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REVIEW

The biological and clinical advances of androgen receptor function in age-related diseases and cancer

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Abstract. Hormonal alterations with aging contribute to the pathogenesis of several diseases. Androgens mediate their effects predominantly through binding to the androgen receptor (AR), a member of the ligand-dependent nuclear receptor superfamily. By androgen treatment, AR is recruited to specific genomic *loci* dependent on tissue specific pioneer factors to regulate target gene expression. Recent studies have revealed the epigenetic modulation by AR-associated histone modifiers and the roles of non-coding RNAs in AR signaling. Androgens are male sex hormone to induce differentiation of the male reproductive system required for the establishment of adult sexual function. As shown by several reports using AR knockout mouse models, androgens also have anabolic functions in several tissues such as bone, muscle and central nervous systems. Notably, AR has a central role in prostate cancer progression. Prostate cancer is the most frequently diagnosed cancer in men. Androgen-deprivation therapy for cancer patients and decline of serum androgen with aging promote several diseases associated with aging and quality of life of older men such as osteoporosis, sarcopenia and dementia. Thus, androgen replacement therapy for treating late onset hypogonadism (LOH) or new epigenetic regulators have the potential to overcome the symptoms caused by the low androgen, although adverse effects for cardiovascular diseases have been reported. Given the increasing longevity and consequent rise of age-related diseases and prostate cancer patients, a more understanding of the AR actions in male health remains a high research priority.

Key words: Androgen receptor, Sarcopenia, Prostate cancer, Osteoporosis, Dementia

Introduction

As global aging and life expectancy increases, strategies to reduce age-related diseases receive increasing attention [1]. Hormonal alterations contribute to the pathogenesis of several conditions and might cause a significant reduction in the sense of well-being. Androgens mediate their effects predominantly through binding to the androgen receptor (AR), a member of the ligand-dependent nuclear receptor superfamily [2, 3]. Two natural steroids, testosterone and dihydrotestosterone (DHT), bind and activate AR to regulate target gene expression [4]. Androgens are male sex hormone in principle inducing differentiation of the male reproductive system and external genitalia during fetal life. They are also required for the establishment of adult sexual function by promoting sexual characteristic during puberty. In addition to the classical activities in the male reproductive system, androgens also have anabolic functions in other tissues such as bone, muscle and central nervous systems [5, 6]. Notably, AR has a central role in prostate cancer progression. Prostate cancer is the most frequently diagnosed cancer in men and the number of patient increases with aging [4]. A number of recent studies have demonstrated that androgen actions are related with development of age-related diseases including osteoporosis, sarcopenia and prostate cancer.

In this review, I summarized the functions of androgens and AR associated with age-related diseases and prostate cancer progression. In particular, I focused on recent advances in the research field of molecular mechanisms of AR in nucleus and pathophysiological roles of AR obtained from various AR knockout (KO) models. The possible impacts of clinical and epidemiological studies were also discussed.

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The androgen receptor (AR) functions as a nuclear receptor to regulate its target genes

AR has an important role in androgen signaling in various tissues such as prostate, bone and muscle by functioning as a nuclear receptor [5, 6]. Nuclear receptors including AR have multiple domains called DNA binding domain (DBD), a ligand-binding domain (LBD), and an N-terminal domain (NTD) (Fig. 1A). AR mRNA can be alternatively spliced to AR-Vs (AR-V7 is a representative isoform) in human tissues and results in prematurely termination of the full AR protein [7]. In the NTD, the transcriptional activation function 1 (AF1) domain promotes transcriptional activation with or without ligand binding, which is associated with enhanced AR function. In the LBD, the binding of androgen hormone to AR is promoted [8]. AF2 domain in the LBD interacts with co-regulators with LXXLL motif [9-11]. In the absence of hormone, AR forms a complex in the cytoplasm with the heat shock protein (Hsp) family that functions as molecular chaperones. After binding to androgen, a conformational change of AR-Hsp complex is induced and then AR can translocate to the nucleus. In the nucleus, AR recognizes and binds to the genomic regions including sequence motifs called androgen response elements (AREs) as a dimer. Most of AR binding sites (ARBSs) have been identified in the promoter/enhancer regions of target genes [4, 12] (Fig. 1B).

Functional ARBSs were not only determined by sequence motifs but also chromatin accessibility. Chromatin-opening transcription factors forkhead box protein A1 (FOXA1) is able to directly bind to the chromatin to open up the local nucleosomal domain. In prostate cells, FOXA1 protein has been shown to physically interact with AR protein and play critical roles in regulating the transcription of prostate genes [13]. Following recent mappings of genome-wide ARBSs, the mechanisms underlying AR recruitment to genomic *loci* have also become increasingly investigated. ChIP-seq analyses of AR-binding sites in prostate, kidney and epididymis showed that *in vivo* AR cistromes and their respective androgen-dependent transcription programs are highly tissue specific biological pathways. Importantly, this tissue specificity is achieved by the use of different collaborating factors in the three androgen-responsive tissues. Thus, two novel collaborating factors for AR, hepatocyte nuclear factor 4α (HNF4α) in mouse kidney and activating enhancer binding protein 2α (AP2α) in mouse epididymis, were found in this study [14]. Taken together, tissue specific function of AR might be correlated with tissue specific pioneer factor expression (Fig. 2).

