Published in final edited form as:

Differentiation. 2011 ; 82(4-5): 211–219. doi:10.1016/j.diff.2011.05.011.

Potential protective mechanisms of aryl hydrocarbon receptor (AHR) signaling in benign prostatic hyperplasia

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Abstract

The aryl hydrocarbon receptor (AHR) is an evolutionarily conserved ligand activated transcription factor best known for its role in mediating toxic responses to dioxin-like environmental contaminants. However, AHR signaling has also emerged as an active participant in processes of normal development and disease progression. Here, we review the role of AHR signaling in prostate development and disease processes, with a particular emphasis on benign prostatic hyperplasia (BPH). Inappropriate AHR activation has recently been associated with a decreased risk of symptomatic BPH in humans and has been shown to impair prostate development and disrupt endocrine signaling in rodents. We highlight known physiological responses to AHR activation in prostate and other tissues and discuss potential mechanisms by which it may act in adult human prostate to protect against symptomatic BPH.

Keywords

AHR; prostate; BPH; development; vasculature; androgen

Overview of the AHR signaling pathway

The mammalian AHR protein is approximately 90 kDa and contains four major structural motifs (Jain et al., 1994; Swanson et al., 1993; Burbach et al., 1992). The first is an N-terminal basic-helix-loop-helix DNA binding domain, the second is a transactivation domain, and the third and fourth are protein-protein interaction and ligand binding PAS domains, named after their homology with the clock protein *period* (*Per*), the xenobiotic and oxygen sensing protein *aryl hydrocarbon receptor nuclear translocator* (*Arnt*), and the neuronal cell lineage regulator *single-minded* (*Sim*). The AHR is one of at least 34 known mammalian PAS family members (McIntosh et al., 2010) and is evolutionarily conserved in species ranging from flies to humans (Hahn, 2002).

The normal AHR biological function is not well understood, in part because it is activated by such a diverse array of anthropogenic and naturally occurring ligands that are structurally but not biologically related. Chemical activators of mammalian AHR signaling include but

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are not limited to dietary indoles, flavinoids, caratinoids, polycyclic aromatic hydrocarbons, polychlorinated biphenyls (PCBs), halogenated dibenzo-*p*-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) (Denison and Nagy, 2003). The environmental contaminant 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) is known as the highest affinity AHR ligand (Denison and Nagy, 2003). TCDD is frequently used as a research tool to activate AHR signaling, to reveal physiological responses to AHR signaling, and to characterize biological mechanisms for AHR-induced pathology. TCDD is an appropriate tool for these studies because nearly all biological actions of TCDD in mammals are mediated through AHR activation (Gonzalez et al., 1996). It should be noted that while AHR is evolutionarily conserved, the invertebrate AHR does not bind TCDD (Powell-Coffman et al., 1998) or require ligand for its activation (Emmons et al., 1999).

Most AHR actions are elicited by transcriptional mechanisms, though there are exceptions such as AHR-mediated enhancement of ubiquitin-mediated proteolysis (Ohtake et al., 2009). The mechanism for AHR-induced transcription is closely related to that of steroid hormone receptors. In its inactive non-ligand bound state, the AHR is sequestered in cytoplasm by a multimeric protein complex containing heat shock protein 90, prostaglandin E synthase 3, and AHR interacting protein (Carver et al., 1998; Cox and Miller, 2004). Upon ligand binding, the AHR undergoes nuclear translocation and binds to ARNT. The AHR/ARNT heterodimeric receptor complex is recruited to AHR response elements (AHREs) in or nearby target genes. DNA binding by the AHR/ARNT heterodimer elicits context-dependent transcriptional transactivation or repression (Okino and Whitlock, 2000). The most prominent members of the AHR-activated gene battery are drug metabolizing enzymes and some of which, including cytochrome P450 (CYP)1A1, CYP1B1, epoxide hydrolase, and glutathione S transferase mu 1, are present in human normal, benign hyperplastic and cancerous prostate (Carnell et al., 2004; Murray et al., 1995; Tokizane et al., 2005).

Evidence that activation of AHR signaling increases prostate cancer risk but decreases symptomatic BPH risk

There have been several industrial or environmental accidents that caused the accidental environmental release of TCDD or other toxic AHR ligands. Our understanding of how inappropriate AHR activation influences prostate disease risk is becoming clear as cohorts of TCDD-exposed men age and begin to develop prostate pathology. Interestingly, indications are that TCDD-induced AHR activation increases risk of developing prostate cancer and decreases risk of developing BPH. Evidence for a role of AHR signaling in both diseases will be presented separately.

