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Developmental Estrogen Exposures Predispose to Prostate Carcinogenesis with Aging

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Abstract

Prostate morphogenesis occurs *in utero* in humans and during the perinatal period in rodents. While largely driven by androgens, there is compelling evidence for a permanent influence of estrogens on prostatic development. If estrogenic exposures are abnormally high during the critical developmental period, permanent alterations in prostate morphology and function are observed, a process referred to as developmental estrogenization. Using the neonatal rodent as an animal model, it has been shown that early exposure to high doses of estradiol results in an increased incidence of prostatic lesions with aging which include hyperplasia, inflammatory cell infiltration and prostatic intraepithelial neoplasia or PIN, believed to be the precursor lesion for prostatic adenocarcinoma. The present review summarizes research performed in our laboratory to characterize developmental estrogenization and identify the molecular pathways involved in mediating this response. Furthermore, recent studies performed with low-dose estradiol exposures during development as well as exposures to environmentally relevant doses of the endocrine disruptor bisphenol A show increased susceptibility to PIN lesions with aging following additional adult exposure to estradiol. Gene methylation analysis revealed a potential epigenetic basis for the estrogen imprinting of the prostate gland. Taken together, our results suggest that a full range of estrogenic exposures during the postnatal critical period – from environmentally relevant bisphenol A exposure to low-dose and pharmacologic estradiol exposures – results in an increased incidence and susceptibility to neoplastic transformation of the prostate gland in the aging male which may provide a fetal basis for this adult disease.

Keywords

Prostate; carcinogenesis; estradiol; estrogens bisphenol A; development; steroid receptor; epigenetics; methylation

Introduction

The prostate is a male accessory sex gland that receives a great deal of interest not because of its physiologic role, but rather due to the high incidence of abnormal growth and tumor

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formation with aging in humans. Currently, prostate cancer is the most common non-skin cancer in males and is the second leading cause of cancer deaths in American men (1). According to the American Cancer Society, prostate cancer rates have been on the rise since 1975. With the 1987 introduction of PSA testing, the newly enhanced ability to diagnose the disease caused incidence to spike to 240 age-adjusted cases per 100,000 men by 1992. After this “catch-up” period, rates dropped for three years, but have been rising again since 1998. Additionally, benign prostatic hyperplasia (BPH) is the most common benign neoplasm, occurring in ~ 50% of all men by the age of 60. Despite extensive research in the field, the basis for these high rates of abnormal prostatic growth is not well understood. It is recognized, however, that steroid hormones play a role in the initiation and progression of prostate cancer which is the basis for hormonal treatment strategies. Eunuchs with low levels of circulating testosterone do not develop prostatic carcinoma (2) and cancer regression can be initially achieved by castration and androgen blockade (3). Although primarily under androgenic control, the prostate gland is also an estrogen target organ. Furthermore, estrogen involvement in the etiology of BPH and prostatic cancer has been postulated (4-6) and the use of antiestrogens has been recently recognized to have a therapeutic role in prostate cancer management (7,8).

It has long been speculated that early developmental events which are regulated by steroids in the prostate gland may be linked to its predisposition to high rates of disease in adult men (9). Thus it is noteworthy that relative to adult estrogenic responses, the prostate gland is particularly sensitive to estrogen exposures during the critical developmental period. In this context, the present review will focus on the potential role of fetal or perinatal estrogens in permanently imprinting the prostate gland during development which in turn sets the stage for increased susceptibility to prostate carcinogenesis with aging.

Prostate Gland Development

Unlike other male accessory sex glands which develop embryologically from the mesodermal Wolffian ducts, the prostate gland originates from the urogenital sinus (UGS) and is endodermal in origin. It has been suggested for decades that the high rates of prostate cancer in men compared to the paucity of carcinoma in the seminal vesicles, vas deferens or epididymis may have a basis in this unique embryologic origin for an accessory sex gland. Prostate development commences *in utero* as UGS epithelial cells form outgrowths or buds that penetrate into the surrounding UGS mesenchyme in the ventral, dorsal and lateral directions posterior to the bladder. In humans, prostate morphogenesis occurs during the second and third trimester and is complete at the time of birth (10,11). This contrasts with the rodent prostate gland where bud initiation commences in late fetal life and at the time of birth, a rudimentary structure is present consisting of a few main epithelial ducts. Extensive branching morphogenesis and cellular differentiation subsequently take place during the first 15 days of life (12). Thus the neonatal rodent prostate gland has emerged as a useful model for fetal prostate development in humans.

