Estrogen and Its Receptors in Cancer

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Abstract: The involvement of estrogen and its receptors in the development of cancer has been known for years. However, the exact mechanism responsible is far from clear. The estrogen-mediated carcinogenic process is complicated by recent findings, which reveal that estrogens have multiple functions in cells, which can be either adverse or beneficial, and that the effects of estrogen may be cell-type or organ dependent. The estrogenic effect may be also greatly influenced by the state of two estrogen receptors, ERα and ERβ. This review will discuss the role and function of estrogens and its receptors in cancers of three categories: (1) Breast cancer and gynecologic cancers, (2) Cancers of endocrine organs, (3) Lung cancer and cancers of digestive system. We will also review some novel treatments aiming to interfere with relevant pathways mediated by estrogens and its receptors. © 2008 Wiley Periodicals, Inc. Med Res Rev, 28, No. 6, 954–974, 2008

Key words: estrogen; estrogen receptor; cancer

1. INTRODUCTION

Estrogen regulates the growth, differentiation, and physiology of the reproductive process. Estrogen also influences the pathological processes of hormone-dependent cancers, such as breast, endometrial, prostate and ovarian and thyroid cancers.1,2 Estrogens are a group of steroid compounds which function as the primary female sex hormones. While estrogens are present in men and women, their levels are significantly higher in women of reproductive age. They are mainly produced by the adrenal cortex and ovary, and three estrogens occur naturally in the female.1,3 In pre-menopausal women, 17β-estradiol (E2), produced by the ovary, is the estrogen produced in the largest quantity and is the most potent as it has the highest affinity for its receptors. In pre-menopausal...
women, circulating estradiol levels fluctuate from 40 to 200–400 pg/mL during the menstrual cycle.\(^3\) After menopause, estradiol levels drop to less than 20 pg/mL. The second endogenous estrogen is estrone, a less potent metabolite of estradiol. Estrone is produced from androstenedione, the immediate precursor of estrone, in adipose tissue. In post-menopausal women, the ovary ceases to produce estradiol while the adrenal gland continues to produce androstenedione. As the result, the level of estrone remains unchanged while the plasma level of estradiol falls significantly. The third endogenous estrogen is estriol (E3), also a metabolite of estradiol. Estriol is the principal estrogen produced by the placenta during pregnancy, and is found in smaller quantities than estradiol and estrone in non-pregnant women.\(^1,3\)

The biological actions of estrogens are traditionally mediated by binding to one of two specific estrogen receptors (ERs), ER\(\alpha\) or ER\(\beta\), which belong to the nuclear receptor (NR) superfamily, a family of ligand-regulated transcription factors. ER\(\alpha\) and ER\(\beta\) are encoded by separate genes, ESR1 and ESR2 respectively. Although binding of estrogens to ERs results in a variety of changes in cell functions, the mechanism of its action can be classified in two pathways: genomic and non-genomic.

### A. Genomic Pathway

Estrogen influences the cellular events through two main pathways, genomic and non-genomic (Fig. 1).\(^1\) In the genomic pathway, estrogen exerts its function via ER\(\alpha\) and ER\(\beta\). In general, this classical pathway of estrogen involves estrogen-dependent formation of nuclear ER homo- or heterodimers, and the subsequent binding of this nuclear estrogen-ER complex binds to estrogen response element (ERE) sequences in the promoter region of estrogen-responsive genes, resulting in the recruitment of coregulatory proteins (co-activators or co-repressors) to the promoter, which leads to an increase or decrease in mRNA levels, the production of associated proteins and finally a physiological response. The genomic pathway typically occurs over the course of hours. There is evidence that ER\(\alpha\) and ER\(\beta\) can regulate transcription of some genes independent of ERE by interacting with other DNA-bound transcription factors, rather than binding directly to DNA,\(^1,2\) which may explain that about one third of estrogen-induced genes lack functional ERE.\(^4\) AP-1, SP-1, forkhead box (Fox), oct, nuclear factor kappaB (NF-\(k\)B) and GATA-3 are some of known non-ERE DNA-bound transcription factors that interact with ERs.\(^5\)–\(^7\) Interestingly, the level of E2 that determines the saturation of liganded/unliganded ERs may have opposite effects on the expression of TNF\(\alpha\) via interacting with glucocorticoid receptor-interacting protein 1 (GRIP1), c-jun and NF-\(k\)B.\(^5\)

### B. Non-Genomic Pathway

In the non-genomic pathway, estrogen acts either through the ER located in or adjacent to the plasma membrane, or through other non-ER plasma membrane-associated estrogen-binding proteins (Fig. 1).\(^1\)–\(^4,8\) The non-genomic action of estrogen results in cellular responses such as increased levels of calcium or nitric oxide and the activation of multiple intracellular kinase cascades including mitogen-activated protein kinase (MAPK), phosphoinositide 3-kinase (PI3K), protein kinase A (PKA), protein kinase C (PKC). The ER may be targeted to the plasma membrane by adaptor proteins such as caveolin-1 or Shc (an SH2 containing proto-oncogene). AP-1 response elements, for instance, may be regulated indirectly through interactions between ERs and the AP-1 transcription factors c-fos and c-jun. On the other hand, AP-1-dependent transcription may also be directly and efficiently activated by binding of E2 to the cytoplasmic ERs that may form a complex with SRC-1, p300, ubiquitin ligase E6-AP, Mdm2, Carm, and pol II.\(^9,10\) These transcription factors regulate genes involved in many cellular processes, including proliferation, differentiation, cell motility, and apoptosis. The non-genomic effects occur within a few minutes, which is too rapid to be mediated by biosynthesis of RNA or new proteins.

