Received: 30 July 2024 | Accepted: 19 September 2024

DOI: 10.1002/rmb2.12611

Reproductive Medicine and Biology

WILEY

REVIEW

Developmental and functional roles of androgen and interactive signals for external genitalia and erectile tissues

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Funding information
Japan Society for the Promotion of Science, Grant/Award Number: 21K06822 and 23KJ1859

Abstract

Background: Recent progress in molecular and signal analyses revealed essential functions of cellular signals including androgen and related growth factors such as Wnt regulators for external genitalia (ExG) development and its pathogenesis. Accumulated data showed their fundamental functions also for erectile tissue (corporal body) development and its abnormalities. The current review focuses on such signals from developmental and functional viewpoints.

Methods: Experimental strategies including histological and molecular signal analyses with conditional mutant mice for androgen and Wnt signals have been extensively utilized.

Main findings: Essential roles of androgen for the development of male-type ExG and urethral formation are shown. Wnt signals are associated with androgen for male-type ExG organogenesis. Androgen plays essential roles in the development of erectile tissue, the corporal body and it also regulates the duration time of erection. Wnt and other signals are essential for the regulation of mesenchymal cells of erectile tissue as shown by its conditional mutant mouse analyses. Stress signals, continuous erection, and the potential of lymphatic characteristics of the erectile vessels with sinusoids are also shown.

Conclusion: Reiterated involvement of androgen, Wnt, and other regulatory factors is stated for the development and pathogenesis of ExG and erectile tissues.

KEYWORDS
androgen, corpus cavernosum, ED, erection, external genitalia

1 | INTRODUCTION

External genitalia (ExG) is one of the central reproductive organs, and its erectile tissue, the corporal body, is the essential tissue for copulation. Key signal affording complex developmental and pathogenic processes of ExG is the male hormone known as androgen. Androgen (circulating testosterone and its converted dihydrotestosterone; DHT) functions during ExG development and its physiological regulation.[1–3] Androgen and its receptor (AR) exert multiple signaling functions for many downstream genes. As such, their associating signal cascades are

essential for regulating the ExG and corporal body formation. Recent progress in molecular and signal analyses revealed essential functions of cellular signals including Wnt and other regulators for such development. Accumulated data also show their critical functions for the corresponding pathogenesis. Of note is the significance of those signals revealed through developmental and functional investigations.

As for developmental processes, the primordium of ExG develops as a bud (genital tubercle; GT) in early-staged embryos around the cloacal region (E10.5 for mouse embryos, Figure 1A). Signals from cloacal membrane (CM) and associating mesenchyme play major roles in GT outgrowth (Figure 1B).[4–6] Such a signal includes an essential Wnt signal and its relayed signal cascades. In late embryogenesis and neonatal stages, part of the mesenchyme of ExG differentiates to erectile tissue composed of corpus cavernosum glandis (CCG), corpus cavernosum (CC), and corpus cavernosum urethra (CCU). In such processes, androgen and essential growth factor signals including Wnt are also integrated for their regulation.[7,8] Reproductive functions for erection are mediated by sinusoidal contraction/relaxation and androgen play a role through their interacting factors.

Due to the broad functions of androgen signals, the associating abnormalities result in significant developmental defects and pathogenesis.[9,10] As a result, a large number of patients are reported for birth defects of male-type urethral formation including hypospadias and erectile dysfunctions (ED).[4,5,8,11] For reproductive abnormalities, ED is one of the central medical topics.[12] ED is not only important as a reproductive disease but is also a predictive risk factor for cardiovascular diseases. Such male reproductive disorders are increasing in the current society. The current review also covers such related research trends.