The initiation of prostatic development is dependent upon androgens produced by the fetal testes (9) and studies with 5 α reductase inhibitors have shown that dihydrotestosterone (DHT) is the active androgen required for prostate formation (13). Normal development, differentiation and onset of secretory activity requires the presence of androgens throughout the developmental process (14). Androgen receptors (AR) are highly expressed in the UGS mesenchyme prior to and during prostate morphogenesis (15,16) and evidence by Cunha and colleagues using mice has demonstrated that androgen-stimulated mesenchymal factors drive the morphogenetic process (17). Since AR are induced in rat prostate epithelium by postnatal day 1-3, it is possible that androgen-driven epithelial signals also contribute to morphogenesis and differentiation of the prostate (15).

The developing prostate gland also expresses other members of the steroid receptor superfamily including estrogen receptors ER α and ER β and retinoic acid receptors RAR α , β and γ which are liganded by all-trans or 9-cis retinoic acid as well as RXR α , β and γ which are activated by 9-cis retinoic acid alone. Studies in rodent prostate glands have shown relatively high stromal cell ER α expression during perinatal morphogenesis of the gland which significantly declines thereafter suggesting a specific role for ER α in prostate development (16,18,19). In the rat and murine prostate, ER β is primarily localized to differentiated luminal epithelial cells (20-22). ER β expression is low at birth, increases as epithelial cells cytodifferentiate and reaches maximal expression with onset of secretory capacity at puberty which suggests a role for ER β in the differentiated function of the prostate (20). In humans, ER α is also consistently observed in stromal cells during fetal development (23). It is noteworthy, however, that the developmental pattern for ER β in the human prostate differs markedly from the rodent. As early as fetal week 7, ER β is expressed throughout the urogenital sinus epithelium and stroma (23). This strong expression is maintained in most epithelial and stromal cells throughout gestation, particularly in the active phase of branching morphogenesis during the second trimester suggesting the involvement of ER β and estrogens in this process (16,23). While this pattern is maintained postnatally for several months, ER β expression declines thereafter with a noticeable decrease in adluminal cells at puberty (16) again suggesting a specific developmental role for estrogens.

Estrogen Imprinting of the Developing Prostate: fetal basis for adult disease

Similar to androgens, circulating levels of estradiol are high during the fetal and early neonatal life in both humans and rodent models (24) and there is compelling evidence that the developing prostate gland is particularly sensitive to these estrogens. During the third trimester of *in utero* development in humans, rising maternal estradiol levels and declining fetal androgen production result in an increased estrogen/testosterone (E/T) ratio. This relative increase in estradiol has been shown to directly stimulate extensive squamous metaplasia within the developing prostatic epithelium which regresses rapidly after birth when estrogen levels drop precipitously (25-27). Although the natural role for estrogens during prostatic development is unclear, it has been proposed that excessive estrogenization during prostatic development may contribute to the high incidence of BPH and prostatic carcinoma currently observed in the aging male population (28,29). African-American men have a two-fold increased risk of prostatic carcinoma as compared to their Caucasian counterparts and it has been postulated that this is related, in part, to elevated levels of maternal estrogens during early gestation in this population (30,31). Indicators of pregnancy estrogen levels such as length of gestation, pre-eclampsia and jaundice indicate a significant correlation between elevated estrogen levels and prostate cancer risk (32,33). Further, maternal exposure to diethylstilbestrol (DES) during pregnancy was found to result in more extensive prostatic squamous metaplasia in male offspring than observed with maternal estradiol alone (34). While prostatic metaplasia eventually resolved following DES withdrawal, ectasia and persistent distortion of ductal architecture remained (35). This has led to the postulation that men exposed prenatally to DES may be at increased risk for prostatic disease later in life although this has not been borne out in the limited population studies conducted to date (36). However, extensive studies with rodent models predict marked abnormalities in the adult prostate including increased susceptibility to adult-onset carcinogenesis following early estrogenic exposures (28,37-39). Although use of DES during pregnancy was discontinued in the early 1970s, the recent realization that certain environmental chemicals have potent estrogenic activities (40) has led to a renewed interest in the effects of exogenous estrogens during prostatic development (41).