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Figure 1. Estrogen-mediated genomic and non-genomic pathways.
C. Non-Genomic Pathway Crosstalk to Genomic Pathway

Though the genomic and non-genomic pathways function differently, studies have indicated that there is a crosstalk between them (Fig. 1). The rapid non-genomic activation of cytoplasmic signaling pathways has been shown to regulate gene expression independent of ERE. For example, ERK activation in the non-genomic pathway enhances AP-1-mediated gene expression. The non-genomic signaling can activate MAPK to stimulate the phosphorylation and recruitment of coactivators such as SRC-1 and the phosphorylation of SRC-1 enhances nuclear ER transcriptional activity.

D. G Protein-Coupled Receptor-30

Although the transcriptional effects of estrogen in many cases can be explained by the binding of estrogen to its traditional receptors, ERα and ERβ, it is now widely accepted that some of its rapid effects cannot be attributed to ERα and ERβ. Recent studies have discovered some novel estrogen receptors. Among them, G protein-coupled receptor-30 (GPR30), an orphan member of the seven transmembrane receptor, has been shown to involve in cancer development. Estrogen through GPR30 can stimulate MAPK and ERK1/2, and transactivation of the epidermal growth factor receptor (EGFR) independent of ERα and ERβ in breast cancer SKBR3 cells. However, GPR30 is not involved in estrogen-mediated MAPK activation in breast cancer MCF-7 cells. The findings suggest that the GPR30-mediated pathway of estrogen might be cell type-dependent. E2-GPR30 interaction has similar characteristics as the non-genomic signaling of E2-ERs and thus it can be regarded as an alternative non-genomic pathway.

E. Affinity of Estrogen Receptors and SERMs: Selective Estrogen Receptor Modulators

ERα and ERβ differ mostly in the N-terminal A/B and F domains, exhibiting a 15% and 18% identity respectively. The ligand binding domain (E domain) is moderately conserved between both receptors and shows a 59% amino acid identity. Despite the identity between ERα and ERβ in the ligand binding domain, the two receptors exhibit differences in ligand binding specificity. A number of compounds have been found to have different binding affinities to ERs. These compounds are capable of acting as ER agonists in some tissues but as antagonists in others. Therefore, they are selective estrogens and antiestrogens at different target tissues and are termed as selective ER modulators (SERM). From the therapeutic point of view, the ideal SERM should be antiestrogenic in cancer tissues but proestrogenic in the vasculature and brain. Chemically, SERM can be classified in five groups: triphenylethylenes, benzothiophenes, tetrahydronaphylens, indoles, and benzopyrans. Chemicals in the latter three groups are still under the development or clinical trials. Triphenylethylenes are developed for the treatment of the estrogen-dependent breast cancer. The representative of this category is tamoxifene (ICI 46474) that is clinically used as a first-line endocrine treatment. However, tamoxifene has proestrogenic effect on the endometrium and its treatment is associated with an increase in the incidence of endometrial cancer. For this reason, its use for the endocrine treatment may be limited though it is still recommended for the treatment of ER-positive breast cancer. Benzo[38]
F. Aromatase and its Inhibitors

The importance of ER in the development and progression of hormonally sensitive tumors has been well recognized. However, it has also been noted that some of these tumors can develop in an ER-free environment. The occurrence of mammary tumor in ER knockout mice is the best evidence to support this concept. People were once puzzled by the fact the incidence of breast cancer is even higher in post-menopausal women when the ovaries have ceased to produce estrogens. It is now known that in post-menopausal women, estrogen levels in breast tissue are 10–50 times the level in blood and concentrations of E2 are higher in malignant tissues than in non-malignant tissues. Therefore, estrogen can be de novo produced in some types of tumors including breast cancer, indicating a significant role of the peripheral aromatization of ovarian and adrenal androgens in cancer development. Evidence also shows that estrogen metabolites may also be carcinogenic. The above information leads to the application of aromatase inhibitors such as anastrozole, letrozole and exemestane to attenuate estrogen biosynthesis and thus to treat certain types of hormonally sensitive tumors. Recent large clinical trial have shown that aromatase inhibitors are superior to tamoxifen for prolonging disease-free survival in post-menopausal women with ER-positive breast cancer in adjuvant scenario. In addition, aromatase inhibitors have been used in the treatment of metastatic breast cancer in post-menopausal women. These agents might also have a potential role in the prevention setting.