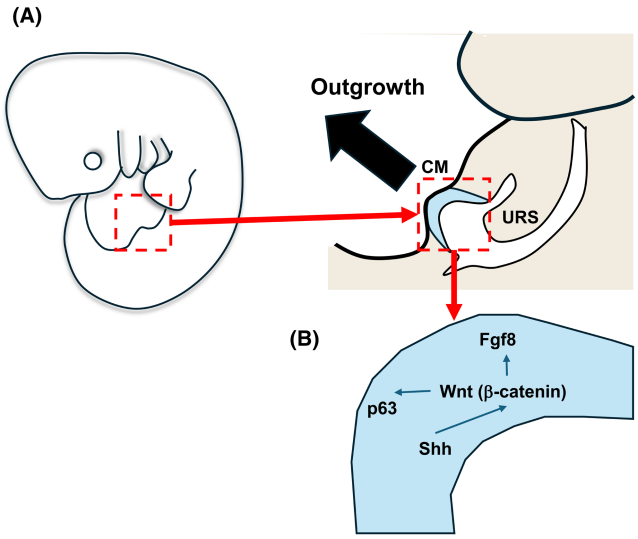

FIGURE 1 Early stage of genital tubercle (GT) development in mouse embryos. (A) The illustration shows the outgrowth of GT (shown by black arrow) from the region of the red dotted box. Magnified red dotted box region showing the cloacal membrane (CM), cloacal cavity, and urorectal septum (URS) by sagittal section of an early mouse embryo at E10.5. (B) Magnified CM region and signal cascades for GT outgrowth. Sonic hedgehog (Shh) expressed in CM located upstream of canonical Wnt/β-catenin signal which regulates Fibroblast growth factor 8 (Fgf8) expression in the distal urethral epithelium (DUE) of GT. p63 is essential for CM development and urethra formation locating potentially downstream of Wnt/β-catenin signal.

2 | ANDROGEN FUNCTIONS FOR THE MASCULINIZATION OF ExG AND URETHRAL DEVELOPMENT

During development, androgen plays a major role in regulating robust cellular differentiation and proliferation necessary for various organ development.[2,8,13] Among them, androgen is necessary for the growth of ExG and augmented cell proliferation has been suggested to occur underlying its organogenesis. As for the corresponding defects, defective masculinization as hypoplastic ExG is described in experimental animal models and also as human birth defects, micropenis.[14] Due to such activities, administration of exogenous androgen by muscular injection or cream has been frequently performed in clinics to treat micropenis patients.

At the cellular level, cell cycle regulators are the essential factors for the above process. The cyclin-dependent kinase inhibitor p21 (also known as CDKN1A) negatively regulates cell growth.[15] For in vivo roles, muscle regeneration in p21 null mutants shows enhanced myoblast proliferation,[16] and supplementation of testosterone is reported to reverse the increased p21 expression in aged muscles[17] suggesting that cell proliferation without androgen signaling was impeded due to the increased p21 expression. In addition, its overexpression in vascular smooth muscles results in growth inhibition through increased apoptosis. Regarding the reproductive muscle system, AR mutation causes defective development of the sexually dimorphic bulbocavernosus (BC) muscle as a result of reduced proliferation of myofibroblasts.[18] In male-type tumor biology, expression of p21 in prostate cancer cells can be upregulated by androgen. Regarding the mesenchymal cell proliferation in GT, it was shown that DHT, the potent androgen converted from T by steroid 5α reductase (SRD), negatively regulates its cell proliferation.[19] Hence, depending on the types of androgens and mesenchymal region, androgen action for cell proliferation is target tissue dependent. Negative regulation of cell proliferation through the DHT is also reported in other tissues including hair which is the frequent site of age-related lesions, androgenetic alopecia (AGA) (see below).

In addition to the ExG outgrowth, prominent developmental features are observed during ExG masculinization as a male type of urethral formation.[2,4,5] The embryonic mesenchyme of GT lateral to the presumptive urethra is referred to as the bilateral mesenchyme (biMs).[2,20] Due to multiple types of epithelia (urethral) and mesenchymal (biMs) interactions, the common embryonic urethral plate eventually forms canalized urethra in the male ExG (Figure 2A). In contrast to male-type development, the female genital tubercle (GT), the anlage of clitoris, does not form a tubular urethra with an enclosed prepuce.[4,5] Such type of canalized urethral formation occurs in the specific embryonal period known as the masculinizing program window (MPW).[3,21] To demonstrate the essential androgen

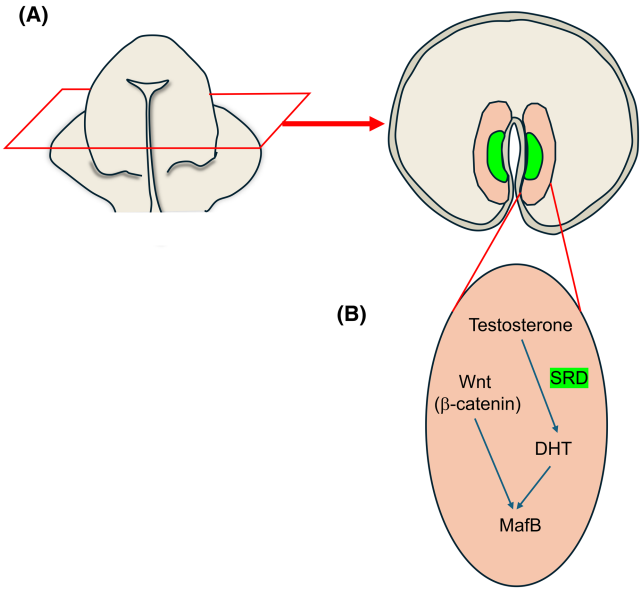

FIGURE 2 (A) During masculinization of male-type urethral formation, bi-lateral mesenchyme (biMs shown by orange color) is essential and its development is regulated by androgen through androgen receptor (AR; its expression shown by beige) and 5α-SRD (green) around E14.5. (B) Magnified view of biMs showing signal cascades necessary for male-type urethral formation. Wnt/β-catenin signal and locally converted androgen (DHT; dihydrotestosterone) are located upstream of the essential male type transcription factor (MafB belonging to AP-1 superfamily). Locally converted androgen is produced through steroid 5α reductase (SRD) from testosterone (T).

