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Estrogen-responsive genes for environmental studies

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HIGHLIGHTS

- A comprehensive list of estrogen-responsive genes is provided.
- Signal transduction pathways related to estrogen-responsive genes are summarized.
- Potential toxicity pathways which could be used for environmental studies are discussed.

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ABSTRACT

Studying the effect of estrogenic chemicals, especially estrogenic endocrine disruptors, needs mechanism-based understanding of toxicity pathways, especially at the level of cell signaling. We first summarized how estrogen action can be monitored through gene expression profiles by means of estrogen-responsive genes, which are associated with or mediate various types of cell signaling through pathways, such as mitogen-activated protein kinase (MAPK), angiogenesis, nuclear receptor, ErbB/HER and ubiquitin/proteasome signaling pathways, and the regulation of cell functions, such as chromatin/epigenesis, apoptosis, autophagy, cellular metabolism, translational control, cell cycle/DNA damage/cytoskeletal formation, immunology/inflammation response, neurological diseases and development/differentiation. The cell signals induced by estrogenic chemicals can be monitored by appropriate sets of estrogen-responsive genes, where the above-mentioned signaling pathways are involved. The association of estrogenic endocrine disruptors and environmental estrogens, such as flavonoids, zearalenone, bisphenol A, perfluorooctane sulfonate and di(2-ethylhexyl) phthalate, with cell signaling is discussed along with a comprehensive list of signaling pathways induced by these chemicals. The signaling pathways identified could be used as candidate toxicity pathways to monitor and evaluate endocrine disruptor action.

Abbreviations: AHR, aryl hydrocarbon receptor; AML, acute myeloid leukemia; AMPK, AMP-activated protein kinase; AR, androgen receptor; ATR, ataxia telangiectasia and Rad3 related; β-BHC, β-benzene hexachloride; BMP, bone morphogenetic protein; CDK, cyclin-dependent kinase; DDT, *o,p′*-dichlorodiphenyltrichloroethane; DEHP, di(2-ethylhexyl) phthalate; DES, diethylstilbestrol; EGF, epidermal growth factor; EGFR, epidermal growth factor receptor; eIF, eukaryotic initiation factor; ER, estrogen receptor; ERK, extracellular-signal-regulated kinase; FasL, Fas ligand; FGF, fibroblast growth factor; FGFR, fibroblast growth factor receptor; FOXO, forkhead box O; GPCR, G-protein-coupled receptor; hIP, human prostacyclin receptor; IFN, interferon; IGF, insulin-like growth factor; IL, interleukin; IL-1R, interleukin-1 receptor; IP, inositol phosphate; JNK, c-Jun N-terminal kinase; LIF, leukemia inhibitory factor; LIMK, LIM domain kinase; MAPK, mitogen-activated protein kinase; MEK, mitogen-activated protein kinase; mTOR/TOR, (mammalian) target of rapamycin; NGF, nerve growth factor; NLK, Nemo-like kinase; NO, nitric oxide; PBDEs, polybrominated diphenyl ethers; PCBs, polychlorinated biphenyls; PI3K, phosphatidylinositol 3-kinase; PKC, protein kinase C; PLC, phospholipase C; PPAR, peroxisome proliferator-activated receptor; ROCK, Rho-associated protein kinase; ROS, reactive oxygen species; RT-PCR, reverse-transcription polymerase chain reaction; TCDD, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin; TCR, T-cell receptor; TGF, transforming growth factor; THPO, thrombopoietin; TK, tyrosine kinase; TLR, Toll-like receptor; TNF, tumor necrosis factor; VEGF, vascular endothelial growth factor; ZAL, zearalanone/zearalanol; ZEA, zearalenone/zearalenol.

