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REVIEW

The androgen-thyroid hormone crosstalk in prostate cancer and the clinical implications

Sepehr Torabinejad[1], **Caterina Miro**[1], **Biagio Barone**[2], **Ciro Imbimbo**[2], **Felice Crocetto**[2,*] and **Monica Dentice**[1,3,*]

[1]Department of Clinical Medicine and Surgery, University of Naples 'Federico II', Naples, Italy
[2]Department of Neurosciences, Reproductive Sciences and Odontostomatology, University of Naples Federico II
[3]CEINGE – Biotecnologie Avanzate Scarl, Naples, Italy

Correspondence should be addressed to M Dentice: monica.dentice@unina.it

[*](F Crocetto and M Dentice contributed equally to this work)

Abstract

There is increasing evidence that thyroid hormones (THs) work in an integrative fashion with androgen receptors (ARs) to regulate gonadal differentiation and reproductive function. Studies reveal that THs have interactions with the AR promoter region and increase AR expression. THs also have a role in the regulation of enzymes involved in the biosynthesis of androgens, such as 5α-reductase, which is essential in the conversion of testosterone into its active form, 5α-dihydrotestosterone. Additionally, the presence of androgen response elements in the promoter regions of TH-related genes, such as deiodinases and TH receptor isoforms, has been identified in some vertebrates, indicating a mutual interaction between THs and ARs. Since the androgen signaling pathway, mediated by ARs, plays a key role in the formation and progression of prostate cancer (PCa), the existence of crosstalk between THs and ARs supports the epidemiologic and experimental evidence indicating a relationship between the high incidence of PCa and hyperthyroidism. This article aims to review the role of androgen-TH crosstalk in PCa and its implication in clinical management. As life expectancy is growing these days, it can increase the number of patients with PCa and the critical relevance of the disease. In order to gain better knowledge about PCa and to improve clinical management, it is essential to get better insight into the key factors related to the formation and progression of this cancer.

Key Words

- thyroid hormone
- androgens
- prostate cancer

Introduction

The prostate cancer

The prostate is a small gland with a key function in male fertility by adding essential secretions to semen and contributing to ejaculation and sperm viability (1). This organ develops from the urogenital sinus and consists of two main parts, the epithelium and the stroma surrounding the epithelium (2). To maintain normal development and homeostasis of the prostate, these two compartments should influence one another via some signaling pathways (3). The epithelium contains prostatic progenitors, stem cells, and also epithelial cells (4) which express androgen receptors (ARs) and the prostate-specific antigen (PSA) (5). In terms of the stroma, it consists of different cell types, including AR-positive smooth-muscle cells, fibroblasts, myofibroblasts, and immune cells (6).

Benign prostatic hyperplasia (BPH) and prostate cancer (PCa) are two prostate-related health problems

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that men can undergo during their lifetime. Today, BPH is a common public health issue around the world. About 50% of men worldwide will suffer from symptoms related to BPH later in their lives. In aging men, in spite of a decrease in the testosterone level, some other systemic and local biological changes, such as increases in the AR number and chronic inflammation, a decrease in prostatic cells apoptosis, and prostatic tissue remodeling, result in a rise in the number of the cells and volume of the prostate (BPH) (7). Despite the fact that BPH is an important prostatic complication, it is not as threatening as PCa for a man's body wellness.

In PCa, the prostatic cells give rise to tumors, mostly in aging men (1). Age, genetics, family history, race, diet, smoking, and obesity are the main risk factors for PCa (8). This cancer was the second most common and the fifth fatal one among men in the year 2020 (9). Sixty percent of patients diagnosed with PCa are over 65 years old with an average of 66 at the time of diagnosis (10). Although most patients do not die from PCa, it can be a cause of death when it spreads to other parts of the body, such as the lymph nodes, bladder, bone, rectum, brain, and spinal cord (10). While prostate adenocarcinoma derives from the epithelial compartment of the prostate gland (4), stromal fibroblasts induce survival signals, transform epithelial cells to a cancerous stage, and contribute to the therapeutic resistance of the cancer cells (5).

PCa arises from molecular changes in the prostatic cells (1), including DNA repairing system deficiency (11), gene amplification and epigenetic alterations of *AR* (12), loss of one copy of the tumor suppressor PTEN that leads to the escalation of PI3K–AKT–mTOR signaling pathway, mutations in the *TP53* and cyclin-dependent kinase inhibitor 1B (*CDKN1B*) genes, promoter hypermethylation (in retinoblastoma (*RB1*), *PTEN* and *cadherin 1* genes), and also the fusion of the androgen-induced transmembrane gene serine 2 protease gene (*TMPRSS2*) with members of the erythroblast transformation-specific related gene (ERG) family of transcription factors (11). Based on the biological alterations in PCa patients, there are some markers (PSA, kallikrein 2 (hK2), prostate cancer antigen 3 (PCA3), and some methods (digital rectal exam, transrectal ultrasound, color Doppler ultrasound, combination of MRI with TRUS-guided biopsy) to screen PCa (10).

Among all the cell signals, the androgen signaling pathway plays a key role in the formation and progression of PCa (1). Based on this fact, the standard treatment for advanced prostatic cancer, which is sensitive to androgens, is androgen deprivation therapy (ADT) leading to apoptosis and cell death; however, a considerable fraction of the cells remain viable. The surviving cells transmit androgen-sensitive PCa to castration-resistant prostate cancer (CRPC), a type of PCa that can continue progression after ADT (13). Nowadays, although multiple drugs for the treatment of patients with CRPC are available and prolong the patients' survival, owing to primary and secondary resistance to these therapies (14), the median survival is only around 42 months (15).