A limited number of studies support the hypothesis that inappropriate AHR activation increases prostate cancer risk in humans. A group of United States Air Force Vietnam War veterans participating in “Operation Ranch Hand” were exposed to TCDD-contaminated Agent Orange. The Institute of Medicine (2007) concluded that there is ‘limited or suggestive evidence’ for an association between Agent Orange exposure and prostate cancer. Ranch Hand veterans in the highest TCDD exposure category and who spent less than two years of service in Southeast Asia displayed an increased risk of developing prostate cancer compared to age-matched controls (Akhtar et al., 2004). In a case-control pilot study conducted at the Department of Veterans Affairs Medical Center in Ann Arbor, Michigan, men with prostate cancer were approximately two times more likely to report previous exposure to Agent Orange, although the results of this pilot study were not statistically significant (Giri et al., 2004). In a separate study, Chamie et al (2008) revealed a two-fold increase in prostate cancer incidence in Vietnam War veterans that self-reported Agent Orange exposure versus veterans who reported no Agent Orange exposure, and a two-fold increase in the proportion of Agent Orange exposed veterans with severe (Gleason

scores 8 through 10) prostate cancer. Yet, results from the latter two studies should be interpreted with caution because of inherent variability associated with the self-reporting methods employed in these studies (Schechter et al., 2009). An additional confounding variable for the Ranch Hand studies is that service time in Southeast Asia, independent of Agent Orange exposure, appears to be associated with increased prostate cancer risk (Pavuk et al., 2006).

Experimental studies in animals corroborate epidemiological evidence that high AHR activity is likely to increase prostate cancer risk. TCDD-induced AHR activation during fetal and perinatal prostate development in C57BL/6J mice increases the incidence of cribriform hyperplasia when mice reach senescence (510 days old) (Fritz et al., 2005b). Though C57BL/6J wild type mice do not develop prostate cancer, cribriform hyperplasia is often considered to be a pre-cancerous lesion.

Paradoxically, complete loss of AHR signaling may also increase prostate cancer risk. *Ahr* deletion in TRansgenic Adenocarcinoma of the Mouse Prostate (TRAMP) mice that are predisposed to neuroendocrine prostate tumor formation accelerated onset and increased frequency of prostate tumor formation compared to TRAMP mice possessing two functional *Ahr* copies (Fritz et al., 2007).

The discussion above supports the hypothesis that low AHR signaling may protect against prostate cancer, while high AHR signaling may promote it. In line with this hypothesis, selective AHR modulators (SAHRMs), which are partial AHR agonists that bind to the AHR but do not elicit the severe toxic responses associated with TCDD-induced AHR activation, have been shown to protect against metastatic prostate cancer in a mouse model. Exposure of TRAMP mice to the SAHRM 6-methyl-1,3,8-trichlorodibenzofuran (40 mg 6-MCDF/kg diet) show a reduced incidence of pelvic lymph node metastases compared to TRAMP mice that were fed a control diet (Fritz et al., 2009). Although the neuroendocrine histotype of TRAMP tumors mimics an aggressive but rare human prostate tumor histotype, it is unclear whether AHR signaling in mice will also modulate prostate adenocarcinoma formation, a tumor more common to human males. This knowledge gap presents an important future research opportunity that will bolster our understanding of AHR signaling in prostate carcinogenesis.

Although inappropriate AHR activation appears to be associated with increased prostate cancer risk, it has paradoxically been linked to decreased BPH risk. A prospective study of 971 Ranch Hand and 1,266 control veterans who did not handle herbicides were evaluated for BPH based on medical history and physical examination. Serum TCDD levels in Ranch Hand veterans were inversely related to the risk of developing symptomatic BPH (Gupta et al., 2006a). In the National Health and Nutritional Examination Survey (Gupta et al., 2006b), serum concentrations of TCDD and other AHR ligands were measured in 42 patients who self-reported a previous medical diagnosis of enlarged prostate and 99 control patients who had not been previously diagnosed with an enlarged prostate. The study revealed an inverse relationship between serum levels of TCDD and other AHR agonists and previous diagnosis of enlarged prostate.

