Molecular biology of beta-estradiol-estrogen receptor complex binding to estrogen response element and the effect on cell proliferation

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Abstract

Group of estrogen pollutants, where the highest estrogen activity is reported at estradiol, is characterized by the fact that even at very low concentrations have potential to cause xenoestrogenic effects. During exposure of excessive amounts of estradiols may be produced undesirable effects resulting in the feminization of males of water organisms. The presence of estradiols in drinking water implies also a risk for the human population in the form of cancers of endocrine systems, abnormalities in reproduction or dysfunctions of neuronal and immune system. Currently, the research is focused mainly to uncover the relationship between the estrogen receptors binding affinity with an estrogen response element and estradiol. In this review we summarized facts about molecular biological principles of β estradiol-estrogen receptor complex binding with estrogen response element and its successive effect on cancer genes expression.

Abbreviations:

E2	- β estradiol
EDC	- Endocrine disrupting compounds
ER	- Estrogen receptor
ERE	- Estrogen response element
HSP 90	- Heat shock protein 90
RA	- Retinoic acid
LBD	- Ligand binding domain
DBD	- DNA binding domain
AF1	- Hormone-independent transcriptional activation function domain
AF2	- Ligand-dependent transcriptional activation domain
MAPK	- Mitogen-activated protein-kinase
SRC	- Steroid receptor coactivator
LSD1	- Lysine-specific demethylase 1
CCAR1	- Cell cycle apoptosis regulator 1
DBC1	- Deleted in breast cancer 1
CARM1	- Coactivator-associated arginine methyltransferase 1
SERMs	- Selective estrogen receptor modulator

REVIEW ARTICLE

INTRODUCTION

Estrogenic compounds related to Endocrine Disrupting Compounds (EDC) belong to pollutants gaining the increasing environmental and social concerns in recent years because of their endocrine-disrupting property and other serious side effects on human health (Liu *et al.* 2004; Ballesteros-Gomez *et al.* 2009; Swedenborg *et al.* 2009). With the rapid economy development, a variety of EDCs have been discharged into the aquatic environment (Jeffries *et al.* 2011), received in large amounts in the urine and excreta of livestock (Tang *et al.* 2013) but also in the urine of people that contains residues of contraceptives, whose consumption increases every year (Preda *et al.* 2012; Qiang *et al.* 2013). Estrogenic pollutants, among which the highest activity was reported at β estradiol (E2) (Boulay & Perdiz, 2005; Heldring *et al.* 2007) are characterized by the fact that even at very low concentrations, the long-term exposure can cause xenoestrogenic effects (Lange *et al.* 2012; Qin *et al.* 2013b). Sewage treatment plants currently do not have a way how to effectively break down these substances from the water. Although a certain amount of these substances remains bound in the sludge (Gagne *et al.* 2013), the rest is released into the receiving waters of the sewage treatment plant (Nie *et al.* 2012). Their adverse effects are caused through the endocrine system by mimicking the action of natural hormones (Bovet *et al.* 2009). As EDCs disrupt the actions of endogenous hormones, they may induce abnormal reproduction, stimulation of cancer growth, dysfunction of neuronal and immune systems (Howdeshell *et al.* 2008; Jenkins *et al.* 2012; Macon & Fenton, 2013; Lee *et al.* 2013; Tang *et al.* 2013). Estrogens provide gene transcription and signaling of plenty processes in cell in a variety of tissues, the bone, breast, and endometrium, through binding and activation of estrogen receptors (ERs) (Ceylan *et al.* 2012; Wolinska-Witort *et al.* 2012; Komm & Mirkin, 2013). Because breast cancer is the most common cancer in women, both in developed and developing countries