G. Estrogen Receptor Variants

The function of estrogens can be influenced by a balance between wild-type ERs and their functional exon-skipping variants, which may encode proteins interfering with the co-expressed wild-type forms in a dominant negative manner or by becoming constitutively and ligand-independently activated. In breast cancer MCF7 cells, in addition to a predominant full-length 66 kDa form of ERα (ERα66), the ERα gene also produces a 46 kDa isoform (ERα46), lacking N-terminal domains. ERα46 has been shown to suppress not only the activation function-1 (AF1) activity of ERα66 but also the transcription of target genes. However, whether the presence of ERα46 has any association with anti-tumor treatment remains unknown. Recently, a report describes the function of...

| Table I. Values of the Relative Binding Affinity of Some SERM to ERα and ERβ |
|-----------------|-----------------|-----------------|
| SERM           | ERα  | ERβ  |
| E2             | 100  | 100  |
| genistein      | 4    | 87   |
| ICI 164,384    | 85   | 166  |
| tamoxifen(4-OHT) | 257  | 232  |
| tamoxifen      | 4    | 3    |
| toremifene     | 10   | 90   |
| raloxifene     | 69   | 16   |
| DPN            | 0    | 18   |
| 8β-VE2         | 0    | 83   |
| ERB-041        | 0    | 72   |
| WAY-200070     | 0    | 180  |
| WAY-202196     | 0    | 133  |
| PPT            | 49   | 0    |
| 16-LE2         | 57   | 0    |

Relative binding affinity was calculated as ratio of concentrations of E2 to reduce the specific radioligand binding by 50%. The value for E2 is arbitrarily set at 100.
ERβ and its variants in breast cancer. Two ERβ splice isoforms, one lacking ERβ1 exons 1, 2, and 5 (ERβδ125) and the other lacking exons 1, 2, 5, and 6 (ERβδ1256) are identified in MDA-MB231 breast cancer cells. Over-expression of wild-type ERβ, but not the two exon-deleted variants exerts strong antitumor effect on either ERα-positive or ERα-negative breast cancer cells. This antitumor effect is reflected by inhibition of E2-stimulated cell growth, enhancement of apoptosis, and increase in the antiproliferative effect of tamoxifen on tumor cells. In addition to the above isoforms or variants of ERs, a number of other variants have been found in different types of tissues or cells, which has been well summarized elsewhere.

2. ESTROGEN AND ESTROGEN RECEPTORS IN CANCERS

Estrogen and its receptors are involved in the development of many types of malignant tumors. These tumors are generally classified in four groups, (1) breast and gynecologic cancers (cervical endometrial, ovarian), (2) endocrine gland cancers (adrenocortical, ovarian, pancreatic, prostate and thyroid), (3) digestive cancers (colorectal, esophageal, liver and pancreatic), and (4) lung carcinoma. Most of these tumors can express both ERα and ERβ (Table II). ERα appears to promote the proliferation of breast, gynecologic cancers and endocrine gland cancers but to inhibit the proliferation of digestive cancers and lung cancer. In contrast to ERα, ERβ suppresses the proliferation of tumor cells in the former groups but increases it in the latter groups. The prognostic significance of ER change is inconsistent among these tumors. However, the decreased level of ERβ is usually associated with poor prognosis (Table II). Most of the above-mentioned tumors are able to de novo biosynthesize E2 through the action of aromatase (Table III). The locally produced E2 may then function in a paracrine manner to stimulate the proliferation and growth of cells or/and to render cells more resistant to apoptosis. However, it is noted that the function of E2 in at least two organs colorectum and esophagus to inhibit proliferation and growth of tumor cells, which is opposite to its function in the most other organs. The differential effects of estrogens on organs are further supported by the epidemiological study on risk of cancers in patients who receiving estrogen-replacement therapy (ERT) or hormone-replacement therapy (HRT)/oral contraceptives (OC) (Table IV). The risk

| Table II. ERα and ERβ in Cancers, and Their Impacts on Cell Proliferation and Prognosis |
|---------------------------------------------|-------------|---------------------------------|--------|-----------------|
| Site of cancer | ERα | ERβ | Increase in proliferation | Poor prognosis | References |
| Adrenocort | + | + | ERβ↑, ERα↑ | ND | 101 |
| Breast | +/- | +/- | ERβ↑ | ERβ↑ | 2,22,61,62,68,69 |
| | | | | | 139 |
| Cervix | + | ND | ERα↑ | ND | 56 |
| Colorectum | ↓, -,-, | ↓ | ERα↑, ERβ↑ | ND | 125-128,140 |
| Endometrium | ↓ | ↓ | ERα↑ | ERα↑ | 57,76,141,142 |
| Esophagus | + | + | ND | ERα↑, ERβ↑ | 131,132 |
| Liver | ↑ | ↑ | ND | vER | 133,134,143 |
| Lung | + | + | ERα↑, ERβ↑ | ERα↑ | 135,136,138,144 |
| Ovary | + | + | ERβ↑ | ERβ↑ | 63,145 |
| Pancreas | + | + | ND | ND | 102,113 |
| Prostate | + | ↓ | ERα↑, ERβ↑ | ERβ↑ | 97,104,107,146 |
| Thyroid | ↑,- | ↓,- | ERα↑, ERβ↑ | ND | 109-112,147 |

+ , expression/positive; − , no expression/negative; ↑ , increase (compared with non-tumor or normal tissues/cells); ↓ , decrease (compared with non-tumor or normal tissues/cells); − , no effect or no change; ND, not data available; vER, variant ERs.
for the development of breast, gynecologic cancers and endocrine gland cancers is generally increased in patients who have female hormone treatments. In contrast, the risk for the development of digestive cancers and lung cancer is generally decreased in patients who receive these treatments. Currently there is no an explanation for these observations of obviously opposite impacts of female hormones on cancers. Nevertheless, the findings strongly indicate that the function of female hormones may be organ-dependent.