The epidemiological studies above reveal associations between inappropriate AHR activation and risk of developing prostate disease. However, there is little information about how endogenous, low-level AHR signaling influences adult prostate homeostasis and disease progression. This is a significant knowledge gap because selective, less toxic, and less potent SAHRMs, currently in development for treatment of breast cancer and other diseases (Safe et al., 1999), may be of therapeutic value for symptomatic BPH. To address potential mechanisms by which AHR signaling could curtail BPH development, and to

highlight specific areas where future research opportunities exist, the remainder of this review will focus on pathways currently believed to contribute to BPH and will explore how these pathways are influenced by AHR signaling in prostate and other tissues.

Role of AHR signaling in prostate development

It has been posited that BPH stems from age-inappropriate activation of prostate developmental signaling pathways (McNeal, 1978). Therefore, an examination of how AHR signaling participates in prostate ductal budding and branching morphogenesis may reveal potential mechanisms by which it acts in the adult prostate to curb BPH disease progression.

Nearly all knowledge of AHR signaling during prostate development has been obtained from the laboratory rodent. The main constituents of the AHR signaling pathway, AHR and ARNT, are likely to be expressed in prostate throughout all stages of male development. Both genes are expressed in fetal mouse and rat urogenital sinus (UGS) from which the prostate derives (Vezina et al., 2008b; Sommer et al., 1999) and have been detected in normal, hyperplastic, and cancerous adult human prostate (Kashani et al., 1998). No single AHR-responsive mRNA reliably reports AHR activity in all cell types, but the presence of certain AHR-responsive transcripts in the UGS during prostatic bud formation allows for the possibility that AHR is active in the UGS during fetal prostate development. The mouse *Cyp1b1* gene is an AHR-responsive gene that contains multiple AHREs in its promoter, at least one of which may be necessary for *Cyp1b1* transcription in some tissues (Tsuchiya et al., 2003). *Cyp1b1* is present in a diffuse domain of mouse UGS mesenchyme during prostatic bud formation and its abundance in UGS mesenchyme and epithelium is greatly increased by TCDD exposure (Vezina et al., 2008b). Reporter gene activity in mice expressing an Ahr-responsive *LacZ* that is under the control of multiple AHREs (Willey et al., 1998) is restricted to a small domain in the dorsal UGS aspect and expands following TCDD exposure (Vezina et al., 2008b). On the other hand, AHR-responsive *Cyp1a1* have not been detected in the control prostate during prostatic bud formation (Vezina et al., 2008b). Together, these results suggest that if the AHR signaling pathway is active during prostatic bud formation, it is likely to be active at a low level or in a limited subset of UGS cells.

The mechanism of AHR action in prostate development is likely to be functionally redundant with other signaling pathways because *Ahr* deletion in mice does not noticeably alter prostatic budding and branching morphogenesis, although anterior and dorsolateral prostate weights are slightly reduced (Lin et al., 2002). The concept of functional redundancy in prostate development is not unique to AHR signaling and has been described for other key prostate development genes including members of the homeobox (HOX) gene family (Warot et al., 1997) and is thought to also occur for the WNT/ β -catenin signaling pathway (Yu et al., 2009). It is unclear whether regeneration of the castrated adult prostate, a process believed to involve reawakening of developmental signaling pathways, requires AHR signaling. Further examination in this area is warranted because TCDD-induced AHR activation impairs tissue regeneration in other organs and species (Mitchell et al., 2006; Mathew et al., 2006) and because it may reveal mechanistic differences between normal prostate development and prostate injury/repair mechanisms that are likely to contribute to BPH.

Although AHR is not necessary for prostate development, its ectopic activation by TCDD is sufficient to disrupt key stages of ductal morphogenesis. TCDD-induced AHR activation during fetal-pubertal development significantly delays prostate growth as assessed by decreased relative prostate lobe weights in rats (Mably et al., 1992; Gray et al., 1997; Simanainen et al., 2004) and mice (Theobald and Peterson, 1997; Lin et al., 2002) and

decreased number of lumenized prostate ducts in monkeys (Arima et al., 2010). TCDD-induced AHR activation during prostate development also impairs prostate ductal differentiation as evidenced by a reduction in prostate secretory gene expression in mice (Lin et al., 2002) and reduced prostate luminal epithelial cell height in monkeys (Arima et al., 2010). Inhibitory effects of TCDD on rodent prostate development are linked to its impairment of prostate branching morphogenesis (Ko et al., 2002) and prostatic bud formation (Lin et al., 2003a). Prostatic bud inhibitory actions of TCDD are *Ahr*-dependent and occur in specific UGS regions: anterior budding is not affected, dorsolateral buds are reduced in number and repositioned towards the anterior UGS surface, and ventral buds do not form (Fig. 1).