Epigenetic regulatory roles of AR by interacting with coregulators and non-coding RNAs

For regulating AR transcriptional function, recent large-scale studies have demonstrated that several factors regulating epigenetic conditions and non-coding RNAs are important [4, 15]. DNA, histones and other

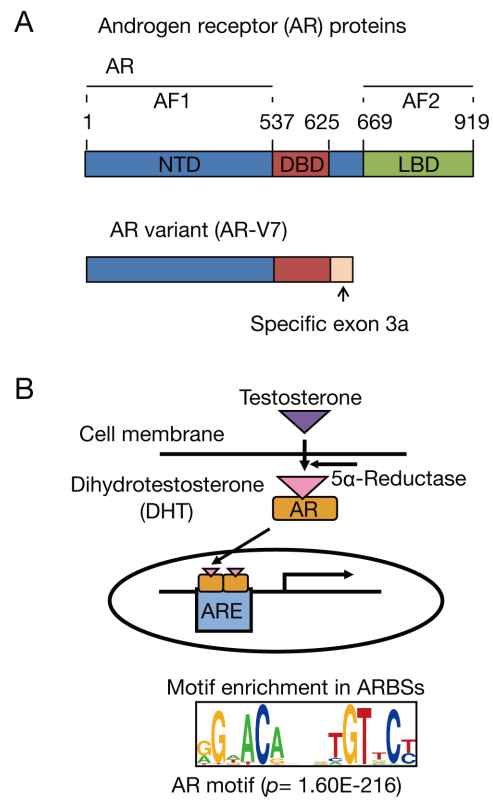

Fig. 1 Molecular mechanism of androgen receptor (AR)
(**A**) The molecular structure of the AR protein showing the domain regions. AR full length and AR-variant, AR-V7, are shown. NTD, N-terminal domain; DBD, DNA binding domain; LBD, ligand binding domain; AF1, activation function 1; AF2, activation function 2. (**B**) The schematic summary of AR as a nuclear receptor. Testosterone is a major androgen produced in testis. Testosterone is converted to dihydrotestosterone by 5α-reductase and binds to AR. AR translocate to the nucleus for binding to the specific genomic regions. AR binding motif, called androgen response element (ARE), is most significantly enriched in the AR binding sites obtained by AR ChIP-seq.

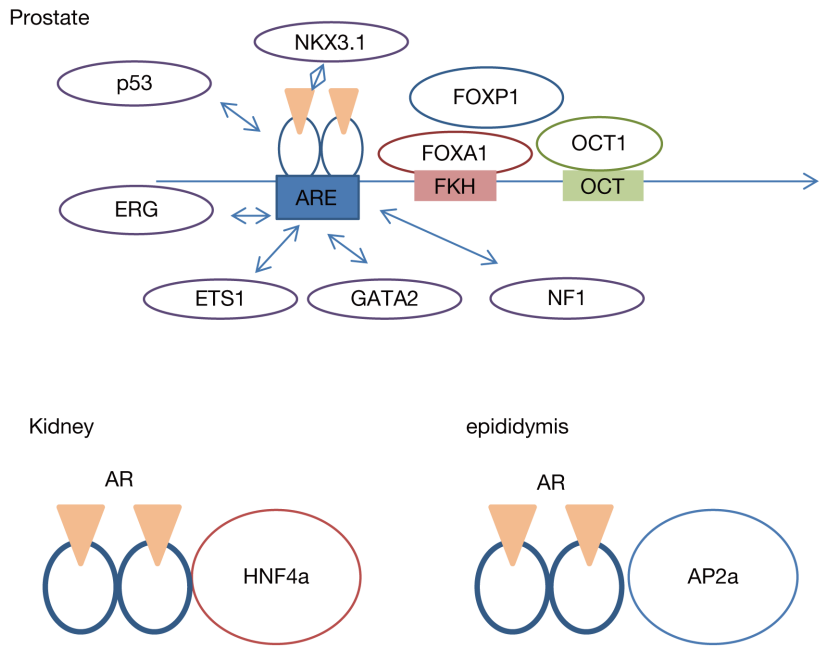

Fig. 2 AR interacts with various tissue specific transcription factors to bind to the specific genomic *loci*

In and around the AR binding peaks identified by ChIP-seq, several transcriptional factor binding motifs were enriched tissue specifically [13, 14]. In prostate, forkhead box protein A1 (FOXA1) or octamer transcription factor 1 (OCT1), nuclear factor 1 (NF1) and GATA transcription factor 2 (GATA2) are significantly enriched [33]. In addition, several transcription factors interact with AR for transactivation. In other tissues, kidney and epididymis specific binding partners have been reported. These tissue specific transcription factors are responsible for opening chromatin to recruit AR ligand-dependently.

proteins formed chromatin as a highly ordered structure. Chromatin forms a unit called the nucleosome consisting of a histone octamer (H2A, H2B, H3 and H4, two pairs of each) and DNA. A tightly wrapped DNA around the histones is packaged in the nucleus of all eukaryotic cells. Histone modifications have a role in conformational changes of the chromatin and affect the interaction of DNA with transcription factors or other proteins binding to DNA to control gene regulation. Lysine, arginine, serine and threonine residues enriched in N-terminal histone tails serve as substrates for post-translational modifications such as acetylation, phosphotylation, methylation, ubiquitination, sumoylation and deamination. This process is called epigenetic control [16]. AR regulates the histone modifications in ARBSs and promotes enhancer activity by directly interacting with many co-regulators including steroid receptor coactivators (SRCs) or other histone histone-modifying enzymes [10, 12, 17].