Rat Model of Developmental Estrogenization

To carefully examine and elucidate a potential role for early-life estrogen exposures in adult prostate disease, we have made extensive use of the rat model for developmental estrogenization. The initial model used in our laboratory is the Sprague-Dawley rat given injections of 25 µg estradiol benzoate on neonatal days 1, 3 and 5 of life (Figure 1). It is important to mention that while this is considered “high-dose”, the majority of neonatally administered estradiol is bound to α -fetoprotein which circulates at high levels in neonatal rat serum (42). Consequently, neonatal estradiol is 75-fold less potent than an equivalent dose of DES (43) or, put another way, 25 µg estradiol/pup is equivalent to 0.33 µg DES/pup. As observed in earlier studies with mice and rats following early DES exposure (44,45), neonatal estradiol exposure consistently led to prominent pathology of the rat prostate gland. Histologic analysis of the young adult (day 90) ventral prostates of neonatally estrogenized prostates revealed disorganization of the epithelium with loss of basal/apical orientation, epithelial hyperplasia, inflammatory cell infiltrates and a relative increase in stromal elements (46-48). Of significant interest, the pathologic lesions of the epithelium progress with aging such that by 18-22 months of age, ventral and dorsal lobes exhibited extensive hyperplasia (epithelial piling and cribriform patterning within the lumens), adenoma formation and moderate -to- high grade prostatic intraepithelial neoplasia (PIN) lesions characterized by nuclear enlargement, anisokaryosis and hyperchromasia (49). Since neonatal estrogen exposure also results in decreased circulating testosterone (T) levels, a group of aged estrogenized rats were given 2 cm T implants for the last 6 months of life which restored T levels to normal. This treatment resulted in a 100% incidence of high-grade PIN throughout the ventral lobes by 18 months of age. Aged male rats exposed neonatally to DES have also been shown to develop profound squamous metaplasia in the dorsolateral prostate and development of solid tumors with highly invasive squamous cell carcinoma in some animals (44). Similar results have been observed in neonatal DES-exposed mice (37,45). Together these findings support the hypothesis that early high-dose estrogen imprinting may be a predisposing factor to malignant transformation of the prostate gland in the aging male.

To better understand the processes by which high-dose neonatal estrogens drive hyperplasia and PIN lesions within the adult prostate gland, we further characterized the estrogenized phenotype at the anatomic and cellular level. Brief neonatal estrogen exposure permanently retards growth and development of the prostate gland such that all lobes are hypomorphic, reaching only 20-50% of normal adult prostate size (48). In the dorsal and lateral lobes, not only is growth reduced but severe branching deficiencies exist such that elongating ducts fail to develop secondary and tertiary branch points and complex morphology (50). While reduced growth is in part a function of reduced circulating T levels following neonatal estrogen exposure (48), organ culture studies also demonstrated a direct effect of estrogens in growth retardation as well as altering prostate differentiation (51,52).

Following neonatal exposure to high-dose estradiol, both epithelial and stromal cell proliferation and differentiation are markedly disturbed leading to defects that persist throughout the lifespan of the animal (15,53-56). For epithelial cells, cytodifferentiation during development is perturbed or, for some end-points, permanently blocked by neonatal estrogens as determined by alterations in basal and luminal cell markers (p63, cytokeratins 5/15 and 8/18) and decreased production of secretory proteins (PBP, DLP proteins, urokinase, 26 kD protease) (15,22,53). Furthermore, alterations in the expression of e-cadherin and the gap junction proteins connexin 32 and connexin 43 in the adult prostate epithelial cells result in impaired cell-cell adhesion and defective cell-cell communication (56). In this regard, it is noteworthy that the epithelial cell differentiation defects are most prominent in the ventral prostate which also has the highest incidence of aging-associated PIN lesions (49).