The fact that TCDD impairs prostatic budding in select mouse UGS regions is a clue to participation of AHR signaling in prostate ductal pattern formation. Rodent anterior, dorsolateral, and ventral buds are specified and initiated at different stages of UGS development (Cunha, 1973). Prostatic buds from each of these UGS regions have different windows of sensitivity to TCDD (Vezina et al., 2008a). At no time is anterior prostatic budding sensitive to inhibition by TCDD at a 5 µg/kg dose. Dorsolateral buds are only inhibited when exposure occurs on or before E14.5. Ventral prostatic buds are inhibited when TCDD exposure occurs on or before E15.5. The significance of these results is that they have framed prostate developmental windows upon which TCDD acts (prostatic bud specification and initiation phases) and have exposed a potential mechanism of TCDD-induced AHR action: disruption of fetal prostate patterning.

A series of studies have been conducted to identify the mechanism by which TCDD-induced AHR action causes prostatic bud inhibition. It is known that androgen receptor (AR) in UGS mesenchyme but not UGS epithelium is needed for prostate ductal bud formation, while AR in UGS epithelium is unnecessary (Lasnitzki and Mizuno, 1980; Cunha and Lung, 1978). Likewise, AHR is needed in UGS mesenchyme but not UGS epithelium for prostatic bud inhibition by TCDD (Ko et al., 2004a). These results have further refined the trajectory of AHR signaling research in the developing prostate to focus on crosstalk between AR and AHR signaling pathways.

The role of AHR signaling in prostate ductal development was further defined by the observation that TCDD-induced AHR activation does not directly impair the activity of an AR-responsive reporter gene transiently transfected into UGS mesenchyme, nor does TCDD decrease UGS mesenchyme abundance of an endogenous AR-responsive gene steroid 5 alpha reductase type 2 (Ko et al., 2004b). Also, prostatic budding inhibition by TCDD-induced AHR activation cannot be overcome by exposing fetal male mice to high levels of the potent androgen, 53 dihydrotestosterone (Lin et al., 2003b). Collectively, these results suggest that AHR acts downstream of AR signaling to pattern ventral prostate development.

Allgeier et al. (2009) compared ventral UGS bud patterning in male and female mouse fetuses. Males normally form large prostatic buds on the ventral UGS surface, while females form much smaller buds. Under conditions of high AR signaling in the male fetus, ventral prostatic buds are completely eliminated by TCDD-induced AHR activation. However, in female UGSs where AR signaling is much lower than in males, ventral buds are distributed differently than in males and are not completely eliminated by TCDD-induced AHR activation. These results further support the concept that AHR-mediated prostate ductal inhibition acts downstream of AR signaling. These results may be important because if AHR signaling activation inhibits BPH in the adult by the same mechanism as it inhibits prostatic bud formation in the fetus, then it is likely to involve interactions with the downstream targets of the AR signaling pathway.

the activated AHR also appears to interact with non-canonical WNT signaling. Non-canonical WNTs bind to cell surface receptors and activate several intracellular signaling cascades including those mediated by phosphatidylinositol 3 kinase/ Ca^{2+} , JNK, and P38/ATF (Ma and Wang, 2007; Slusarski et al., 1997; Yamanaka et al., 2002). While the full complement of non-canonical WNT actions has not yet been elucidated, there is evidence that non-canonical WNTs are functional antagonists of canonical WNTs in some tissues and contexts (Mikels and Nusse, 2006).

