroid hormone 17 β -estradiol (E2) is the most potent estrogen produced in the body. The physiological actions of estrogens are largely mediated through the activities of estrogen receptors α and β (ER α/β), belonging to the nuclear receptor superfamily of transcription factors.^{1,2)} Since the DNA-binding domain, involved in DNA recognition and binding, is highly conserved between ER α and ER β ,²⁾ both ERs can recognize the same estrogen-response element (ERE).³⁾ ER α and ER β regulate the transcription of a large number of genes. Thus, perturbations to ER-mediated estrogen signaling not only disrupts normal function and development, but also initiates the progression of breast cancer.

Human breast cancers are sub-grouped based on the expression of ER α , progesterone receptor, and human epidermal growth factor receptor 2. However, ER status (positive or negative) in human breast cancer is defined only by the measurement of ER α ,⁴⁾ regardless of the status of ER β expression. ER α affects both tumor development and progression and is

significantly associated with poor breast cancer prognosis and malignancy.⁵⁾ In ER-positive breast cancer, ER α promotes tumor cell proliferation and metastasis through estrogenic action.⁵⁾ Most breast cancer tumors co-express both ER α and ER β .^{6,7)} ER β inhibits ER α -mediated transcriptional activity (Fig. 1, left panel), both *in vitro* and *in vivo*.⁸⁻¹¹⁾ Especially, ER β reduces cell proliferation in ER α/β -positive breast cancer cell lines and inhibits tumor formation in mice.¹²⁻¹⁴⁾ Moreover, increased ER β expression levels in breast cancer have been reported to be positively correlated with good prognosis.^{15,16)} Therefore, ER β is attracting attention as a possible tumor suppressor and is considered as a biomarker and therapeutic target in ER α/β -positive breast cancer.

It is generally accepted that endocrine-disrupting chemicals (EDCs) can exhibit estrogenic/anti-estrogenic activities through ER α and ER β .¹⁷⁾ It has been shown that i) EDCs, such as bisphenols and cadmium, can directly/indirectly interact with ER α and ER β as ligands, and ii) EDCs, such as bisphenols and Δ^9 -tetrahydrocannabinol (Δ^9 -THC), can up-/down-regulate the ER expression levels. EDCs have been shown to disrupt ER-mediated estrogen signaling through these modula-

Fig. 1. Effects of Exposure to EDCs on ERα and ERβ Expression in ERα/β-Positive Breast Cancer Cells

In general, in ERα/β-positive breast cancer, ERα promotes tumor cell proliferation, whereas ERβ inhibits ERα-mediated transcriptional activity, resulting in abrogation of cell growth (left panel). Some EDCs can perturb the balance between ERα and ERβ expression. MBP stimulates cell proliferation through down-regulation of ERα expression and activation of ERβ-mediated transcription (right upper panel),³³⁾ whereas Δ⁹-THC and BPAF inhibits cell proliferation by up-regulation of ERβ expression (right lower panel).^{34–37)} The functional role of ERβ is altered by exposure to EDCs in ERα/β-positive breast cancer cells.

tions. Therefore, we have hypothesized that some EDCs that can perturb the balanced expressions of ERα and ERβ in breast cancer cells might exert adverse effects on the breast cancer cell growth. In this review, we discuss the exposure effects of EDCs on the ERα and ERβ receptor expressions and the alteration mechanisms of the ERβ role in ERα/β-positive breast cancer by exposure to EDCs.

2. IMPACTS OF EXPOSURE TO EDCS ON ERα AND ERβ EXPRESSION IN ERα/β-POSITIVE BREAST CANCER CELLS

Since EDCs are defined by the Endocrine Society of the United States as “exogenous chemicals, or mixture of chemicals, that interfere with any aspect of hormone action,”¹⁸⁾ EDCs include natural products as well as environmental chemicals. Some EDCs, including bisphenols, phthalates, cadmium, and Δ⁹-THC, can modify ER-mediated estrogen signaling through direct/indirect interaction with ERα and ERβ.^{19–30)} We have recently investigated the effects of exposure to 4-methyl-2,4-bis(4-hydroxyphenyl)pent-1-ene (MBP), an active metabolite of bisphenol A (BPA),^{31,32)} at concentrations similar to the environmental levels on ERα/β-positive breast cancer MCF-7 cell proliferation. MBP positively stimulated cell proliferation and strongly activated the ERs/ERE-mediated transcription. On analysis, 1 nM MBP significantly down-regulated ERα expression and slightly up-regulated ERβ expression. Moreover, MBP-stimulated transcriptional activity and cell proliferation were completely abrogated by co-treatment with ERβ selective antagonist (PHTPP). Taken together, we demonstrated for the first time that MBP significantly down-

regulates ERα expression, but stimulates the proliferation of MCF-7 cells through the activation of ERβ-mediated transcription (Fig. 1, right upper panel),³³⁾ indicating that exposure to MBP alters the functional role of ERβ in breast cancer cells from suppressor to promoter.

