Gestational Exposure to the AhR Agonist 2,3,7,8-Tetrachlorodibenzo-p-dioxin Induces BRCA-1 Promoter Hypermethylation and Reduces BRCA-1 Expression in Mammary Tissue of Rat Offspring: Preventive Effects of Resveratrol

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Studies with murine models suggest that maternal exposure to aromatic hydrocarbon receptor (AhR) agonists may impair mammary gland differentiation and increase the susceptibility to mammary carcinogenesis in offspring. However, the molecular mechanisms responsible for these perturbations remain largely unknown. Previously, we reported that the AhR agonists 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) induced CpG methylation of the breast cancer-1 (BRCA-1) gene and reduced BRCA-1 expression in breast cancer cell lines. Based on the information both the human and rat BRCA-1 genes harbor xenobiotic responsive elements (XRE = 5'-GCGTG-3'), which are binding targets for the AhR, we extended our studies to the analysis of offspring of pregnant Sprague–Dawley rats treated during gestation with TCDD alone or in combination with the dietary AhR antagonist resveratrol (Res). We report that the in utero exposure to TCDD increased the number of terminal end buds (TEB) and reduced BRCA-1 expression in mammary tissue of offspring. The treatment with TCDD induced occupancy of the BRCA-1 promoter by DNA methyltransferase-1 (DNMT-1), CpG methylation of the BRCA-1 promoter, and expression of cyclin D1 and cyclin-dependent kinase-4 (CDK4). These changes were partially overridden by pre-exposure to Res, which stimulated the expression of the AhR repressor (AhRR) and its recruitment to the BRCA-1 gene. These findings point to maternal exposure to AhR agonists as a risk factor for breast cancer in offspring through epigenetic inhibition of BRCA-1 expression, whereas dietary antagonists of the AhR may exert protective effects.

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Key words: epigenetics; BRCA-1; mammary carcinogenesis; aromatic hydrocarbon receptor; resveratrol

INTRODUCTION

Perturbations in epigenetic regulation may contribute to the development of sporadic cancers [1]. For example, sporadic breast tumors have absent or markedly reduced levels of BRCA-1 in the absence of mutations in the BRCA-1 gene [2–4], but the BRCA-1 promoter is hypermethylated [5–11]. The mechanisms that contribute to the differential expression and epigenetic state of the BRCA-1 gene in familial and sporadic breast cancers remain largely unknown [12]. Previously, we reported that exposure of breast cancer cells to the AhR agonist TCDD reduced the association of acetylated histone-4 (AcH4) with the BRCA-1 promoter, and BRCA-1 mRNA and protein levels [13,14]. Also, TCDD increased the association of trimethylated histone-3 lysine-9 (H3K9me3), DNMT-1, and methyl-binding domain protein 2 (MBD2) [15] with the BRCA-1 gene, and CpG methylation of the BRCA-1 promoter [16]. These epigenetic alterations were antagonized in cell culture by pre-exposure to the diet phytoalexin Res, which possesses antagonistic properties towards the AhR [17,18].

Three important lines of evidence suggest a role for prenatal exposure to AhR agonists in the etiology of breast cancer in the offspring. First, preclinical studies with rodent models illustrated that maternal exposure to TCDD increased the number of TEB in mammary tissue and susceptibility to chemically induced tumorigenesis in female offspring [19–21]. Second, both the human [14–16] and rat [22] BRCA-1 promoters harbor

**Abbreviations** GD, gestation day; PND, post-natal day; TD, terminal ducts; AB, alveolar buds; LOB, lobules; U, unmethylated; M, methylated; CHIP, chromatin immunoprecipitation; IgG, immunoglobulin; QPCR, quantitative real-time PCR; GAPDH, glyceraldehyde 3-phosphate; ESR1, estrogen receptor-α; ARNT, AhR nuclear translocator.

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Received 25 March 2013; Revised 11 September 2013; Accepted 24 September 2013

DOI 10.1002/mc.22095

Published online in Wiley Online Library (wileyonlinelibrary.com).
putative XREs suggesting the BRCA-1 gene is a molecular target for regulation by AhR-regulated pathways. Third, both human [23] and murine [24] mammary tumors express higher levels of AhR-regulated genes. However, whether gestational exposure to AhR agonists alters BRCA-1 expression in offspring has not been investigated. Here, we report that in utero exposure to TCDD reduces mammary gland differentiation and BRCA-1 expression, while enhancing CpG methylation of the BRCA-1 gene, and cyclin D1 and CDK4 expression, in mammary tissue of female offspring. These changes are partially overridden by pre-exposure to Res, which induces the expression of the AhRR and its recruitment to the BRCA-1 gene.