In addition to the role of the androgen signaling pathway and other mentioned factors affecting the incidence and progression of PCa, it has been shown that thyroid hormones (THs) have distinct effects on PCa cells (16). One piece of evidence for the impact of THs on the prostate is that the PSA gene (*KLK3*) is not only sensitive to androgens but also to THs (17, 18). Although population-based studies suggest that subclinical and clinical hyperthyroidism increase the risk of solid malignancies, there are still controversies on the effects of hypo- and hyperthyroidism in PCa, partially due to the highly spatiotemporally dependent effects of THs (19). Moreover, there are some studies that show crosstalk between androgens and THs in the normal and cancerous prostate (20, 21).

The current paper aims at reviewing the studies on the effects of androgens, THs, and the crosstalk between them on PCa, as well as their implications. To this aim, we describe the role of androgens and their receptors (ARs) in PCa, as well as the controversial results regarding the relationship between hypo- and hyperthyroidism and PCa and evidence indicating a positive association between hyperthyroidism and PCa. Additionally, we speculate about the putative mechanism of interaction between THs and ARs in normal cells. Finally, the implications of this interaction in clinical approaches are reviewed.

Androgens and prostate cancer

Testosterone and its dynamic metabolite, dihydrotestosterone (DHT), are the main androgens (male sex hormones) in the circulation of male adults (22). Leydig cells synthesize androgens, such as testosterone, in the testis under the pulsatile control of pituitary luteinizing hormone (LH), produced by the anterior pituitary gland. Of note, LH is itself regulated by the hypothalamic gonadotropin-releasing hormone (23). After secretion by the endocrine glands, androgens are transported to the target tissues via blood and their lipophilic character enables them to dissociate spontaneously from the carriers and enter the target cells by passive diffusion and also by specific transporters (24,

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25). Inside the cell, testosterone is metabolized into its more biologically active form, 5α-dihydrotestosterone (DHT), by 5α-reductase, and to estradiol by aromatase. Both testosterone and DHT bind to ARs expressed by prostate cells and dissociate them from heat shock proteins. Testosterone levels change during life. In a male fetus, testosterone levels are high, while they decrease post-natally. During puberty and pre-puberty, testosterone production increases again and stimulates prostate growth. Since then, prostate size is constant, due to a balance between proliferation and cell death.

The structure and function of the prostate depend on the action of androgens. The proliferation and differentiation of epithelial and stromal cells, as well as the metabolic and secretory functions of the prostate, are all regulated by androgens and their receptor response. Therefore, dysregulation and malfunction of these molecules can result in prostate disorders. A deficit of androgen causes the loss of epithelial cells through apoptosis, and the administration of androgens reverses the process and regains prostate function (26). On the contrary, increases in androgen concentration in the blood serum rise the risk of PCa. Nevertheless, there is a saturation level (240 ng/dL), where despite the rise of testosterone level in the blood serum, the risk of PCa remains constant (26). The reason is the fact that AR numbers determine the intensity of AR signaling; hence, as all receptors are bound to androgen, excess androgens are useless and cannot increase the risk of cancer. According to this theory, named saturation theory, patients who suffer from hypogonadism as well as those patients who have been relieved from PCa can receive androgen replacement therapy without having a concern about cancer risk enhancement/cancer recurrence (26). Of note, exogenous administration of androgen for these patients does not increase intra-prostatic androgens but results in elevation of its serum level (27), which has a saturation level above the one that can raise the cancer risk. As indicated previously, the gold standard therapy for most of PCa patients is ADT. Surgical castration implies testes removal, while in medical castration, the production of the gonadotropin-releasing hormone is inhibited. The response to the treatment is favorable, but not persistent, and after 2–3 years, treatment resistance, CRPC, begins. Several mechanisms are involved in CRPC: (i) *AR* gene amplification and post-translational modification raise the AR production/stability, consequently increasing the androgen response by ARs. (ii) AR point mutations change the ligand-binding domain (LBD) of AR and result in broadening ligand specificity, leading to AR activation by alternative non-androgen ligands, such as estrogen, progesterone, and even some androgen antagonists. (iii) AR splice variants lose their C-terminal LBD region and remain constitutively activated independently of androgen or antagonist action. (iv) Intra-tumoral androgen biosynthesis also neutralizes androgen reduction induced by ADT. (v) Due to plasticity, prostate epithelial cells convert into neuroendocrine cells and induce signals without AR requirement. (vi) Tumor microenvironment and signaling alteration of hormones, growth factors, kinases, cytokines, and enzymes are also involved in CRPC (14). In patients with CRPC, resistance to androgen antagonists can also be due to the conversion of these factors in AR agonists, thus supporting PCa instead of suppressing it. In this case, anti-androgen withdrawal results in better clinical conditions (28). Therefore, challenging efforts have been made, in recent years, to invent more effective drugs.

Thyroid hormones and prostate cancer

THs control a variety of biological events, such as metabolism, cellular proliferation, differentiation, and apoptosis. The action of THs is mainly regulated by their internalization via specific membrane transporters, including the monocarboxylate transporters MCT8 and MCT10 that transport THs into the cells (29, 30) where through the binding to nuclear receptors, act as transcription factors. Apart from this genomic action, THs function is also regulated by their interaction with receptors on the cell membrane, as in the case of the αvβ3 integrin, or with intracellular molecules to activate intracellular cascade pathways (31, 32). The iodothyronine deiodinases D1, D2, and D3, expressed, respectively, by the *DIO1*, *DIO2*, and *DIO3* genes, are involved in the activation/inactivation of THs. D1 and D2 catalyze the conversion of the thyroxine (T4) into the tri-iodothyronine (T3), with more affinity than T4 for nuclear receptors, whereas, D1 and D3 inactivate both T4 and T3 (33, 34).