**Table III.** Estrogen Synthesis in Cancers and its Influence on Cell Functions

<table>
<thead>
<tr>
<th>Estrogen synthesis</th>
<th>Estrogen function</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Proliferation</td>
<td>Apoptosis</td>
</tr>
<tr>
<td>Adrenocort</td>
<td>+</td>
<td>↑</td>
</tr>
<tr>
<td>Breast</td>
<td>+</td>
<td>↑</td>
</tr>
<tr>
<td>Cervix</td>
<td>+</td>
<td>↑</td>
</tr>
<tr>
<td>Colorectum</td>
<td>+</td>
<td>↓ or ↑</td>
</tr>
<tr>
<td>Endometrium</td>
<td>+</td>
<td>↑</td>
</tr>
<tr>
<td>Esophagus</td>
<td>ND</td>
<td>↓, ~</td>
</tr>
<tr>
<td>Liver</td>
<td>+</td>
<td>ND</td>
</tr>
<tr>
<td>Lung</td>
<td>+</td>
<td>ND</td>
</tr>
<tr>
<td>Ovary</td>
<td>+</td>
<td>↑</td>
</tr>
<tr>
<td>Pancreas</td>
<td>ND</td>
<td>↑</td>
</tr>
<tr>
<td>Prostate</td>
<td>+</td>
<td>↑</td>
</tr>
<tr>
<td>Thyroid</td>
<td>+</td>
<td>↑</td>
</tr>
</tbody>
</table>

+, positive; −, negative; ↑, increase (compared with non-treated group); ↓, decrease (compared with non-treated group); ~, no change; ND, not data available.

**Table IV.** Relationship Between Female Hormone Use and Cancer

<table>
<thead>
<tr>
<th>cancer</th>
<th>ERT</th>
<th>EP (HRT, OC)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adrenocort</td>
<td>ND</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>Breast</td>
<td>↑</td>
<td>↑</td>
<td>32-35</td>
</tr>
<tr>
<td>Cervix</td>
<td>↑</td>
<td>↑, -</td>
<td>38,159,160</td>
</tr>
<tr>
<td>Colorectum</td>
<td>↓</td>
<td>↓</td>
<td>34,35,115,118-121</td>
</tr>
<tr>
<td>Endometrium</td>
<td>↑</td>
<td>↑</td>
<td>33-35,120,161</td>
</tr>
<tr>
<td>Esophagus</td>
<td>ND</td>
<td>↓</td>
<td>122</td>
</tr>
<tr>
<td>Liver</td>
<td>ND</td>
<td>↓</td>
<td>35</td>
</tr>
<tr>
<td>Lung</td>
<td>↓</td>
<td>↓</td>
<td>123,124</td>
</tr>
<tr>
<td>Ovary</td>
<td>↑</td>
<td>↑</td>
<td>34,120,121,162-164</td>
</tr>
<tr>
<td>Pancreas</td>
<td>-</td>
<td>-</td>
<td>165</td>
</tr>
<tr>
<td>Prostate</td>
<td>ND</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>Thyroid</td>
<td>↑</td>
<td>↑</td>
<td>87-90</td>
</tr>
</tbody>
</table>

-, no relationship; ↑, increase (compared with non-treated group); ↓, decrease (compared with non-treated group); ND, not data available.

ERT, estrogen-replacement therapy; EP, estrogen + progestin; HRT, hormone-replacement therapy; OC, oral contraceptives.

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A. Breast and Gynecologic Cancers (Cervical, Endometrial, Ovarian)

The role of estrogens and ERs has been extensively investigated in breast cancer. Epidemiologically, the incidence of breast cancer is significantly higher in women who receive ERT or HRT, especially in post-menopausal women with long-term of hormone treatments.32–35 There is increasing evidence to show that breast tumors express a high level of aromatase to biosynthesize estrogen locally.22,23 The consequence of such a high level of aromatase is that the tumor is able to produce estrogens locally in an autocrine fashion, resulting in much higher estrogen levels in the tumor tissue than non-tumor tissues or circulation system.22,23 The involvement of estrogen in the breast cancer development is further evident by study using aromatase inhibitors. The inhibition of aromatase by its chemical inhibitors or siRNA has been shown to suppress the proliferation of breast cancer cells in culture and reduce the growth of breast tumor in animal experiments as well as patients with breast cancer.22,23,36,37 Similar to breast cancer, there are also solid supports of the involvement of estrogen in the development of gynecologic tumors including cervical, endometrial, ovarian cancers. Female hormone therapies are positively associated with these cancers (Table IV). Hormone therapy may also help to create a carcinogenic environment for certain gynecologic tumors. For example, HPV infection in cervical cancer may benefit from HRT.38 The majority of these gynecologic tumors also has an autocrine mechanism to produce intratumoral estrogen via aromatase.39–43 The expression of aromatase in the tumor can also affect the survival of patients. For example, there is an association between intratumoral aromatase expression and poor survival in endometrial cancer.44