MATERIALS AND METHODS

TCDD was supplied by the National Cancer Institute, Division of Cancer Biology, Chemical and Physical Carcinogenesis Branch, and distributed by Midwest Research Institute under contract (64 CFR 72090, 64 CFR 28205). Resveratrol (98% purity by thin-layer chromatography, Lot No. 31JGM11J1) was obtained from Axxora (San Diego, CA) and mixed with purified diet (AIN-76A) to a concentration of 7 ppm following manufacturer’s (Harlan Laboratories, Madison, WI) standardized quality control protocols. Diet was air dried, kept at 4°C, and protected from light exposure until use.

Materials and Gestational Diets

Pregnant female Sprague-Dawley rats were purchased from Harlan Laboratories (Houston, TX). All dams were placed on basal AIN-76A diet and assigned to one of four groups: (1) Dams were gavaged with vehicle control (~0.2 ml sesame oil) or (2) TCDD (1 μg TCDD/kg body weight) at gestation day (GD) 15 as described previously [19]; (3) Dams in the Res group were started on the AIN-76-A diet containing Res at GD7 until the end of pregnancy, and gavaged at GD15 with vehicle (~0.2 ml sesame oil); (4) Dams assigned to the TCDD plus Res group received the AIN-76A diet containing Res at GD7 until the end of pregnancy and gavaged at GD15 with 1 μg TCDD/kg body weight. All dams were allowed free access to chow and drinking water throughout the experiment as described previously [25]. At ~10 h after birth, female pups were switched to foster mothers and weaned at post-natal day (PND) 21. Female offspring were sacrificed at PND46 and PND71 according to protocols approved by the IACUC of the University of Arizona. Mammary gland tissues were collected for biochemical analyses. The protocol for collection of mammary glands and preparation of whole mounts is described elsewhere [19]. Briefly, mammary glands were dissected at necropsy from each rat, stretched on a glass slide, and placed into Carnoy’s fixative for 2 d. Then, mammary glands were washed in 70% ethanol for 1 h, rinsed in water for 30 min, and stained in carmine aluminum for 2 d. Glands were washed in ethanol and placed in xylene for 2 d. Finally, glands were covered with Permount (Fisher Scientific, Pittsburg, PA) and cover-slipped. Morphological analyses of whole mounts were performed blindly by two independent investigators using an Olympus CK30 microscope with a 40× magnification objective. Terminal end bud, terminal duct (TD), alveolar bud (AB), and lobule (LOB) structures were counted in the 5 mm most exterior zone opposite to the nipple, which is the most actively growing region of the mammary gland [26], as described previously [27].

BRCA-1 Promoter CpG Methylation

Analysis of BRCA-1 promoter methylation was carried out as described previously [16]. Briefly, genomic DNA was isolated from 30 mg of mammary tissue using the DNeasy Blood & Tissue Kit (Qiagen, Valencia, CA). DNA (1 μg) was subjected to bisulfite modification using the CpGenome DNA Modification Kit (Millipore, Billerica, MA). In preliminary experiments, we verified that the number of cycles for semiquantitative amplification of the rat BRCA-1 promoter fragment with unmethylated (U) and methylated (M)-specific primers was in the linear range. The bisulfite-modified DNA was analyzed by PCR as follows: 1 cycle at 95°C for 5 sec; 37 cycles at 95°C for 45 s, 55°C (U) and 59°C (M) for 45 s, and 72°C for 1 min; and 1 cycle at 72°C for 5 min. Briefly, reactions were carried out at a final volume of 25 μl consisting of the following master mix: bisulfite-modified DNA, JumpStart Taq DNA polymerase, 1× PCR buffer, 2.0 mM MgCl₂, 200 mM dNTPs, 1 μl each of forward and reverse primers. The PCR amplification products were separated on 2% agarose gels and visualized using ethidium bromide staining. The rat BRCA-1 amplicon was of the expected size (142 bp) and its authenticity to the rat BRCA-1 gene [22] was confirmed by direct sequencing. The rat BRCA-1 primers synthesized by Sigma-Aldrich (St. Louis, MO) were: U-sense: 5′-GTGAGAAGGTTTTTGTTGTATT-3′, and U-antisense: 5′-CCAAATTCCACATACATTACA-3′; M-sense: 5′-GGAGGAGTGTGTGTTGATTCG-3′, and M-antisense: 5′-ACCAAATCAGACATACATTACG-3′.