actions, mice with tissue-specific mutations in AR are frequently used. The testicular feminization mouse (Tfm) model without functional ARs, is useful for analyzing the phenotypes of null mutations in AR corresponding to human complete androgen-insensitivity syndrome (CAIS).[22–26] As for the above male type urethral formation, AR is prominently expressed in biMs and its conditional knockout of AR of male mice causes female-like developmental features including the reduced size of the GT, and defective urethral fusion.[3] In such processes, GT mesenchyme shows directed cellular migration to the midline and mesenchymal differentiation including their condensation and extracellular matrix (ECM) remodeling.[27–29] A defective male type of urethral formation, with abnormal urethral meatus located in several proximal and distal regions of the penis, is characterized as hypospadias, which is a frequent birth defect.[30–34] Such hypospadias phenotypes include underdeveloped foreskin, bending of the penis (known as chordee), and undescended testes.[35]

3 | FROM ANDROGEN SIGNAL TO CELLULAR Wnt SIGNALS FOR MASCULINIZATION

Wnt signals are involved in the genesis of organs and their pathological processes.[36] Regulatory factors involved in canonical Wnt signaling have been identified in GT development.[3,37] Such Wnt signals are mediated by factors including β-catenin and its activity is detected during the initiation of GT development at the CM in early mouse embryos (Figure 1A). CM and its interaction with the surrounding mesenchyme is the key developmental event regulating the early phase of GT outgrowth and other urogenital organ formation (Black arrows; Figure 1A).[20,38–41] Hedgehog signal, another essential growth factor, is expressed in the CM and its disruption leads to GT agenesis shown by Sonic hedgehog (Shh) knockout mouse studies.[38] Signal interaction with early Wnt as the downstream of cloacal hedgehog signals has been suggested, which is necessary for subsequent GT development (Figure 1B).[37,42]

Wnt signal for organogenesis has been known as repeatedly involved from the initiation and organogenesis. An example is known for regulating limb initiation in the body[36,43] to its late-stage differentiation including the digit formation. Key events, including such initiation and digit formation, are limb bud outgrowth from the body of lateral plate mesoderm (LPM) and cell death in the interdigit region.[36,44] In addition to such early Wnt/β-catenin function for GT development, the signal is also shown as essential for GT masculinization.[3] β-catenin expression is prominent during masculinization of the mesenchyme (biMs). Its gain-of-function mutation specifically in such mesenchyme results in male-like ExG formation in female (XX) embryos showing masculinization by modulating local growth factor signals (Figure 2A,B).[3]

Of note is the involvement of the essential function of the Wnt modulator, the antagonist genes. Such genes include Dkk and Sfrp, which attenuate the Wnt signal in various tissue contexts also for the case of masculinization. Expression of canonical Wnt signaling antagonist, Dkk2, is increased in prospective female-type GT.[3] Possible involvement of antagonistic factors including Dkk has been also suggested in other hormone-dependent processes such as mammary gland tumors and hair-related pathogenesis including AGA. Upregulation of Dkk1 and the reduced Wnt/β-catenin signal is responsible for the alopecia (see below).[45] Thus, attenuation of canonical Wnt signal by such antagonists could be one of the processes for male/female type of organogenesis and pathogenesis.

How canonical Wnt signaling and AR signaling interact with regard to the masculinization of ExG has not been extensively studied. Previous investigation suggested that β-catenin can bind to the AR ligand binding domain, increasing its transcriptional activity in GnRH neuronal cells.[46] Modulations in AR-induced transcription have been also attributed to a polyglutamine stretch of variable length in its amino-terminal domain. A variable number of CAG triplets in exon 1 of AR, located on the X chromosome, encode this polyglutamine stretch, and pathologically elongated such repeats are reported in Kennedy syndrome, a neurodegenerative disease.[47] Generally, the formation of an AR transcriptional complex requires the functional and structural interaction of AR with its co-regulators, including the CREB-binding protein (CBP)–P300 family.[48]