Notably, THs are involved in the growth, invasion, and metastasis of cancer (35, 36). Therefore, many studies have investigated the role of THs in PCa, but the results are controversial. In a number of studies, it has been observed that hypothyroidism prevents PCa (37, 38, 39), whereas others indicate that there is no relationship between hypothyroidism and PCa (40, 41). The role of high levels of T3 on PCa is still contradictory; however, the majority of the studies show that high levels of T4 are associated with enhanced PCa progression (38, 42, 43).

Trying to address the molecular basis of the clinically observed THs-dependent effects on PCa, our group recently

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studied the role of the intracellular metabolism of THs mediated by the deiodinases D2 and D3 in the formation and progression of BPH and PCa. We demonstrated that the expression of the *DIO2* gene, the TH receptor TRα1, and transporter MCT8 in PCa cells were increased in comparison with normal prostate cells. We also showed that the treatment of an androgen-sensitive PCa cell line (LNCaP) with THs resulted in proliferation (up-regulation of Cyclin D1) and epithelial-to-mesenchymal transition (a switch from E-Cadherin to N-Cadherin expression), while the same effect was not observed in the AR-negative PC3 cells. Moreover, tumors inoculated in mice receiving exogenous THs grew faster than tumors inoculated in control mice. Additionally, by assessment of different metabolic enzymes' expression, it was disclosed that THs sustain aerobic-glycolysis in LNCaP cells (21). Consistently, among several normal and PCa cell lines, T3 could raise the proliferation of only LNCaP cells (21, 44) in a dose-dependent manner and stop the action at a saturation concentration of 10^{-9} M (44). In another study, it was demonstrated that also T4 has a positive effect on LNCaP cell proliferation; however, in spite of the proliferation induced by T3, it was blocked by integrin-binding inhibitor peptide; indicating that the effect of T4, not T3, is mediated through binding with integrin αvβ3 (42). Finally, in two human studies, both free T4 and T3 were associated with increased PCa incidence (38, 45). It was also shown that in men with BPH, the level of T3 was more elevated than in men with PCa, and in the latter, the T3 level was more than in healthy men (46).

Furthermore, another study demonstrated a T4 inhibitory effect on detachment-induced apoptosis and also a T4 stimulatory effect on PCa cell migration (43). However, these effects were observed only in an anoikis-resistant PCa cell line (PC3), and not in DU145 cell line. Tetraiodothyroacetic acid, a competitive antagonist of T4 at integrin αvβ3, suppressed T4 actions in PC3 cell line by blocking MAPK/ERK pathway, which lowered the expression of *XIAP* and *MMP-2* genes involved in cell migration and invasion, as well as the expression of *VEGF* gene implicated in angiogenesis (43).

Interestingly, in our recent study, we observed that THs also influence the tumor stroma by acting on BPH-derived fibroblasts (BPFs). This observation implies that THs influence both the tumor and its microenvironment. Treatment of BPFs with THs led to a rise in Cyclin D1 protein level and phospho-ERK/ERK ratio, indicating an enhanced proliferation rate. Furthermore, significant increases in mRNA expression of typical mesenchymal markers including N-Cadherin and Vimentin, inflammatory cytokine (interleukin-6), COL3A1 collagen, platelet-derived growth factor, as well as key metabolic enzymes involved in aerobic-glycolysis were observed in THs-treated BPFs. Besides, we observed that THs activated NF-κB inflammatory signaling in BPFs (21).

Crosstalk between thyroid hormones and androgens in prostate cancer

The family of nuclear receptors

Nuclear receptors are typically defined as ligand-regulated transcription factors able to directly modulate the expression of target genes involved in the control of different biological processes, such as cell proliferation, development, metabolism, and reproduction (47). Their ligands are small lipophilic compounds that can cross the plasma membrane (freely for some ligands or mediated by specific transport mechanisms for the others) and bind to the nuclear receptors inside the cell. In detail, nuclear receptors are activated by steroid hormones, such as estrogen, progesterone, and various other lipid-soluble compounds, including retinoic acid, oxysterols, and THs. Based on their ligand, they can be classified into three different categories. The most extensively characterized class is that of steroid- and thyroid-hormone receptors (TRs) (48). The second class is defined as the orphan receptors that function in a ligand-independent manner, and lastly, the third class of nuclear receptors is represented by the so-called 'adopted' orphan receptors, initially fell into the second category, until specific ligands were identified, highlighting their specific physiological function (49). Nuclear receptors share high sequence identity and a well-conserved functional domain organization, comprising the A/B domain that includes the highly variable amino (N)-terminal domain (NTD) and the first of two transactivation domains (AF-1). The C region is a central DNA-binding domain (DBD) that consists of two zinc finger binding motifs; the D domain is responsible for nuclear localization and is also known as the hinge region. Next, there is a well-conserved carboxy (C)-terminal LBD and a second transactivation domain (AF-2) (47, 48) (Fig. 1).