Chromatin Immunoprecipitation Assay

Chromatin immunoprecipitation (ChIP) assays were performed using the EZ ChIP tissue kit (Millipore) as previously described [16]. Chromatin preparations were immunoprecipitated with antibodies
against DNMT-1 (Cell Signaling Technology, Danvers, MA), AhR (Santa Cruz Biotechnology, Santa Cruz, CA), and AhRR (Abcam Inc. Cambridge, MA). Non-specific immunoglobulin G (IgG) was used as an internal control for ChIP assays. DNA fragments were amplified by quantitative real-time PCR (QPCR) using the SYBR Green PCR Reagents kit (Applied Biosystems, Carlsbad, CA). Briefly, reactions were carried out at a final volume of 25 μl consisting of the following master mix: 12.5 μl of SYBR Green buffer, 1 μl each of the following primers (Sigma–Aldrich): rat BRCA-1 forward 5′-TACCACGCAAATGACATAACCAGGAG-3′ (sense) and reverse 5′-TACACCAATTCCAGCTGCAATTCGGGAA-3′ (antisense); human BRCA-1 forward 5′-AGCTCGCTGAGACTTCCTGGAC-3′ (sense) and reverse 5′-GTCAGCTTCCGAAAATCTCCTCTC-3′ (antisense); 8.5 μl nuclease free water, and 2 μl DNA purified from the ChIP assay. Bound DNA was normalized to input DNA.

mRNA Analysis

Mammary tissues were homogenized in 1 ml of QIAzol Reagent (Invitrogen, Carlsbad, CA)/40 mg of tissue. Total RNA was extracted from mammary tissue and purified using RNeasy Lipid Tissue Mini Kit as per manufacturer’s instructions (QiaGen). Concentrations and quality of RNA were verified using the Nanodrop1000 Spectrophotometer (Thermo Scientific, Wilmington, DE). Equal amounts of total RNA (500 ng) were transcribed into cDNA using ISCRIPT supermix kit (Bio-Rad Laboratories, Hercules, CA). Next, PCR products were amplified from the cDNA fragments by QPCR using the SYBR Green PCR Reagents kit (Applied Biosystems). Briefly, reactions were run at a final volume of 25 μl consisting of the following master mix: 12.5 μl of SYBR Green buffer, 1 μl each of forward and reverse primers, 9.5 μl nuclease-free water, and 1 μl cDNA. Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) amplification was used for normalization of mRNA expression. The rat primer (Sigma–Aldrich) sequences were: BRCA-1, sense: 5′-GGGAAACCTACTGAACCATC-3′, antisense: 5′-GACGATGTGCTCTCCAGTA-3′; CYP1A1, sense: 5′-CCCTACATGACCCACAGA-3′, antisense: 5′-TTGTCACCTCTAACCACCCAGAATC-3′; CDK4, sense: 5′-TGAACGCCCTGTTGATATTG-3′, antisense: 5′-CAGATTCCTCCTCTCCGCGC-3′; cyclin D1, sense: 5′-CTGCGATGAGAATCTCCCTGA-3′, antisense: 5′-GTCACAGTGTGCACTCTCAG-3′; GAPDH, sense: 5′-TGTTGAAGTCTGGTGTA-3′, antisense: 5′-AGGGTGGTGGTGATGGCACA-3′.

Western Blot Analysis

Western blot analysis was performed as previously described [16]. Immunoblotting was carried out with antibodies against BRCA-1, GAPDH (Cell Signaling Technology), AhR, estrogen receptor-α (ERα), AhR nuclear translocator (ARNT) (Santa Cruz Biotechnology Inc.), and AhRR (Abcam Inc.). The immunocomplexes were detected using enhanced chemiluminescence (GE Healthcare Life Sciences, Little Chalfont, UK). GAPDH protein was used for normalization of protein expression.