The carcinogenic effect of estrogen is executed at least in three ways (Fig. 2). First, estrogens promote the proliferation of cells via ER-mediated genomic or/and non-genomic pathways. A increasing number of estrogen-induced molecules have been identified and these molecules can function to promote cell proliferation, growth, to reduce sensitivity to apoptotic stimuli, to enhance invasiveness. For example, E2 can stimulate the LRP16 gene expression via ERx in breast cancer and the induced LRP16 can interfere with ERx-mediated transcription of E-cadherin, resulting in the reduction of E-cadherin and subsequently the invasive growth of breast cancer and endometrial cancer.45,46 Similarly, E2 may up-regulate the level of Wnt11 via an ER-dependent mechanism in breast cancer.47 The induced Wnt offers the resistance of tumor cells to apoptosis and thus increases the survival.48 Second, estrogen promotes cell proliferation via cell membrane-related but ER-independent phosphorylation of target molecules. This pathway is first shows in ER-negative breast cancer cells (MDA-MB-435 and MAD-MD-231).49 Estrogen can activate Akt by inducing rapid phosphorylation at Ser(473) of this protein and this activation can be blocked by the inhibitors of PI3K and Src kinase but not by the ER antagonist ICI 182780 (fulvestrant).49 A similar finding is also demonstrated in endometrial cancer cells, and neither ERE activation nor ICI 182780 can affect E2-mediated Akt or Erk1/2 activation and cell proliferation.50,51 In another study, effects of E2 and E2-BSA that cannot enter the cell on protein kinase C (PKC) is investigated in ER-negative HCC38 breast cancer cells.52 It shows that both E2 and E2-BSA can rapidly increase PKC via phosphatidylinositol-dependent phospholipase C and G protein and that the action is not affected by ER-agonist diethylstilbestrol, and antibodies to ERx and ERβ, indicating that E2-mediated PKC activity is via membrane pathways which is involving neither ERx nor ERβ. Interestingly, PKC activity is found to be positively correlated with the severity of breast cancer and such a correlation is even greater in ER-negative tumors.52 Third, direct genotoxic effects of estrogen metabolites on DNA damage, mutation and cell transformation. It has been well known that some of estrogen metabolites are carcinogens due to their toxicity to DNA and the toxic effect is independent of ER. The toxicity is mainly related to the feature of reactive oxygen species in these metabolites. The details of this aspect are well summarized by three review articles published recently.22,23,53

The relationship and the balance between ERx and ERβ may greatly influence the development of tumors and the treatment. The early study has suggested that as normal breast tissues become...
tumorigenic, the amount of ERα RNA increases whereas the amount of ERβ decreases.\textsuperscript{54} However, probably due to the reliability or accuracy of ERβ antibodies that were available in the market of the early 2000s, results of ERβ protein in tumors are inconsistent as to the prognostic value of ERβ, showing that ERβ is a good survival marker of breast cancer in some studies but a bad one in other reports.\textsuperscript{2} Studies into the ERβ function in breast and gynecologic cancers are few until recently. A recent study of 512 breast cancer tissue samples reveals that 78% of the tumors are ERα positive and 50% are ERβ positive.\textsuperscript{55} Functionally, the activation of ERα is associated with the proliferation and growth of tumor cells,\textsuperscript{48,56–60} whereas the activation of ERβ promotes apoptosis, suppresses the malignant transformation and inhibits the growth of the tumor cells.\textsuperscript{61–67} Over-expression of ERβ prevents establishment and growth of breast tumors in a subcutaneous xenograft mouse model.\textsuperscript{62} Restoration of ERβ in ovarian cancer cells results in enhancement of apoptosis of tumor cells, and a strong inhibition of their proliferation and invasion.\textsuperscript{65} E2-mediated proliferation of endometrial cells is blocked by transfection with ERα antisense DNA\textsuperscript{57,59} but not with ERβ antisense DNA.\textsuperscript{59} The inhibitory effect of ERβ may associate with its ability to decrease the expression of c-myc, cyclin A, cyclin D1 and cyclin E and to increase the levels of p21(cip1) and p27(Kip1).\textsuperscript{66,67} ERβ may also act as an ERβ antagonist to directly interact with ERα since ERβ has shown to negatively regulate the transactivation of ERα in breast cancer cells (MCF7).\textsuperscript{68,69} The experiment on ER-knockout mice has also suggested a stimulatory role of ERα and an inhibitory effect of ERβ in the proliferation of different estrogen-target tissues.\textsuperscript{27,70,71} Taken together, the functional experiments have demonstrated that ERα and ERβ have completely different roles in the breast and gynecologic cancers, in which ERα functions as a tumor promoter whereas ERβ as a tumor suppressor. This concept is in agreement with the clinical values of ERβ detected in tumor tissues. The presence of ERβ in breast, endometrial and ovarian tumors is associated with better prognosis or a longer disease-free survival,\textsuperscript{55,69,72–75} and the level of ERβ is significantly decreased in higher grade endometrial

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cancers. Therefore, ERβ is not a surrogate for ERα in breast and gynecologic cancers. ERα and ERβ can interact with each other and they both have their own pathological and clinical relevance in tumor progression and prognosis. The activity or the level of ERs, especially ERα can be also regulated by hypermethylation/hypomethylation and phosphorylation. The hypomethylation or the phosphorylation of ERα may lead to resistance of breast cancer to antiestrogen treatments. The details of the aspect has been recently reviewed.