Another important characteristic of these receptors is that they can exist as monomers, homodimers, or heterodimers and recognize DNA sequences termed hormone response elements (HREs) consisting of 6-meric repeats with the consensus RGGTCA (R is a purine). Based on their mode of action, they can be subdivided into four

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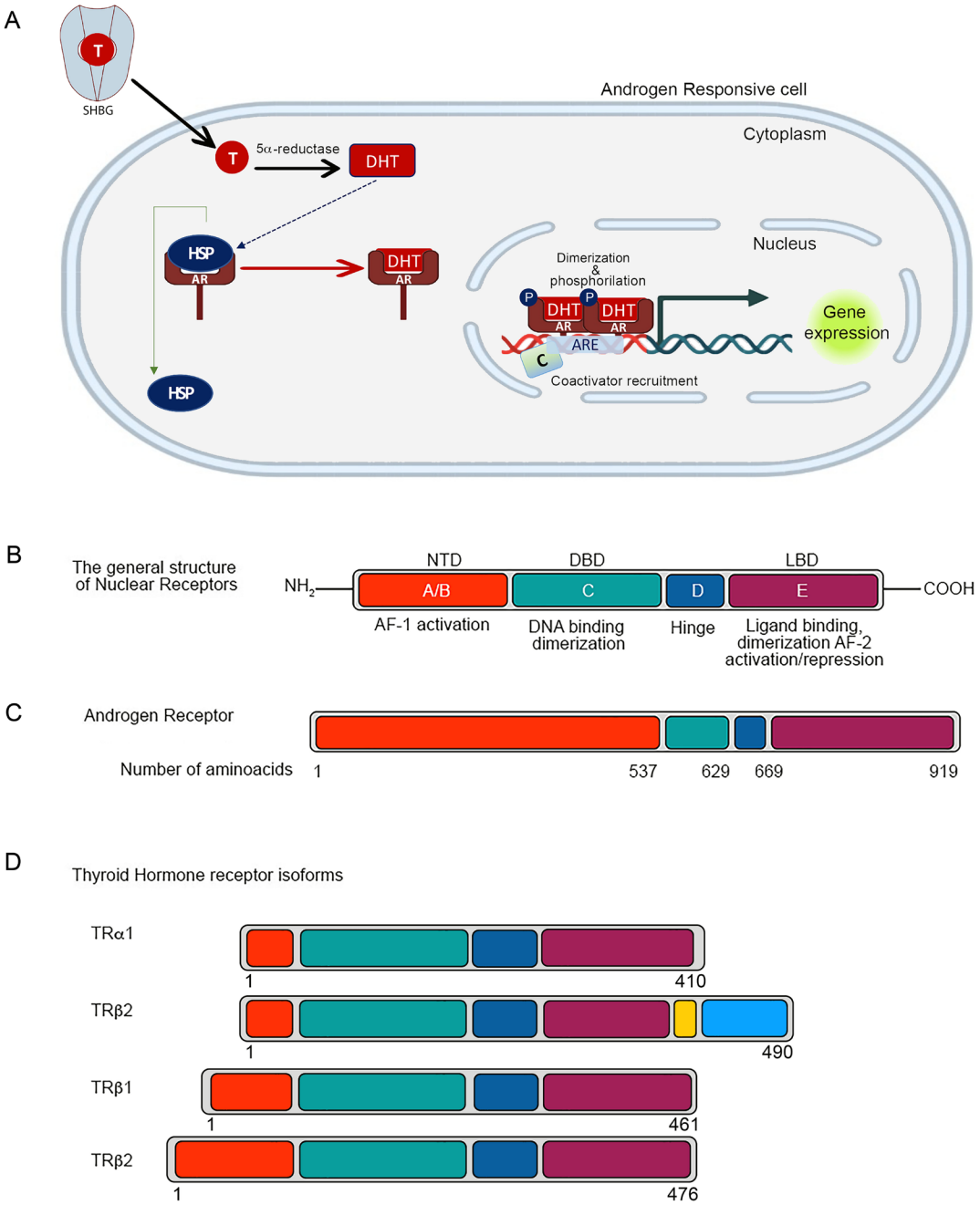

Figure 1

Mechanism of action of androgen receptor and structure of androgen receptor (AR) and thyroid receptor. (A) AR action. After dissociation from the carrier (SHBG) and entering the cell, free testosterone is metabolized into 5α-dihydrotestosterone (DHT) by the enzymatic action of 5α-reductase. DHT binds to the AR and dissociates it from heat-shock protein (HSP). The androgen/AR complex translocates to the nucleus, dimerizes, and binds to the promoter of the target genes (ARE). Then, the complex recruits coactivators and other transcription factors (coregulator proteins) to modulate gene transcription. (B) Nuclear receptors comprise four sections, including the A/B region which consists of the N-terminal domain (NTD) and the first transactivation domain (AF-1); the C region or the DNA-binding domain (DBD); the D domain known as the hinge region, which promotes DBD binding to DNA; and the E region including C-terminal ligand-binding domain (LBD) and the second transactivation domain (AF-2). (C) The structure of the AR containing the regions enumerated for nuclear receptors as a whole. (D) The structure of four thyroid hormone isoforms. TRβ1 and TRβ2, the most known TR isoforms, differ in the NTD, but TRα1 and TRα2 vary in the LBD. TRα2 with longer LBD is unable to bind to the thyroid hormones. The digits indicate the number of beginning and ending human amino acids in each section. AF-1 activation domain is indicated in red, the DBD is indicated in green; the hinge domain is indicated in dark blue; the LBD is indicated in violet and TRα2 carboxy termini are indicated in yellow and light blue. AR, androgen receptor; DBD, DNA-binding domain, DHT, dihydrotestosterone; HSP, heat-shock protein; LBD, ligand-binding domain; NTD: N-Terminal Domain; SHBG, sex hormone binding globulin.