Statistical Analysis

Densitometry after Western blotting and methylation analyses were performed using Kodak ID Image Analysis Software (Eastman Kodak Company, Rochester, NY). Statistical analysis was performed using Prism 5.0 (GraphPad Software Inc., La Jolla, CA) [16]. Data were analyzed using 1-way ANOVA. Post-hoc multiple comparisons among all means were conducted using Tukey’s test after main effects and interactions were found to be significant at P<0.05. Data were presented as means ± SEM and statistical differences highlighted with different letters.

RESULTS

Maternal Exposure to TCDD Increases the Number of Proliferative Structures in Mammary Tissue of Female Offspring

To minimize lactational effects associated with exposure to TCDD and Res, offspring were transferred to foster mothers within 10 h post-partum, as described previously [19]. We examined the effects of in utero exposure to TCDD, Res, and their combination on mammary gland morphology (Figure 1A and B) at two time-points, PND46 and PND71. The gestational exposure to TCDD, Res, and their combination did not alter the number of TD plus TEB (Figure 1C) and AB plus LOB (Figure 1D) in mammary tissue of offspring at PND46. Conversely, TCDD induced (~4.0-fold) the number of proliferative TD plus TEB, and reduced by ~70% the number of differentiated AB plus LOB at PND71. The pretreatment with Res reduced by ~50% the stimulatory effects of TCDD on the number of proliferative TD + TEB, and maintained to near control levels the number of AB plus LOB structures. These data confirmed earlier observations [19,28–30] that gestational exposure to TCDD disrupted mammary gland morphology in offspring.

In Utero Exposure to TCDD Reduces BRCA-1 Expression in Mammary Tissue of Female Offspring

Quantitative PCR and Western blotting analyses of mammary tissue harvested at PND46 revealed that the in utero treatment with TCDD reduced respectively BRCA-1 mRNA (~50%) (Figure 2A) and protein (~40%) (Figure 2B) levels. Conversely, TCDD induced CDK4 (2.5-fold, Figure 2C) cyclin D1 (2.5-fold, Figure 2D), and CYP1A1 (4.8-fold, Figure 2E) mRNA expression. Compared to control tissue, the pre-exposure to Res antagonized the adverse (BRCA-1) and stimulatory (CDK4, cyclin D1, CYP1A1) effects of TCDD on gene expression. Interestingly, the gestational treatment with Res alone increased BRCA-1 mRNA (0.45-fold) and protein (0.75-fold) (Figure 2A
Figure 1. Maternal exposure to TCDD alters mammary gland architecture in offspring. (A) Five (n = 5) dams were gavaged with vehicle (Control) or TCDD (n = 7) at GD15 as described in Materials and Methods Section. Dams in Res (n = 7) or TCDD plus Res (n = 6) groups were started on diet containing Res from GD7 until end of pregnancy. Female pups were switched to foster mothers and mammary tissue harvested at PND46 or PND71; (B) Representative whole-mounts at PND71; In (C) and (D), bars represent quantitation of respectively, TD plus TEB, and AB plus LOB structures. Means ± SEM with a different letter (a, b, c) differ (P < 0.05).

Figure 2. Maternal exposure to TCDD lowers BRCA-1 expression in mammary tissue of PND46 offspring. Data are from female offspring exposed in utero to Control (n = 5), TCDD (n = 7), Res (n = 7), and TCDD plus Res (n = 6) diet. Bars represent quantitation (fold of control) at PND46 of (A) BRCA-1 mRNA; (B) BRCA-1 protein; (C) CDK4 mRNA; (D) cyclin D1 mRNA; and (E) CYP1A1 mRNA. Changes in mRNA were determined twice from each sample in triplicate. Changes in protein levels were determined in whole cells in triplicate. Levels of GADPH protein and mRNA were used as internal controls for Western blotting and QPCR, respectively. Means ± SEM with a different letter (a, b, c) differ (P < 0.05).

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and B), and AhRR protein (1.0-fold) (Figure 3A and B) levels. Conversely, neither TCDD nor Res influenced the expression of AhR, ARNT, and ERα protein (Figure 3A).

In mammary tissue of offspring at PND71, the in utero treatment with TCDD brought about a reduction in BRCA-1 protein (~40%) (Figure 4A) and CYP1A1 mRNA (~80%) (Figure 4B) expression, but it stimulated CDK4 mRNA levels by 17.0-fold (Figure 4C). The pretreatment with Res antagonized the effects of TCDD on expression of BRCA-1, CYP1A1, and CDK4.