The different functions of ERα and ERβ may complicate the carcinogenic aspects of estrogen via above-mentioned three pathways that can act in an additive or synergistic fashion to induce tumors and promote their growth. Such multiple channels and different layers of complexity in estrogen carcinogenesis have required anti-tumor treatments to be finely tuned and more specifically targeted. The treatment established earlier such as tamoxifen to target ER non-selectively appears to be not specific enough to block the carcinogenic effect of estrogen in a given organ (Fig. 2). Pure antiestrogens such as ICI 182780 that bind the receptor to impair ER dimerization are also limited in its efficacy and specificity. Recently, ER-selective agonists or antagonists such as DPN (an ERβ agonist and MPP (an ERα antagonist) have been shown to inhibit the cell proliferation or to enhance apoptosis. The ERα-selective antagonist or ERβ-selective agonist may have potential to be more specific in target tumor cells. Aromatase inhibitors that completely erase estrogen seem to be the most effective therapeutic agents since it blocks all three proposed processes (Fig. 2). However, estrogen-deprivation therapy may associate with potential serious side-effects as estrogens in physiological concentrations are required for numerous functions of a cell. Therefore, the usefulness of this therapeutic strategy remains to be further evaluated.

B. Endocrine Gland Cancers (Adrenocortical, Pancreatic, Prostate, and Thyroid)

There is ample evidence to support that estrogens play a role in the development of endocrine gland cancers. Epidemically, the incidence of thyroid cancer is three to five times more frequent in woman than in men and the gender difference in the incidence is particularly obvious for women of reproductive age relative to men. The use of oral contraceptives or female hormone therapy appears to result in a moderately increased risk of developing thyroid cancer or pancreatic cancer. Although prostate cancer only occurs in men, the estrogenic factor is still evident. Prostate cancer develops in older men at a time when the level of serum testosterone is in decline, and the level of estrogen remains unchanged or increases with age. The net result is a significant increase the ratio of testosterone to estrogen allowing the regulation of prostate gland to be shifted pre-dominately under the control of estrogen. African-American men have a twofold increased risk of prostate cancer as compared to their Caucasian counterparts and this increased incidence is correlated with a higher level of estrogens in the former than in the latter. There is not relevant information about adrenocortical cancer probably due to this tumor is very rare. Animal studies agree with the epidemiological data and suggest that exogenous E2 may promote thyroid tumors. Ovary intact rats with the highest level of bioavailable E2 has the highest incidence of thyroid tumor development, and the ovariecromized rats with reduced concentrations of bioavailable E2 show a decrease in the incidence of thyroid tumors. Importantly, when ovariecromized rats are supplemented with E2, they develop a higher incidence of thyroid tumors than ovariecromized rats that are not supplemented with E2. The prostate of the adult male animals (rats, dogs, and monkeys) can develop carcinogenic features when the animal is treated with estrogen together with testosterone, and aromatase knockout mice, which are estrogen deficient but have an increase in androgens, do not develop prostate cancer. Similar to breast and gynecologic cancers, most endocrine gland tumors are now known to be able to biosynthesize estrogen in a paracrine manner since these malignant tissues can express a high level of aromatase.

Estrogen may carry out its carcinogenic function in endocrine gland tumors via the three channels described in breast and gynecologic cancers. However, few studies have been performed to
investigate the genotoxic role of estrogens as well as its phosphorylation function in endocrine gland tumors, leaving these two possible pathways unproved. Estrogen-mediated genomic and non-genomic pathways in endocrine gland tumors have been well examined and the study has been extensively focused on the shift in balance between ERα and ERβ in tumor cells with a decreased level of ERβ. In prostate cancer, E2-mediated the activation of ERα is associated with aberrant proliferation, inflammation and the development of malignancy, whereas E2-mediated the activation of ERβ is associated with anti-proliferation, differentiation and apoptosis. The importance of ERα rather than ERβ during the hormonal induction of prostate cancer is well demonstrated in the following animal study though it has not been proved in human. Pre-malignant prostatic intraepithelial neoplasia (PIN) lesions observed in the mouse model of prostate tumors induced by testosterone and E2 are characterized by significantly increased ERα expression within the lesion itself, and interestingly, PIN lesions can be induced in ERβ knockout but not ERα knockout mice. There is also strong evidence to support the inhibitory function of ERβ in prostate tumors. Knockdown of ERβ by its siRNA in prostate cells increases the expression of genes highly relevant to tumor cell proliferation such as prostate-derived Ets factor, the catalytic subunit of the telomerase, and ERα. In contrast, the up-regulation of ERβ by histone deacetylase inhibitor valproic acid (VPA) or tectorigenin results in antiproliferative effects by down-regulation of these genes. Therefore, the loss or decreased ERβ has been consistently associated with the progression of prostate tumors. The decreased level of ERβ may attribute to the apoptosis of ERβ cells which have been shown to have increased levels of Bax, poly(ADP-ribose) polymerase and caspase-3.

