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Endocrine Disrupting Compounds and Prostate Cell Proliferation

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Abstract

Epidemiological, *in vitro* and animal studies have indicated that Endocrine Disrupting Compounds (EDCs) influence the normal growth and development of the prostate as well as development and progression of prostate cancer. This has been linked to an increased presence of environmental chemicals that interfere with hormonal signaling. Many of these effects appear to be associated with interferences with steroid hormone receptor signaling or by affecting steroidogenesis. Currently, there is abundant evidence from epidemiological studies linking pesticides and EDCs with elevated prostate cancer risk. Bisphenol-A, a known EDC, has been shown to promote induction of Prostate Specific Antigen (PSA), which is a biomarker for prostate cancer, in the LNCaP human prostate carcinoma cells, and to increase prostate carcinogenesis in animal models. Our research has focused on AR signaling and identification of novel androgenic and anti-androgenic EDCs. Using prostate cancer cell lines we have shown that the AR agonist, TBECH, and antagonist ATE, which is also a partial AR_{T877A} agonist, induce PSA expression. With the increasing presence of these EDCs in indoor and outdoor air, follows an increased risk of disturbed prostate development, regulation and function. Hence, identification of the contribution of EDCs to prostate cancer development should be considered a high priority.

Keywords: Endocrine disrupting compounds; Androgen receptor; Prostate cancer

Abbbreviations

AF: Activating Function; AR: Androgen Receptor; ATE/TBP-AE: Allyl 2, 4, 6-tribromophenyl ether; BATE/TBP-BAE: 2-bromoallyl 2, 4, 6-tribromophenyl ether; DPTE/TBP-DBPE: 2, 3-dibromopropyl-2, 4, 6-tribromophenyl ether; BFR: Brominated Flame Retardant; DBD: DNA Binding Domain; DHT: Dihydrotestosterone; EDC: Endocrine Disrupting Compound; LBD: Ligand Binding Domain; NTD: N-terminal Domain; PCa: Prostate Cancer; PSA: Prostate Specific Antigen; T: Testosterone; TAU: Transcription Activation Unit; TBECH: 1, 2-dibromo-4-(1, 2 dibromoethyl) cyclohexane; AD: Androgen Dependent; AI: Androgen Independent; BPA: Bisphenol A; ER: Estrogen Receptor; PR: Progesterone Receptor; TR: Thyroid Receptor; PPAR: Peroxisome-Proliferator Activated Receptor; PBDE: Polybrominateddiphenyl Ether; H874Y: Histidine 874 Tyrosine, T877A: Threonine 877 Alanine; T877S: Threonine 877 Serine; W741C: Tryphtohan 741 Cysteine; F876L: Phenylalanine 876 Leucine; DHEA: Dehydroepiandrosterone

Androgen Receptor, Mutations and Prostate Cancer

Testosterone (T) and its metabolite 5α-dihydrotestosterone (DHT) are the main male sex steroids. They are indispensable for male development as well as sexual behavior, and mediate their biological effects via the Androgen Receptor (AR) [1]. AR is expressed in many organs such as hypothalamus, liver, pituitary, prostate and testes [2]. AR belongs to the nuclear receptor subfamily 3, group C, member 4 (NR3C4), a member of the steroid receptor family of ligand-inducible transcription factors [3]. The human AR cDNA was first cloned in 1988 [4], and localized on X chromosome at Xq11-12 [5]. Human AR consists of 8 exons that encode a 110 kDa protein consisting of 919 amino acids [6]. AR protein has a modular structure, consisting of four structural domains each harboring an independent function that is crucial for AR action (Figure 1). These are the NH2-terminal or A/B domain (NTD), the DNA-binding or C domain (DBD), the hinge region or D domain, and the ligand-binding or E domain (LBD) [7-9].

The NTD, encoded by exon 1 makes up more than half the size of AR (residues 1-555) [6]. The first 30 residues are highly conserved and crucial for interactions with the LBD, a property that is unique to AR among the steroid receptor family [10]. The human NTD comprises of polyglutamine

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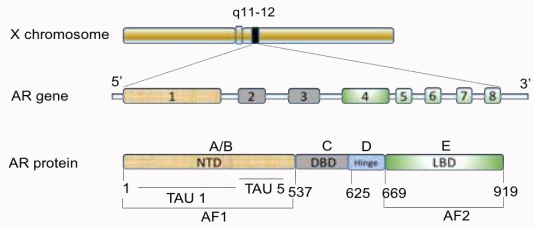

Figure 1: Structural and functional organization of the human AR gene and protein. A schematic mapping of the AR gene on the long arm of the X-chromosome, displaying the eight exons that codes for a 919 amino acids long protein made up of different functional domains.