Gestational Exposure to TCDD Induces CpG methylation of the BRCA-1 Promoter in Mammary Tissue of Female Offspring

Using bisulfite-treated genomic DNA, we examined whether the in utero treatment with TCDD altered the CpG methylation status of the BRCA-1 gene in mammary tissue of offspring. We targeted a region of the rat BRCA-1 promoter sequence upstream of exon 1 (Figure 5A), which harbors an XRE and nine CpGs (Figure 5B). Results of control experiments with U- and M-specific primers confirmed that PCR amplification occurred in the linear range, and the rat BRCA-1 products were of the expected size (142 bp) (Figure 5C). Compared to control tissue, TCDD increased by ~0.75-fold at PND46, and ~0.40-fold at PND71, the BRCA-1 M/U ratio (Figure 5D and E). The pretreatment with Res lowered the BRCA-1 M/U ratio by 23% at PND46, and 40% at PND71, whereas no changes were observed in response to Res alone.

The authenticity of the BRCA-1 amplicons to the rat BRCA-1 promoter [22], and presence of methylated CpG at consensus Kaiso and MBD1-binding domains (Figure 5B) were ascertained by direct sequencing.

In Utero exposure to TCDD and Res Differentially Alter BRCA-1 Promoter Occupancy in Mammary Tissue of Offspring

In preliminary experiments, we used genomic DNA obtained from breast cancer MCF-7 cells cultured in control medium or in the presence of TCDD to validate the conditions for preparation of input DNA and ChIP assay with an AhR antibody and control IgG (Figure 6A). For PCR amplification, we targeted a human BRCA-1 promoter fragment containing an XRE, and found that TCDD did not alter the occupancy of the rat BRCA-1 promoter by the AhR in mammary tissue of PND46 offspring (Figure 6B). However, TCDD
induced the recruitment of DNMT-1 (5.8-fold) and AhRR (4.0-fold). The treatment with Res alone reduced the association of the AhR (75%) and DNMT-1 (65%), but stimulated a large increase (17.0-fold) in the recruitment of the AhRR (Figure 6D). Compared to TCDD alone, the combination of pretreatment with Res plus TCDD reduced the occupancy of the BRCA-1 promoter by DNMT-1 by ~50% (Figure 6C). These cumulative data suggested that the protective effects of Res against the TCDD-dependent increase in CpG methylation of the rat BRCA-1 promoter were related to lower occupancy of DNMT-1, and increased association of the rat BRCA-1 gene with the AhRR.

**DISCUSSION**

Decreased expression of BRCA-1 and accelerated growth are hallmarks of sporadic breast cancers [2]. Although hypermethylation of the BRCA-1 promoter and reduced BRCA-1 expression have been linked to increased mortality among women with breast cancer [31], the stimuli responsible for the placement of repressive chromatin marks on the BRCA-1 gene remain largely unknown. In this study, we hypothesized that gestational exposure to AhR agonists alters BRCA-1 expression in mammary tissue of female offspring through epigenetic mechanisms. This hypothesis is supported by several lines of evidence. First, our published data suggest that TCDD induces the placement of repressive histone (H3K9me3) and DNA (DNMT-1, MBD-2, and CpG methylation) marks on the BRCA-1 gene in breast cancer cells [15,16]. Second, prenatal exposure to TCDD impairs mammary gland differentiation, while increasing TEB formation and mammary cancer risk, in offspring of rodent models [32]. In contrast, knock-out of the AhR in the mammary gland reduces the formation of TEB [33], which are undifferentiated structures equivalent to human lobules type 1 (Lob 1). A preponderance of Lob 1 structures are found in breast cancers of BRCA-1 mutation carriers [34]. Third, premalignant and malignant human mammary tissues express constitutive higher levels of AhR-regulated genes [35]. Finally, prenatal exposure to dioxin-like compounds delays the initiation of breast development in girls [36].