Understanding the correlation of androgen and Wnt signal for urethral masculinization is also achieved by the identification of new regulatory factors. Regulatory genes such as v-maf avian

musculoaponeurotic fibrosarcoma oncogene homolog B (*Mafb*), and 5α-reductase type 2 (*Srd5α2*) are expressed in male biMs under androgen signaling. *MafB* is identified as a direct androgen target that is essential for urethral masculinization.[49] The role of AR-androgen responsive element (ARE) binding in its masculinized regulation is also described. ARE sites in the 3′ UTR of *Mafb* are necessary for the androgen-AR-induced expression in GT.[50]

The expression of *MafB* shows a sexually dimorphic pattern from embryonic 14.5 days (E14.5), before the onset of male-type urethral formation. Male *MafB* mutant mice show an abnormal, open urethra like the cases for knockout mice of *β-catenin* in the biMs. It was found that locally active canonical Wnt signals also regulate the downstream genetic cascades and constitutively active β-catenin augments the *MafB* expression and the β-catenin conditional knockout mutant GT shows reduced expression, indicating the *MafB* expression is β-catenin dependent.[50] Thus, *MafB* is under two phases of regulation by androgen and Wnt/β-catenin regulating biMs masculinization (Figure 2B). Further works are necessary to examine the order of androgen and Wnt/β-catenin signal as the upstream.

4 | ANDROGEN AND Wnt SIGNALS FOR CORPORAL TISSUE FORMATION

ExG contains three corporal bodies, CC, CCG, and CCU (Figure 3A). In mice, the embryonic primordium of CC (CC anlagen) develops in the upper (dorsal) part of GT[7] as condensed a mesenchymal mass around E14.5. Androgen plays major roles from late embryogenesis to the adult erectile tissue formation postnatally and it is produced in the testes from E14.5 reaching its peak at E18.5.[51,52] The robust morphological sex differences in CC size are observed after birth.[7]

As for androgenic regulation, it is reported that exposure of pregnant rats to androgens enlarges ExG, typically for the region of Os-penis including the distal region, the male urogenital mating protuberance (MUMP) cartilage.[53,54] However, it has been poorly understood about the regulatory genes for CC and CCG formation. It is demonstrated that androgen-mediated development of a male reproductive organ size such as testis and Os-penis occurs within a specific fetal time window (MPW).[21] It is possible that androgen shows a critical function in response to the above timing for regulating CC size by enhancing cell proliferation and further analyses are required for such MPW and CC development (Figure 3B).

As above, Wnt signals have been proposed as one of the central signals for the masculinization of ExG and they are also involved in CC formation. Modulation of its signal leads to augmented mesenchymal cell proliferation in CC.[7] Dkk2, an inhibitor of β-catenin signaling, is predominantly expressed in female CC compared with male. Furthermore, administration of androgens resulted in activation of β-catenin signaling.[7] Works are thus necessary to investigate the potential similarity of androgenic responses between biMs and mesenchymal primordia for CC/CCG. It was found the Sox9 gene, one of the essential markers for chondrocyte and mesenchymal cells, is specifically expressed in the embryonic CC.[7,8] In fact, CC-specific, Sox9-CreERT2, β-catenin conditional mutant mice showed defective cell proliferation and activated form of β-catenin mutants (gain of function mutation for Wnt/β-catenin signaling) showed augmented cell proliferation.[7] Sox9 is thus one of the essential embryonic mesenchymal genes for CC, and the reiterated involvement of mesenchymal Wnt signals as a masculinization factor is shown for both mid and late embryogenesis for GT (biMs) and CC masculinization.[7,37] Further works are necessary to investigate the potential similarity of such reiterated functions of androgen and Wnt signal cascades.

It has been recently performed introducing immature type mesenchymal cells for CC including stem cells (MSCs) for ExG and CC for potential ED treatment.[55] Such types of cells include mesenchymal stem cells (MSC) derived from various tissues. Some reports indicated the incorporation of such cells inside the injected corporal tissue. Characterization and co-injection of the factors including Wnt signal affecting such immature cells have not been