Abbreviations: NTD: NH_2-Terminal Domain; DBD: DNA Binding Domain; LBD: Ligand Binding Domain; AF: Activation Function; TAU: Transcriptional Activation Unit

Exon 1 encodes for the NTD, exons 2 and 3 encode the DBD, and exons 4 to 8 encode both the hinge and LBD. Adapted from Lonergan PE, Tindall, DJ. J Carcinog. 2011; 10: 20.

(CAG) and polyglycine (GGC) repeats that is highly variable but absent in lower organisms like zebrafish [11-13]. In addition to its variable sequence property, the NTD also contains the transcriptional activation function AF-1 that is made up of two highly modular transcriptional units (TAUs) TAU 1 (residues 100-370) and TAU 5 (residues 360-485) [14]. Transcriptional activation of AR occurs when AF-1 is separated from the LBD. Additionally phosphorylation of NTD via several intracellular kinases is a well-known post-translational modification permitting ligand-independent AR activation and function [6,10].

The DBD (residues 556-623) is encoded by exons 2 and 3, and is the most conserved region within the nuclear receptor family. The DBD region consists of eight cysteine residues forming two zinc fingers, each made up of four cysteines and a Zn^{2+} ion that binds to the major groove of DNA [15]. The first zinc finger contains the proximal box (P-box) that determines the specificity of DNA sequence recognition of the AR protein as well as formation of a "recognition helix" [16]. The second zinc finger form the "D-box" (distal box) and is involved in DNA-dependent AR dimerization [16]. AR binds to target androgen response elements (AREs) in a head-to-head dimer-like manner [17]. Apart from DNA binding, the DBD also play a vital role in mediating nuclear localization and dimerization of AR. The hinge region or D domain serves as a flexible linker connecting DBD and LBD, and also contains the nuclear localization signal that influences AR subcellular location. The hinge region is also involved in DNA binding, coactivator recruitment and AR dimerization [18,19].

The AR LBD (666-919) is a well characterized structure and serves primarily to bind androgens [20]. The AR LBD is made up of only 11 α-helices, rather than 12, due to the absence of helix 2, found in other nuclear receptors. It also contain four short β strands forming two anti-parallel β-sheets [7,20-22].The AR LBD is identical for humans, rats and mice and offers high affinity binding of the two endogenous androgens, T and DHT [23]. Like other steroid receptors, the LBD contains a transcriptional activation function 2 (AF-2) that is ligand dependent. The AF-2 co-activator surface serves to recruit co-activators such as p160 thereby promoting transcriptional activity [22]. In contrast, binding of AR antagonists do not induce a similar repositioning of helix-12 thereby leading to recruitment of co-repressors such as the nuclear receptor co-repressor (NCoR) and the silencing mediator of retinoic acid and thyroid hormone receptor (SMRT) [24].

The prostate depends on circulating androgens for normal growth, development and function [25]. In rodents and humans, the loss of AR is associated with failure of prostate development [26,27]. The development and metastasis of prostate cancer (PCa) is dependent on AR activation by androgens. Globally, PCa is the second most frequently diagnosed cancer in men [28]. Treatment of metastatic PCa involves either androgen ablation monotherapy or anti-androgen drug treatment. AR positive PCa tumors can either display androgen-dependent or androgen-independent characteristics. Occasionally PCa can show heterogeneity within a tumor, with the presence of both androgen-dependent and androgen-independent cells [29,30]. Initially most PCa tumors are dependent on circulating androgens for growth, and treatment is therefore aimed at lowering serum androgen levels [31]. In some patients it has been observed that the anti-androgens convert to AR agonists when PCa has reached advanced stages [32]. AR is prone to mutations and so far 159 mutations have been detected in PCa tissue with a majority of them being single base substitutions [33]. Among these 45% are present within the AR-LBD. The most frequently detected substitution mutation in PCa tumors is that of codon 877 which encode threonine and is substituted by alanine (AR_{T877A}). This mutation comprises 25-31% of the mutations in advanced PCa patients treated with androgen ablation therapy [34,35]. The presence of AR_{T877A} mutations within the LBD render AR non-specifically activated by different ligands that include dehydroepiandrosterone (DHEA), estrogens, phytoestrogens, progestogens, anti-androgens such as cyproterone acetate, hydroxyflutamide and nilutamide [36,37]. Another PCa associated mutation within the codon 877 where threonineis substituted by serine (AR_{T877S}) exhibited a potent activation by estradiol, progesterone and cyproterone acetate [38]. Another mutation, AR_{H874Y} showed a similar response as AR_{T877A} to a diverse group of ligands [36,38]. The anti-androgenic drug bicalutamide exhibited agonist activity to AR with the W741C mutation within the LBD that is detected in advanced PCa patients [39,40]. Recent studies have also shown that the mutation AR_{F876L} within the LBD convert the second-generation PCa drug enzalutamide into an agonist [41-43].