Methylations of H3K4 (mono-, di- or tri-methylation) indicate the active promoter or enhancer regions [18] and are promoted by the SET1/MLL histone methyltransferase (HMTase) complex. MLL complex plays an important role for androgen-mediated gene induction and its activity is regulated finely. After androgen stimulation, protein kinase C-related kinase 1 (PRK1) promotes histone H3 threonine 11 phosphorylation (H3T11P) [19]. WD repeat containing protein 5 (WDR5), a subunit of the SET1/MLL complex, associates with H3T11P and then promotes the recruitment of the MLL complex for H3K4 tri-methylation (H3K4me3) in ARBSs [20]. PRK1 kinase activity facilitates demethylation of H3K9 by cooperating with lysine-specific demethylase 1 (LSD1) [19, 21]. Furthermore, Protein kinase C beta 1 (PKCβ1) phosphorylates histone H3T6 prevents lysine specific demethylase including LSD1 from histone H3K4 demethylation [22]. Thus, these findings have a clinical relevance to develop new drugs for treatment by regulating epigenetic status [23] (Fig. 3A).

In addition to histone modification, DNA methylation is also the representative epigenetic mark adding a methyl group to the 5' position of cytosine (5-mC). DNA methylation is added or removed in a spatially and temporally defined context throughout the genome including enhancer/promoter regions. DNA methyltransferases

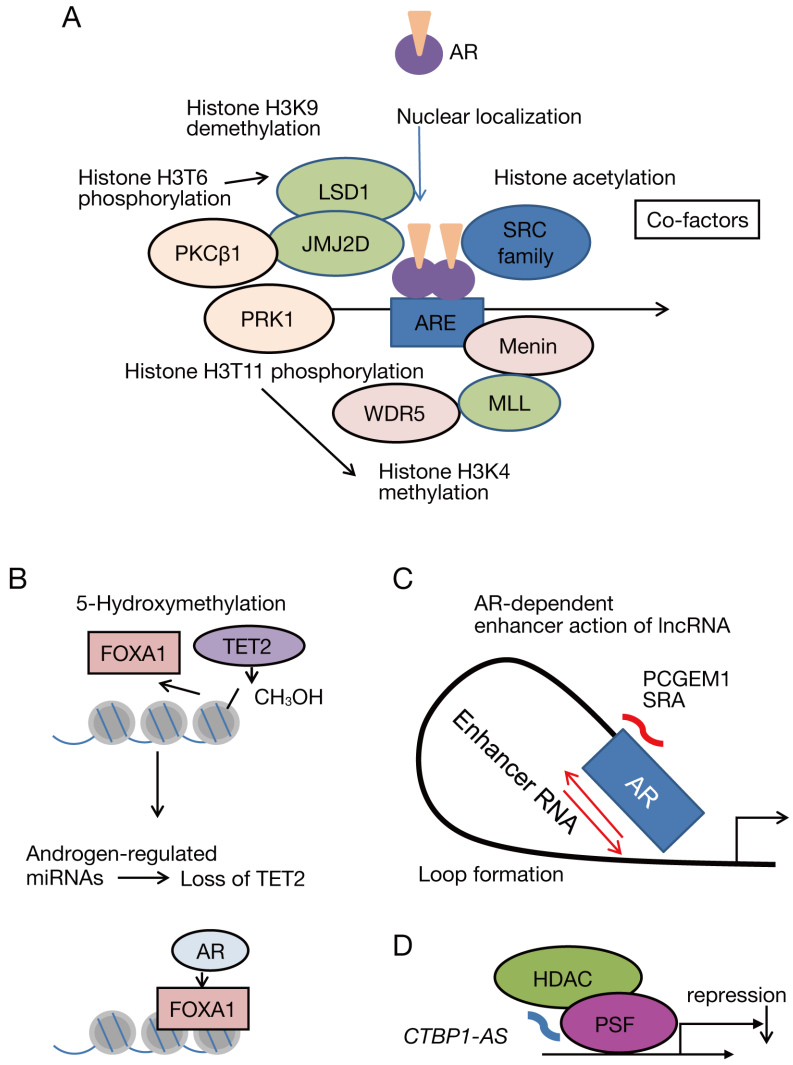

Fig. 3 Epigenetic regulation of AR binding sites (ARBSs) and the role of ncRNAs in AR action

