Estrogen plays key role in the development and maintenance of mammary glands and its signaling is associated with breast cancer growth. Estrogen can exert physiological actions via estrogen receptors α/β (ERα/β). There is experimental evidence suggesting that in ERα/β-positive breast cancer, ERα promotes tumor cell proliferation and ERβ inhibits ERα-mediated transcriptional activity, resulting in abrogation of cell growth. Therefore, ERβ is attracting attention as a potential tumor suppressor, and as a biomarker and therapeutic target in the ERα/β-positive breast cancer. Based on this information, we have hypothesized that some endocrine-disrupting chemicals (EDCs) that can perturb the balance between ERα and ERβ 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 ERα/β-positive human breast cancer significantly down-regulates ERα expression, yet stimulates cell proliferation through the activation of ERβ-mediated transcription. These results support our hypothesis by demonstrating that exposure to MBP altered the functional role of ERβ in breast cancer cells from suppressor to promoter. In contrast, some EDCs, such as Δ⁹-tetrahydrocannabinol and bisphenol AF, can exhibit anti-estrogenic effects through up-regulation of ERβ expression without affecting the ERα expression levels. However, there is no consensus on the correlation between ERβ 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 ERβ in ERα/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 17β-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 (ERα/β), belonging to the nuclear receptor superfamily of transcription factors. Since the DNA-binding domain, involved in DNA recognition and binding, is highly conserved between ERα and ERβ, both ERs can recognize the same estrogen-response element (ERE). ERα and ERβ 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 ERα, 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 ERα, regardless of the status of ERβ expression. ERα affects both tumor development and progression and is significantly associated with poor breast cancer prognosis and malignancy. In ER-positive breast cancer, ERα promotes tumor cell proliferation and metastasis through estrogen action. Most breast cancer tumors co-express both ERα and ERβ. ERβ inhibits ERα-mediated transcriptional activity (Fig. 1, left panel), both in vitro and in vivo. Especially, ERβ reduces cell proliferation in ERα/β-positive breast cancer cell lines and inhibits tumor formation in mice. Moreover, increased ERβ expression levels in breast cancer have been reported to be positively correlated with good prognosis. Therefore, ERβ is attracting attention as a possible tumor suppressor and is considered as a biomarker and therapeutic target in ERα/β-positive breast cancer.

It is generally accepted that endocrine-disrupting chemicals (EDCs) can exhibit estrogenic/anti-estrogenic activities through ERα and ERβ. It has been shown that i) EDCs, such as bisphenols and cadmium, can directly/indirectly interact with ERα and ERβ as ligands, and ii) EDCs, such as bisphenols and Δ⁹-tetrahydrocannabinol (Δ⁹-THC), can up-/down-regulate the ER expression levels. EDCs have been shown to disrupt ER-mediated estrogen signaling through these modula-
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), whereas Δ9-THC and BPAF inhibits cell proliferation by up-regulation of ERβ expression (right lower panel). The functional role of ERβ is altered by exposure to EDCs in ERα/β-positive breast cancer cells.

3. A MECHANISM OF ALTERATION OF ERβ ROLE IN ERαβ-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. 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. ERβ1 and ERβ2 can heterodimerize with ERα, which suppress ERα-mediated transcription, whereas Δ9-THC and BPAF form heterodimer with ERβ1 and enhance the activity of ERβ1 in a ligand-dependent manner. 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. 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 ERαβ-positive breast cancer cells.

4. CONCLUSION

Several immunohistochemistry-based studies have demonstrated conflicting data in relation to ERβ expression in breast...
Breast cancer cell proliferation.

ERα and ERβ suppress ERα-mediated transcription activity by heterodimerization with ERα. ERβ is also able to heterodimerize with ERβ and enhance the activity of ERα/β in a ligand-dependent manner. The ERα/ERβ and ERα/ERβ/β heteromers, as suppressors in breast cancer cell proliferation, whereas the homodimers of ERβ (i.e., ERβ/ERβ, ERβ/ERα, and ERβ/ERα) in addition to ERα/ERα homodimer, play a role in promotion of breast cancer cell proliferation.

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

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Conflict of Interest The author declares no conflict of interest.

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