Stromal-epithelial communication is also perturbed in the estrogen-exposed prostate through increased proliferation of periductal fibroblasts immediately adjacent to the outgrowing ducts during early development (53). Direct cross-talk between epithelial cells and adjacent smooth muscle cells via secreted growth factors and extracellular matrix components is essential for normal prostate development (57). The immediate proliferation and differentiation of mesenchymal cells into a multicellular fibroblast layer between the epithelial and smooth muscle cells effectively blocks this critical stromal-epithelial cell interaction and interrupts growth factor communication as has been shown specifically for transforming growth factor β 1 (*Tgfb1*) (53). Altogether, the above findings indicate that neonatal estrogen exposure interrupts intercellular communication and blocks certain epithelial cells within the rat prostate from entering a normal differentiation pathway. These persistent alterations in differentiation and gene expression may be a key mechanism through which changes towards a dysplastic state are mediated.

Molecular Pathways for Developmental Estrogenization of the Prostate

We next sought to determine the molecular pathways which mediate permanent alterations in prostate growth and function long after the hormone is withdrawn. It was observed in early studies that the activational response to androgens during adulthood is permanently blunted in estrogenized rats (46) and we determined that this effect is mediated, in part, through an immediate and permanent reduction in prostatic AR expression (15,48,58,59). Furthermore, the temporal expression patterns and quantitative levels of several other members of the steroid receptor superfamily are dysregulated by early exposure to high doses of estradiol. Thus ER α and progesterone receptor (PR) are transiently up-regulated in stromal cells (18,38), ER β is permanently down-regulated in luminal epithelial cells (20), RAR β is up-regulated in basal cells while RAR α is up-regulated in both epithelial and stromal cells (60). This has led us to propose that early estrogen exposure effectively switches the developing prostate gland from an androgen-AR dominated tissue to one that is primarily regulated by estrogens and retinoids. We further hypothesize that this irretrievably alters the prostate by changing organizational signals that determine prostate behavior throughout life.

Developmental genes that dictate normal prostate gland morphogenesis were next examined as potential direct targets of the altered steroid signaling *milieu*. Specific perturbations in expression of key homeobox transcription factors as well as secreted morphoregulatory genes were observed which serves to explain some of the common and lobe-specific estrogenized phenotypes. As this has been the subject of two recent reviews (39,61), the findings will be briefly summarized here. In the prostate gland, the posterior *Hox13* genes are involved in positional identity and differentiation. Of these, *Hoxb13* is expressed in the epithelium where it plays a specific role in differentiation (62,63). In the rat prostate, *Hoxb13* epithelial expression increased postnatally and was expressed at the highest levels in the ventral lobe (38). Following neonatal exposure to high-dose estrogen, *Hoxb13* expression was immediately and permanently suppressed in all prostate lobes with the most significant reduction (80%) observed in the ventral prostate gland. Another critical homeobox gene, the androgen-regulated *Nkx3.1*, is normally expressed in UGS-derived prostate epithelium where it plays a role in differentiation and growth (64,65). In control rats, a marked peak in *Nkx3.1* expression was observed postnatally between days 6-15 of life which subsequently declined to steady-state levels thereafter. While adult levels were not disturbed, the transient postnatal *Nkx3.1* peak was completely abolished following high-dose neonatal estrogen exposure (61). We propose that estrogen-initiated loss of prostatic epithelial *Hoxb-13* and *Nkx3.1* genes may play a critical role in mediating the differentiation defects observed in the developmentally estrogenized prostate gland.

