Molecular Pathways: Environmental Estrogens Activate Nongenomic Signaling to Developmentally Reprogram the Epigenome

Rebecca Lee Yean Wong and Cheryl Lyn Walker

Abstract

Exposure to environmental xenoestrogens is a major health concern because of the ability of these compounds to perturb estrogen receptor (ER) signaling and act as endocrine disrupting compounds (EDC). Inappropriate exposure to EDCs during development, even at low doses, can predispose individuals to an increased lifetime risk of disease, including cancer. Recent data indicate that perinatal exposure to EDCs increases cancer risk by (re)programming the epigenome via alterations in DNA and histone methylation. We and others have begun to dissect the mechanisms by which xenoestrogens disrupt the epigenetic machinery to reprogram the epigenome and induce developmental reprogramming. Our studies revealed that xenoestrogens induce nongenomic ER signaling to activate PI3K/AKT, resulting in AKT phosphorylation and inactivation of the histone methyltransferase EZH2, thus providing a direct link to disruption of the epigenome. Other epigenetic "readers, writers, and erasers" may also be targeted by nongenomic signaling, suggesting this is a central mechanism by which xenoestrogens and other EDCs disrupt the epigenome to induce developmental reprogramming. Elucidating mechanisms of developmental reprogramming of the epigenome is important for understanding how environmental exposures increase cancer risk, and provides a rationale for developing epigenetic interventions that can reverse the effects of environmental exposures to reduce cancer risk. *Clin Cancer Res; 19(14); 3732–7. ©2013 AACR.*

Background

Nongenomic signaling by nuclear hormone receptors

The nuclear hormone receptor (NHR) superfamily comprises receptors for thyroid and steroid hormones, retinoids and vitamin D, and also orphan receptors with unknown ligands. For steroids, NHR activity via the genomic pathway involves hormone binding to cytosolic receptors, nuclear translocation, and the subsequent binding of the ligand-activated hormone receptors to hormone-responsive elements in chromatin to modulate gene transcription. In addition to this classic genomic mechanism, it is now appreciated that when liganded to NHRs including the estrogen, progesterone, androgen, aryl hydrocarbon, and peroxisome proliferator-activated receptors (PPAR), endogenous and environmental hormones activate nongenomic or membrane-initiated signaling pathways that activate several kinase cascades. This rapid and transient nongenomic

signaling occurs in seconds to minutes, compared with genomic signaling, which occurs over hours to days (1–8).

Many of the kinases and pathways involved in nongenomic signaling have been identified. Activation of nongenomic signaling by NHRs primarily initiates at the cell membrane. Estrogen receptors (ERα and ERβ), progesterone receptors (PR-A and PR-B), and the androgen receptor (AR) have been shown to localize to the cell membrane in various cell types (9). The E domain of the NHRs has a conserved palmitoylation motif, which is responsible for membrane localization (9). In the case of the ER, palmitoylation enhances membrane localization, interaction with caveolin-1, and nongenomic activities including activation of mitogen-activated protein kinase (MAPK) and phosphoinositide 3-kinase (PI3K) signaling (10).

Activation of PI3K/AKT and MAPK signaling are well-characterized examples of nongenomic signaling pathways activated by many NHRs. Several studies have shown that PI3K/AKT and MAPK signaling mediates numerous functions evoked by activation of NHRs such as cell growth, motility, differentiation, survival, and apoptosis (11, 12). ERs have been shown to reside in the lipid rafts (13, 14) where association between ER and PI3K occur to activate PI3K/AKT (15). In the case of PI3K signaling, ER directly interacts with the p85α regulatory subunit of PI3K and this interaction is required to induce nongenomic signaling in breast and endothelial cells (16–18). Complex interactions between AR, p85α, and Src are involved in AR activation of

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PI3K signaling (4). Membrane-localized AR is found in caveolae, where this NHR interacts with caveolin-1 and c-Src to facilitate activation of c-Src/PI3K/AKT cascade and subsequent activation of endothelial nitric oxide synthase (eNOS; ref. 19). Other studies have also shown that AR and ER associate with Src to activate MAPK signaling leading to cell survival and proliferation (20–22). In addition to PI3K and MAPK, numerous studies have shown that nongenomic signaling of membrane-localized ERs activates other signaling cascades including PKA; PLC/PKC; JAK/STAT; Pak1; casein kinase I-γ2; sphingosine kinase; calcium calmodulin-dependent protein kinase IV (CaMKIV), tyrosine kinase Src, p21ras, adenylyl cyclase, and PKG (23–25).