Wnt5a is the prototype for non-canonical WNT signaling and is abundant in the male mouse UGS (Zhang et al., 2006), neonatal rat prostate (Huang et al., 2009), and adult human prostate (Yamamoto et al., 2010). Like TCDD, WNT5A negatively affects mouse prostate ductal bud formation (Allgeier et al., 2008), rat prostate ductal branching morphogenesis, and rat prostate luminal epithelial cell differentiation (Huang et al., 2009). WNT5A actions on prostatic budding closely resemble those of TCDD-induced AHR activation because ventral prostatic buds are the most susceptible to inhibition by both signaling pathways (Allgeier et al., 2008). Further evidence for their interaction is the fact that a WNT5A inhibitory antibody protects against ventral prostatic budding inhibition by TCDD (Allgeier et al., 2008). Whether WNT5A signaling contributes to LUTS symptoms in BPH is unknown and is an important subject for future investigation.

FGF10 is synthesized in UGS mesenchyme of mice and rats (Thomson and Cunha, 1999) and is present in primary cultured human prostate fibroblasts from men with BPH (Nakano et al., 1999). FGF10 is a critically important paracrine signaling pathway for prostatic bud formation (Donjacour et al., 2003) and AHR signaling antagonizes FGF10-induced DNA synthesis in basal epithelial cells and impairs FGF10-induced prostatic bud formation (Vezina et al., 2009). However, TCDD does not alter the abundance of the FGF10 receptor in UGS basal epithelium and does not interfere with the ability of FGF10 to stimulate extracellular regulated kinases 1 and 2 in UGS basal epithelium (Vezina et al., 2009). Therefore, it appears that TCDD acts downstream of FGF10 signaling in UGS basal epithelial cells to impair prostatic bud formation. The significance of this study is that it further refines the mechanism of AHR action in prostatic bud formation to AR-dependent signaling downstream of FGF10. What remains to be determined is how TCDD-induced AHR signaling interacts with AR, WNT5A, FGF10, and other growth-stimulatory and inhibitory signals to impair prostatic bud formation. It is also unclear whether these same signaling pathways participate in BPH etiology and whether AHR signaling interfaces with them in adult prostate. Mechanisms of prostatic bud formation and BPH progression are likely to overlap (McNeal, 1978). Since TCDD-induced AHR activation interferes with both processes, additional studies on how it inhibits prostatic bud formation in mice may inform studies on the mechanism by which it curtails BPH progression in men.

Role of AHR signaling in vasculogenesis

There appears to be an association between inappropriate prostate vasculogenesis and BPH. Vascular density is greater in epithelial and stromal BPH nodules compared to adjacent normal tissue (Deering et al., 1995). Prostatic microvessel density is positively associated with BPH symptoms (Kojima et al., 1997), particularly with hematuria secondary to BPH (Foley and Bailey, 2000). Prostate microvessel density is greater in men with recurrent BPH compared to those whose symptoms were alleviated by a single transurethral resection of the prostate (Sun et al., 2010). The mechanism for inappropriate vasculogenesis in BPH is unclear and warrants further investigation. The AHR signaling pathway influences vascular development in a wide spectrum of tissues and species and its regulation of prostate vascular development would provide a rationale for why AHR activation protects against BPH.

At least two opposing forces are known to collaborate during vascular development. Angiogenesis involves new vessel sprouting from preexisting vessels and is controlled in part by the hypoxia inducible factor 1 alpha subunit (HIF1A)/ARNT PAS protein transcription complex (Maxwell et al., 1997). Vascular pruning removes smaller microvessels to integrate the mature vascular system (Ashton, 1966) and is regulated in part by AHR/ARNT signaling. *Ahr* null mice exhibit defects in vascular pruning, resulting in the persistence of fetal vascular structures that are normally pruned in late gestation, including the hepatic ductus venosus, the optic hyaloid and limbal arteries, and renal vessels (Lahvis et al., 2005). The fact that TCDD-induced AHR hyperactivity also causes vascular defects reveals the importance of titrating AHR signaling for normal vasculogenesis. TCDD-induced AHR activation represses vascular remodeling in a wide variety of tissues and species, including rat placenta (Ishimura et al., 2006) zebrafish regenerating fin (Andreassen et al., 2007) zebrafish cranial vasculature (Dong et al., 2004), chicken embryo coronary vasculature (Ivnitski-Steele and Walker, 2003), and monkey hepatic vasculature (Korenaga et al., 2007).