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different sub-categories: (i) Type I receptors, comprising the AR, the estrogen receptor (ER), and the progesterone receptor (PR), are anchored in the cytoplasm by chaperone proteins (e.g. HSP90) (50). Once activated by the ligand binding, they make up a homodimer, exhibit the nuclear localization sequence and translocate into the nucleus, in which they promote the expression of specific target genes (51). (ii) Type II receptors, such as the TR and the retinoic acid receptor, already reside in the nucleus and, even in the absence of ligands, bind to their specific DNA response elements. They generally form heterodimers with the retinoid X receptor and, in the absence of ligand, interact with co-repressor complexes (NCoR and SMRT) that, in turn, are associated with histone deacetylases (52, 53, 54). The ligand binding promotes the co-repressors' dissociation and their replacement with co-activator complexes, such as histone acetyltransferases that help open chromatin and lead to the activation of target genes. (iii) Type III receptors function like the type I receptors save for the HRE that is a direct repeat rather than inverted, while (iv) type IV receptors instead bind as monomers to HREs (47). In each case, the activation of nuclear receptors implicates a complex multistep program that starts with the ligand binding and culminates in the modulation of the transcriptional machinery to produce tissue-specific responses.

The androgen receptor

The action of androgens is mediated by the AR, which is a ligand-dependent transcription factor belonging to the steroid hormone nuclear receptors family, comprising also the ER, glucocorticoid receptor, PR, and mineralocorticoid receptor (47, 55).

AR gene is located on the X chromosome and expressed in different tissues, in which it covers important biological roles in the development and maintenance of the reproductive, musculoskeletal, cardiovascular, immune, neural, and hemopoietic systems (56). Given its wide expression in many tissues, it is not surprising that AR signaling may also be involved in the development of tumors in the prostate, bladder, liver, kidney, and lung.

Like the other members of the nuclear receptor family, AR has three functional domains, including the NTD, the DBD, and the C-terminal LBD (Fig. 1). DBD recognizes the AR-regulated genes and tethers the AR to their promoter and enhancer regions. The NTD is constitutively active, but the activation of the LBD is ligand-dependent (57). Normally, in the absence of androgen, AR is localized to the cytoplasm and it shuttles between the nucleus and the cytoplasm itself in an inactive state (58). Upon ligand binding, the androgen/AR complex translocates to the nucleus where it dimerizes and binds to the androgen response elements (AREs) in the promoter regions of the target genes involved in the development, cell growth, and cell cycle regulation and metabolism (59). The receptor complex can then recruit co-regulator proteins (co-activators or co-repressors), which modify the chromatin structure in order to modulate gene transcription (60).

The thyroid hormone receptors

The principal physiological action of THs is determined by the binding of T3 to the nuclear receptors, TRs. Indeed, T3 has a higher affinity for the nuclear receptors than T4, and its main mechanism of action is mediated by genomic regulation of target genes transcription, via nuclear TRs binding. The T3–TR complex recognizes specific DNA-binding regions (thyroid response elements, TREs) in the promoter of their target genes, enabling the recruitment of regulatory complexes which modify histones leading to the transactivation of TH target genes (61). TRs are produced by two different genes, *THRA* on chromosome 17 and *THRB* on chromosome 3, that encode TRα and TRβ, respectively (62). The two kinds of TRs share a highly homologous protein structure, similar to the general structure of the nuclear receptors, with a short amino acid sequence within the DBD domain that defines the target sequence specificity and mediates TR binding to TRE regions (Fig. 1). As a consequence of the alternative splicing, various TR isoforms exist: the most studied isoforms are TRα1, TRα2, and TRβ1, which diverge in the NTD; whereas, TRα1 and TRα2 differ in the carboxy (C)-terminal LBD. Differences in the C-terminal region in length and aminoacidic sequence in the two TRα isoforms, render TRα2 unable to bind to TH (T3), and capable of acting as an endogenous inhibitor of T3 action (61).

In the last few years, apart from the well-known genomic action of THs, the focus has also been pointed to the emerging roles of THs in the cytoplasm. Indeed, in addition to the nuclear function of TRs, extranuclear or nongenomic actions of THs have been widely reported in a variety of cells. Non-genomic actions of TH do not require the formation of a nuclear complex between T3 and TRs, thus exhibiting rapid effects, mediated by different interactions between TH or TR with cytoplasmatic proteins (31, 63).

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The androgen-thyroid hormone crosstalk

Since both TRs and ARs are located within the target cells and undergo dimerization upon the binding with their ligands, different research groups suggested that the THs and the androgen-dependent endocrine axis might work in an integrative fashion, hypothesizing the existence of direct crosstalk between TRs and ARs (Fig. 2). However, the molecular basis of this crosstalk is still largely unexplored and has not been extensively discussed. Several authors have proposed that TH signaling coordinates and responds to feedback from other hormone pathways rather than functioning in parallel with them. Moreover, other studies have conferred TH a pivotal role in the hormonal network, defining it as a key regulator of other hormones (e.g. estrogen, testosterone, growth hormones, etc.) that together cooperate to induce developmental changes in anurans (64).