Several reports from our group and others have shown that in addition to endogenous estrogen (17-β estradiol), low concentrations of xenoestrogens such as bisphenol A (BPA), genistein (GEN), and diethylstilbestrol (DES), can induce nongenomic ER signaling. Acting in pico to nanomolar concentrations, these xenoestrogens are potent activators of nongenomic signaling as exemplified by raising intracellular calcium levels, activating eNOS and signaling cascades such as PI3K/AKT and MAPK in cells and tissues, including the uterus and prostate (5, 26–34). Importantly, these experimental xenoestrogen doses fall within the range of what had been reported in human studies. For example, 0.3 to 5 ng/mL (~1–20 nmol/L) BPA has been detected in adult and fetal human plasma, urine, and breast milk (35). In the case of GEN, serum levels ranged from 2 (7 nmol/L) to 25 μg/L (92.5 nmol/L) in Asian women and nonvegetarian women, respectively (36). Xenoestrogen exposure at critical windows of development can have widespread deleterious effects. Xenoestrogens such as BPA, GEN, and DES that function as endocrine disrupting compounds (EDC) can disrupt normal organogenesis, reduce fecundity, alter sexual behavior and memory, and cause malformations and decreased sperm mobility. Early life exposures to xenoestrogens and other EDCs can increase susceptibility to chronic diseases such as obesity, diabetes mellitus, asthma, and cancer (37).

The role of nongenomic signaling by xenoestrogens in mediating these adverse effects is just becoming known. Importantly, nongenomic signaling provides a mechanism by which NHR activation by xenoestrogens and other EDCs can induce phosphorylation [and perhaps other posttranslational modifications (PTM)] of numerous downstream proteins and alter their activity. For example, recent studies from our group have shown that 17-β estradiol, BPA, GEN, and DES evoke rapid nongenomic signaling that regulates the histone methyltransferase (HMT) enhancer of Zeste homolog 2 (EZH2) in breast, uterus, and prostate cells and tissues (26, 27). Interestingly, activation of nongenomic signaling by xenoestrogens exhibits tissue-specificity; BPA for example, activates nongenomic PI3K/AKT signaling in the prostate but not in the uterus (26).

Xenoestrogen-induced developmental reprogramming increases susceptibility to cancer

Exposure of developing tissues or organs to an adverse stimulus or insult during critical periods of development can permanently reprogram normal physiologic responses in a manner that promotes diseases later in life. This process, termed developmental reprogramming, is now known to increase risk in adulthood for many diseases, including cancer. Multiple lines of evidence from human and animal studies have established that epigenetic alterations induced by developmental reprogramming are responsible for the altered patterns of gene expression in adulthood that underlie this increased cancer risk. For example, in the uterus, perinatal EDC exposure reprograms the expression of many estrogen-responsive genes, so that in the adult uterus these genes become hyperresponsive to estrogen, increasing the risk for development of hormone-dependent tumors such as endometrial hyperplasia/carcinoma and uterine leiomyoma (38, 39). Developmental reprogramming of cancer susceptibility by environmental exposures has recently been reviewed (40), and will not be covered in detail here where we focus on the signaling pathways by which EDCs engage the cell's epigenetic machinery to induce developmental reprogramming of the epigenome to increase cancer risk.

Nongenomic signaling regulates the activity of "readers, writers, and erasers" of the epigenome during developmental reprogramming

Epigenetic alterations are now appreciated to contribute to the underlying mechanisms by which environmental exposures influence health and disease, including susceptibility to cancer induced by developmental reprogramming. Histone modification, one of the best characterized epigenetic modifications, directly impacts DNA accessibility and chromatin structure to regulate gene expression. It is now thought that combinatorial sets of specific histone modifications are written by HMTs (writers), removed by histone demethylases (HDM; erasers), and recognized by effector proteins (readers) that are recruited and bind to histone modifications via specific domains, to constitute a "histone code" (41, 42). These "reading, writing, and erasing" activities remodel chromatin to regulate biologic processes such as transcription, DNA replication, and repair.

The mechanisms that regulate epigenetic "readers, writers, and erasers" are only now beginning to be understood. We initially postulated that these epigenetic programmers were targets for nongenomic signaling cascades, which would provide a direct link between environmental exposures and alterations in the epigenome. For instance, insulin-like growth factor receptor (IGF-R) signaling to PI3K/AKT induces EZH2 phosphorylation at serine 21, which inhibits its HMT activity, decreasing lysine 27 trimethylation of histone H3 (H3K27me3) and, consequently, increasing gene expression due to loss of this repressive methyl mark (43). Importantly, we showed that xenoestrogen-induced activation of PI3K/AKT by nongenomic signaling also caused AKT to phosphorylate EZH2 at serine 21, inactivating its HMT activity and reducing global levels of H3K27me3. This discovery provided a direct linkage between xenoestrogen-induced NHR signaling and modulation of the cell's epigenetic machinery.