Further evidence for a role of AHR signaling in prostate angiogenesis comes from studies of prostate carcinogenesis in TRAMP mice. One of the first known events in prostate tumor angiogenesis is HIF1A/ARNT mediated transcription of vascular endothelial growth factor, *Vegf* (Shukla et al., 2005). Since ARNT is a common dimerization partner of HIF1A and AHR, it was hypothesized by Fritz et al (2008) that AHR would compete with HIF1A for ARNT dimerization and thereby inhibit *Vegf* transcription and angiogenesis in TRAMP tumors. Wild type control and *Ahr* null TRAMP mouse prostate explants were cultured in media containing graded concentrations of sodium ortho-vanadate, an inducer of HIF1A/ARNT dimerization. Vanadate increased VEGF protein abundance in *Ahr* null TRAMP prostates explants but not in prostates containing both copies of functional *Ahr*. The results of this study suggest that AHR sequesters ARNT in TRAMP prostate and thereby decreases VEGF transcription. Results from this and other studies support the hypothesis that AHR signaling activation is likely to impair vasculogenesis in prostate and non-prostate tissues.

There is very little known about vasculogenesis in prostate development and whether vascular neogenesis or vascular pruning defects are responsible for increased microvessel density in BPH. We recently identified a rich vascular bed in the developing mouse prostate that is intimately associated with developing prostate buds (Fig. 2.). Therefore the developing prostate provides a research model for investigating paracrine signaling mechanisms between androgen-responsive UGS stroma, UGS epithelium, and UGS vascular endothelium. The developing prostate will also be a useful research tool for testing hypotheses about crosstalk between AR and AHR during prostate vasculogenesis. In fact, AHR induced inhibition of vascular development may in part be responsible for TCDD-induced prostatic budding defects and this is an important hypothesis to test in future studies.

BPH-associated inflammation: a potential therapeutic target for AHR agonists?

Acute and/or chronic prostatic inflammation is associated with increased prostate size, urinary retention and other LUTS that accompany symptomatic BPH (Mishra et al., 2007; Asgari and Mohammadi, 2011; Song et al., 2011; Nickel et al., 2008; Robert et al., 2009). BPH stroma secretes cytokines and chemokines that encourage pro-inflammatory cell invasion (Begley et al., 2008; Penna et al., 2009; Kohnen and Drach, 1979), thereby enhancing prostate inflammation and possibly resulting in fibrosis (Steiner et al., 2003; Kramer et al., 2002). Pro-inflammatory infiltrate, particularly the presence of macrophages and T cells in prostate interstitium, is positively associated with age (Bianchi-Frias et al.,

2010), suggesting that the potential for an enhanced prostate inflammatory response during aging.

One mechanism by which AHR signaling could provide therapeutic benefit for symptomatic BPH is by suppressing the prostate immune response. A prominent physiological response of AHR activation is suppression of innate and humoral immunity. The AHR is expressed in a diverse spectrum of immune cells including macrophages, cytotoxic and regulatory T cells, dendritic cells, and neutrophils (Komura et al., 2001; Vorderstrasse and Kerkvliet, 2001; Ackermann et al., 1989; Lawrence et al., 1996). TCDD-induced AHR activation has been shown to suppress inflammatory cytokine production by macrophages stimulated with lipopolysaccharide (Kimura et al., 2009), and inhibit polymorphonucleocyte cytolytic and cytostatic activity (Ackermann et al., 1989).

Immunosuppressive activity of the AHR signaling pathway has drawn recent attention as a therapeutic target for immune disorders. TCDD-induced AHR activation decreases the latency of recovery from chemically-induced colitis in mice, in part by reducing abundance of interferon gamma and TNF alpha signaling (Benson and Shepherd, 2011). TCDD-induced AHR activation decreases graft versus host response in mice, in part by increasing the abundance of T regulatory cells (also known as suppressor T cells) (Funatake et al., 2005). The AHR may in fact play an unappreciated mechanistic role in anti-inflammatory drug action. It was recently discovered that activation of AHR signaling may be responsible for some of the therapeutic effects of leflunomide, a drug approved for treatment of rheumatoid arthritis (O'Donnell et al., 2010).

The discussion above suggests a potential beneficial role for AHR signaling in suppressing the inflammatory response associated with BPH. Accordingly Lin *et. al* (2007) tested the hypothesis that functional AHR signaling would curtail mouse prostate inflammation secondary to inducible prostatitis. Ten-week-old wild type and *Ahr* null C57Bl/6J mice were infected via intra-urethral installation with uropathogenic *E. coli* 1677, resulting in a sub-acute prostate inflammatory reaction that receded by 20 weeks. The presence or absence of functional AHR did not affect onset of inflammation-induced prostate epithelial cell dysplasia. However, future research is still needed to determine whether pharmacological AHR activation protects against the prostatitis-induced prostate inflammatory response and whether AHR signaling represses the accumulation of pro-inflammatory cells in the aging prostate.

