

# Estrogen Receptor—Tumor Suppressor Protein p53 Signaling Crosstalk as Potential Targets of Xenoestrogens

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**Abstract** Excessive exposure to endogenous estrogens can lead to adverse health effects including higher risk for diseases such as breast cancer. Many exogenous chemicals have been known to affect the normal physiology of organisms including human beings. Among these are compounds that affect the endocrine system. These compounds, collectively called xenoestrogens, are either synthetic or naturally occurring in plants (phytoestrogens). The cellular effect of xenoestrogens has been studied mostly in the context of nuclear gene regulation. Although there is ample evidence of the important roles of crosstalk between signaling pathways and nuclear–mitochondrial communication in normal and pathophysiology, these have been largely ignored as potential targets for xenoestrogens. However, available evidence points to the importance of analyzing the effect of xenoestrogens from this angle for better understanding of both harmful as well as beneficial health effects of xenoestrogens including phytoestrogens.

**Keywords** Estrogen receptor • Tumor suppressor • p53 • Xenoestrogens • Phytoestrogens • Mitochondria • Apoptosis • Cell cycle • MicroRNA

## Introduction

Communication and coordination between nuclear and mitochondrial genomes is required for the regulated expression of proteins required for mitochondrial biogenesis and function, which are essential in maintaining the fate of the cell (Leigh-Brown et al. 2010). Therefore, environmental chemicals or agents that affect nuclear function could affect mitochondrial functions and vice versa. Among these chemicals, endocrine disruptors are of major health concern as they can interfere with normal endocrine signaling in the body. As per the International Program for Chemical Safety, endocrine disruptors are “exogenous substances that alter function(s) of the endocrine system and consequently cause adverse health

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effects in an intact organism, its progeny, or (sub)populations.” The endocrine disruptors affect normal physiology by (i) mimicking the endogenous hormones by binding to their receptors, (ii) blocking the endogenous hormone receptors from binding the endogenous hormones, and (iii) altering the synthesis and function of endogenous hormone receptors and hormones (Roper et al. 2006). This chapter provides a brief description of how xenoestrogens affect cellular estrogen and p53 signaling that might involve both nuclear and nonnuclear compartments such as mitochondria.

## Nuclear and Mitochondrial ER and p53

Synthetic and plant-derived xenoestrogens include estrogenic, antiestrogenic, and selective estrogen receptor modulator (SERM)-like compounds that are capable of interfering with genomic and nongenomic signaling (Safe and Papineni 2006; Wong and Walker 2013). Xenoestrogens are reported to mediate their biological effects by binding estrogen receptor- $\alpha$  (ER  $\alpha$ ) and estrogen receptor- $\beta$  (ER $\beta$ ) and regulating their transcriptional functions. The current bioassays for xenoestrogens are based on this concept; for example, gene transcription reporter assays and ER competitive binding assays are used frequently. Therefore, although there have been several studies on the effect of xenoestrogens on the nuclear functions of ERs, very little, if any, information is available on the impact of these chemicals on signaling crosstalk involving ERs and other important proteins such as tumor suppressor p53. Moreover, the effect of these chemicals on signaling in nonnuclear locations such as mitochondria has remained largely unclear.

ERs are nuclear hormone receptors that act as transcription factors to regulate genes involved in growth, development, and differentiation of secondary sex characteristics, homeostasis, and metabolism and play a fundamental role in proliferation of breast cancer cells (Ali and Coombes 2002; Pearce and Jordan 2004; Shao and Brown 2004; Osborne and Schiff 2005).

p53 is a key tumor suppressor protein that serves as a sensor of cellular stress, and by integrating various signaling pathways, plays a central role in cellular processes such as cell cycle arrest, apoptosis, senescence, and differentiation. Since its discovery in 1979, reports have been continuously emerging on multiple functions of p53 in normal and cancer cells. In addition to its ability to initiate cell-cycle arrest and apoptosis, it has been shown to regulate metabolism, autophagy, and oxidative status of the cell (Bensaad et al. 2006; Matoba et al. 2006; Bensaad and Vousden 2007; Cheung and Vousden 2010). Of note, p53 elicits protective, prosurvival cellular responses to maintain genome integrity and viability in cells that sustain limited and reversible damage. These various responses rely on the ability of p53 to function as a transcriptional regulator of an increasing array of target genes as well as on its transcription-independent activities including those that occur in the cytosol and mitochondria.