In thyroid cancer, the functions of ERα and ERβ mimic that in prostate cancer. The expression of ERα appears to be increased in thyroid tumor tissues while ERβ is frequently undetected. E2 can promote the proliferation of thyroid cells and this effect is positively related to ERα but negatively to ERβ, as ERα agonist PPT enhances cell proliferation whereas ERβ agonist DPN inhibits it. The E2-mediated cell proliferation is associated with a rapid (within minutes) increase in the phosphorylation of Erk1/2 and subsequently a reduced Bcl-2 but a decreased Bax level. The knockdown of ERα significantly attenuates the E2-mediated Bcl-2 and pERK1/2 expression. In contrast, the knockdown of ERβ markedly enhanced them. The E2-mediated regulation of Erk1/2 and Bcl-2 family molecules is believed to be via both genomic and non-genomic pathways since its effects can be achieved in minutes and also maintained for a few days. The inhibitory role of ERα is re-inforced using ERα selective antagonist MPP, which can effectively block E2-mediated proliferation to a similar level by ICI182780. The pro-proliferative function of ERα and the anti-proliferative function of ERβ have been further evident in another study using an adenoviral vector carrying ERα (Ad-ERα) or ERβ (Ad-ERβ). Ad-ERalpha infection stimulates thyroid cancer cell growth, in contrast, Ad-ERbeta infection suppresses their growth by inducing apoptosis. Furthermore, estrogen and anti-estrogen suppresses AP1 activity in Ad-ERalpha-infected cells, whereas upon Ad-ERbeta infection estrogen further stimulates AP1 activity which in turn is suppressed by anti-estrogen, suggesting that each ER acts differently through a non-ERE-mediated pathway.

In pancreatic cancer, E2 has been shown to stimulate the growth of tumor cells probably in a ER-related pathway. The expression of ERα mRNA is increased whereas ERβ is significantly decreased in the advantage of the tumor, suggesting a positive role of ERα but a negative role for ERβ in pancreatic cancer. In adrenocortical cancer, E2 enhances the proliferation of cancer cells. Compared with the normal adrenal cortex and adrenocortical adenomas, carcinomas are characterized by significantly lower ERβ levels, ERα upregulation, and aromatase overexpression.

Taken together, the above findings demonstrate the opposite function of ERα and ERβ in the tumors of endocrine system and most of these tumors have been shown to express a high level of pro-proliferative ERα but a low level of anti-proliferative ERβ. These findings raise the possibility of targeting ERα or stimulating ERβ as a possible therapy. In this aspect, the selective ERα antagonist Toremifene has been used to reduce the incidence of prostate cancer in men with high grade prostatic...
intraepithelial neoplasia, showing that Toremifene decreases the incidence of prostate cancer by 1 year.\textsuperscript{114} In the strategy to activate ER\(\beta\), ER\(\beta\) selective agonist DPN has successfully reduced the proliferation of prostate cancer cells and thyroid cancer cells,\textsuperscript{80,110} suggesting that ER\(\beta\) specific agonists might be valid candidates for new pharmacological approaches against tumors of endocrine system.

\textbf{C. Lung Cancer and Cancers of Digestive System (Colorectal, Esophageal, and Liver)}

The role of estrogen in lung cancer and digestive cancers (colorectal, esophageal, and liver) appears to be different from the other two categories described in the previous sections. The protective role of estrogen in lung cancer and digestive cancers is strongly suggested by the epidemiological observations. The current HRT users shows approximately 30\% reduced incidence of colorectal cancers\textsuperscript{115} and similar observations have been reported by a number of other reports.\textsuperscript{34,116--121} The mechanism of protection may associate with selective regulation of apoptotic genes in colon cancer cells by E2.\textsuperscript{119} The E2-protective role is also noted in liver, esophageal, and lung cancers.\textsuperscript{35,122--124}