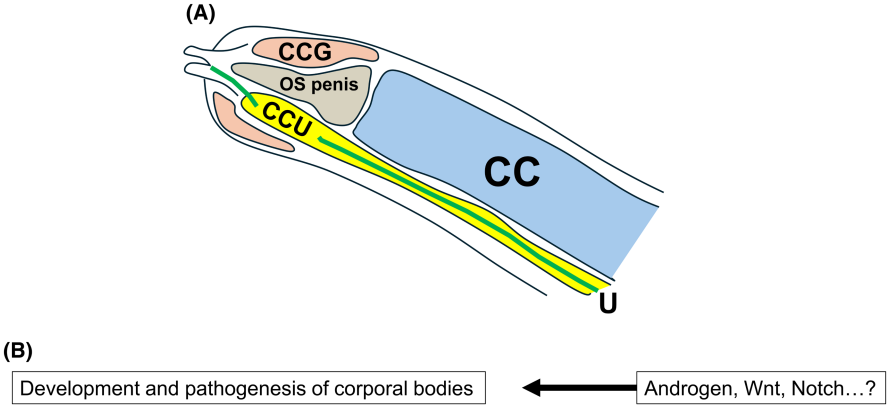

FIGURE 3 (A) Sagittal section of adult male mouse erectile tissues containing corpus cavernosum (CC), corpus cavernosum glandis (CCG), corpus cavernosum urethra (CCU), urethra (U), and OS penis. Unlike human penis, many animal species including rodents display androgen-dependent OS penis development. (B) Signals involved in the development of sinusoids of corporal tissue and its pathogenic conditions. Androgen and its interacting signals including Wnt and transcription factors are necessary for the formation of late embryonic to newborn erectile tissues. Involvement of androgen, Wnt, and Notch is suggested for the pathogenesis of erectile tissues.

reported. Of interest is the SRD expression in the presumptive embryonic CC region, which suggests the involvement of Wnt and DHT signals in embryonic CC mesenchyme, reminiscent of the above case of biMs.[2,7]

Related to such mesenchymal cells, recent works suggested the importance of immature peri-vascular fibroblast for CC/CCG and erectile activities.[56–60] Peri-vascular cells including pericytes have been reported as androgen-dependent penile vascular cells.[61] In the case of erectile tissue formation, the regulation of cellular migration and cellular attachment are also essential like other vascular organs.[61,62] One of the important cell components for the vascular system, pericytes as migratory cells, adhere to the vessel structure including endothelium to stabilize vascular networks.[61,63] Utilizing organ culture and androgen administration experiments, pericytes show unique androgen-dependent cellular migration. Recent single-cell RNA-seq analyses showed the importance of peri-vascular fibroblasts as supporting and playing roles for penile structure and functions.[56,64] Another work suggested few cells lodge close to the peri-vascular region in CC by injected MSCs. Further works are necessary to investigate the relationship between peri-vascular fibroblasts and MSCs. Understanding the androgen and related cell signals, such as Wnt, is generally required for such mesenchymal cell populations including MSCs. Possible manipulation of Wnt signal for the therapeutic application of CC mesenchymal cell proliferation may be considered. In response to the augmented stress signals, the resultant Wnt signal might induce the mesenchymal cell proliferation possibly contributing to the homeostatic control of damaged CC (Figure 3B).

As for cell migratory regulation, androgen-mediated cytoskeletal behaviors such as mesenchymal cell migration and differentiation play roles during urethral masculinization. Various cytoskeletal essential components have been revealed acting during ExG development. In such topics, mesenchymal F-actin shows sexually dimorphic expression pattern in the ExG biMs.[28] Currently, it is still unclear how androgen regulates actomyosin contractility, cellular migration, and other behaviors during urethral masculinization.[29] One possible mechanism is through the RhoA-ROCK pathway, which is a major regulatory mechanism for actomyosin contractility.

5 | ERECTION BY BLOOD DRAINAGE AND STRESS/CONTRACTION-RELATED FACTORS

When CC is filled with blood, the microvascular complex termed sinusoids expands during erection.[11,65,66] Sinusoid structure is detected in several organs including liver, spleen, and bone marrow, and they show structural variations performing different functions such as nutritious absorptions, waste product exclusion, and sinusoidal space expansion. In liver, sinusoidal structure basically shows a unit-like structure between major blood vessels, hepatic artery, and central and portal veins. In contrast, the penile sinusoid structures showed varied sizes revealed by reconstructed three-dimensional (3D) histological images of the entire CC.[8,61] In addition, it has been reported that mouse CC contains collagen-rich prominent trabeculae, "island like" central area, adjacent to the deep artery in the central region. Such peculiar fibrous structure does not contain sinusoids but mesenchymal-rich ECM region, which is considered as supporting to erection process.

As for the blood supply to them, anatomical blood vessel connection is described. In the middle of CC, the deep artery delivers blood through the helicine artery to the surrounding sinusoids. In response to sexual stimulation, the release of nitric oxide (NO) results in the formation of cGMP relaxing the smooth muscles and allowing increased blood inflow from the artery.[12,67,68] The storage of blood during erection through the occlusive function by the surrounding tissue of tunica albuginea is also essential.[65] Thus, sinusoids require functions for veno-occlusion necessary to increase inner pressure for erection and such proper drainage enables expansion for erection. More recently, blood drainage status has been analyzed with a new experimental system revealing its important blood regulatory abnormalities in priapism, the continuous erection, mouse model (Figure 4).[69] Such a system revealed that the presence of abnormal blood drainage from CC in the mutant (Figure 4).