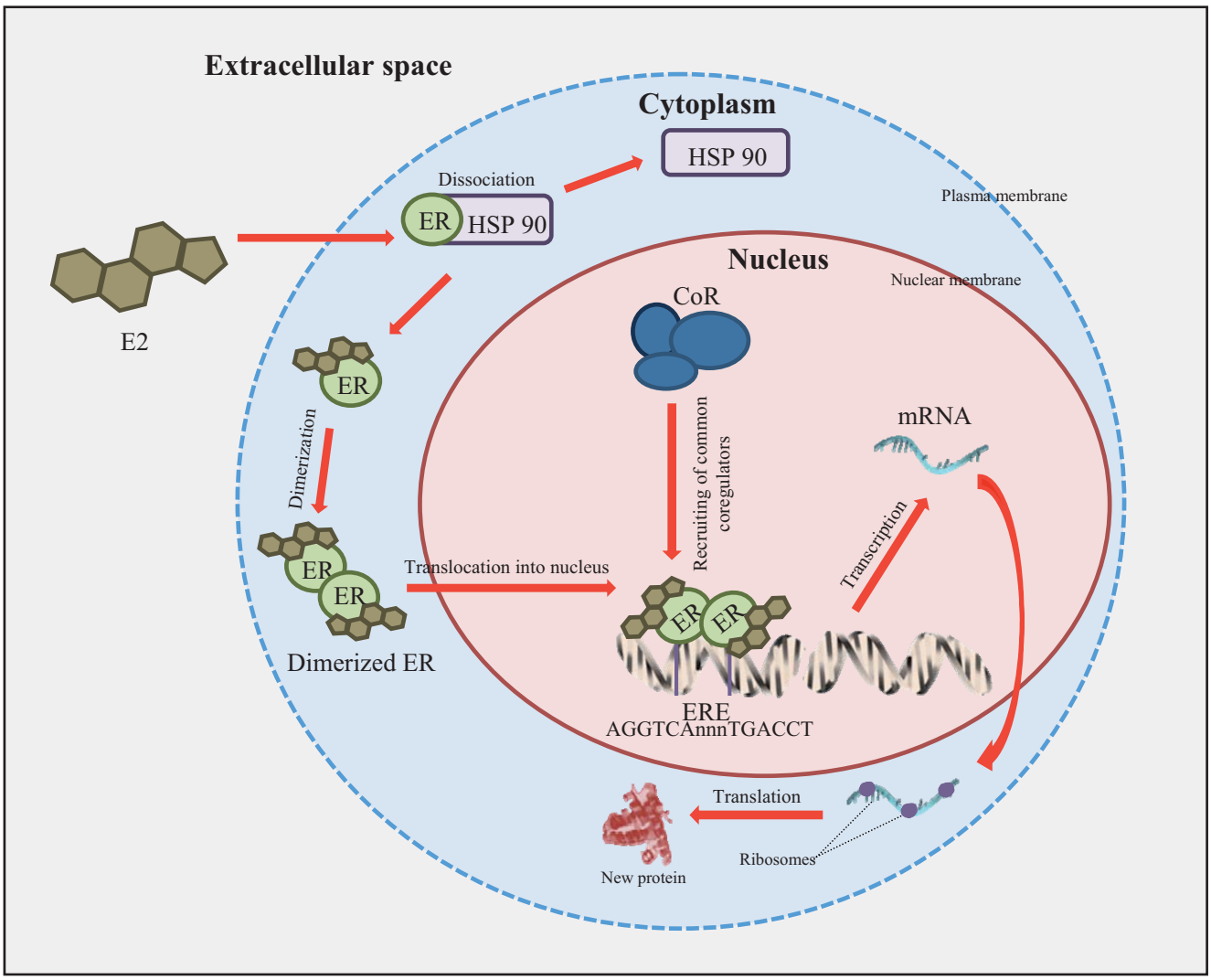

Fig. 1. Scheme of basic molecular biological way of estradiol physiological function, where E2 stays for estradiol, ER for estrogen receptor, HSP 90 for heat shock protein 90, CoR for coregulators and ERE for estrogen response element.

(van Duursen *et al.* 2013), the issue of reproductive toxicants is a major scientific challenge for human health (Bechi *et al.* 2013). The aims of this review are to summarize the facts about the molecular biological principles of estradiol pathways in complex with estrogen receptor, it is binding with estrogen response element and its effect on target genes involved in cell proliferation.

MOLECULAR BIOLOGY OF ESTRADIOL

Currently, research covering this issue is applied especially to clarify the relationship between estradiol (Figure 1), estrogen response element (ERE) and estrogen receptor (ER), a member of hormone receptors of the nuclear receptor family (Gronemeyer *et al.* 2004; Srinivasan *et al.* 2013). There are known two subtypes of estrogen receptors ERα and ERβ (Muyan *et al.* 2012; Chieffi & Chieffi, 2013; Oh & Chung, 2013) encoded by distinct genes, *Esr1* and *Esr2*, respectively (Billon-Gales *et al.* 2011). These steroid transcription factors remain nowadays the most informative biomarker in breast cancer diagnosis (Patani *et al.* 2013), because more than three quarters of breast tumors are ERα-positive (Dunnwald *et al.* 2007). ERs are composed of the polypeptide chain and located in the cell cytoplasm in multiprotein complex containing the molecular chaperone HSP 90 (heat shock protein 90, Figure 1) (Cheng *et al.* 2012). Ligand binding triggers the conformational changes that lead to dissociation and receptor dimerization (Jeong *et al.* 2012). The proliferative or pro-survival action of estrogens is antagonized in most cases by retinoic acid (RA) (Ombra *et al.* 2013). Activated receptor-ligand complex is replaced into the nucleus of cell, where interactions with coactivators (CoA) or corepressors (CoR) of transcription are undergoing (McDonnell & Wardell, 2010; Coughlan *et al.* 2013).

PRINCIPLES OF E2-ER-ERE BINDING

Because of steroidal hormone properties, estrogens can pass through the phospholipid membranes of the cell to realize the binding with ER (Oh & Chung, 2013; Qin *et al.* 2013a). ER proteins are composed of six functional domains labeled as A through F (Ascenzi *et al.* 2006; Han *et al.* 2007). The E ligand-binding domain (LBD) provides ligand specificity to the receptor and contains ligand-dependent transcriptional activation function (AF2) (MacPherson *et al.* 2009; Jiang *et al.* 2010), localized in its conformationally dynamic region (Figure 2). It was found that ERs conformation changes are leading to a helix 12 realignment with helices 3, 5–6 and 11 and thereby forming a lid on the LBD for the surrounded E2 (Figure 2) (Endler *et al.* 2012). Agonist ligands stabilize a receptor conformation that is optimal for efficient interaction with coactivators and the direct (or indirect) binding to *cis*-acting elements and thereby triggering of a transcriptional activation (Heldring *et al.* 2007; Chen *et al.* 2009; Billon-Gales *et al.* 2011). The C domain responsible for targeting the receptor to DNA possesses two zinc fingers forming a helix-loop-helix motif and primarily functions in binding of the receptor to the hormone response elements. Zinc finger contains a P box for identification of the specific DNA sequence. Second zinc finger includes at its base a D box. Its main role is the recognition of the distance between hexamers constituting the ERE in the promoter of the target gene (Puzianowska-Kuznicka *et al.* 2013). In the N-terminus, ER contains a hormone-independent transcriptional activation function domain (AF1) (Alimirah *et al.* 2012), regulated by growth factors acting through the MAPK signaling pathway (Kato *et al.* 1995). Both AF domains recruit a range of coregulatory protein complexes to the DNA-bound receptor, altering the chromatin structure and facilitate recruitment of the RNA polymerase II transcription system (Heldring *et al.* 2007).