One of the first pieces of evidence of THs-ARs interaction was identified in male reproductive

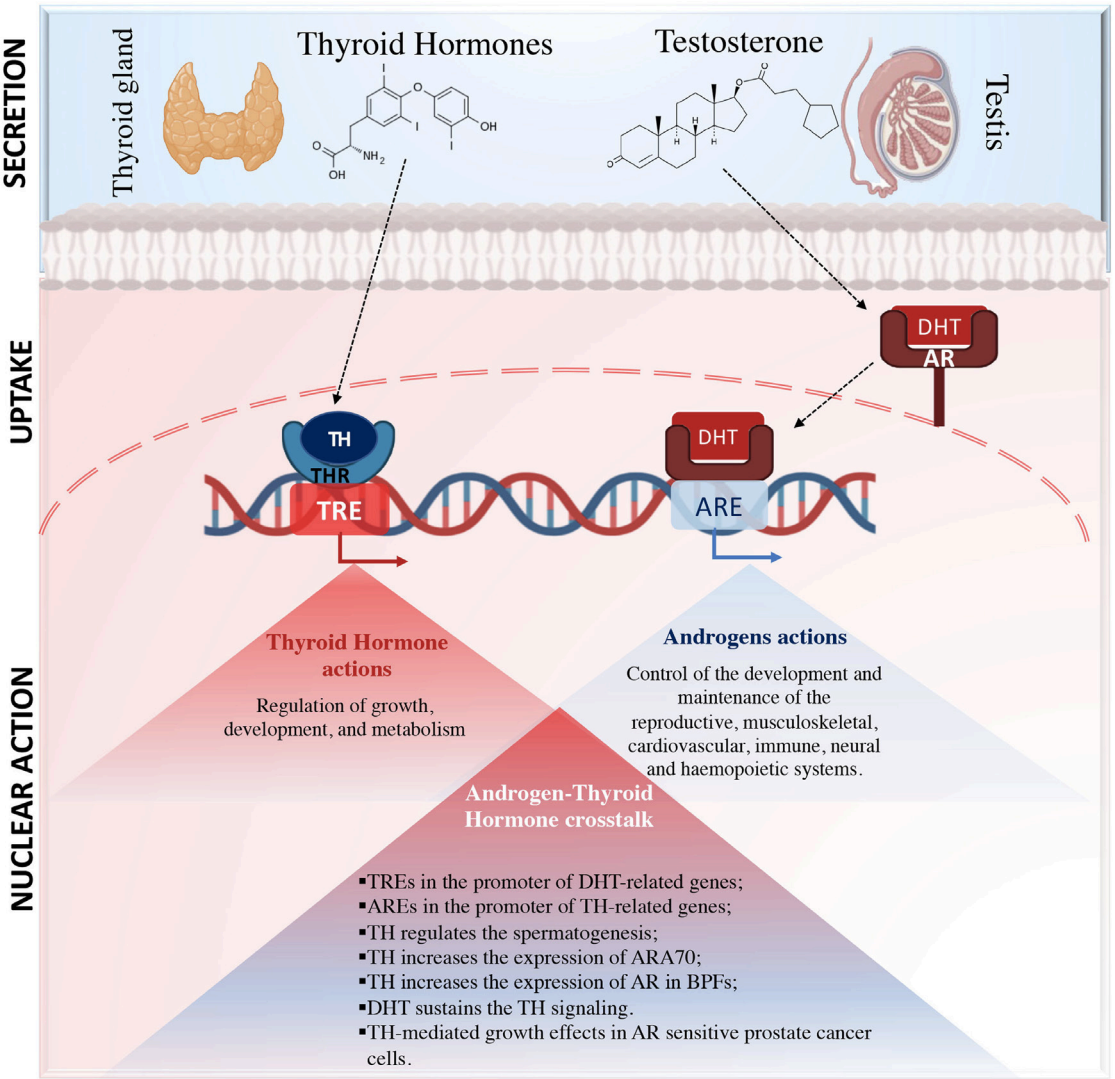

Figure 2
The androgen-thyroid hormone (TH) crosstalk. (Top) Graphical representation of TH and 5α-dihydrotestosterone (DHT) secretion. TH is produced through the hypothalamic–pituitary–thyroid (HPT) axis and exerts its function mainly through the regulation of gene expression by binding the nuclear receptors (TRs). The TH–TR complex recognizes specific DNA-binding regions (TREs, thyroid response elements) in the promoter of target genes. Leydig cells synthesize androgens, such as testosterone, in the testis; DHT binds to ARs and the DHT–AR complex translocates into the nucleus and binds the promoter region of AR-target genes to induce gene transcription. (Bottom) Nuclear actions of TH–TRs and DHT–AR complex and the combined effects of TH and DHT. AR, androgen receptor; ARE, androgen response element; DHT, 5α-dihydrotestosterone; TH, thyroid hormone; TR, thyroid hormone receptor; TRE, thyroid hormone response element.

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organs, during the postnatal Sertoli cell proliferation and differentiation (65). In this study, the authors demonstrated that T3 treatment increased the mRNA levels of AR in cultured Sertoli cells derived from 5-day-old rats, defining T3 as an important regulator of AR expression in these cells (65). Moreover, previous work reported that the follicle-stimulating hormone (FSH) is an important tropic hormone for Sertoli cells, capable to increase the expression of AR in this cellular population (66). Since both T3 and FSH have stimulatory effects on Sertoli cells proliferation and differentiation, the authors have studied and next identified also additive combined effects between the two endocrine pathways on AR mRNA in neonatal and juvenile Sertoli cells, with a major effect compared to each hormone alone (65). Further studies demonstrated that T3 treatment increases the AR expression in different vertebrate testes *Silurana tropicalis* (67), Tungara frog, *Physalaemus pustulosus* (68), Italian wall lizard, *Podarcis sicula* (69), *Mus musculus* (70), *Rattus norvegicus* (65). Also, Sertoli and Leydig cells, responsible for androgen biosynthesis and spermatogenesis in vertebrates, are co-regulated by AR and THs (71).