(**A**) Upon androgen treatment, several histone modifying enzymes were recruited to AR binding sites. PKCβ1-mediated histone H3T6 phosphorylation directs LSD1 for not H3K4 but H3K9 demethylation by cooperating with JMJ2D. H3T11 phosphorylation also accelerates WDR5-mediated MLL recruitments and LSD1 activity. MLL complex interacts with AR through menin and promotes histone H3K4 methylation to enhance AR dependent gene expression. SRC family or ARA70 are AR interacting cofactors for histone acetylation [18-23]. (**B**) Androgen-induced miRNA mediated TET2 repression inhibits 5-hmC modifications in FOXA1 occupied enhancer regions. By removal of 5-hmC, FOXA1 is activated and induce FOXA1 or AR-regulated genes [25]. (**C**) The role of enhancer RNA (eRNA) or other AR interacting lncRNA. These lncRNAs (*PCGEM1*, *PRNCR1* or *SRA*) promotes loop formation for promoter/enhancer interaction and epigenetic controls [15]. (**D**) Epigenetic regulation by androgen-regulated lncRNA, *CTBP1-AS*, which interacts with RNA-binding protein, PSF. This complex binds to and deacetylates specific gene regions in prostate cancer [29].

(DNMTs) contribute to the process as enzymes. DNMTs include DNMT3A/DNMT3B for de novo and DNMT1 for maintenance of methylation. The ten-eleven translocation (TET) family proteins catalyze the production of 5-hydroxymethylcytosine (5-hmC), an oxidation product of 5-mC. Several studies have demonstrated that 5-hmC is not only an intermediate product of a demethylation process, but can also function as a stable epigenetic mark. Recently several studies have shown that the production of TET-mediated 5-hmC regulates the activity of these elements and 5-hmC modifications can be highly enriched at poised and active enhancers [24].

Non-coding RNAs (ncRNAs) are RNA transcripts that do not code for proteins [15]. They can be divided into two major groups: small ncRNAs between 18 and 200 nucleotides (nt) in length, and long non-coding RNAs (lncRNAs), which are larger than 200 nt. MicroRNAs (miRNAs) are evolutionally conserved single-stranded small non-protein coding transcripts of approximately 18-22 nt that post-transcriptionally regulate gene expression. Several studies have shown the importance of ncRNAs as modulators of key cellular processes in diseases such as cancer, metabolic diseases, and age-related diseases as well as in normal physiology. Generally miRNAs binds to the 3' untranslated region (UTR) of mRNAs to inhibit their translation. For example, dysregulation of miRNA expression profiles during the progression of prostate cancer have been discussed [15]. In these studies, miR-21, miR-29a/b, miR-32, miR-99a, miR-148a, miR-125b and miR-141 were found to be androgen-regulated miRNAs and dysregulated in prostate cancer.

Importantly, I have demonstrated that miR-29 family and miR-22 are highly induced by androgen in hormone-therapy resistant prostate cancer [25]. In prostate cancer clinical samples the expression level of miR-29a/b is negatively associated with that of its target gene, TET2. Importantly, *in situ* hybridization (ISH) study of clinical samples indicated that miR-29a/b is highly expressed in a subset of prostate cancers with poor prognoses. Mechanistically, TET2 repression by miR-29 family decreased 5-hmC levels, which is correlated with FOXA1 transcriptional activity. FOXA1 activation induced expressions of prostate cancer related genes. My experimental and clinical data suggested a novel oncogenic role of miR-29 family in prostate cancer progression. Thus, the role of TET2 and 5-hmC modifications in prostate cancer deserves additional analysis and may define a subset of metastatic disease (Fig. 3B).

Unlike miRNAs, lncRNAs are able to fold into secondary and tertiary structures by which they perform their function. Direct regulation of AR epigenetic function by lncRNA is strikingly receiving attention among all. *Prostate cancer gene expression marker 1 (PCGEM1)* was originally found as an androgen-regulated and prostate tissue-specific lncRNA [26]. *Prostate cancer noncoding RNA 1 (PRNCR1)* was identified by investigating the surrounding region of SNPs (single nucleotide polymorphisms) correlated with prostate cancer susceptibility. Importantly, both *PCGEM1* and *PRNCR1* cooperatively function for AR-mediated gene regulation [27]. The associations of *PCGEM1* and *PRNCR1* with AR were shown to be important in the mechanism of AR activation (Fig. 3C). By modulating interactions of AR proteins with several enzymes, these two lncRNAs were shown to be responsible for AR-associated loop formation between enhancer and promoter [27]. Another lncRNA, *steroid receptor RNA activator (SRA)* modulates the functions of various nuclear receptors. *SRA* associates with a coactivator SRC-1 (steroid receptor coactivator) and six stem-loop motifs in SRA are required for co-activation. Interestingly, overexpression of *SRA* was found in various tumors [28].

Genome-wide androgen-regulated transcriptome analysis identified a new androgen-responsive lncRNA, *CTBP1-AS* [29]. C-terminal binding protein 1 (CTBP1) functions as a transcriptional repressor for AR and negatively regulates AR downstream signals. It was demonstrated that *CTBP1-AS* is induced by AR-binding to its promoter region. In addition, *CTBP1-AS* associates with a RNA binding protein, PSF (PTB-associated splicing factor) to transcriptionally repress its target genes *via* histone deacetylation [15, 29]. Thus, androgen-regulated lncRNAs mediates AR function by modulating epigenetic status and gene expression (Fig. 3D).