ER COREGULATORS

Transcription inhibition or enhancement is achieved by ligand-regulated coactivators and corepressors (Aust *et al.* 2013). Hundreds of potential coregulators with diverse functions, from histone modification and chromatin remodeling to RNA polymerase II recruitment and mRNA splicing, have been identified (Lonard & O'Malley, 2006). Corepressors are crucial regulators of ERα-mediated action, and that their loss could promote breast cancer development and resistance to endocrine therapy (Dobrzycka *et al.* 2003). To the novel coregulators with a multitude functions belongs proline-, glutamic acid-, and leucine-rich protein (PELP1) which expression is deregulated in hormonal tumors, and functions as a proto-oncogene, however, the mechanism by which PELP1 promotes oncogenesis remains unclear (Gonugunta *et al.* 2011). In study by Kim *et al.* (2013) it was reported first evidence about protein CAC1, associated with LSD1, working as an ERα corepressor, implicating a potential antitumor target like Paclitaxel in ERα-positive breast cancer. Jeong *et al.* (2011) demonstrated that interactions with the protein acetyltransferase (TIP 60) are also one of the required coregulators for estrogen-induced transcription of a subset of ERα target genes in human cells. Coactivators, where among the best-characterized belong the steroid receptor coactivators (SRC) or p160 family (SRC-1, SRC-2, SRC-3), work as a scaffold proteins for other coregulators (Jeong *et al.* 2012). Data published by Karmakar *et al.* (2009) indicate that the closely related p160 coactivators are not functionally redundant in breast cancer cells because they play gene-specific roles in regulating mRNA and protein expression, and they therefore are likely making unique contributions to breast tumor genesis. Among important coactivators belong also cell cycle and apoptosis regulator 1 (CCAR1), deleted in breast cancer 1 (DBC1) (Yu *et*