Endocrine Disrupting Compounds

According to the Endocrine Society, an EDC is defined as "an exogenous chemical, or mixture of chemicals, that interferes with any aspect of hormone action" [44]. EDCs interact with receptors by mimicking hormones and exert effects on target cells via activation or repression of target genes. An example of this is bisphenol A (BPA), an estrogen mimic that binds to the Estrogen Receptor (ER) [45,46]. Originally EDCs were thought to be mediating their effects mainly through nuclear receptors such as the ER, AR, Progesterone Receptor (PR), Thyroid Receptor (TR), and retinoid receptor [47]. However, it is now understood that the EDC mechanisms of action are more diverse than originally believed. Apart from steroid nuclear receptors, EDCs exert their effects through other nuclear receptors such as the Peroxisome Proliferator Activated Receptor (PPAR). Phthalates have been shown to exert adverse effects on reproductive functions through exposure of direct activation of PPARs [48,49]. EDCs have also been reported to mediate their actions via neurotransmitter receptors, aryl hydrocarbon receptors, different enzymes involved in steroid metabolism, and other mechanisms related with endocrine regulation and reproduction [47,50].

BPA which is a well-known ER agonist, is also a TR antagonist and an AR wild type antagonist and an agonist to AR_{T877A} [51,52].

EDCs can also exert their effects directly or through metabolic products like OH/Me OH polybrominateddiphenyl ethers (PBDEs), polychlorobiphenylols, dichlorodiphenyldichloroethylene and dichlorodiphenyl-dichloroethane [53-56]. Apart from interfering with hormonal signaling pathways, recent reports have also shown that EDCs can induce epigenetic changes in target tissues, for instance alteration in DNA methylation patterns in prostate and testicular cells following exposure to BPA, the antiandrogen vinclozolin and diethylhexyl phthalate [57-59].

Androgenic and Anti-Androgenic Compounds

There are a large number of compounds that have either been discovered or synthesized to interact with AR [60-63]. AR mediated EDCs have been identified via *in silico* approach as well as *in vitro* experiments. Hence, on the basis of their ability to activate or repress AR transcriptional activity, EDCs can be grouped into androgenic/agonist and anti-androgenic/antagonist compounds. Naturally occurring androgens consist of T and its metabolite DHT (Figure 2). AR agonists mimic endogenous androgens and trigger androgenic responses, while AR antagonist repress AR transactivation. Some of the well-known anti-androgenic compounds are steroidal in nature such as chlormadinoneacetate, cyproterone acetate and allylestrenol, while others are non-steroidal like bicalutamide, flutamide, hydroxyflutamide and enzalutamide (Figure 2) [64,65]. There are numerous studies where *in silico* screening or reporter assays or both has been used to identify compounds with AR binding activity. A majority of the reported compounds exhibit anti-androgenic activity while only a few show androgenic activity [60,62,63,66-73]. From the systematic screening of different compounds, the first reported case of environmental chemicals exhibiting AR agonistic properties were 2-tert-butylanthraquinone and benzoanthrone (Figure 2), which were relatively weak partial agonists with only 10% maximal activation relative to the natural ligand DHT [62].