Prostate aging and cancer progression is prominent in aged men depending on AR function

Androgens play a key role in the development of the male genital tract favoring differentiation and proliferation of stromal and epithelial cells of the prostate gland. In spite of the decline in testosterone with aging, development of prostatic diseases such as benign prostatic hyperplasia (BPH) or prostate cancer in aged men is promoted by androgen signaling [30, 31]. Androgens induce proliferation of prostate epithelial cells or tumor growth of prostate cancer [32]. Prostate cancer is one of the leading causes of cancer morbidity and mortality. The measurement of serum prostate-specific antigen (PSA), a representative AR-target gene, is a biomarker for diagnosing prostate cancer. Thus, AR and its downstream signaling are fundamental for the development and progression of both localized and advanced metastatic prostate cancer. Hormone therapy is a first-line and useful strategy for treating advanced prostate cancer. Blocking AR

activity by castration or using antagonists of AR elicits a favorable response.

However, some tumors will become hormone refractory following androgen deprivation therapy (ADT), featured by increasing PSA levels in blood and upregulation of the AR in cancer cells. Over a period of time of 12–36 months, a disease state called castration-resistant prostate cancer (CRPC) evolves in almost every patient [33]. The ineffectiveness of conventional ADT in these CRPC is a result of androgen-independent activation of the AR and its downstream pathways [34]. Abiraterone acetate, a potent inhibitor of CYP17 reduces testosterone synthesis from cholesterol [35]. Clinical studies showed that abiraterone improved overall survival, progression free survival, delayed initiation of chemotherapy and doubled the time to first skeletal event. Enzalutamide (formerly MDV3100) is another novel endocrine treatment with reported significant anti-tumor activity [36]. It is an AR-receptor-signaling inhibitor, blocking nuclear translocation, DNA binding, and co-activator recruitment. Enzalutamide significantly prolonged the survival of men with metastatic CRPC after chemotherapy [37]. Interestingly, AR-V7 (Fig. 1A) was discovered to be the most frequently expressed in HRPC/CRPCs among AR variants. Although AR-V7 is missing LBD, it retains the NTD to drive transcription and promotes resistance of tumors to existing therapies directed to androgen/AR [7, 38]. Therefore, identification of AR downstream signals and new molecular mechanisms for AR activation are important to improve the treatment of CRPC.

Recently a group demonstrated the potential for bromodomain inhibitors of Bromodomain and Extra-Terminal motif (BET) proteins as a novel epigenetic approach to treat CRPC [39]. BET bromodomain inhibitor, JQ1, was shown to induce apoptosis in prostate cancer cell lines. BET subfamily of human bromodomain (BRD) proteins with a focus on BRD4 were shown to play a major role in AR signaling and interact with AR via bromodomain. JQ1 inhibits this BRD4-AR bond, resulting in removal of RNA polymerase II from AR target genes, causing reduced AR gene transcription and subsequent diminished AR signaling [39]. This study suggests for the first time that modulating epigenetic function of AR could be a useful strategy to overcome clinical problems associated with AR signaling. Thus, epigenetic regulation could be the next-generation challenge to develop new drugs.

Androgen-deprivation therapy, late-onset hypogonadism (LOH) and age-related diseases

A system of endocrine regulating the homeostasis of male body and mind is complex. Although the consequence of ADT is both immediate and long lasting, it can influence on health and quality of life in patients with prostate cancer [40]. ADT induces severe adverse effects in cognitive function, bone, cardiovascular, and metabolic health. Sexual dysfunction and mental disorders such as depression are also reported to be the most significant adverse effects for patients [41]. By analyzing the symptoms induced by ADT in patients, it is possible to estimate the physiological effect of androgens on human health.

ADT treatment is associated with more metabolic diseases, atherosclerosis, coronary artery disease, and cardiovascular events [42-45]. However, treating hypogonadism in the aging male has resulted in discrepant results in regard to its effect on cardiovascular events. Some studies suggest that testosterone may have a future role in treating heart failure, angina, and myocardial ischemia [46]. In addition, several studies showed that testosterone exerted beneficial effects in brain function, including preventing neuronal cell death, balancing brain oxidative stress and antioxidant activity, improving synaptic plasticity and involving cognitive formation [47]. ADT has been found to impair memory, attention and executive functions [48]. Although previous studies showed that testosterone deficiency is positively correlated with cognitive impairment, several studies demonstrated contradictory findings [49]. Moreover, ADT reduces muscle mass and strength. Receiving ADT changes body composition in men, leading to a significant decrease in lean body mass and a significant increase in fat mass [41]. Exercise can mitigate such changes in physical functioning, fatigue and body composition to improve quality of life [41].

The prevalence of osteoporosis is high in prostate cancer patients treated with ADT [50]. Within the first year of ADT, absolute bone mineral density (BMD) loss is about 5%. The temporal relationship of ADT and incidence of osteoporosis is demonstrated over 4 and 10 years at 49.2% and 80.6%, respectively. However, dual-energy X-ray absorptiometry (DEXA) scans underestimate osteoporosis, as trabecular numbers also decline with the actual density of the bone. This underscores the importance of preventing the initial loss early. Decline

of BMD is a surrogate for fracture risk, which in turn is associated with increased risk of mortality. In a large retrospective study, ADT was shown to be associated with increased rate of fracture, and mortality risk doubled after a fracture [50, 51].