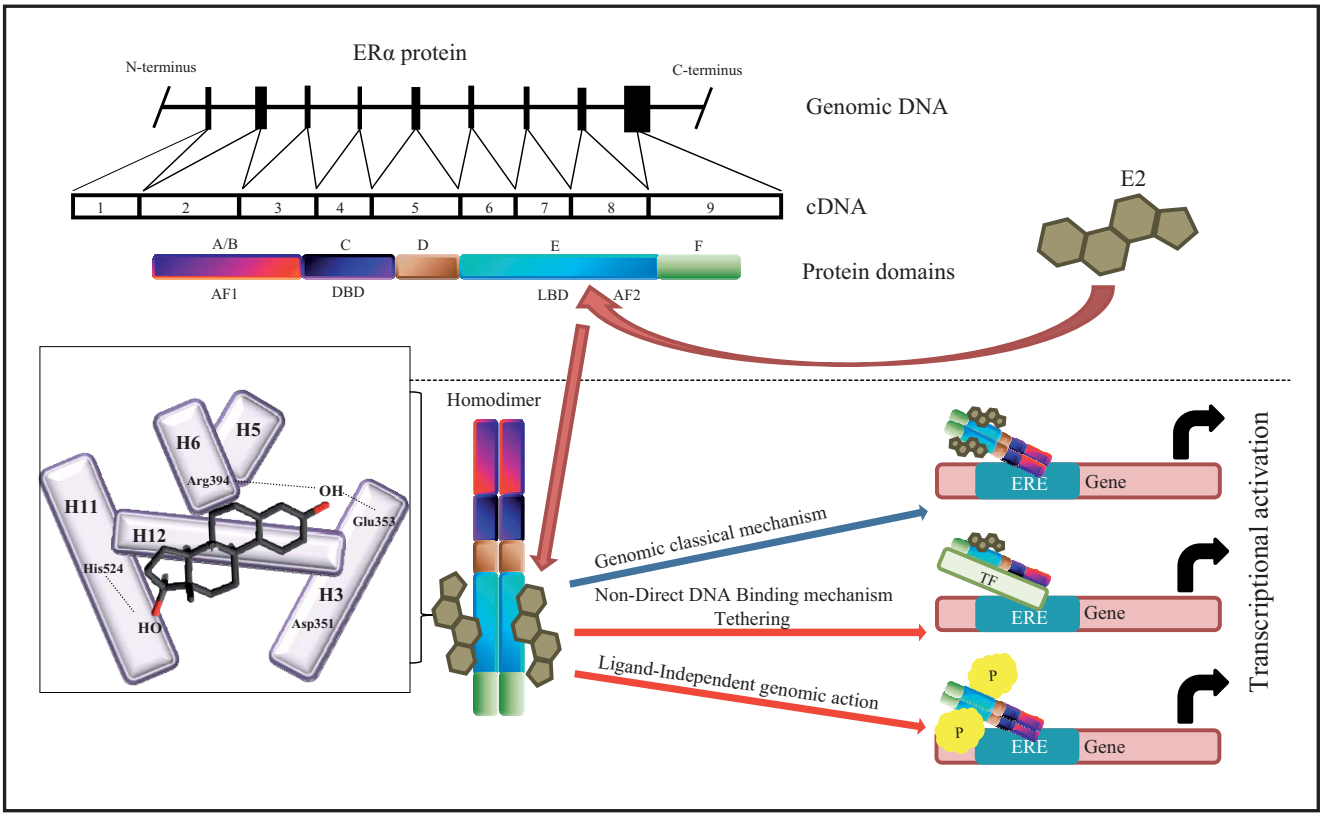

Fig. 2. Scheme of ER protein, its cDNA, gene and its binding mechanisms. DBD stays for DNA binding domain, LBD for ligand binding domain, TF for another protein – transcription factor, interacting with ER during Non-Direct DNA binding mechanism, P for phosphorylation induced by various kinases, AF1 for hormone-independent transcriptional activation function domain and AF2 for ligand-dependent transcriptional activation domain.

al. 2011) and coactivator-associated arginine methyltransferase 1 (CARM1) (Coughlan *et al.* 2013; Zeng *et al.* 2013) important co-activators for estrogen-induced gene expression and estrogen-dependent growth of breast cancer cells.

MOLECULAR PATHWAYS INVOLVING E2-ER COMPLEX BINDING TO ERE

The proliferative effect of estrogens is exerted via genomic and non-genomic pathways (Abbondanza *et al.* 2012). Non-genomic pathway is not as well understood as the genomic mechanism, but has been observed in many tissues. Probably signaling cascades are initiated via second messengers affecting ion channels or increasing nitric oxide levels in the cytoplasm, leading to a physiological responses without involving gene regulation (Heldring *et al.* 2007). In the genomic pathways in the endocrine glands as a mammary gland, proliferating cells express low levels of ERα, which is down regulated through a ubiquitin proteasome pathway, in the presence of β estradiol by direct binding to ERE (Cirillo *et al.* 2013). ERs can also be recruited to genomic DNA by indirect by tethering through other DNA-bound transcription factors, including members of the activating protein-1 (AP-1) and cAMP response element-binding protein family members creating a heterodimers (Heldring *et al.* 2011). The last of genomic pathways works through ER phosphorylation. Mitogen activated protein kinase (MAPK) is known to directly phosphorylate ER alpha at serine 118 in a ligand-independent manner (McGlynn *et al.* 2013). As a crucial target or ligand-independent pathway is AF1 region that forms the target for receptor phosphorylation. In addition the AF1 and AF2 regions can interact with different coactivators and corepressors, triggering gene transcription separately or synergistically (Acconcia & Kumar, 2006).

GENES EXPRESSED IN CONNECTION WITH ER-INDUCED CANCER

Tumor development occurs when a buildup of genetic mutations in genes, mostly controlling cell growth and division or the damaged DNA repairment, allows cells to grow and divide uncontrollably and to form a tumorous tissue subsequently. For example tumor suppressive p53 gene encodes a same name protein stopping tumor growth. Mutations in p53 gene cause a disorders with potential to develop soft tissue cancers like breast cancer (Chang *et al.* 2013). Many genes involved in cell growth, growth factor signaling, and cell cycle control are estrogen responsive (e.g. *fos*, *myc*, *myb*, *cdc25a*, *A2*, *p53* and *stk15*). Inappropriate expression or activ-

ity of a number of these genes has been implicated in breast cancer (Hodges *et al.* 2003). At *in vitro* study by Shahmoradgoli *et al.* (2013) significantly higher levels of *PPP1R15B* mRNA expression in luminal (ERα-positive) and normal-like subtypes of breast tumors were observed. High levels of *Runx2* expressed in some invasive breast cancer cell lines were observed by Ferrari and colleagues, but intriguingly, *Runx2* was also expressed in the ER negative population (Ferrari *et al.* 2013). Genetic alterations in the *TopBP1* gene influencing the risk of breast cancer were also observed (Forma *et al.* 2013). *ROS1* mRNA expression connection with decreased ER expression was confirmed by Eom *et al.* (2013). Their real-time PCR results showed that *ROS1* expression was decreased in patients with the increased histologic grade and increased mitotic counts. Among genes that are commonly a part of a ERα-positive breast tumor signature belong *GATA-3* (Wilson & Giguere, 2008; Gaynor *et al.* 2013), *FOXA-1* (Schneider *et al.* 2006), *XBP-1* (van't Veer *et al.* 2002) and *TFF3* (Chen *et al.* 2011). In study of Lattrich *et al.* (2013) it was mentioned also the connection between luminal breast tumors exerted by unspecific activation of ERα and proliferative effects accompanied by overexpression of *cyclin B1*, *PR* and *PS2* genes. E2-induced *Bcl-2* transcription effects on demethylation of lysine driven by the LSD1 demethylase, producing reactive oxygen species (ROS) with consequent influence on DNA strands were observed (Perillo *et al.* 2008). Estradiol was proved as a downregulator of *ST8SIA1* mRNA expression as well as *ST8SIA1* core promoter activity linked with luminal breast cancer (Bobowski *et al.* 2013). There exist far more genes involved in progression of cancers, connected with ER observed by different methods, but nowadays the largest discussion arises according to genes *BRCA1* and *BRCA2*, whose mutations are highly linked with different types of breast tumors (Meric-Bernstam *et al.* 2013). The absence of ER in *BRCA1/2* (69.1/19 % of patients, according to (Meric-Bernstam *et al.* 2013)) often induced breast tumors deteriorates the benefits from hormonal therapy, but still sensitivity to cytotoxic treatment regimens remains (Brunello *et al.* 2013).