Thus, considering the presence of TH machinery in testicular tissues, the TH axis exerts an important regulatory role in testicular functioning and sperm production. Additional studies on the combined role of these hormones will be necessary to understand the mechanism by which T3 treatment increases AR mRNA levels regulating the overall developmental sequence of male reproductive organs.

In accordance, other studies have revealed that THs interact with the AR promoter region and affect the androgen responsiveness by increasing AR expression. Indeed, in silico analysis allowed researchers to identify the presence of TREs in the promoter regions of AR and in several androgen-related genes (*M. musculus*, *S. tropicalis*, and *Oryzias latipes*) (72). Hence, the TH–TR complexes directly bind to the response elements within androgen-related genes promoting their expression in vertebrate species. Therefore, THs can also regulate enzymes involved in androgen biosynthesis, such as 5α- reductases, essential to convert testosterone into its active form 5α dihydrotestosterone (5α-DHT) (73). By using the same approach, the authors have identified the presence of AREs in the promoter regions of TH-related genes, such as deiodinase and TR isoforms in *M. musculus*, *S. tropicalis*, and *O. latipes*, whereas the AREs are absent in the *Dio3* promoter region of *M. musculus* and *O. latipes*, and in that of *Dio2* in *S. tropicalis* (72).

Interestingly, a study by Pei-Ju Tai and colleagues provided other molecular evidence of an interesting linkage between T3 and AR-associated protein 70 (ARA70). ARA70 is the first ligand-dependent coactivator of AR, able to interact with AR and promote its activity (74). In this study, the authors demonstrated that T3 treatment increases the expression of ARA70 by 4- to 5-fold in HepG2 cells, acting through the binding of T3 to a TRE site in the ARA70 promoter region. Contrarily, ARA70 negatively regulates T3 signaling in a TRE-dependent manner. Aksoy O. and coauthors investigated the crosstalk among μ-Crystallin (CRYM), T3 and AR signaling in PCa (75). CRYM is a strong antagonist of TH signaling and binds T3 with a high affinity, buffering the elevated amount of T3 freely available in the cytosol and so the expression of T3-responsive genes (76). In this study, the authors revealed that CRYM is inversely correlated to PCa progression, indeed the levels of CRYM are lower in PCa compared to normal prostate tissue and further reduced in metastatic disease. Moreover, they demonstrated that lower levels of CRYM are also associated with poor prognosis in men with PCa, defining CRYM as a negative prognostic factor of PCa. In the same study, the authors have explained that the growth-promoting and invasive effect of T3 on PCa cells can be attenuated by the intracellular action of CRYM that counteract the T3 action, decreasing the intracellular availability of T3 (75, 77). Moreover, the RNA-Seq analysis in AR-expressing PCa cells demonstrated that the over-expression of CRYM induces an important down-regulation of the TH/androgen-regulated gene network, comprising PSA. Therefore, this is another important evidence of the possible interaction of THs and androgen signaling pathways in PCa (75) (Fig. 3). In agreement with the previously cited works, as also described earlier, our group demonstrated that only the AR-positive cells were sensitive to THs-induced proliferation, while the same effect was absent in the AR-negative PC3 cells and similarly, lost in the AR-positive cells after AR silencing (21) (Fig. 2).

The clinical point of view

Albeit the role of THs and their crosstalk with androgens *in vivo* is understudied and controversial, different pieces of evidence are shining a light on the complex interaction between THs and AR. The influence of THs on the prostate has indeed been recognized over the last 50 years, but the clinical information has remained unlinked the common practice. Regarding BPH, if no relationship has been initially found between thyroid status and risk

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of developing BPH (78), hyperthyroidism apparently increases the risk of BPH, reporting an incidence of 18.51% compared to 15.53% of controls, with a hazard ratio (HR) of 1.24 for hyperthyroid patients. Nevertheless, when these data were adjusted for other covariates such as age and clinical conditions such as diabetes, this relationship failed to remain significant (79). A positive correlation between prostate volume and elevated levels of free T3–T4 was similarly found in a study by Lee and colleagues (80); moreover, TSH seems to be negatively correlated with prostate size, improving the scores related to the lower urinary tract symptoms, which were even more evident when BPH patients were treated with dutasteride, an inhibitor of the local conversion of testosterone to DHT (81). A surrogate data were similarly found by Senel and colleagues who reported, in a small study involving 50 patients, how decreased TSH and increased serum T3 and T4 were associated with increased PSA levels (82). As a result, the complex crosstalk between the thyroid and prostate, despite still obscure and fragmentary, should be further analyzed in a multidisciplinary manner, in order to integrate those data into the clinical practice of BPH treatment and management.