Furthermore, longitudinal studies have clearly documented the decline in testosterone with aging [1, 52]. Testosterone levels decrease steadily and continuously during aging, resulting in late-onset hypogonadism (LOH). The decline is correlated with alterations in body composition, diminished energy expenditure, diminished muscle strength, reduced sexual function, and depression [53, 54]. Vasomotor instability, reduced muscle strength/mass and decreased bone mineral density are associated with 'fraility' of aged men. The fall in testosterone levels with age is an important factor for these disease progressions. Declining testicular function and hypothalamic dysfunction would be the mechanisms explaining the fall in testosterone levels with age. The increased prevalence of obesity and chronic illness in ageing men are responsible for the large reduction of testosterone levels [55].

For frail older men with low testosterone levels, testosterone replacement therapy (TRT) may improve QOL and physical function [49]. However, TRT in aged men is often limited owing to the side effects including hyperstimulation of prostate. In contrast to other age-related diseases, prostate cancer, which is the most prevalent cancer and one of the leading causes of cancer death in men, could be promoted by androgen [4]. More importantly, a greater awareness of the potential risks associated with the treatment in aged men, particularly in regard to cardiovascular risk and mortality, have been shown [56, 57]. There is an urgent need for randomized clinical trials with sufficient size, duration and power to determine specific risks and benefits of TRT in older men. The physiology of normal aging is represented by complex symptoms of hypogonadism. Moreover, a highly prevalent burden of medical conditions and polypharmacy complicates the differentiation of signs and symptoms of hypogonadism from those of normal aging in older men [1].

Osteoporosis in aging men

As men are less likely than women to develop osteoporosis, male osteoporosis remains poorly understood. However, elderly men have a clearly reduced BMD and increased risk for fractures [58]. Both androgens and estrogens are associated with skeletal development and maintenance in males. Male osteoporosis differs from postmenopausal osteoporosis in that decreased bone formation is involved and that age-related changes in cortical bone structure and perforation of the trabeculae of cancellous bone are unlikely to occur [59, 60]. Studies of the effect of testosterone on bone metabolism in castrated mice indicated that testosterone increased tranbecular bone number, width, volume and BMD. In contrast, it reduced bone turnover in an AR-dependent manner.

Moreover, various ARKO mouse models have showed the importance of AR in bone metabolism [61, 62]. In the first report of ARKO mice, Yeh *et al.* observed lower cancellous bone volumes than male and female wild type mice. The osteopenia in ARKO mice was attributed to an enhanced bone resorption rate than bone formation. Male ARKO mice had higher osteoblast numbers, higher mineral deposition, and bone formation rates in the femoral metaphysis. In addition, ablation of AR suppresses new bone formation and decreased osteoblast activity required for bone differentiation [63, 64]. DHT stimulated the expression of *AKP2* gene, which is a tissue non-specific alkaline phosphatase (TNSALP). Androgen increased TNSALP activity and intracellular inorganic phosphate levels in osteoblast differentiation. Small integrain-binding ligand, N-linked glycoprotein (SIBLING) was also found to be AR target gene and decreased in calvarial cells from ARKO [64]. ARKO bone marrow stromal cells had higher numbers of colony formation unit-fibrobrasts (CFU-F), a heterogenous population of stem and progenitor cells than wild type cells [65]. Higher population of cells with CD44 (marker of mesenchymal stem cells) was included in CFU-F. Thus, AR is assumed to regulate cell proliferation of mesenchymal stem cells and osteogenic differentiation. Moreover, either inactivation of AR or loss of ERα or both resulted in reduced BMD in femoral bone and cortical width of tibia compared to wild type male mice, indicating both AR and ERα activities are important for maintaining bone mineralization in males [66, 67] (Fig. 4).

Osteoblast-specific exon 3-truncated AR mouse model by crossing Col2.3-Cre mice with their exon3-floxed AR mice was generated [68]. The Cre-mediated recombination resulted in truncation of AR exon 3 in mature osteoblasts and the resultant transgenic mice exhibited reduced trabecular bone volume and a reduction in connectivity

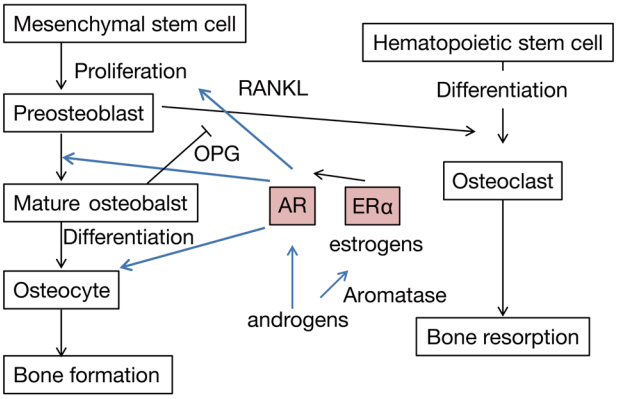

Fig. 4 The roles of AR in bone turnover to prevent osteoporosis
AR promotes cell proliferation of pre-osteoblast and differentiation of osteoblast to osteocyte to activate bone formation and increase BMD. Estrogen is also produced from androgen by aromatase. Experiments using mouse model revealed the importance of ERα in bone formation. The differentiation of osteoclast is not directly regulated by AR in male mice. OPG, osteoprotegerin; RANKL, receptor activator of NF-κB ligand.