In contrast, Δ⁹-THC and bisphenol AF (BPAF) up-regulate ERβ expression without affecting ERα expression levels in MCF-7 cells, and they exhibit anti-estrogenic effects (Fig. 1, right lower panel).^{34–37)} Based on these findings, it has been suggested that Δ⁹-THC and BPAF enhance the function of ERβ as a tumor suppressor. Δ⁹-THC has no ability to bind to ERβ,³⁴⁾ whereas BPAF can act as a ligand for ERβ.²⁵⁾ In order to exhibit ERβ-mediated anti-estrogenic activity, it is not essential that these exposed EDCs bind to ERβ as ligands, whereas it is important to up-regulate ERβ expression. Moreover, the difference in the effects to exposure between these EDCs and MBP is the presence or absence of an effect on the expression level of ERα. In addition, it has been reported that ERβ functions as a mediator of estrogenic action in ERα/β-positive breast cancer cells when ERα expression is reduced, in certain situations without exposure to EDCs.³⁸⁾ Therefore, although the detailed mechanism is still unknown, it might be necessary to down-regulate ERα expression and directly activate ERβ as a ligand in order to act as a mediator of estrogenic action in ERα/β-positive breast cancer cells.

3. A MECHANISM OF ALTERATION OF ERβ ROLE IN ERα/β-POSITIVE BREAST CANCER CELLS BY EXPOSURE TO EDCS

It is known that alternatively spliced ERβ variants, ERβ1, ERβ2 (also called ERβcx), ERβ4, and ERβ5, are expressed in breast cancer cells.^{39–42)} ERβ1, the full-length human ERβ, has a ligand binding pocket and is the only full-functional isoform, whereas ERβ2, ERβ4, and ERβ5 have no affinity for the ligand.⁴²⁾ ERβ1 and ERβ2 can heterodimerize with ERα, which suppress ERα-mediated transcription.^{10,11)} ERβ2, ERβ4, and ERβ5 form heterodimer with ERβ1 and enhance the activity of ERβ1 in a ligand-dependent manner.⁴²⁾ In other words, while the ERα/ERβ1 and ERα/ERβ2 heterodimers function as suppressors, the homodimers of ERβ (*i.e.*, ERβ1/ERβ1, ERβ1/ERβ2, ERβ1/ERβ4, and ERβ1/ERβ5) play a role as promoters of breast cancer cell proliferation (Fig. 2). Therefore, it is necessary to consider the effect of exposure to EDCs on the expression of alternative splicing variants of ERβ as well. We investigated the effect of MBP on expression of ERβ variants in MCF-7 cells. MBP tended to up-regulate the mRNA expression of ERβ2 and ERβ5, but not ERβ1 (Hirao-Suzuki *et al.*, unpublished observations). Conversely, we reported that BPAF can up-regulate mRNA expression of ERβ1 and ERβ2, although the study used ERα-negative breast cancer MDA-MB-231 cells.²⁵⁾ These findings suggest that exposure to EDCs can disrupt the balanced expression of ERα and ERβ including alternative splicing variants of ERβ, resulting in an alteration of the ERβ role through altered composition of the ER dimers presented in ERα/β-positive breast cancer cells.

4. CONCLUSION

Several immunohistochemistry-based studies have demonstrated conflicting data in relation to ERβ expression in breast

Fig. 2. Proposed Model of the Balance of ER Dimers That Promote/Suppress Breast Cancer Cell Proliferation

ER β 1 and ER β 2 suppress ER α -mediated transcription activity by heterodimerization with ER α .^{11,10} ER β 2, ER β 4, and ER β 5 heterodimerize with ER β 1 and enhance the activity of ER β 1 in a ligand-dependent manner.⁴² The ER α /ER β 1 and ER α /ER β 2 heterodimers, act as suppressors in breast cancer cell proliferation, whereas the homodimers of ER β (i.e., ER β 1/ER β 1, ER β 1/ER β 2, ER β 1/ER β 4, and ER β 1/ER β 5), in addition to ER α /ER α homodimer, play a role in promotion of breast cancer cell proliferation.

tumors. In many published reports on clinical outcomes, ER β expression levels in different classes of breast cancer are positively correlated with overall survival (OS) and disease-free survival (DFS).^{15,16} However, there are also reports where ER β expression levels are not correlated or negatively correlated with OS and DFS.^{43–45} Moreover, although we are constantly exposed to various chemicals including EDCs, there are no reports that have examined the correlation between ER β expression levels and clinical outcomes accounting the effects of exposure to EDCs. Investigations of the effects of EDC exposure on ER α and ER β expression in ER α /ER β -positive breast cancer have demonstrated that i) exposure to EDCs that down-regulate ER α expression and activate ER β , can change the role of ER β from a suppressor to a promotor, and ii) exposure to EDCs that up-regulate ER β expression, can enhance its function as a suppressor. Exposure to EDCs causes inconsistencies in the functional role of ER β in ER α /ER β -positive breast cancer. Therefore, elucidating the effects of exposure to EDCs is expected to reveal the exact functional role of ER β in ER α /ER β -positive breast cancer.

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Conflict of Interest The author declares no conflict of interest.

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