CONCLUSION

β-estradiol as the most potent estrogen pollutant represents currently significant scientific challenge due to its endocrine-disrupting properties and other serious undesirable effects on health. Although the classical pathway of estradiol-ER direct binding to ERE has been known for quite long period of time, recently there were showed additional possible bindings in the interaction with proteins or after phosphorylation. Knowledge of the molecular pathways of estradiol may help to develop increasingly efficient antitumor drugs, such as SERMs granting the possibility to selectively inhibit or stimulate estrogen-like actions in different tissues.

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REFERENCES

1 Abbondanza C, De Rosa C, D'arcangelo A, Pacifico M, Spizuoco C, Piluso G, , et al (2012). Identification of a functional estrogen-responsive enhancer element in the promoter 2 of PRDM2 gene in breast cancer cell lines. J Cell Physiol. **227**: 964–975.
2 Acconcia F, Kumar R (2006). Signaling regulation of genomic and nongenomic functions of estrogen receptors. Cancer Lett. **238**: 1–14.
3 Alimirah F, Peng XJ, Yuan L, Mehta RR, Von Knethen A, Choubey D, et al (2012). Crosstalk between the peroxisome proliferator-activated receptor gamma (PPAR gamma) and the vitamin D receptor (VDR) in human breast cancer cells: PPAR gamma binds to VDR and inhibits 1 alpha,25-dihydroxyvitamin D-3 mediated transactivation. Exp Cell Res. **318**: 2490–2497.
4 Ascenzi P, Bocedi A, Marino M (2006). Structure-function relationship of estrogen receptor α and β: Impact on human health. Mol Asp Med. **27**: 299–402.
5 Aust S, Horak P, Pils D, Pils S, Grimm C, Horvat R, et al (2013). The prognostic value of estrogen receptor beta and proline-, glutamic acid- and leucine-rich protein 1 (PELP1) expression in ovarian cancer. BMC Cancer. **13**: 1–7.
6 Ballesteros-Gomez A, Rubio S, Perez-Bendito D (2009). Analytical methods for the determination of bisphenol A in food. J Chromatogr A. **1216**: 449–469.
7 Bechi N, Sorda G, Spagnoletti A, Bhattacharjee J, Ferro EaV, Barbosa BD, et al (2013). Toxicity assessment on trophoblast cells for some environment polluting chemicals and 17 beta-estradiol. Toxicol Vitro. **27**: 995–1000.
8 Billon-Gales A, Krust A, Fontaine C, Abot A, Flouriot G, Toutain C, Berges H, Gadeau AP, et al (2011). Activation function 2 (AF2) of estrogen receptor-alpha is required for the atheroprotective action of estradiol but not to accelerate endothelial healing. Proc Natl Acad Sci U S A. **108**: 13311–13316.
9 Bobowski M, Vincent A, Steenackers A, Colomb F, Van Seuningen I, Julien S, et al (2013). Estradiol Represses the G(D3) Synthase Gene ST8SIA1 Expression in Human Breast Cancer Cells by Preventing NF kappa B Binding to ST8SIA1 Promoter. PLoS One. **8**: 1–12.
10 Boulay F, Perdiz D (2005). 17 beta-Estradiol modulates UVB-induced cellular responses in estrogen receptors positive human breast cancer cells. J Photochem Photobiol B-Biol. **81**: 143–153.
11 Bovet C, Plet B, Ruff M, Eiler S, Granger F, Panagiotidis A, et al (2009). Towards high-throughput identification of endocrine disrupting compounds with mass spectrometry. Toxicol Vitro. **23**: 704–709.
12 Brunello A, Borgato L, Basso U, Lumachi F, Zagonel V (2013). Targeted Approaches to Triple-Negative Breast Cancer: Current Practice and Future Directions. Curr Med Chem. **20**: 605–612.
13 Ceylan U, Akhan SE, Bastu E, Gungor-Ugurlucan F, Iyibozkurt AC, Topuz S (2012). Comparison of tibolone and 17beta-estradiol administration on the expression of zonula occludens-1, occludin, glial fibrillary acidic protein and c-fos levels in the brain cortex and hippocampus of female rats. Neuroendocrinol Lett. **33**: 505–510.
14 Chang HL, Chen CY, Hsu YF, Kuo WS, Ou G, Chiu PT, et al (2013). Simvastatin induced HCT116 colorectal cancer cell apoptosis through p38MAPK-p53-survivin signaling cascade. Biochim Biophys Acta-Gen Subj. **1830**: 4053–4064.