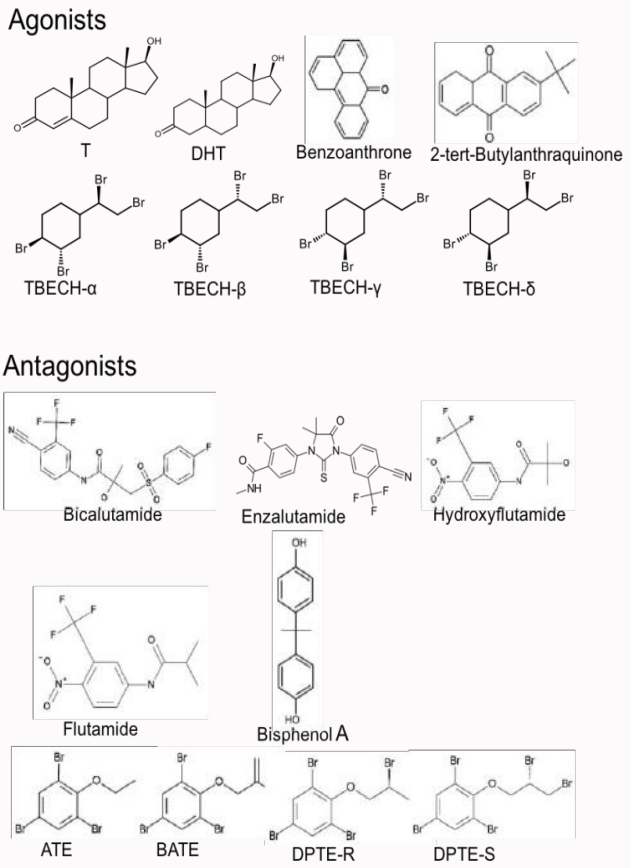

Figure 2: Molecular structures of androgens and EDCs that interact with AR. Agonists: T, DHT, Benzoanthrne, 2-tert-Butylanthraquinone, TBECH isoforms α, β, γ and δ; and Antagonists: Bicalutamide, Enzalutamide, Hydroxyfutamide, Flutamide, Bisphenol A, ATE, BATE, and DPTE-R and –S isoforms.

The first potent environmental AR agonist was 1,2-dibromo-4-(1,2 dibromoethyl) cyclohexane (TBECH/DBE-DBCH) [74], and TBECH (Figure 2) was also later shown to act as AR agonist for chicken and zebrafish AR using *in silico*, *in vitro*, and *in vivo* approaches [75,76]. 2-tert-Butylanthraquinone and Benzanthrone are fused polycyclic hydrocarbons and were identified as AR agonists from a screen of 253 industrial chemicals in a study using the AR EcoScreen assay [62]. However, these agonists displayed weak responses, being approximately 1000-10,000 fold less potent than DHT. TBECH is a Brominated Flame Retardant (BFR) used as an additive in electrical appliances, plastics, fabric adhesives, and in polystyrene and polyurethane [77]. It exist as four diastereomers (α, β, γ and δ) each with its own enantiomer [77]. Due to the potency of TBECH in activating AR at nanomolar concentrations, it has been ranked as one of the 10% most hazardous compounds to ecosystems [78]. TBECH has been identified in sediments, living organisms, and in indoor and outdoor air [79]. TBECH is commercially available as a mixture of α:β in the marketed flame retardant Saytex BCL 462 (Albemarle Corporation), but at temperature >120°C the α and β forms are converted to γ and δ [77]. Among the four diastereomers, γ and δ are more potent at activating AR across species [75,76,80]. TBECH has been shown to be maternally transferred and cause delayed hatching in zebrafish [76,81]. TBECH has also been shown to be mutagenic in the L5178Y tk+/tk- mouse lymphoma-cell forward-mutation assay [82].

While few androgen agonists have been identified, there are more reports on environmental and industrial compounds exhibiting anti-androgenic properties. The most common AR antagonists are agricultural products such as pesticides, herbicides, insecticides, and fungicides. AR antagonists have also been identified from products used in plastics and other industrial products.

PBDEs and their congeners, are brominated flame retardants used in commercial products such as electronic equipment and textiles [83]. The PBDE congeners DE-71, BDE-47 and BDE-100 have been identified as AR antagonists through *in vitro* and *in vivo* analyses. Exposure to these PBDEs resulted in decreased size of male accessory genital glands [84]. In a study on male Wistar rats, exposure to DE-71 resulted in delayed puberty as well as decreased size of the prostate and seminal vesicle [85].