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1. Genes involved in endocrine disruptor action

Endocrine disruptors (or endocrine disrupting chemicals) are a class of chemicals that mimic hormones and disrupt hormonal action (Safe, 2000; Hutchinson et al., 2000; Moggs, 2005; Marino et al., 2012). Because of the abundance of estrogenic chemicals among synthetic and natural chemicals (Kiyama and Zhu, 2014), they have the most significant impact as potential endocrine disruptors. Their impact has been discussed in terms of the methodology of detection/evaluation/risk assessment (Stokes, 2004; Rodriguez-Mozaz et al., 2004; Charles, 2004), impact on animals/tissues and organs/cells (Bigsby et al., 1999; Iguchi et al., 2006; Sikka and Wang, 2008), and the outcomes of the effects on epigenetics/evolution/health (Marino et al., 2012; Crews and McLachlan, 2006; McLachlan et al., 2006). Meanwhile, the mechanisms of the effects of endocrine disruptors have been discussed at the level of genes of specific tissues/organs/cells (Caserta et al., 2008; Taylor et al., 2011; Macon and Fenton, 2013), owing to the development of gene/genome-based technologies. Several marker genes have been reported along with the detection systems (Jung et al., 2012), including previously identified vitellogenin, pS2 (or *TFF1*) and the progesterone receptor (*PGR*) genes.

The National Research Council (2007) of USA has recommended toxicity testing based on toxicity pathways and targeted testing, which will provide more information about the effect of potentially toxic chemicals at low levels and reduce the need for animal testing. New *in vitro* toxicity tests, based on primary cells, transformed culture cells or cells derived from tissues or stem cells, need to adequately mimic the features focused on *in vivo* tests by using *in vivo* data as a gold standard and understanding the mechanisms occurring both in humans *in vivo* and cells *in vitro* (Roggen, 2011). So, other than the development of test systems based on new technology, understanding new toxicity pathways along with appropriate markers is needed. Furthermore, reducing animal tests is an urgent and important issue and animal protection laws or rules have been revised in many countries, including Japan (Inomata, 2014), USA (Bishop et al., 2012), Korea (Choe and Lee, 2013) and EU (Lorenzetti et al., 2011), to replace animal tests with other cell/molecular biology-based, omics-based or computational technologies.

There are two major pathways of transducing estrogenic signaling within cells: genomic and non-genomic pathways (summarized in Fig. 1; also see Kiyama and Zhu (2014)). While the non-genomic pathway is transcription-independent and mediates signals through protein–protein interaction and modification of proteins, the genomic pathway involves estrogen receptors (ERs) as transcription factors and affects the transcription of the target genes, collectively called estrogen-responsive genes. Estrogen-responsive genes mediate signals through transcription-dependent modulation of estrogenic effects in the genomic pathway. Meanwhile, the non-genomic pathway, or the extranuclear signaling, consists of various metabolic networks including those for glucose/lipid homeostasis and tissue-specific estrogenic effects, which are balanced by the action of ERα and ERβ (Barros and Gustafsson, 2011) and/or regulated by splice variants of ERα (Taylor et al., 2010). Recently, the involvement of non-genomic estrogen signaling has been discussed to understand the mechanisms of endocrine disruptor action (Tilghman et al., 2010; Shanle and Xu, 2011; Hu et al., 2012). Note that, even in the non-genomic pathway, signaling could be influenced by gene expression; an increase or decrease in the kinase/phosphatase activity or the amount of a regulatory protein resulting in the modulation of signaling.

While estrogen-responsive genes were found in early days among the genes functionally related to estrogen action, they were identified and characterized more recently by techniques for monitoring gene expression, such as *PGR* by Northern blotting (Conneely et al., 1986), the EGFR gene (*EGFR*) by quantitative RT-PCR (Paria et al., 1993), a hypoxia inducible factor gene (*EGLN2*) by SAGE (Serial Analysis of Gene Expression) (Seth et al., 2002), the early growth response 3 gene (*EGR3*) by DNA microarray assay (Inoue et al., 2004), the core histone genes (proteins) by mass spectrometry-based proteomics (Zhu et al., 2009), and ER target genes such as *TPD52L1* by next-generation sequencing-based transcriptomics (Yamaga et al., 2013). Here, we summarize the estrogen-responsive genes identified by DNA microarray-based gene expression profiling.