As for "the drainage type" characters, lymphatic vessels function for liquid drainage from various tissues containing waste products.[70] Recently, Lyve-1, the major lymphatic endothelial marker, is found as expressed in some endothelial sinusoids.[57,69] It is still unclear how such Lyve-1-positive endothelial cells function in penile sinusoids. Lyve-1 is prominently expressed in areas rather close to tunica albuginea and

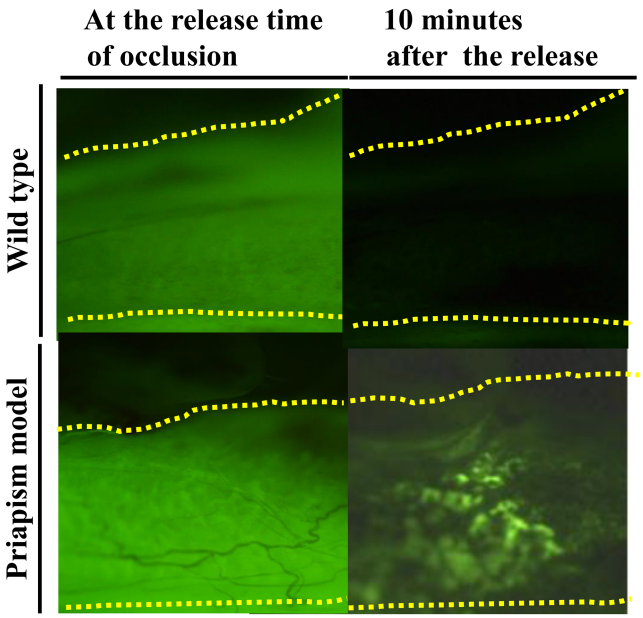

FIGURE 4 The FITC-Dextran injection filled the sinusoidal spaces of CC. The occlusion was released and the alteration of fluorescence intensity (FI) of the CC was observed. Time-lapse images show the dynamic change of FI analyzed under a stereomicroscope. After 10 min of one-time administration, the FI disappears in wild-type mice. On the other hand, the FI remains in the priapism model mice (lower right). CC, corpus cavernosum, SS, sinusoidal space, and Yellow dotted line indicate the CC region. The original data were published in Reprod Med Biol 23, e12570 (2024).

the positive endothelial cells might be involved in drainage functions (Figure 5C). It was also shown that such positive sinusoids may contribute to the drainage function by their ability to take up high-molecular-weight dye tracers.[57] Alternatively, such Lyve-1 epxression might reflect to sort of stressed cellular condtions which are suggested in part of the liver sinusoids. Endothelial Lyve-1 expression might be regulated by mesenchyme-derived Wnt.[71,72] As for such regulation, various mesenchymal cells including smooth muscle cells and fibroblasts express several Wnt signals that may affect the nearby expression.

In addition to such erectile functional viewpoints, the observed Lyve-1 expression may be also correlated with penile defensive mechanisms.[69] It is also expressed in the upper region close to urethra. Penis is a frequent site of infection due to improper sexual intercourse known as sexually transmitted disease (STD) including human papilloma virus (HPV) infection (Figure 5B). For such STD infections, the surface of glans and urethra play key roles in the infection. Thus, defense mechanisms around urethra and para-urethral region are intriguing and its augmented expression is observed by LPS administration.[69] Lyve-1 has been also described as prominently induced in various pathological conditions[73–75] and cumulative function of Lyve-1 for erectile drainage and anti-inflammation should be thus further studied (Figure 5B,C).

The functions and tissue homeostasis depend on vascular capillaries of the body and the essential roles of microcirculations are recognized. ED is a common and complex disorder as the inability to obtain and maintain an erection for satisfactory sexual intercourse.[11] Since systemic vascular disease and ED share common risk factors, it is noted that ED is an early marker for atherosclerosis and various cardiovascular diseases. The disruption of sinusoidal microcirculation is one of the causative factors for possible tissue damage in ED patients.[76] It is also reported that vasoactive stress substances affect the relaxation/contraction of vascular smooth muscles.[76–79] Various stress signals including oxidative, mechanical, and aging related factors have been implicated in the onset of ED (Figure 5A). Because of the wide spectrum of such signals, the frequency of ED is increasing in the current society.

Stress signal is regarded as one of the risk factors of ED also via Ras homolog family member A (RhoA)/Rho-associated protein kinase 1 (Rock1) signals. The essential cellular skeletal components including Rho, RhoA system are involved in the regulation of erection with contraction.[80,81] Recent studies revealed that this signaling pathway contributes in maintaining a flaccid state of penis and its augmented signal for ED patients. The involvement of Rho-ROCK system has been also implicated in pathogenic erectile functions of diabetic animal models.[82] Repeated contraction/relaxation of CC also showed augmented RhoA and Rock mRNA expression.[66] These results suggest the in vitro system contributes to analyses of repeated erectile stress and investigation of the regulatory mechanisms of androgen-induced skeletal regulations, during erection and urethral masculinization (see the previous chapter) is necessary.