BPA is a synthetic polymer widely used for manufacturing of polycarbonate plastics and epoxy resins [86]. While BPA primarily act as an ER agonist, recent studies have shown that it binds to AR and display AR antagonistic activity [51]. A study on rodents have shown that it reduces sperm count [87] and it has also been linked to erectile dysfunction in men having high levels of BPA in their urine [88]. Studies from rodent models and human PCa cell lines indicate that BPA is carcinogenic and stimulate tumors progression [89-91]. BPA activate the mutated AR_{T877A} frequently found in PCa patients who relapse following androgen ablation therapy [52]. This indicates

that further studies are needed to better understand the role of BPA in PCa progression.

Allyl 2, 4, 6-tribromophenyl ether (ATE/TBP-AE), 2-bromoallyl 2, 4, 6-tribromophenyl ether (BATE/TBP-BAE) and 2, 3-dibromopropyl-2, 4, 6-tribromophenyl ether (DPTE/TBP-DBPE) (Figure 2) are a novel group of BFRs that we recently identified as AR antagonists [92]. These compounds have been detected in the environment, house dust and in aquatic animals. Among these, only ATE and DPTE, that exist in two iso-forms DPTE-R and DPTE-S, were used as flame retardants, whereas BATE is a by-product of DPTE biotransformation and has never been used as a BFR [93]. ATE is the main constituent of the BFR PHE-65 (Great Lakes Chemical Corporation) and is currently still in use, while DPTE was the main constituent of the BFR Bromkal 73-5PE (Chemische Fabrik Kalk) until the mid 1980s [94,95]. These AR antagonists are equally potent at inhibiting AR transcriptional activity across different species, including human, chicken and zebrafish [92,96,97].

TBECH and Androgen Receptor Mutations

TBECH is present in the commercially available product Saytex BCL 462 as an equimolar mixture of TBECH α and β [77]. We have shown that all four TBECH diastereomers induce expression of prostate specific antigen (PSA) in human prostate carcinoma LNCaP cells harboring the AR_{T877A} mutation [80]. The TBECH diastereomers were more potent at transcriptionally activating the mutated AR_{W741C} and AR_{T877A} than the wild type AR [98]. TBECH γ and δ are much more potent than α and β at activating AR [80]. While TBECH γ and δ are as potent as DHT, only high concentrations of α:β and β (1 μM and 10 μM) are able to induce transcriptional activation of AR_{W741C}. The α:β and β iso-forms are also more potent at activating AR_{T877A} when compared to AR_{W741C} and AR_{WT}. Both α:β and γ:δ induced PSA expression to comparable levels in the human LNCaP cell line. Although currently no epidemiological studies have reported a link between the TBECH and PCa progression, the mechanistic results indicate that TBECH could interfere with PCa progression.

ATE/DPTE and Prostate Cancer Drugs, Comparison of Potency

Currently only ATE is in use as a BFR, as the production of DPTE ceased in the mid 1980s due to a fire incidence in the factory where it was manufactured [94,95]. The two BFRs were equally potent at inhibiting DHT-induced AR transcriptional activity in HeLa cells [92], while DPTE is more potent at inhibiting DHT-induced PSA expression in LNCaP cell lines when compared to ATE. Interestingly, when LNCaP cells were exposed to ATE alone there was a low level induction of PSA expression, indicating that ATE acts as a partial agonist to AR_{T877A} present in LNCaP cells. Co-exposure of HeLa cells to DHT with DPTE or either of the three PCa drugs bicalutamide, flutamide and hydroxyflutamide, resulted in comparable dose dependent inhibition of DHT-induced AR transcriptional activity and PSA expression. This indicates that DPTE is equally potent as the PCa drugs at inhibiting AR activity [92]. Exposure of LNCaP cells harboring the AR_{T877A} mutation to hydroxyflutamide resulted in induction of PSA expression. This showed that both ATE and hydroxyflutamide are partial agonists to LNCaP AR_{T877A}, suggesting that exposure of PCa patients harboring this mutation could lead to PCa progression.

Conclusion on Possible Involvement of EDC in Prostate Cancer Progression

TBECH, apart from activating AR, exhibit mutagenic activity, thereby suggesting that it may act as a carcinogen. In addition, as the AR antagonist ATE exhibit partial agonistic property towards AR_{T877A} it may also be an EDC capable of stimulating PCa growth and progression. In order to establish a direct link between EDCs and PCa, epidemiological determination of correlations between exposure and health are clearly needed.

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