Other links between cell signaling pathways and epigenetic programmers also exist. Wnt5a has been shown to activate Nemo-like kinase (NLK) through CaMKII and MAPKKK TAK1/TAB2 signaling. Activated NLK can phosphorylate the HMT SETDB1, which leads to the formation of an active corepressor complex, an increase in the H3K9me3 SETDB1 repressive methyl mark and silencing of target genes such as PPARγ (44). Neurotrophic factors such as BDNF and NGF activate neuronal NOS, which nitrosylates glyceraldehyde 3-phosphate dehydrogenase (GAPDH), enabling it to bind to Siah and translocate to the nucleus. In a ternary complex that comprises GAPDH-Siah and the HMT SUV39H1, Siah ubiquitinates SUV39H1, resulting in loss of SUV39H1 HMT activity and a decrease in the repressive H3K9me3 methyl mark, thus facilitating activation of target genes (45).

Several other HMTs can also be regulated by phosphorylation. For example, PR-SET7 is phosphorylated by cyclin-dependent kinase 1 (CDK1)/cyclin B complex at serine 29 and though this phosphorylation has no impact on its HMT activity, it removes PR-SET7 from mitotic chromosomes and confers protein stability during mitosis (46). SU (VAR)3–9 is phosphorylated by chromosomal kinase JIL-1 at the N-terminus but this PTM does not alter its ability to repress transcription (47). CDK1 phosphorylates EZH2 at threonine 345 and 487 to target EZH2 for ubiquitination and subsequent proteasomal degradation (48). The latter PTM is also critical for EZH2 interaction with PRC2 components SUZ12 and EED and its HMT activity (43). CARM1 is phosphorylated at serine 217 by an unidentified kinase but this PTM is essential for S-adenosylmethionine binding and HMT activity (49). ENX2 is phosphorylated $in\ vivo$ but neither the site nor the biologic function of this PTM is understood (50).

Mammalian HDMs can also be posttranslationally modified via phosphorylation (51). One recent example is PHF2, a JmjC demethylase, which converts to an active H3K9me2 demethylase when phosphorylated by PKA. Activated PHF2 then associates with ARID5B to induce demethylation of methylated ARID5B. This PTM also mediates targeting of PHF2–ARID5B complex to promoters where it removes the dimethyl H3K9 mark (52). Another example is PHF8, which is a H4K20me1 demethylase that can function as a cell-cycle regulator, partially based on its demethylase activity. When phosphorylated by CDK1, PHF8 dissociates from chromatin in prophase. Concomitantly, increased expression of Pr-Set7 is observed, which leads to a surge of H4K20me1 mark that has the ability to interact with the Condensin II complex (53). The H3K36 demethylase RPH1 has been reported to repress transcription of $PHR1$ gene in a HDM-dependent manner. RPH1 is phosphorylated at serine 652 by a RAD53 kinase-dependent manner and this PTM potentially triggers the dissociation of RPH1 from chromatin and modulates transcriptional derepression of $PHR1$ gene during DNA damage (54).

Phosphorylation of chromatin effector proteins (epigenetic "readers") has been described as in the case for

Heterochromatin protein 1γ (HP1γ). HP1γ binds to H3K9me3 via its chromodomain, and has been shown to be targeted by PKA for phosphorylation at serine 83. This phosphorylation event is essential for HP1γ's localization in the euchromatin and interaction with Ku70 but functionally impaired its silencing activity (55). Another HP1 homologue, HP1β, is phosphorylated by Casein kinase 2 in response to DNA damage. Phosphorylation at its chromodomain leads to rapid release of HP1β from H3K9me3, followed by reassociation at later times (56, 57).