In addition, the assessment of stress responses of CC after contraction/relaxation processes has been performed. It has been known that oxygen and/or tension-mediated stress in CC increases during erection and it is necessary to recover from various damages for proper erectile functions. Signal pathways have been suggested for the potential recoveries of CC and after its contraction/relaxation process.[65,66] Hypoxia-inducible factor 1α (Hif1α) is one of the major genes responding to hypoxia. The effect of contraction/relaxation processes and blood supply has been assumed to play roles in

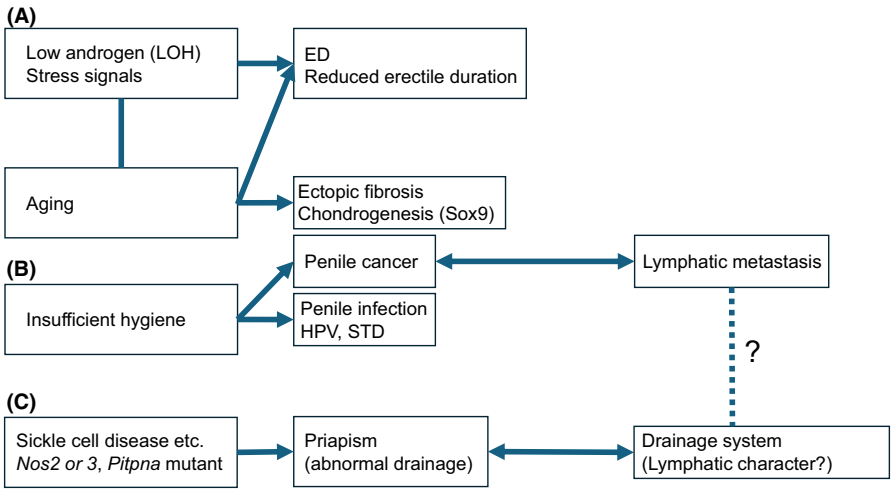

FIGURE 5 (A, B) Signals involved for the erection and pathogenic conditions underlying erectile dysfunction (ED), fibrosis, infection (Sexually transmitted disease, STD), and penile cancer. (A) Androgen and its dysregulation (LOH) are responsible for the onset of ED and also for the duration time of erection. In aged conditions, ectopic chondrogenesis is often observed predominantly in mouse CC associated with upregulated Sox9 expression. Fibrosis is often observed in aged human corporal bodies. (B) Insufficient hygiene and improper sexual intercourse induce penile infection (STD) and a higher incidence of penile cancer. The correlation of cancer metastasis to penile lymph nodes is a critical issue for its prognosis. (C) Persistent erection termed priapism is caused by some conditions including sickle cell disease and mutation of *Nos2*, *Nos3*, and *Pitpna*. Such priapism has recently been shown to be associated with abnormal blood drainage, which might be influenced by the lymphatic-type of characteristics of the drainage system.

the damaged CC. Hif1α and RhoA/Rock signals are also suggested as related to penile fibrosis.[83,84]

6 | LOW LEVEL OF ANDROGEN AND PENILE PATHOLOGY

Previous studies have shown that the influx of blood into the penile CC increases intracavernosal pressure (ICP) and castrated animals treated with 5.0 mg/kg testosterone pellets show higher ICP indicating that erection is androgen-dependent.[85] In addition, time-lapse imaging is performed in castrated mice to evaluate such conditions, and no prominent differences are observed in the rate of relaxation/contraction.[65] Generally, studies on aging-related reproductive functions including CC abnormalities require long-term observation, and application of such in vitro explant system shows the castrated mice with a shorter erectile duration time (Figure 5A).[65] Such results are consistent with reports that the neuroelectric stimulation of chronically castrated animals increases ICP and that erection is not completely lost in such conditions.[86] Castration is considered to cause veno-occlusive erectile dysfunction,[85,86] and the observed shortened erectile duration time also suggests a reduced erectile capacity.

As for the structural changes, it is suggested that castration results in the absence of significant histological changes for short-term periods, suggesting the androgen less dependent effects for erection. In the recent aging society, elderly patients with lower levels of androgen production (LOH syndrome) have been increasing with higher incidence of ED,[87] and the lower level of androgen is responsible for the improper regulation of erection (Figure 5A). How androgen regulates such complex events including the regulation of cellular components of sinusoids requires further analysis. Low degree of androgen with other pathogenic states such as diabetes should be analyzed (see below).