In addition to developmental regulation by homeobox genes, branching morphogenesis occurs as a complex interplay between epithelial and mesenchymal cells through secreted morphoregulatory genes (66). We have recently examined the ontogeny and localization of bone morphogenic protein 4 (*Bmp4*), sonic hedgehog (*Shh*) and fibroblast growth factor-10 (*Fgf10*) in the normal developing rat prostate lobes and those exposed neonatally to estradiol to determine if alterations in their signaling pathways are involved in mediating specific aspects of the estrogenized phenotype. *Bmp4* has been implicated as a negative regulator of prostate growth (67) and levels in the rat prostate lobes rapidly decline postnatally. Following estrogen exposure however, *Bmp4* expression remained high through postnatal day 30 and we propose that this contributes to hypomorphic growth throughout the prostatic complex (61). Our recent studies on epithelial *Shh* (50) and mesenchymal *Fgf10* (68) demonstrated a critical role for these two genes in regulating branching morphogenesis of the prostate gland. Interestingly, early estrogen exposure led to a lobe-specific reduction in *Shh* and *Fgf10* signaling in the dorsolateral prostate which is the site of severe branching deficiencies in response to estrogenization. Furthermore, the data suggest that reduced *Fgf10* expression in the stromal cells by estrogens is the proximate cause of *Shh* reductions and branching deficiencies (68). Since a precise temporal expression pattern of these and other morphoregulatory genes is normally required for appropriate growth and differentiation of the prostatic epithelium and stroma, the estrogen-initiated disruption in this pattern would lead to permanent growth, branching and differentiation defects of the prostate gland. In summary, we propose that these and other yet unidentified molecular defects as a result of developmental estrogenization initiate permanent disturbances in prostate homeostasis which contributes to the development of prostatic neoplasia, PIN lesions and carcinoma as the animals age.

Neonatal Exposure to Low-dose Estradiol and Bisphenol A

The above effects of developmental estrogen exposures were in response to pharmacologic levels of estrogens as a model for early maternal exposures to agents such as DES or continued contraceptive use of ethinyl estradiol during pregnancy. A separate yet equally important issue is whether lower estrogenic exposures during development, such as elevated maternal estrogens or environmental estrogenic exposures, produce permanent prostatic abnormalities. Initial studies by vom Saal and colleagues to address the low-dose estrogen effects on the prostate gland found that in contrast to high-dose exposures, low-doses of estradiol or bisphenol A (BPA) during fetal life increased prostatic bud number, cell proliferation and adult prostate size in mice (69,70). However, no histopathologic abnormalities were observed in young adulthood. Furthermore, the low-dose estrogenic response in the prostate gland has not been consistently reported (71), due in part to species/strain differences, background estrogen levels and other experimental variables, and is a matter of considerable debate (72,73). To examine this issue in the neonatal rat model, we administered estradiol over a 7-log range of doses on neonatal days 1, 3 and 5 in both Sprague-Dawley rats and the more estrogen-sensitive Fischer 344 rats (74). We observed that only high-dose neonatal estradiol produced consistent prostatic pathology at 3 months whereas exposure to lower estradiol levels produced no permanent prostatic weight change or pathologic alterations (74) despite the advancement of puberty (75).

Although low-dose estrogens by themselves did not appear to drive prostate pathology in early adulthood, we asked whether low-dose exposures during development might shift the sensitivity of the prostate gland to adult estrogenic exposures as has been recently observed for some female reproductive endpoints (76,77). This question is biologically relevant since circulating estradiol levels and the serum estrogen: testosterone ratio increase in aging men partly due to increased body fat content and aromatase activity, at a time when prostate cancer incidence rises (78). Furthermore, prolonged adult exposure to estradiol at levels within a physiologic range is capable of driving prostatic carcinogenesis in the Noble rat model (4). To

address this possibility, we established a “second-hit” model as schematized in Figure 2. Briefly, newborn male rats were exposed to either high-dose estradiol (2.5 mg/kg BW), low-dose estradiol (0.1 µg/kg BW), an environmentally relevant dose of BPA (10 µg/kg BW) or oil as controls on neonatal days 1, 3 and 5 as a “first hit”. At day 90 of life, a “second hit” of estradiol (E) was given by implanting T+E (or empty) capsules for 16 weeks. The T capsules result in 3ng/ml serum T levels (79) and were necessary to maintain prostate homeostasis since E treatment alone results in feedback inhibition of endogenous T secretion with resultant prostatic involution. The E capsules produce serum levels of ~ 75 pg/ml in rats which, although elevated for males, is not considered pharmacologic (79). These T+E capsule for 16 weeks produce PIN in the dorsolateral prostates at 100% incidence in Noble rats (4) but only 33% incidence in Sprague-Dawley rats (80). At 28 weeks of age, the prostates were examined for hyperplasia, inflammation and PIN, the presumed precursor lesion of prostate cancer.