The examples above show that many epigenetic "readers, writers, and erasers" can be targeted by kinases activated by nongenomic signaling, suggesting that nongenomic signaling may be a central mechanism by which EDCs engage the developing epigenome to induce developmental reprogramming (Fig. 1). Because we still know relatively little about how epigenetic programmers are regulated, conceivably, most, if not all, "readers, writer, and erasers" may be regulated by PTMs such as phosphorylation, making them targets for nongenomic signaling. A summary is provided
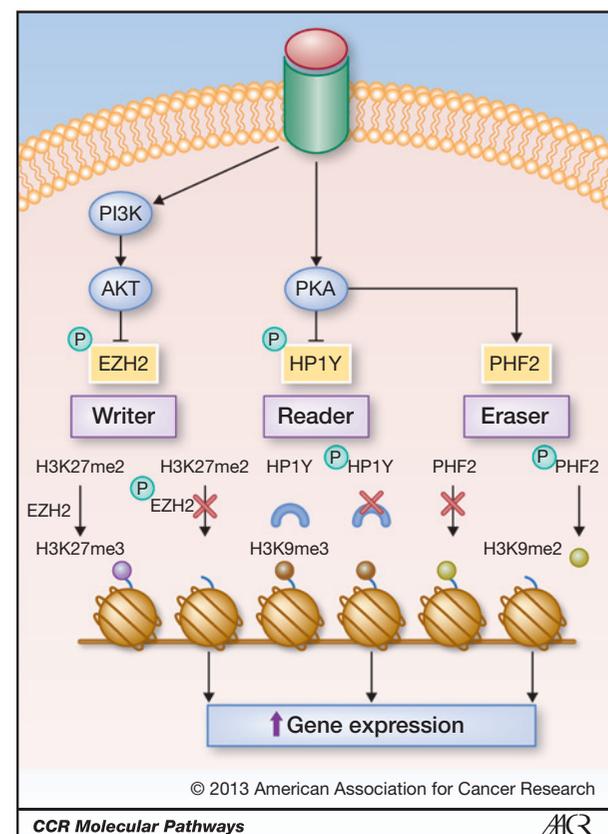

Figure 1. Nongenomic signaling pathways that modulate the activity of epigenetic "readers, writers, and erasers." Endogenous and environmental ligands bind to NHRs to activate nongenomic signaling and kinase cascades. Activated kinases such as AKT and PKA phosphorylate and inhibit the activity of epigenetic "writers" such as the HMT EZH2 and "readers" such as HP1γ, respectively. The "eraser" HDM PHF2, also a PKA substrate, becomes activated when phosphorylated. In all three scenarios, the result is increased gene expression, due to loss of, or inability to read, repressive histone methyl marks.

Table 1. Examples of signaling cascades activated by ligand-activated hormone receptors that impinge on epigenetic regulators, altering gene expression to result in increased tumor susceptibility

	Examples
Ligand	17-β Estradiol, xenoestrogens (e.g., BPA, DES, Genistein)
$\downarrow$	
Nuclear hormone receptor	ER, PR, AR, Ahr, PPARγ
$\downarrow$	
Nongenomic signaling	PI3K/AKT, PKA, MAPK, PLC/PKC, PKG, JAK/STAT, Pak1, casein kinase I-γ2, sphingosine kinase, CaMKIV, tyrosine kinase Src, p21ras, adenylyl cyclase, etc.
$\downarrow$	
Writers, erasers, readers	EZH2, SETDB1, SUV39H1, PR-Set7, SU(VAR)3-9, Carm1, ENX2, PHF2, PHF8, Rph1, HP1Y, HP1β
$\downarrow$	
(Re)Programming of the epigenome	Altered H3K27me3, H3K9me2/3, H4K20me1, H3R17me2, H3K36me2/3
$\downarrow$	
Altered gene expression	*HOXA10, PDE4D4, LTF, FOS, HMGN5, CALB3, GRIA2, GDF10, MMP3*
$\downarrow$	
Increased susceptibility to tumorigenesis	Uterine and prostate cancer

in Table 1 of key nongenomic signaling pathways and "readers, writers and erasers" known to be, or potentially, regulated by kinases in nongenomic signaling pathways.

Clinical–Translational Advances

Exposures to EDCs such as xenoestrogens are a major health concern because of the ubiquitous nature of exposures to some of these compounds, and the potential for these exposures, especially when they occur during key developmental windows, to cause life-long changes in the epigenome that increase susceptibility to diseases of adulthood including diabetes, obesity, metabolic syndrome, infertility, and cancer. Importantly, the persistent epigenetic changes induced by developmental reprogramming are present before disease onset, presenting an opportunity for developing screens for epigenetic alterations that identify individuals at increased risk, and the development of effective interventions to reduce cancer risk. Just as importantly, the epigenetic alterations induced by developmental reprogramming may be reversible with epigenetic therapy, or lifestyle interventions such as diet and exercise. In this regard, now is the time for additional effort geared toward identifying tissue- and disease-specific epigenetic signatures induced by developmental reprogramming to determine whether these epigenetic "fingerprints" can be used as reliable biomarkers of environmental exposure and disease risk. The identification of such epigenetic biomarkers will be useful not only for identifying at-risk individuals, but also to determine whether epigenetic (or other) therapies can reverse the effects of developmental reprogramming to decrease cancer risk.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors' Contributions

Conception and design: R.L.Y. Wong, C.L. Walker
Writing, review, and/or revision of the manuscript: R.L.Y. Wong, C.L. Walker

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