density. Thus, AR genomic function in mature osteoblasts is involved in maintaining trabecular bone volume by reducing bone resorption [68, 69]. However, osteoclast specific ARKO mice displayed no differences in BMD, bone formation and bone resorption, suggesting the AR expressed in osteoclast is not functional to inhibit osteoclast activity [70]. Ostecyte specific ARKO displayed lower trabecular number and volume in femora and tibia at 32 weeks old, whereas no difference was observed in trabecular bone formation or cortical structure [71]. The femora of Osteocyte-ARKO were more sensitive to mechanical force-induced failure, suggesting the importance of AR in osteocyte to maintain integrity of trabecular bone (Fig. 4).

The downstream signals of AR in bone have been poorly understood. Sclerostin, a glycoprotein secreted by osteocytes, is known to inhibit bone formation. In human cultured osteocytes, stimulation with DHT decreased sclerostin expression in cultured osteocytes in a time- and dose-dependent manner [72]. Hypogonadal patients showed higher serum sclerostin levels compared with controls. Serum testosterone levels were negatively correlated with sclerostin. This study supports the emerging clinical significance of sclerostin as a therapeutic target for osteoporosis.

In the study using an osteoblastic model with enhanced androgen responsiveness, MC3T3-E1 cells stably transfected with AR under the control of the type I collagen promoter (colAR-MC3T3), genes related with MAP kinase-mediated signaling were observed to be repressed by androgen with the most dramatic effect on Elk1 (ETS Transcription Factor, Elk1) expression. DHT treatment inhibited MAP kinase activation such as phosphor-ERK1/2 levels, Elk1 protein and phosphor-Elk1 levels, and downstream AP1/luciferase reporter activity, suggesting the hypothesis that the MAP cascade may be a specific downstream target of AR in osteoblast [64].

The relationship between androgen and muscle mass and strength

Frailty, a functional status that precedes disability, is characterized by decreased functional reserve and increased vulnerability [73]. In addition to disability, the frailty phenotype predicts falls, institutionalization, hospitalization and mortality. Frailty is promoted by the interaction between the aging process and some chronic diseases and conditions that compromise functional systems. Many of the clinical manifestations of frailty are explained by sarcopenia, which is closely related to poor physical performance due to muscle atrophy and fatigue [73]. Clinical studies indicate that testosterone replacement in hypogonadal men and men with sarcopenia associated with chronic illness has the potential to increase skeletal muscle mass and strength [74]. However, other clinical studies of hormone replacement therapy in older men indicated that androgens do not stimulate muscle strength [75].

Perineal skeletal muscles, which are bulbocavernosus (BC) and levator ani (LA), collectively BC/LA, are highly androgen sensitive in rodents [76]. Postnatal gonadal testosterone production in males increases BC/LA muscle fiber number and muscle fiber hypertrophy at puberty. At adulthood BC/LA fiber size decreases markedly after castration and increases with androgen treatment, but fiber number remains unchanged [77-79]. It has been reported that androgen withdrawal by castration in mice reduces fast-twitch hind limb muscle mass and maximum force production, whereas testosterone administration prevents hind limb skeletal muscle atrophy and enhances fatigue resistance of soleus muscle in male orchidectomized mice [80].

As AR is expressed in various cell types of skeletal muscle including fibroblasts, satellite cells, and myofibers in mammals, all these cells are potentially

regulated by AR signaling. In comparing hind limb skeletal muscle from ARKO mice to wild type mice, the levels of myosin and troponin specific for slow-twich muscle fibers were decreased. In contrast, that of troponin T specific for fast-twich muscle fibers was increased in quadricep muscle of ARKO. Thus, AR might control the balance of muscle fiber type through upregulation of slow-twich fibers and downregulation of fast-twich fibers [77]. Another group observed muscle mass was decreased in ARKO, suggesting that AR signaling is important for growth of muscle mass. Contractile analyses indicated that fast-twich muscles from male mutants produced less force, whereas their slow-twich muscles had increased fatigue resistance [81]. In the analysis of a myocyte-specific ARKO (M-ARKO) mouse, it was found that myocytic AR transduces androgen-dependent postnatal fiber hypertrophy in perineal but not in limb skeletal muscles [82]. Elevation of slow-twich fibers and reduced fast-twich muscle fibers were also displayed in M-ARKO, suggeating the myocytic AR functions to generate maximum force of fast- and intermediary-twitch leg muscles by controlling myofibrillar organization of androgen-induced hypertrophic myofibers. However, whether muscle strength and fatigue were regulated by AR or not is still controversial [82, 83].