PIN scores, based on grade and frequency, and PIN incidence showed marked differences across treatment groups (81). Neonatal exposure to either high-dose estradiol or to low-dose estradiol alone resulted in elevated PIN scores and incidence with aging (66% and 55%, respectively) while BPA alone had no effect on prostate pathology. As expected, prolonged adult T+E exposure increased PIN incidence to 40% in control rats given oil neonatally. This was further increased to a 100% incidence with significantly elevated PIN scores by initial early exposure to high-dose estradiol. Neonatal low-dose estradiol prior to adult hormones, however, did not augment PIN lesions further than that seen with neonatal low-dose estradiol alone. In contrast, neonatal exposure to an environmentally relevant dose of BPA produced a significant augmentation of PIN lesions to 100% incidence when followed by adult T+E exposure. The overall PIN score was significantly higher than both oil-treated rats ($P<0.05$) and those given BPA alone ($P<0.01$) and was equivalent to the PIN score observed following high-dose estradiol exposure. Histologically, severe atypia was common with nuclear elongation and irregular size, cellular piling, and adenoma formation. These findings are highly significant since they are the *first* observation of a link between developmental low-dose BPA exposure and adult prostatic pathologic lesions. Together, this new experimental paradigm suggests that low-dose exposures to estradiol alone increase susceptibility to adult onset prostate dysplasia while environmentally relevant doses of BPA increase the sensitivity of the prostate gland to carcinogenesis following additional adult insults such as elevated circulating estrogens.

Epigenetic Changes in DNA Methylation as a Molecular Mediator for Estrogen Imprinting of the Prostate Gland

While we have identified both transient and permanent alterations in the expression of multiple cell signaling pathways following high-dose estradiol exposure (see above), the molecular basis of these changes has remained elusive. One distinct possibility is through epigenetic modifications of DNA via cytosine methylation or demethylation which would result in aberrant and heritable silencing or activation of genes. Importantly, there is evidence that early hormonal exposures during developmental sensitive periods can permanently alter DNA methylation of specific genes. McLachlan showed demethylation of CpG/-464 in the lactoferrin promoter of mouse uteri following neonatal high-dose DES exposure which persisted as tumors developed (82). Similarly, neonatal phytoestrogen exposure was associated with hypermethylation of c-H-*ras* in the rat pancreas (83) while fetal DES and methoxychlor exposure resulted in altered methylation of ribosomal DNA in mouse uteri (84).

To examine whether epigenomic alterations in DNA methylation play a role in prostate imprinting by estrogens, we screened for global methylation changes using methylation sensitive restriction fingerprinting, or MSRF, using prostates exposed neonatally to high-dose estradiol, low-dose estradiol or environmentally relevant doses of BPA without or with adult

T+E exposure (see above). As recently described (81), over 50 DNA candidates were identified as potential leads with repeatable methylation alterations across multiple samples and lobes. Of the identified candidates, 16 showed no homology with known rat genes, six were identified one time (PLC β 3, HPCAL1, CARK, GPCR14, PDE4D4 and PDGFR α) and two were identified multiple times (CAR-X1 and SLC12A2) with similar methylation patterns observed each time. It is noteworthy that several candidate genes involve signal transduction pathways: Na-K-Cl cotransport (SLC12A2), serotonin receptor/G-protein coupled receptor (GPCR14), MAPK/ERK pathway (PDGFR α), phosphokinase C pathway (PLC β 3), cAMP pathways (PDE4D4 and HPCAL1) and neural or cardiac development (CARX1, CARK). Furthermore, these signaling pathways are involved in cell cycle and/or apoptosis, suggesting that neonatal estrogen exposures may perturb proliferation/apoptosis equilibrium through epigenetic gene (de)regulation.

We initiated further studies to determine whether altered DNA methylation due to neonatal estrogenic exposures results in altered gene expression. Our initial studies focused on phosphodiesterase type4, variant 4 (PDE4D4), an intracellular enzyme that specifically degrades cAMP (85). This gene was chosen since the differentially methylated DNA fragment identified by MSRF corresponded to the 5' flanking region of the gene, it was consistently hypomethylated by all neonatal estrogenic exposures and the changes were identified by day 10 of life. Importantly, PDE4D4 controls the intracellular levels of cAMP which activates multiple downstream cell signalling pathways, regulating transcription of genes involved in cell growth and differentiation (86). Thus persistent activation of cAMP pathways may contribute to neoplastic transformation. In this regard, recent studies have shown a tight association between PDE4 expression and cancer cell proliferation, including glioma cells (87), osteosarcomas (88) and chronic lymphocytic leukemia (89). Furthermore, PDE4 is currently being pursued as a possible chemotherapeutic target (90).