2. Estrogen-responsive genes and their signaling pathways

Estrogen-responsive genes can be obtained by selecting genes that show increased or decreased expression after treatment with estrogen (summarized in Kiyama and Zhu (2014)). This selection can be performed by DNA microarray-based gene expression profiling due to its advantage of comprehensive and reproducible analysis of the response of estrogen-responsive cells. We obtained a set of genes responding to estrogen (17β-estradiol or E_2) in human breast cancer MCF-7 cells by means of large-scale microarray assays, where 172 estrogen-responsive genes were screened from approximately 20,000 genes (Inoue et al., 2002; Terasaka et al., 2004). The genes showing significant up-regulation or down-regulation are summarized in Table 1. These genes have been used to evaluate the estrogenic activity of 53 pure chemicals (such as phenols, phthalates, parabens, zearalenone/zearalanone, flavonoids, and natural and synthetic estrogens) and 7 mixtures of chemicals (such as plant extracts and oil-degradation products) in MCF-7 cells and 6 pure chemicals in human breast cancer T-47D cells (a complete list is shown in Kiyama and Zhu (2014)).

Thirty-three significantly up-regulated and 68 significantly down-regulated genes have been categorized according to their functions, such as enzymatic activity, cell signaling, cell growth/proliferation, transcription, and cellular transport. When these estrogen-responsive genes were first identified by microarray analysis (Inoue et al., 2002), it was rather strange how genes such as those encoding metabolic enzymes (*fucosyltransferase 8* or *FUT8* for example; Table 1) responded to estrogen, because the functions of most of the genes were not known at all or were very limited. However, we now know that many of them are related to specific signal transduction pathways, such as MAPK signaling (*RAP1GAP*, *RPS6KA3*, *CAMK2N1*, *EFEMP1*, *IER3*, *MGP* and *NF1*), apoptotic signaling (*CCNA1*, *EGR3*, *KRT8*, *PSAT1*, *TAF9*, *TP53I11*), receptor-mediated signaling (*AKR1C4*, *CFB*, *CLIC4*, *ENO1*, *ILK*, *LCN2*, *PEG10*, *SCD*, *TACSTD2*, *ARNT2*, *ARHGDIA*, *BCL2L11*, *CPT1A*, *DAZAP2*, *DDEF1*, *DHCR24*, *ENO3*, *ESR1*, *ESR2*, *FUT8*, *GDF15*, *HSP90B1*, *IGFBP5*, *IL1R1*, *MAN1A1*, *PRKCSH*, *SHMT2*, *SLC7A11* and *SORD*), cell-cycle-related signaling (*CCND1*, *CDC14B*, *NCOA3* and *STC2*), actin/microtubule/adherence junction-related signaling (*CDH18*, *MAP1B*, *C19orf 21*, *ERBB2*, *HMMR*, *NCOA1*, *PMP22*, *TM4SF1* and *TSPAN1*), autophagy-related signaling (*CTSD*, *ULK1*, *ASS1*, *PIK3C3* and *XPOT*), stress-induced signaling (*ACO2*, *RHOC*, *CBX1*, *GARS*, *GOT1*, *IFRD1*, *IMP4*, *PCYT1A*, *QSOX1*, *RCN1*, *RDH11* and *SLC12A2*), disease-related signaling (*CDSN*), immune/inflammation response (*ADORA2A*, *CDIPT* and *HSPA5*) and chromatin-related signaling (*ASNS*) (see the left column in Table 1). Other than MAPK signaling, specific signaling such as Wnt (*RPS6KA3*, *SCD*, *SERPINA3*, *BSN*, *IFRD1* and *RACGAP1*), Notch (*BCL2L11* and *TRIB3*), PI3K/ERK (*SECTM1*, *SLC12A2* and *WARS*), JNK (*JUN*), NF-κB (*SH3BGR*) and GSK-3β (*CTNND2*) signaling pathways are involved. The genes associated with receptor-mediated signaling include those responding to estrogen (*AKR1C4*, *ARNT2*, *ESR1*, *ESR2*, *MAN1A1* and *SLC7A11*), androgen (*AKR1C4*, *DHCR24*