Crosstalk between AHR and estrogen receptor signaling could modulate BPH development

Circulating estradiol levels are low in healthy young men but during aging increase in both absolute concentration and in relation to circulating testosterone (Rubens et al., 1974; Seppelt, 1978; Shibata et al., 2000). It has been proposed that this aging-related increase in the relative estradiol:testosterone ratio contributes to prostate hypertrophy. This hypothesis is supported in part by whole animal (Ricke et al., 2006) and cell culture studies (Ho et al., 2008). TCDD-induced AHR activation during fetal and neonatal female rat development leads to decreased circulating estradiol levels when rats reach sexual maturity (Jablonska et al., 2010). Chronic TCDD-induced AHR activation in adult rats also decreased circulating estradiol levels (Franczak et al., 2006). There is also epidemiological evidence to support an AHR-associated change in estradiol levels in humans. A 1976 explosion at a trichlorophenol herbicide manufacturing plant near Seveso, Italy resulted in the accidental release of up to 30 kg of TCDD (Needham et al., 1997). Serum estradiol levels appear to be decreased in adult men living near Seveso that were exposed to TCDD during pre-pubertal or pubertal

development (Mocarelli et al., 2008). It has not yet been determined whether TCDD-induced reductions in male circulating estradiol concentrations protect against BPH.

The discussion above reveals several future research opportunities. It has not yet been determined whether TCDD-induced AHR activation in male mice would increase absolute concentrations of circulating estradiol during young adulthood, whether estradiol concentrations would be further increased during aging, and whether the relative proportion of estradiol to testosterone concentrations would be reduced by TCDD exposure compared to vehicle-exposed control mice. These studies are important because they may provide mechanistic support for epidemiological evidence that AHR activation in adulthood protects against BPH.

Crosstalk between AHR and AR signaling could modulate BPH development

Pharmacological inhibition of androgen signaling with the steroid 5 α reductase inhibitors finasteride and dutasteride remains the standard of care for severe symptomatic BPH that is refractory to tamsulosin treatment. In prostate and other tissues, AHR signaling influences androgen signaling directly at the level of AR and indirectly through actions on the endocrine system. TCDD-induced AHR activation decreased AR-dependent transcription and reduces AR protein levels in cultured human prostate LNCaP epithelial cells (Morrow et al., 2004; Jana et al., 2000). TCDD exposure during rat perinatal development reduced prostate *Ar* mRNA abundance when rats reached puberty (Ohsako et al., 2001).

There are at least two hypothesized mechanisms by which AHR directly decreases AR signaling. One mechanism is that AHR competes with AR for transcriptional co-activators that are used by both signaling pathways, such as four and a half LIM domains 2 (Kollara and Brown, 2010). Another mechanism for AHR-mediated repression of *Ar*-dependent transcription is through AHR's role as an E3 ligand-dependent ubiquitin ligase complex which regulates AR protein stability (Ohtake et al., 2007).

AHR signaling has also been shown to exert anti-androgenic actions through indirect mechanisms. PCB126-induced AHR signaling inhibits steroid 5 α reductase activity in cultured prostate epithelial LNCaP cells (Endo et al., 2003). TCDD also decreased luteinizing hormone receptor expression in adult mice (Fukuzawa et al., 2004) and repressed circulating testosterone in mice (Fukuzawa et al., 2004) and possibly also in men (Dhooge et al., 2006; Gupta et al., 2006a). Perinatal TCDD-induced AHR activation in mice was associated with an age-inappropriate androgen dependence when mice reached old age (Fritz et al., 2005a), but it is unclear whether AHR activation in adulthood would cause the same response during aging and further investigation is warranted. The discussion above reveals direct and indirect mechanisms by which AHR activation has been shown to interfere with AR signaling. These anti-androgenic actions of AHR signaling, in conjunction with anti-estrogenic actions of AHR signaling discussed earlier, may protect against the aging-related increase in the ratio of circulating estradiol:testosterone which has been associated with symptomatic BPH in men.