The 5' flanking/promoter region of PDE4D4 was identified with a 700-bp CpG island that encompassed the transcription/translation start site (85). Using bisulfite genomic sequencing, a specific methylation cluster was identified in the 5'-flanking region of this PDE4D4 CpG island that was gradually hypermethylated with aging in the normal prostates. It is significant that this age-related PDE4D4 hypermethylation was directly associated with loss of gene expression as determined by real-time RT-PCR. In contrast to normal prostates, the PDE4D4 CpG island became hypomethylated with aging in all prostates exposed neonatally to high or low-dose estradiol or to BPA. Furthermore, this was directly associated with continued elevated PDE4D4 expression throughout life. Cell line studies confirmed that site-specific methylation is involved in transcriptional silencing of the PDE4D4 gene and showed hypomethylation of this gene in the prostate cancer cells. Importantly, PDE4D4 hypomethylation with increased gene expression was distinguishable in all neonatally estrogen/BPA-exposed prostates as early as day 90 of age before any secondary exposure to estrogens had commenced. This raises the possibility that PDE4D4 could be used as a molecular marker for prostate cancer risk assessment as a result of endocrine disruptors.

Summary and Conclusions

In summary, we have shown that a full range of estrogenic exposures during the developmental critical period – from environmentally relevant BPA exposure to low-dose and pharmacologic estradiol exposures – results in an increased incidence and susceptibility to neoplastic transformation of the prostate gland in the aging male which may provide a fetal basis for this adult disease. Our working hypothesis is that abnormal estrogenic exposures during developmental critical periods initiate permanent molecular and cellular changes early in life which predispose the prostate to neoplasia in adulthood. Further, our recent findings provides evidence that developmental exposures to environmental endocrine disruptors (such as BPA),

pharmacologic and natural estrogens (E_2) impact the prostate epigenome during early life which suggests an epigenomic basis for estrogen imprinting of the prostate gland. Based upon these findings, we propose a model for prostate cancer susceptibility due to developmental estrogen exposures in Figure 3. As depicted on the time-line, prostate development normally proceeds from fetal initiation and prostatic budding of progenitor cells, to neonatal morphogenesis and cytodifferentiation to pubertal growth and maturation with functional secretions which continue into adulthood. Developmental exposure to estrogens {high-dose pharmacologic exposure, low-dose exposures such as elevated maternal E_2 , or environmentally relevant xenoestrogens (e.g. BPA exposure)} lead to epigenetic changes (altered DNA methylation) which are heritable through subsequent cell divisions. This initially results in the expansion of progenitor cells with differentiation defects as the prostate develops. Subsequent adult insults such as rising E_2 with aging (or injury, inflammation or other mutation-generating events) are then required to promote these initial alterations leading to prostatic dysplasia and tumor-formation with aging.

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Figure 1.

Schematic representation of rat animal model for developmental estrogenization of the prostate gland. Day of birth (DOB) is considered day 0. Newborn male pups were given S.C. injections of high dose estradiol benzoate (25 µg) or oil as controls on day 1, 3 and 5. Rats are weaned on day 25. In young adulthood, significant differentiation defects and dysplasia are observed in the prostate gland as described in the text.

Figure 2.

Schematic representation of “two-hit” rat animal model for low-dose exposure to estradiol or bisphenol A (BPA) followed by second exposure to T+E implants on day 90. Newborn rats were injected with oil, high-dose estradiol benzoate (EB, 2500 µg/kg BW), low-dose EB (0.1 µg/kg BW) or BPA (10 µg/kg BW) on days 1, 3 and 5. At day 90, they were implanted with empty capsules or T+E capsules for 16 weeks. Arrowheads indicate times for tissue collection.

Figure 3. Proposed model for epigenetic mechanism of developmental estrogenization of the prostate gland by exposures to estradiol, E₂ or environmental disruptors such as BPA. See text for description.