Similarly to the benign counterpart, the role of THs in PCa, and in particular in the initiation and progression of the malignancy has been poorly studied *in vivo*, albeit some hints have been proposed regarding the effects of THs and hormone therapy on cardiovascular safety in PCa patients (83). As stated earlier, there is evidence that subclinical or clinical hypothyroidism does not modify the risk of PCa while hyperthyroidism or reduced levels of TSH could increase this risk. Theodossiou and Schwarzenberger observed that Propylthiouracil, a compound inducing hypothyroidism, slowed down the growth of transplanted PCa cells in the mice (37). Consistently, a higher TSH level was associated with a lower risk of PCa, with a 30% lower risk for every 1 IU/L increase in TSH (38). Besides, results of a randomized controlled trial demonstrated that a lower PCa incidence is associated with hypothyroid status in smoker men (39). However, in a recent meta-analysis study, investigators evaluated the site-specific cancer risks associated with hyperthyroidism or hypothyroidism. They found that PCa was among the cancers positively affected by hyperthyroidism, but not affected by hypothyroidism (40). In a large prospective study involving 29,691 subjects with no previous thyroid diseases for a mean follow-up of 9 years, low TSH levels were associated with increased PCa risk, reporting an HR of 1.97 which increased to 2.60 after the exclusion of the first 2 years of follow-up (41). Analogous data were reported by a large Israeli study involving 375,635 patients with no prior history of cancer. A total of 23,808 cases of cancer were detected over a median follow-up of 10 years. Interestingly, among patients who developed PCa (2406 over 169,554 males), elevated TSH levels were associated with decreased risk

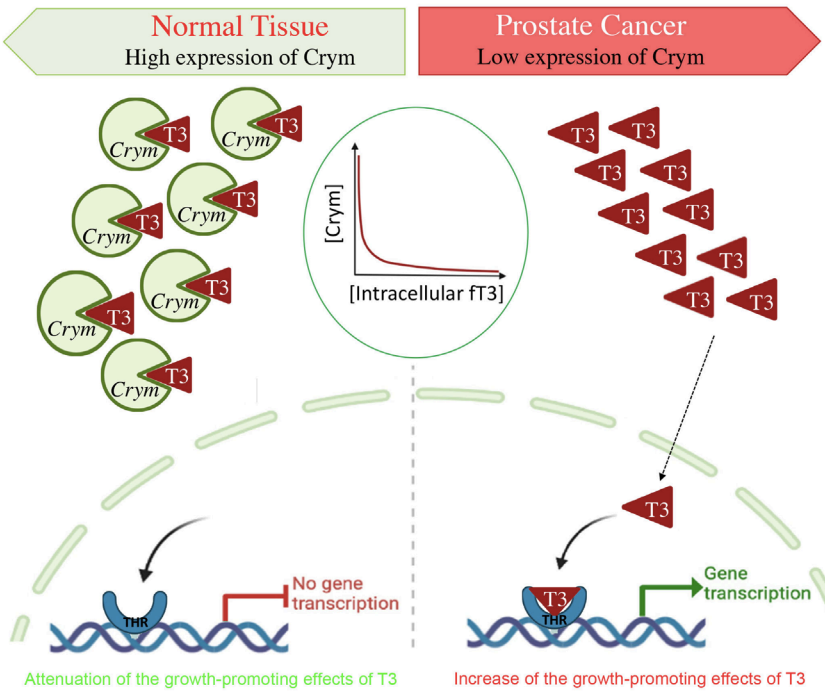

Figure 3
CRYM is inversely correlated with cytoplasmatic free T3 levels. The cytoplasmatic thyroid hormone binding protein μ-Crystallin (CRYM) binds T3 with high affinity. (Left) In normal tissues, the overexpression of T3-buffering CRYM leads to attenuating the growth-promoting effect of T3 by blocking its binding to thyroid hormone nuclear receptors (THR). (Right) In prostate cancer (PCa), the reduction of CRYM levels causes an enhanced cytoplasmatic availability of T3 resulting in an increase in the growth-promoting effect of T3 mediated by the binding to THR. CRYM, thyroid hormone binding protein μ-Crystallin; T3, tri-iodothyronine; THR, thyroid hormone receptor.

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of PCa, yielding an HR of 0.67 (84). Tran and colleagues found similar data, reporting a significant association between hyperthyroidism and PCa (HR = 1.35) (40) and Mondul and colleagues reported a decreased risk of PCa in men with higher serum TSH compared to men with lower TSH, yielding an odds ratio (OR) = 0.70. Additionally, hypothyroid men had an even lower risk of PCa compared to euthyroid men (OR = 0.48) (39). The role of THs was, however, not limited to the risk of PCa. In an older study by Lehrer and colleagues, T3 levels and disease recurrence in men treated for localized PCa were assessed, reporting a correlating increasingly higher T3 levels for higher-risk groups (45). THs were also involved in PCa aggressiveness; higher T3 levels were associated with higher PCa grade groups and with higher tumor percentage involvement as well as pT stage on definitive pathology (85).

Despite all the reported evidence, the role of THs in prostate function and carcinogenesis has to be still evaluated. Additionally, the data related to the potential role of Propylthiouracil and other drugs used in thyroid diseases in PCa should be further investigated. Lastly, there is no current consensus on dedicated diagnostic screening in males affected by thyroid diseases, despite several epidemiological studies that have hypothesized a potentially important role of THs in BPH and PCa.

Conclusions

The ability of THs to foster PCa aggressiveness, as described with some heterogeneity by the majority of the cited works, supports the existence of a thyroid–prostate axis. In particular, the enhancement of THs nuclear availability induced by the D2-mediated TH intracellular activation in PCa suggests that, at least in the late stages of tumorigenesis, hyperthyroidism increases the cancer progression of the prostate gland. However, there are many outstanding questions that remain to be resolved. For example, which specific steps of prostate tumorigenesis are influenced by THs? Do THs promote the hyperproliferation of fibroblasts *in vivo*, thus favoring the BPH? Does the AR physically bind to TRs in the promoter region of TH- and AR-target genes?

To address these issues, it will be important to understand the fingerprint downstream of the TR and AR genomics. Moreover, a fundamental question will be whether there is a direct or indirect interaction between TR and AR. Finally, efforts should also be directed toward multicenter studies which could reveal additional information on this complex interaction between not-so-distant organs.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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