Summary

We have highlighted several physiological processes believed to participate in BPH and have revealed mechanisms by which AHR signaling activation is known to interfere with these processes in prostate or other tissues. A summary of five different AHR-responsive processes that could be targeted for therapeutic treatment of BPH is shown in Fig. 3. TCDD is certainly a potent AHR agonist and while there is limited epidemiological evidence

supporting its protective role in BPH, it is of little therapeutic value because of its toxicity and its resistance to metabolism and elimination. Efforts are ongoing to identify SAHRMS for use in breast cancer treatment and these drug candidates may also be of therapeutic value for symptomatic BPH. However, since evidence in humans suggests that TCDD-induced AHR activation may offer protection against BPH but not against prostate cancer, it is important to identify and segregate protective AHR agonist properties from those that cause unwanted toxic effects on the prostate.

This review has highlighted several knowledge gaps in basic science research that must be addressed to advance our understanding of AHR signaling in the normal and diseased prostate. Although AHR signaling interferes with ductal bud formation in the fetal prostate, it is unclear whether ductal neoplasia known to exist in some BPH lesions will be inhibited by AHR signaling activation. It is unclear whether AHR signaling is active in vascular endothelium of the prostate and whether it influences vasculogenesis or vascular remodeling during prostate development, during androgen-induced regeneration of the castrated prostate, or during vascular neogenesis in BPH. It is not known whether activation of AHR signaling will suppress aging-related prostate inflammation or whether crosstalk among the activated AHR, AR, and ER signaling pathways will protect against prostate hyperplasia in animal models susceptible to BPH. Finally, AHR signaling activation has been tested for its role to modulate prostate cancer progression in susceptible animal models but there have been no parallel studies to determine whether AHR signaling alters urodynamic or neurovascular responses to aging or to bladder outlet obstruction in laboratory rodents. Future studies to address these knowledge gaps are likely to reveal AHR signaling as a more prominent player in prostate biology and disease mechanisms than is currently appreciated.

Acknowledgments

This publication was supported by NIH grants ES001332, ES01332, and DK083425.

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Figure 1. TCDD-induced AHR activation interferes with prostatic bud formation in the fetal male mouse

Wild type and *Ahr* null C57BL/6J mice were exposed on embryonic day 13.5 to vehicle (5 ml/kg corn oil, *po* maternal dose) or TCDD (5 µg/kg maternal dose). Mesenchyme was removed from male UGSs on E18.5 and scanning electron microscopy was used to visualize the lateral surface of the UGS epithelium. Functional *Ahr* is required for TCDD-mediated inhibition of ventral prostatic bud formation, reduction of dorsolateral bud number and displacement of dorsolateral buds to the anterior UGS budding surface. Ventral prostatic buds are pseudocolored blue, dorsolateral buds are green, and anterior buds are red. Abbreviations used are bn: bladder neck, dd: ductus deferens, sv: seminal vesicle, u: urethra. Images are modified from (Vezina et al., 2008b).

Figure 2. Microvasculature in fetal mouse prostate

An embryonic day 17.5 male UGS near mid-sagittal section (50 μm) was assessed by ISH to visualize mRNA expression (purple) of the endothelial marker E26 avian leukemia oncogene 1, 5' domain (*Ets1*). Arrowheads indicate prostatic bud epithelium. The dashed line demarcates the boundary between the inner UGS epithelium and outer UGS mesenchyme. Images are representative of $n=3$ litter-independent mice. Abbreviations are bl: bladder, sv: seminal vesicle, u: urethra.

Figure 3. Hypothesized mechanisms by which the aryl hydrocarbon receptor (AHR) could protect against symptomatic benign prostatic hyperplasia

(1) Activation of AHR signaling has been associated with anti-androgen activity in isolated prostate cells and with reduced testosterone levels in men and rodents. (2) TCDD-induced AHR signaling impairs genes and signaling pathways that are needed for prostate ductal development and thought to be reawakened during BPH. (3) Activation of AHR signaling causes immunosuppression that may alleviate inflammation during BPH. (4) AHR signaling has been shown to impair vasculogenesis in non-prostatic tissues and could protect against increased prostate microvessel density during BPH. (5) Activation of AHR signaling has been shown to elicit anti-estrogenic activity and reduce circulating estradiol levels in rodent species, suggesting that it may protect against estrogen induced prostate hyperplasia.