15 Chen HQ, Yang KH, Choi SY, Fischer JH, Jeong HY (2009). Up-Regulation of UDP-Glucuronosyltransferase (UGT) 1A4 by 17 beta-Estradiol: A Potential Mechanism of Increased Lamotrigine Elimination in Pregnancy. Drug Metab Dispos. **37**: 1841–1847.
16 Chen YZ, Chen CM, Yang BL, Xu QH, Wu F, Liu F, et al (2011). Estrogen receptor-related genes as an important panel of predictors for breast cancer response to neoadjuvant chemotherapy. Cancer Lett. **302**: 63–68.
17 Cheng Q, Chang JT, Geradts J, Neckers LM, Haystead T, Spector NL, et al (2012). Amplification and high-level expression of heat shock protein 90 marks aggressive phenotypes of human epidermal growth factor receptor 2 negative breast cancer. Breast Cancer Res. **14**: 1–15.
18 Chieffi P, Chieffi S (2013). Molecular biomarkers as potential targets for therapeutic strategies in human testicular germ cell tumors: An overview. J Cell Physiol. **228**: 1641–1646.
19 Cirillo F, Nassa G, Taralla R, Stellato C, De Filippo MR, Arribrosino C, et al (2013). Molecular Mechanisms of Selective Estrogen Receptor Modulator Activity in Human Breast Cancer Cells: Identification of Novel Nuclear Cofactors of Antiestrogen-ER alpha Complexes by Interaction Proteomics. J Proteome Res. **12**: 421–431.
20 Coughlan N, Thillainadesan G, Andrews J, Isovic M, Torchia J (2013). beta-Estradiol-dependent activation of the JAK/STAT pathway requires p/CIP and CARM1. Biochim Biophys Acta-Mol Cell Res. **1833**: 1463–1475.
21 Dobrzycka KM, Townson SM, Jiang S, Oesterreich S (2003). Estrogen receptor corepressors - A role in human breast cancer? Endocrine-Rel Cancer. **10**: 517–536.
22 Dunnwald LK, Rossing MA, Li CI (2007). Hormone receptor status, tumor characteristics, and prognosis: a prospective cohort of breast cancer patients. Breast Cancer Res. **9**: 1–10.
23 Endler A, Chen L, Zhang J, Xu GT, Shibasaki F (2012). Binding of the ER alpha and ARNT1 AF2 domains to exon 21 of the SRC1 isoform SRC1e is essential for estrogen- and dioxin-related transcription. J Cell Sci. **125**: 2004–2016.
24 Eom M, Lkhagyadorj S, Oh SS, Han A, Park KH (2013). ROS1 Expression in Invasive Ductal Carcinoma of the Breast Related to Proliferation Activity. Yonsei Med J. **54**: 650–657.
25 Ferrari N, Mcdonald L, Morris JS, Cameron ER, Blyth K (2013). RUNX2 in mammary gland development and breast cancer. J Cell Physiol. **228**: 1137–1142.
26 Forma E, Brzezianska E, Krzeslak A, Chwatko G, Jozwiak P, Szymczyk A, et al (2013). Association between the c.*229C > T polymorphism of the topoisomerase II beta binding protein 1 (TopBP1) gene and breast cancer. Mol Biol Rep. **40**: 3493–3502.
27 Gagne F, Smyth SA, Andre C, Douville M, Gelinas M, Barclay K (2013). Stress-related gene expression changes in rainbow trout hepatocytes exposed to various municipal wastewater treatment influents and effluents. Environ Sci Pollut Res. **20**: 1706–1718.
28 Gaynor KU, Grigorieva IV, Allen MD, Esapa CT, Head RA, Gopinath P, et al (2013). GATA3 Mutations Found in Breast Cancers May Be Associated with Aberrant Nuclear Localization, Reduced Transactivation and Cell Invasiveness. Horm Cancer. **4**: 123–139.
29 Gonugunta VK, Nair BC, Rajhans R, Sareddy GR, Nair SS, Vadlamudi RK (2011). Regulation of rDNA Transcription by Proto-Oncogene PELP1. PLoS One. **6**: 1–9.
30 Gronemeyer H, Gustafsson JA, Laudet V (2004). Principles for modulation of the nuclear receptor superfamily. Nat Rev Drug Discov. **3**: 950–964.
31 Han WD, Zhao YL, Meng YG, Zang L, Wu ZQ, Li Q, et al (2007). Estrogenically regulated LRP16 interacts with estrogen receptor α and enhances the receptor's transcriptional activity. Endocrine-Rel Cancer. **14**: 741–753.
32 Heldring N, Isaacs GD, Diehl AG, Sun M, Cheung E, Ranish JA, et al (2011). Multiple Sequence- Specific DNA-Binding Proteins Mediate Estrogen Receptor Signaling through a Tethering Pathway. Mol Endocrinol. **25**: 564–574.
33 Heldring N, Pike A, Andersson S, Matthews J, Cheng G, Hartman J, et al (2007). Estrogen receptors: How do they signal and what are their targets. Physiol Rev. **87**: 905–931.