Based on these observations, we selected GD15 for the gestational treatment with TCDD because it coincides with maximum expression of the AhR in rat placentas [37]. Also, the exposure to AhR agonists at GD15 exerts long-lasting developmental effects on the mammary gland [38]. We found that in the offspring of pregnant Sprague–Dawley rats treated with TCDD mammary tissue at PND41 and PND71 had higher BRCA-1 promoter CpG methylation. Also, in conjunction with the TCDD exposure, we observed increased recruitment of DNMT-1 to the BRCA-1 promoter and methylation of cytosines within 5'-CGCG-3' and 5'-TCGCA-3' sequences, which are core-
binding sites, respectively, for the transcription repressor factor Kaiso [39] and MBD1 [40]. The Kaiso protein recognizes preferentially methylated CpGs and forms complexes with the corepressor protein N-CoR [41]. The latter interacts with histone deacetylases and contributes to DNA methylation-dependent silencing of tumor suppressor genes [42]. MBD-1 is a member of the MBD family of proteins, which through interactions with H3K9 methyltransferase enzymes [43] contributes to gene silencing [44]. Therefore, through stimulation of CpG methylation at the Kaiso and MBD-1 binding sites, the in utero exposure to AhR agonists may fix the BRCA-1 gene in a hypermethylated state, thus reducing BRCA-1 expression in the offspring. In turn, reduced BRCA-1 functions may predispose to poor differentiation and tumor formation [45,46] sustained by higher cyclin D1 and CDK4 expression. The ability of cyclin D1 to activate CDK4 and repress several transcription factors has been previously associated with increased susceptibility to breast carcinogenesis [47].

Previous studies that examined the gestational effects of TCDD on CYP1A1 expression in offspring reported stimulatory effects in early life (PND25). Conversely, CYP1A1 was nearly undetectable at later time points (PND120) [48]. Similarly, we found that the in utero treatment with TCDD had a stimulatory effect on CYP1A1 mRNA levels at PND41, whereas CYP1A1 expression was repressed at PND71. The mechanisms through which gestational exposure to AhR agonists may contribute to transient elevation of CYP1A1 are unknown. Nevertheless, previous studies attributed postnatal fluctuations in CYP1A1 expression to epigenetic regulation of CYP1A1 promoter regions flanking an XRE [49]. Conversely, we noted that the gestational exposure to TCDD did not alter the expression levels of ERα, AhR, and ARNT protein. In keeping with these findings, other studies reported no changes in expression of AhR and ARNT in human placental tissue from smoking mothers, and cultured choriocarcinoma cells treated in culture with TCDD [37].

In experimental animals, the gestational supplementation with Res was reported to be safe [50], exert protective effects in embryos of diabetic dams [51], reduce renal malformation [52] and protect pregnant mother and fetus from the immunotoxic effects of TCDD [53] without affecting reproductive end points in male offspring [54]. Moreover, recent studies documented that supplemental Res crossed the placenta and protected the fetus in a rat model of severe hypoxia [55]. In the current study, the concentration of Res in the prenatal diet (7 ppm) approximated the level (10 ppm) used in previous studies to prevent mammary carcinogenesis induced by agonists of the AhR (25). We observed that the in utero pretreatment with Res reduced the TCDD-dependent increase in BRCA-1 promoter CpG methylation and occupancy of the BRCA-1 genes by DNMT-1. On the other hand, Res alone exerted partial agonistic effects on BRCA-1 mRNA and protein expression. The latter effects may be related to estrogenic actions of Res on the ERα [56]. Finally, Res increased BRCA-1 promoter occupancy by the AhRR, which through competition for the cofactor ARNT [57–59] may repress AhR functions at the BRCA-1 promoter.

In summary, we report that gestational exposure to the AhR agonist TCDD compromises normal mammary gland development and reduces BRCA-1 expression in the offspring. These alterations are paralleled by increased CpG methylation at the BRCA-1 gene, and stimulation of factors (cyclin D1, CDK4) involved in breast tumor development. Thus, epigenetic repression of BRCA-1 by AhR agonists during gestation may favor the transition to a proliferative
phenotype and augment the risk of breast tumorigenesis in the offspring [19]. Conversely, gestational exposure to AhR antagonists such as Res may exert protective effects against breast tumor development in the newborn.

ACKNOWLEDGMENTS

This work was supported by grants from the US ARMY Medical Research and Materiel Command (DAMD 10-1-0215 to D.F.R.), The American Institute for Cancer Research (10A058 to D.F.R.), and the Arizona Cancer Center Support Grant P30CA23074.

REFERENCES