In experiments, E2 induces colon cancer cell apoptosis in an ER\(\alpha\)-related pathway.\textsuperscript{125} It increases hTNF-alpha gene expression, which in turn activates caspase-8, -9, and caspase-3 and leads to the DNA fragmentation and apoptosis. In the other hand, E2 plus ERalpha down-regulates beta-catenin signalings to suppress proliferation and metastasis of colorectal cells.\textsuperscript{125} The study suggests that efforts aiming at enhancing ER\(\alpha\) level or activity may be an alternative therapy against colorectal cancer.\textsuperscript{125} The finding is somewhat supported by the fact that ER\(\alpha\) is either reduced or undetected in colorectal cancer cells.\textsuperscript{126--128} However, the activation of ER\(\beta\) has also shown to be associated with the inhibition of colon cancer.\textsuperscript{127} The relationship between ER\(\alpha\) and ER\(\beta\) in colorectal cancer remains unknown and the exact function of ER\(\alpha\) and ER\(\beta\) in colorectal cancers also needs further experiments to confirm. However, it appears that the function of ERs in colorectal cancers differs from that in breast cancer, gynecological and endocrine cancers. Similar to colorectal cancer, E2 also inhibits the growth of esophageal cancer cells in an ER\(\alpha\)-dependent pathway since the inhibitory effect is lost in ER\(\alpha\)-negative esophageal cancer cells.\textsuperscript{129,130} Though esophageal cancer cells also express ER\(\beta\),\textsuperscript{131} the role of ER\(\beta\) is unknown. However, positive expression of ER\(\alpha\) in addition to negative expression of ER\(\beta\) is an unfavorable independent prognostic indicator in squamous cell carcinoma of the esophagus.\textsuperscript{132} There are very limited reports on the function of ER\(\alpha\) and ER\(\beta\) in hepatocellular carcinoma (HCC) and their role remain unclear, though HCC cells are known to express both.\textsuperscript{133} There is a study indicating that the presence of variant liver ER transcripts in the tumor is the strongest negative predictor of survival in inoperable HCC and their presence is associated with spontaneous survival significantly worse than in patients with wild-type ERs.\textsuperscript{134}

Non-small cell lung cancer cells (NSLCC) express both ER\(\alpha\) and ER\(\beta\) in the nucleus as well as extra-nuclear sites,\textsuperscript{135} and the block of either of them by their siRNA can results in a significant reduction in the proliferation of cells.\textsuperscript{136} The finding is supported by application of ER antagonist ICI 182780 leading to the inhibition of NSLCC proliferation.\textsuperscript{137} Therefore, the functional role of ER\(\alpha\) and ER\(\beta\) seems to benefit the growth of the tumor since the inhibition or block of both leads to the arrest the tumor growth. The functional relationship between both ERs is complicated by the observations that ER\(\alpha\) expression and the absence of ER\(\beta\) expression are associated with a poorer prognosis for NSCLC patients, and that the absence of ER\(\beta\) serves as a marker identifying patients at high risk even at an early clinical stage.\textsuperscript{138} Obviously, further studies need to clarify this point as well as the signaling pathway of E2 in lung cancer.

\textbf{3. FUTURE PROSPECTS}

A better understanding of the molecular mechanisms by which estrogen stimulates cell growth can provide new insights into diagnosis, treatment and prevention in estrogen-associated tumors. Loss of
Estrogen or its receptors contributes to the development or progression of various tumors. Both activation (via estrogen agonists) and inhibition (via estrogen antagonists) of ER action are therapeutic strategies currently used in the clinical setting. ER antagonists, SERM and aromatase inhibitors are effective for the treatment of breast cancer and endometrial cancer, and their usefulness and efficacy in the treatment of other hormone-dependent cancers such as prostate cancer and thyroid cancer awaits further study. At the same time, other novel strategies to selectively target or stimulate ERα or ERβ may appear to be more effective for certain tumors such as prostate and thyroid cancers. Since both the genomic and non-genomic responses of ERα and ERβ can be exquisitely coordinated and be co-functional in physiological and pathological conditions, it will be certainly a great advantage if a treatment can specifically block adverse effects of ERs but leave beneficial effects of ERs intact. Nevertheless, any single therapy or therapies in combination should be thought to optimally induce apoptosis or death of cancer cells without damaging healthy cells. Clearly, estrogen and its receptors have been implicated in the pathogenesis of several cancers but their definitive role has yet to be fully established, especially in lung cancer and cancer of digestive system. Understanding the role that estrogen and its receptors may play in the risk or severity of the tumor will no doubt increase our ever-expanding knowledge of the relationship among estrogen, ERs, and cancers.

4. Abbreviations

16-LE2 3,17-dihydroxy-19-nor-17-pregna-1,3,5(10)-triene-21,16-lactone
8β-VE2 8-vinylestra-1,3,5(10)-triene-3,17β-diol
Ad-ERα adnoviral vector carrying ERα
Ad-ERβ adnoviral vector carrying ERβ
AF1 activation function-1
AP-1 activator protein 1
DPN diarylpropionitrile
E2 17β-estradiol
E3 estriol
EGFR epidermal growth factor receptor
ERB-041 2-(3-fluoro-4-hydroxyphenyl)-7-vinyl-1,3-benzoxazol-5-ol
ERE estrogen response element
ERs estrogen receptors
ERT estrogen-replacement therapy
ERα46 46 kDa isoform
ERα66 66 kDa form of ERα
ERβδ125 ERβ1 exons 1, 2, and 5
ERβδ1256 ERβ1 exons 1, 2, 5, and 6
Fox forkhead box
GPR30 G protein-coupled receptor-30
GRIP1 glucocorticoid receptor-interacting protein 1
HCC hepatocellular carcinoma
HPA hypothalamic-pituitary-adrenal
HRT hormone-replacement therapy
MAPK mitogen-activated protein kinase
NF-κB nuclear factor kappaB
NR nuclear receptor
NSLCC non-small cell lung cancer cells
OC oral contraceptives
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