34 Hodges LC, Cook JD, Lobenhofer EK, Li LP, Bennett L, Bushel PR, et al (2003). Tamoxifen functions as a molecular agonist inducing cell cycle-associated genes in breast cancer cells. Mol Cancer Res. **1**: 300–311.
35 Howdeshell KI, Wilson VS, Furr J, Lambright CR, Rider CV, Blystone CR, et al (2008). A mixture of five phthalate esters inhibits fetal testicular testosterone production in the Sprague-Dawley rat in a cumulative, dose-additive manner. Toxicol Sci. **105**: 153–165.
36 Jeffries MKS, Conoan NH, Cox MB, Sangster JL, Balsiger HA, Bridges AA, et al (2011). The anti-estrogenic activity of sediments from agriculturally intense watersheds: Assessment using in vivo and in vitro assays. Aquat Toxicol. **105**: 189–198.
37 Jenkins S, Betancourt AM, Wang J, Lamartiniere CA (2012). Endocrine-active chemicals in mammary cancer causation and prevention. J Steroid Biochem Mol Biol. **129**: 191–200.
38 Jeong KW, Kim K, Situ AJ, Ulmer TS, An WJ, Stallcup MR (2011). Recognition of enhancer element-specific histone methylation by TIP60 in transcriptional activation. Nat Struct Mol Biol. **18**: 1358–1365.
39 Jeong KW, Chodankar R, Purcell DJ, Bittencourt D, Stallcup MR (2012). Gene-Specific Patterns of Coregulator Requirements by Estrogen Receptor-alpha in Breast Cancer Cells. Mol Endocrinol. **26**: 955–966.
40 Jiang PP, Hu QP, Ito M, Meyer S, Waltz S, Khan S, et al (2010). Key roles for MED1 LxxLL motifs in pubertal mammary gland development and luminal-cell differentiation. Proc Natl Acad Sci U S A. **107**: 6765–6770.
41 Karmakar S, Foster EA, Smith CL (2009). Unique Roles of p160 Coactivators for Regulation of Breast Cancer Cell Proliferation and Estrogen Receptor-alpha Transcriptional Activity. Endocrinology. **150**: 1588–1596.
42 Kato S, Endoh H, Masuhiro Y, Kitamoto T, Uchiyama S, Sasaki H, et al (1995). Activation of the estrogen-receptor through phosphorylatin by mitogen-activated protein-kinase. Science. **270**: 1491–1494.
43 Kim J, Park UH, Moon M, Um SJ, Kim EJ (2013). Negative regulation of ER alpha by a novel protein CAC1 through association with histone demethylase LSD1. FEBS Lett. **587**: 17–22.
44 Komm BS, Mirkin S (2013). Evolution of the tissue selective estrogen complex (TSEC). J Cell Physiol. **228**: 1423–1427.
45 Lange A, Katsu Y, Miyagawa S, Ogino Y, Urushitani H, Kobayashi T, et al (2012). Comparative responsiveness to natural and synthetic estrogens of fish species commonly used in the laboratory and field monitoring. Aquat Toxicol. **109**: 250–258.
46 Lattrich C, Stegerer A, Haring J, Schuler S, Ortmann O, Treeck O (2013). Estrogen receptor beta agonists affect growth and gene expression of human breast cancer cell lines. Steroids. **78**: 195–202.
47 Lee HR, Jeung EB, Cho MH, Kim TH, Leung PCK, Choi KC (2013). Molecular mechanism(s) of endocrine-disrupting chemicals and their potent oestrogenicity in diverse cells and tissues that express oestrogen receptors. J Cell Mol Med. **17**: 1–11.
48 Liu R, Zhou JL, Wilding A (2004). Simultaneous determination of endocrine disrupting phenolic compounds and steroids in water by solid-phase extraction-gas chromatography-mass spectrometry. J Chromatogr A. **1022**: 179–189.
49 Lonard DM, O'Malley BW (2006). The expanding cosmos of nuclear receptor coactivators. Cell. **125**: 411–414.
50 Macon MB, Fenton SE (2013). Endocrine Disruptors and the Breast: Early Life Effects and Later Life Disease. J Mammary Gland Biol Neoplasia. **18**: 43–61.
51 Macpherson L, Lo R, Ahmed S, Pansoy A, Matthews J (2009). Activation function 2 mediates dioxin-induced recruitment of estrogen receptor alpha to CYP1A1 and CYP1B1. Biochem Biophys Res Commun. **385**: 263–268.
52 Mcdonnell DP, Wardell SE (2010). The molecular mechanisms underlying the pharmacological actions of ER modulators: implications for new drug discovery in breast cancer. Curr Opin Pharmacol. **10**: 620–628.
53 McGlynn LM, Tovey S, Bartlett JMS, Doughty J, Cooke TG, Edwards J (2013). Interactions between MAP kinase and oestrogen receptor in human breast cancer. Eur J Cancer. **49**: 1176–1186.