## ER and p53 Signaling Crosstalk in Normal and Cancer Cells

ER and p53 have largely opposite roles in normal and cancer cells. Estrogen receptor  $\alpha$  (ER $\alpha$ ) plays an important role in the onset and progression of breast cancer, whereas p53 functions as a major tumor suppressor. Most of the information about ER–p53 crosstalk in cancers comes from studies in breast cancer. In comparison to other cancers, overall frequency of p53 mutation in breast cancer is about 20%; however, wild-type p53 is functionally incapacitated. The novel mechanism by which ER $\alpha$ , generally upregulated in luminal breast cancer, suppresses the p53 function was discovered in our laboratory (Liu et al. 2006, 2009; Sayeed et al. 2007; Konduri et al. 2010). Consistent with this finding, clinical studies by us and others showed that ER $\alpha$ -positive patients expressing wild-type p53 were more responsive to tamoxifen therapy (Bergh et al. 1995; Berns et al. 2000; Miller et al. 2005; Yamashita et al. 2006; Konduri et al. 2010). Various other studies have documented the delicate relationship of estrogen signaling and ER $\alpha$  with p53 (Diaz-Cruz and Furth 2010; Liu et al. 2000; Duong et al. 2007; D'Assoro et al. 2008; Katayama and Sen 2011). Genetic support for this idea comes from the longstanding clinical observation that ER $\alpha$ -positive breast cancers express wild-type p53 whereas ER $\alpha$ -negative ones harbor mutant p53 (Cattoretti et al. 1988; Miller et al. 2005). These observations suggest that functional suppression of p53 is an important step in breast oncogenesis. In addition to the functional regulation by protein–protein interaction, ER $\alpha$  and p53 regulate each other at the transcriptional level as well. p53 has been shown to be recruited to the ER $\alpha$  gene promoter resulting in increased transcription of ER $\alpha$  (Angeloni et al. 2004; Shirley et al. 2009). On the other hand, ER $\alpha$  was reported to activate p53 transcription by binding to ERE half-sites within the promoter and knockdown of ER $\alpha$  decreases expression of p53 and its downstream targets (Berger et al. 2012). Together, these observations suggest the existence of a feedback loop between ER $\alpha$  and p53.

## Impact of Xenoestrogens on Gene Transcription

Transcriptional signature profiles (TSPs) determined by Affymetrix microarray when breast cancer cells were proliferating at comparable rates in the presence of various estrogens showed that TSPs of cells treated with xenoestrogens were distinct from those of cells treated with 17 $\beta$ -estradiol; the former strongly enhanced expression of the genes involved in mitochondrial oxidative phosphorylation, whereas the latter showed minimal effects (Shioda et al. 2006). Treatment of progenitor-containing mammospheres with xenoestrogen diethylstilbestrol downregulated *microRNA-9-3* (*miR-9-3*) and there was aberrant methylation of its promoter. Intriguingly, *miR-9-3* is involved in the p53-dependent apoptotic pathway. Therefore, epigenetic silencing of *miR-9-3* in the nucleus promoted cell proliferation by inhibiting apoptosis

(Hsu et al. 2009). Another target of inhibition by diethylstilbestrol is *miRNA-34b* (*mir-34b*). The inhibition of *mir-34b* resulted in restoration of the protein levels of the *mir-34b* targets cyclin D1 (*Ccnd1*) and Jag1 in MCF-7 cells (Lee et al. 2011). It is important to note that the inhibition of *mir-34b* was observed only in cells expressing both ER $\alpha$  and wild-type p53 and treatment with 17 $\beta$ -estradiol disrupted binding of p53 to the promoter. Furthermore, xenoestrogens have been reported to regulate the activity of arginine methyl transferases (PRMTs; Cheng and Bedford 2011). PRMTs are important transcriptional coregulators of various proteins including ER and p53 (Jansson et al. 2008; Le Romancer et al. 2008).

It has been reported that dichlorodiphenyltrichloethane (DDT) exposure activated ER $\alpha$  in mouse liver leading to increased expression of target genes including Cyp2b10, Gadd45 $\beta$ , cMyc, Mdm2, *Ccnd1*, CDK4, and E2F1. In addition, DDT exposure increased Rb phosphorylation, and decrease in p53 protein level and transcriptional activity. Consistent with these observations, there was higher expression of proteins mediating increased cell-cycle progression and decreased apoptosis (Kazantseva et al. 2013).

## Conclusion and Future Perspectives

The reports mentioned above, although few in number, are suggestive of the importance of nuclear–mitochondrial communication and ER and p53 signaling as targets of xenoestrogens in mediating their multiple cellular effects leading to diseases such as cancer. The vast majority of investigations done on tumor development have focused on events in understanding the molecular and genetic hallmarks of the disease (Hanahan and Weinberg 2011). There is increasing evidence of the important role of mitochondria, bioenergetics, and metabolism in the pathophysiology of various diseases including cancer (Wallace 2013). Therefore, the importance of analyzing the effects of xenoestrogens on ER and p53 signaling in the nucleus and mitochondria cannot be overstated. Future research will unravel important information in this exciting area.

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