AR-regulated genes expressed in skeletal muscle were investigated using a mouse model lacking AR DNA binding activity by microarray technique. Biosynthesis pathway such as Odc1 (ornithine decarboxylase1) is differentially regulated by androgen. *In vitro*, myogenic regulatory factors (MRFs) such as Myf5, Myf6 and MyoD1 were identified to be AR-target genes. MRFs are important for muscle development [84]. Another group identified AR-binding sites in primary human muscle cells using ChIP-on-Chip (chromatin immunoprecipitation coupled with tiling microarray detection of genomic fragments) [85]. Sequence analysis of these regions indicated that approximately 90% possess a consensus ARE or half-site. Interestingly, they found that binding sequences for the Myocyte enhancer factor 2 (Mef2) family of transcription factors were enriched in the AR-bound regions, and that several Mef2c-dependent genes are direct targets of AR, suggesting a functional interaction between Mef2c and AR in skeletal muscle. MiR-221/22 and miR-133 were identified to be AR-regulated miRNAs in muscle. MyoM1, MyoT and MyoZ2 were also identified to be putative AR-direct target genes in muscle cells.

The role of AR in central nervous system function

Nowadays the number of elderly people is the most growing segment of the population and could increase dramatically over the next decade. Effective treatment of neurodegenerative disorders is crucial because the incidence of dementia increases rapidly with aging in both men and women [86]. Endogenous testosterone in the aging man has effects on many cognitive functions, especially verbal fluency, visuospatial and visuoperceptual abilities, memory, and executive function. Promising associations have been found between decline of cognitive function and low testosterone levels [87-89]. However, a wide range of results was obtained due to the lack of consensus on methods for testosterone measurement and supplementation [90, 91].

It has been proposed that androgens may exert neuroprotective effects in the brain mainly through neural stem cell stimulation, genomic activity modulation, and upregulation of androgen receptor levels. Several studies investigated the mechanistic role of androgen and found inhibition of pro-inflammatory factor production, such as tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6) that are involved in the pathogenesis of the amyloid plaques of Alzheimer disease [92] or activation of endothelial nitric oxise synthase (eNOS) to produce vascular nitric oxide (NO), resulting in anti-inflammatory impact by inducing the expression of Sirtuin 1 (SIRT1) [93]. However, the molecular mechanisms or downstream target genes responsible for these androgen effects are poorly unknown.

The *in vivo* AR roles in neural effects of androgens in males have been investigated by studying various ARKO mouse models. Neural-specific ARKO resulted in less vigorous aggressive behaviors in addition to reduced erectile activity and sexual motivation [94, 95]. Thus, central AR function in brain is involved in modulation of masculine behaviors. However, regulation of neuroendocrine functions by gonadotropic and somatotropic axes remains to be clarified. In addition, very little AR was expressed in the medial amygdala, bed of nucleus of stria terminalis (BNST), and the hypothalamic medial preoptic area, which are important in the regulation of sexual behaviors. These findings suggest that the role of AR in brain is only modulating the execution of these behaviors and not regulating organization and differentiation of neural circuits controlling masculine behaviors [96, 97].

Furthermore, the brain is an insulin target organ that plays a key role in regulation of energy balance and glucose homeostasis [98]. Higher levels of the AR are found in the hypothalamus of male than female mice and the AR was found to colonize with insulin receptor, suggesting that the brain AR might be associated with the regulation of insulin sensitivity. By generating neural-specific ARKO (N-ARKO), the investigators observed that administration of insulin in the fasting state reduced food intake in wild type, but not in ARKO, suggesting that the insulin resistance is regulated by AR [99]. In addition, glucose intolerance was increased in aged N-ARKO. Aged N-ARKO mice showed greater body weight with increased visceral fat, liver lipid deposition, and hepatic glucose production, suggesting that hypothalamic insulin signaling, which suppresses hepatic glucose production, is blocked by AR depletion. Moreover, the inhibition of hypothalamic role of AR in insulin signaling was found to be associated with reduced expression of Protein-Tyrosine Phosphatase 1B (PTP1B), which is suppressed by AR. These observations indicated the importance of AR in systematic insulin resistance and obesity in aging [99]. However, whether the brain AR is involved in cognitive disorders or depressive mood is remains to be further studied.

Conclusion

Over the recent past years, the massive utilization of biological techniques and functional genomics increased dramatically our knowledge on the regulatory networks of AR signaling. AR modulates its target gene expression or epigenetic status, leading to control of disease progression such as prostate cancer. AR associated histone modifiers such as MLL complex are found to be key molecules for activating AR-dependent gene regulation. In addition, ncRNAs including both miRNAs and lncRNAs regulates AR genomic function in the nucleus or functions as a downstream of AR signaling. These epigenetic modifiers could be promising targets to regulate AR activity. Physiologically, studies using AR knockout mouse models have indicated the importance of AR proteins in osteoblast cells to prevent osteoporosis or in muscle development or strength. Current clinical modalities with androgen supplements do not comply with evidence-based medicine. Additional large multi-centric clinical studies would probably give us a better understanding of its clinical utility in the management of ageing-related disorders.

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Conflict of interest

The author declares no conflict of interest.

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