54 Meric-Bernstam F, Gutierrez-Barrera AM, Litton J, Mellor-Crummey L, Ready K, Gonzalez-Angulo AM, *et al.* (2013). Genotype in BRCA-associated Breast Cancers. Breast J. **19**: 87–91.
55 Muyan M, Callahan LM, Huang YF, Lee AJ (2012). The ligand-mediated nuclear mobility and interaction with estrogen-responsive elements of estrogen receptors are subtype specific. J Mol Endocrinol. **49**: 249–266.
56 Nie YF, Qiang ZM, Zhang HQ, Ben WW (2012). Fate and seasonal variation of endocrine-disrupting chemicals in a sewage treatment plant with A/A/O process. Sep Purif Technol. **84**: 9–15.
57 Oh Y, Chung KC (2013). Zinc finger protein 131 inhibits estrogen signaling by suppressing estrogen receptor alpha homo-dimerization. Biochem Biophys Res Commun. **430**: 400–405.
58 Ombra MN, Di Santi A, Abbondanza C, Migliaccio A, Avvedimento EV, Perillo B (2013). Retinoic acid impairs estrogen signaling in breast cancer cells by interfering with activation of LSD1 via PKA. Biochim Biophys Acta-Gene Regul Mech. **1829**: 480–486.
59 Patani N, Martin LA, Dowsett M (2013). Biomarkers for the clinical management of breast cancer: International perspective. Int J Cancer. **133**: 1–13.
60 Perillo B, Ombra MN, Bertoni A, Cuozzo C, Sacchetti S, Sasso A, et al (2008). DNA oxidation as triggered by H3K9me2 demethylation drives estrogen-induced gene expression. Science. **319**: 202–206.
61 Preda C, Ungureanu MC, Vulpoi C (2012). Endocrine disruptors in the environment and their impact on human health. Environ Eng Manag J. **11**: 1697–1706.
62 Puzianowska-Kuznicka M, Pawlik-Pachucka E, Owczarz M, Budzinska M, Polosak J (2013). Small-Molecule Hormones: Molecular Mechanisms of Action. Int J Endocrinol. **2013**: 1–21.
63 Qiang ZM, Nie YF, Ben WW, Qu JH, Zhang HQ (2013). Degradation of endocrine-disrupting chemicals during activated sludge reduction by ozone. Chemosphere. **91**: 366–373.
64 Qin CX, Lan XL, He J, Xia XT, Tian YL, Pei ZJ, et al (2013a). An In Vitro and In Vivo Evaluation of a Reporter Gene/Probe System hERL/F-18-FES. PLoS One. **8**: 1–8.
65 Qin F, Wang LH, Wang XQ, Liu SZ, Xu P, Wang HP, et al (2013b). Bisphenol A affects gene expression of gonadotropin-releasing hormones and type I GnRH receptors in brains of adult rare minnow Gobiocypris rarus. Comp Biochem Physiol C-Toxicol Pharmacol. **157**: 192–202.
66 Shahmoradgoli M, Riazalhosseini Y, Haag D, Becker N, Hovestadt V, Heck S, et al (2013). Protein phosphatase 1, regulatory subunit 15B is a survival factor for ER-positive breast cancer. Int J Cancer. **132**: 2714–2719.
67 Schneider J, Ruschhaupt M, Buness A, Asslaber M, Regitnig P, Zatloukal K, et al (2006). Identification and meta-analysis of a small gene expression signature for the diagnosis of estrogen receptor status in invasive ductal breast cancer. Int J Cancer. **119**: 2974–2979.
68 Srinivasan S, Nwachukwu JC, Parent AA, Cavett V, Nowak J, Hughes TS, et al (2013). Ligand-binding dynamics rewire cellular signaling via estrogen receptor-alpha. Nat Chem Bio.l **9**: 326–332.
69 Swedenborg E, Ruegg J, Makela S, Pongratz I (2009). Endocrine disruptive chemicals: mechanisms of action and involvement in metabolic disorders. J Mol Endocrinol. **43**: 1–10.
70 Tang XJ, Naveedullah, Hashmi MZ, Zhang H, Qian MR, Yu CN, et al (2013). A Preliminary Study on the Occurrence and Dissipation of Estrogen in Livestock Wastewater. Bull Environ Contam Toxicol. **90**: 391–396.
71 Van't Veer LJ, Dai HY, Van De Vijver MJ, He YDD, Hart AaM, Mao M, et al (2002). Gene expression profiling predicts clinical outcome of breast cancer. Nature. **415**: 530–536.
72 Van Duursen MBM, Smeets E, Rijk JCW, Nijmeijer SM, Van Den Berg M (2013). Phytoestrogens in menopausal supplements induce ER-dependent cell proliferation and overcome breast cancer treatment in an in vitro breast cancer model. Toxicol Appl Pharmacol. **269**: 132–140.
73 Wilson BJ, Giguere V (2008). Meta-analysis of human cancer microarrays reveals GATA3 is integral to the estrogen receptor alpha pathway. Mol Cancer. **7**: 1–8.
74 Wolinska-Witort E, Martynska L, Chmielowska M, Wasilewska-Dziubinska E, Bik W, Baranowska B (2012). The evaluation of estradiol and leptin action on the activity of the somatotropic and gonadotropic axes in peripubertal female rats. Neuroendocrinol Lett. **33**: 138–148.
75 Yu EJ, Kim SH, Heo K, Ou CY, Stallcup MR, Kim JH (2011). Reciprocal roles of DBC1 and SIRT1 in regulating estrogen receptor alpha activity and co-activator synergy. Nucleic Acids Res. **39**: 6932–6943.
76 Zeng H, Wu JC, Bedford MT, Sbardella G, Hoffmann FM, Bi K, Xu W (2013). A TR-FRET-Based Functional Assay for Screening Activators of CARM1. ChemBioChem. **14**: 827–835.