In addition to the increased onset of ED during aging, other corporal abnormalities have been reported including the abnormally curved penile structure of the Peyronie disease.[88] Androgens play an important role in the maintenance of the erectile tissue structure, smooth muscle, and endothelium.[7,89,90] Various histological abnormalities of aged sinusoids are reported. Ectopic chondrogenesis with augmented Sox9 expression is reported in the aged mouse CC tissue.[8] Abnormal mesenchymal differentiation in ExG is intriguing showing various types of abnormalities depending on the animal species.[1,91] Unlike such observation in mice, the frequency of such ectopic chondrogenesis for the aged human penis is rather scarce. Human penile fibrosis associated with aging and/or diabetes is reported (Figure 5A).[92]

7 | MALE TYPE CHARACTERISTICS AND PATHOPHYSIOLOGY

Other than erectile responses, various male-related sexual characteristics are observed. Sexual dimorphism in vertebrates has been extensively studied in rodents, also in the context of analyzing male-biased sexual characteristics. In addition to androgen, various growth factor signals including Wnt have been identified as cross-talking with androgen for such character establishment.

Hair and associated tissues show prominent sex-dependent characteristics including the marriage period of colors. Birds display striking male-type sexual characters in appendicular and reproductive development. Some birds show well developed male-type hairs and feather development.[1] Other than birds, a prominent degree of pigmentation is observed in perineum region including scrotum for humans and other species. Such perineum pigmentation is regulated by androgen shown by castration and testosterone supplementation. As such, perineum region shows sexual characters with colored cutaneous phenotypes.[93] Wnt signal has been shown as one of the central signals for hair growth and its modulation by augmented β-catenin leads to hyperplasia of hair follicles in mouse experimental model.[94] β-catenin also stimulates melanocyte numbers as shown by mouse mutants and other studies.[94,95]

Various stress-related signals including Hif are also noted in male characteristics and pathology. Recently, the stress signal Hif responses located downstream of hair follicles are shown. Stress conditions including the Hif expression lead to several signal outcomes including Wnt signal and a lower level of Hif expression is reported for the case of alopecia skin. Such cascades are essential for normal and pathogenic hair growth underlying the alopecia.[96] As above, various stress-induced factors and Hif signal have been described in sinusoidal contraction/relaxation condition (Figure 5A).[66] Thus, a possible signal cascade of stress-related Hif and Wnt signals could be involved in hair follicle and penile sinusoidal regulation. Several drugs have been known to improve the pathogenesis of alopecia and male urogenital abnormalities. Of interest is the application of drugs for the treatment of alopecia. Sildenafil is known as one of the effective drugs for treating ED due to PDE5 inhibition[97] and is also applied for urogenital disorders including Benign Prostate Hyperplasia (BPH). It can augment the function of Hif in the case of hair growth for the alopecia patient through modulating such a signal. Therefore, application of sildenafil for urogenital disorders and for alopecia has been stated. Furthermore, the oxygen sensor Hif1/2α and the angiogenic factor vascular endothelial growth factor (VEGF) are highly expressed in the lungs of sildenafil-treated rats.[98] Alopecia has been frequently described as the dysregulation of male hormones especially, DHT and SRD. Potential involvement of the androgen and the related signals should be further studied.

ACKNOWLEDGMENTS

This work was supported by the Japan Society for the Promotion of Science 21K06822 and 23KJ1859. The authors thank A. Burnett, T Kataoka, H. Yamamura, A Yamamura, M Kajimoto, A Murashima, D Matsumaru, R Haraguchi, and Y Ogino for their encouragement. The authors also thank all laboratory colleagues for their assistance.

CONFLICT OF INTEREST STATEMENT

Daiki Hashimoto, Kota Fujimoto, Masanori Nakata, Takuya Suzuki, Shinichi Kumegawa, Kentaro Suzuki, Shinichi Asamura, and Gen Yamada declare that they have no conflict of interest.

ETHICS STATEMENT

All procedures and protocols were approved by the committee on animal research at Wakayama Medical University, Wakayama, Japan (approval number: 993, 1166).

ANIMAL STUDIES

All institutional and national guidelines for the care and use of laboratory animals were followed.

HUMAN RIGHTS STATEMENTS AND INFORMED CONSENT

This work does not contain human subjects.

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How to cite this article: Hashimoto D, Fujimoto K, Nakata M, Suzuki T, Kumegawa S, Ueda Y, et al. Developmental and functional roles of androgen and interactive signals for external genitalia and erectile tissues. Reprod Med Biol. 2024;23:e12611. https://doi.org/10.1002/rmb2.12611