Biochimica et Biophysica Acta 1849 (2015) 163–170

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbagrm

Review

Androgens and mammalian male reproductive tract development ☆

Aki Murashima [a], Satoshi Kishigami [b], Axel Thomson [c], Gen Yamada [a,*]

[a] Department of Developmental Genetics, Institute of Advanced Medicine, Wakayama Medical University, Kimiidera 811-1, Wakayama 641-8509, Wakayama, Japan
[b] Faculty of Biology-Oriented Science and Technology, Kinki University, Kinokawa 649-6493, Wakayama, Japan
[c] Department of Urology, McGill University Health Centre, 1650 Cedar Av, Montreal, Québec, H3A 1A4, Canada

ARTICLE INFO

Article history:
Received 10 February 2014
Received in revised form 28 April 2014
Accepted 19 May 2014
Available online 27 May 2014

Keywords:
androgen receptor
growth factor
knockout mouse
external genitalia
epididymis
prostate

ABSTRACT

One of the main functions of androgen is in the sexually dimorphic development of the male reproductive tissues. During embryogenesis, androgen determines the morphogenesis of male specific organs, such as the epididymis, seminal vesicle, prostate and penis. Despite the critical function of androgens in masculinization, the downstream molecular mechanisms of androgen signaling are poorly understood. Tissue recombination experiments and tissue specific androgen receptor (AR) knockout mouse studies have revealed epithelial or mesenchymal specific androgen-AR signaling functions. These findings also indicate that epithelial–mesenchymal interactions are a key feature of AR specific activity, and paracrine growth factor action may mediate some of the effects of androgens. This review focuses on mouse models showing the interactions of androgen and growth factor pathways that promote the sexual differentiation of reproductive organs. Recent studies investigating context dependent AR target genes are also discussed. This article is part of a Special Issue entitled: Nuclear receptors in animal development.

1. Introduction

One of the most important functions of sex steroid receptors is in the sexually dimorphic development of the male and female reproductive tissues. The reproductive tissues arise from 'anlagen' or precursor structures which are identical in both male and female during early development. Subsequent male sexual differentiation of reproductive tract anlage starts after testicular differentiation and androgen production. Embryonic developmental programs control the formation of reproductive anlagen and these are not hormone-regulated, however subsequent growth and sex specific development are controlled by sex hormones. Several signaling cascades control reproductive organogenesis and this is a versatile system to study how hormones regulate organ growth and differentiation. Some molecular pathways have been identified in initial anlagen formation as well as later hormone driven development. These include fibroblast growth factor (FGF), hedgehog (HH), Wnt, transforming growth factor (TGF) signals and other "effector" genes. Androgen dependent signaling and downstream events are involved in not only developmental processes but also disease processes — such as hypospadias and prostate cancer.

2. Androgens regulate male reproductive tract masculinization

A characteristic feature of sexual reproduction is sexually dimorphic adult reproductive organs and these are formed during embryonic development and mature during post-natal puberty. In vertebrates, sex is defined by genetic determination of gonad type followed by the production of gonadal hormones that pattern the rest of the body into a male or a female phenotype and physiology. In mammals and other vertebrates, males are masculinized by androgens produced by the differentiated testes, which regulate reproductive tract patterning and other male characteristics.

In 1953, Alfred Jost castrated rabbit fetuses *in utero* before sexual differentiation of the genital tract and observed that they developed a feminine reproductive tract. Implantation of testosterone propionate crystals into the castrated fetuses led to male reproductive tract stabilization and differentiation [1]. It appeared that the testosterone may not be distributed through the bloodstream during early male reproductive development because male specific differentiation was rescued only in the side where testosterone crystals were placed [2,3]. Recently, circulating androgens have been also shown as important to induce Wolffian duct (WD) stabilization and subsequent formation of the epididymis, as well as the prostatic formation and masculinization of the genital tubercle (GT) and other male traits [4].

Androgen function is dependent on signaling through androgen receptor (AR), a member of the nuclear receptor superfamily [5–7]. Like other members of nuclear receptor superfamily, the AR structure

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* Corresponding author at: Department of Developmental Genetics, Institute of Advanced Medicine, Wakayama Medical University, Kimiidera 811-1, Wakayama 641-8509, Wakayama, Japan. Tel./fax: +81 73 499 5026.
E-mail addresses: genyama77@yahoo.co.jp, transg8@wakayama-med.ac.jp (G. Yamada).

http://dx.doi.org/10.1016/j.bbagrm.2014.05.020
1874-9399

is organized into functional domains, consisting of an N-terminal domain (NTD), a DNA binding domain (DBD), a C-terminal ligand binding domain (LBD), and a small hinge region between DBD and LBD. The DBD and LBD are highly conserved, whereas the NTD sequence varies among species which may underly different homeostatic control and signaling between species [8–10]. The NTD mediates the majority of AR transcriptional activity and is the most active co-regulator interaction surface. Spontaneous AR mutants with an androgen insensitivity phenotype have been known as *testicular feminized* (*Tfm*) in animals, and complete or partial androgen insensitivity in man (CAIS, PAIS) [11,12]. Various AR gene mutations have been identified in mouse, rat and man [13–15]. Rodent Tfm males lack a vas deferens, an epididymis and male accessory sex glands and are a valuable experimental model to investigate the mechanism of androgen receptor-mediated sex differentiation.

3. Differential AR signaling between epithelium and mesenchyme

The male reproductive tract develops principally from two embryonic anlagen: the WD and urogenital sinus (UGS) (Fig. 1). The WD, whose epithelium is mesodermal in origin, gives rise to the epididymis, ductus deferens, and seminal vesicle (SV). The UGS, whose epithelium is derived from the endoderm, gives rise to the bladder, prostate, bulbourethral glands, urethra, and periurethral glands. The epididymis functions in storing and preparing sperm for fertilization, including the resorption of fluid to concentrate sperm. The prostate and SV are male accessory sex glands that secrete proteins, zinc and sugars into seminal plasma and which provide a high proportion of the seminal fluid. Because of their secretory or resorptive functions, the epithelia within these organs are highly convoluted to provide a large surface area. The epididymis is a single epithelial tube that is highly coiled within the mesenchymal matrix. The prostate and SV are organs with branched or infolded epithelia surrounded by the mesenchyme. The development and patterning of these organs depend on androgen signaling, particularly, within the mesenchymal compartment.

At the beginning of the ambisexual stage of sex differentiation, AR expression is present in the mesenchyme of urogenital anlagen [16,17] and is absent from the epithelia. Despite the absence of AR in the epithelium, several androgen-dependent processes are observed in the epithelia during male reproductive tract development. For example, WD epithelia survive and avoid from cell death, SV and prostate epithelia bud or branch, and male mammary epithelial anlagen regresses. These observations suggest the paracrine interaction between mesenchyme (AR-positive) and epithelia (AR-negative) controls androgen dependent epithelial development.

The work of Cunha and colleagues using tissue recombination techniques has demonstrated that AR signaling in mesenchymal tissue is important for epithelial growth and morphogenesis (reviewed in [18–20]). For example, embryonic mesenchyme from the SV (AR-positive) induced cell proliferation and SV-like morphological differentiation of AR-negative ureter epithelium [21]. Conversely, Tfm mesenchyme that lacks AR did not induce epithelial morphogenesis, cell proliferation and cytodifferentiation despite the presence of wild type AR in the epithelia [18]. These experiments support the idea that mesenchymal androgen signaling plays a major role in epithelial cell proliferation and morphological differentiation during masculinization. These results led to the hypothesis that there are paracrine signaling

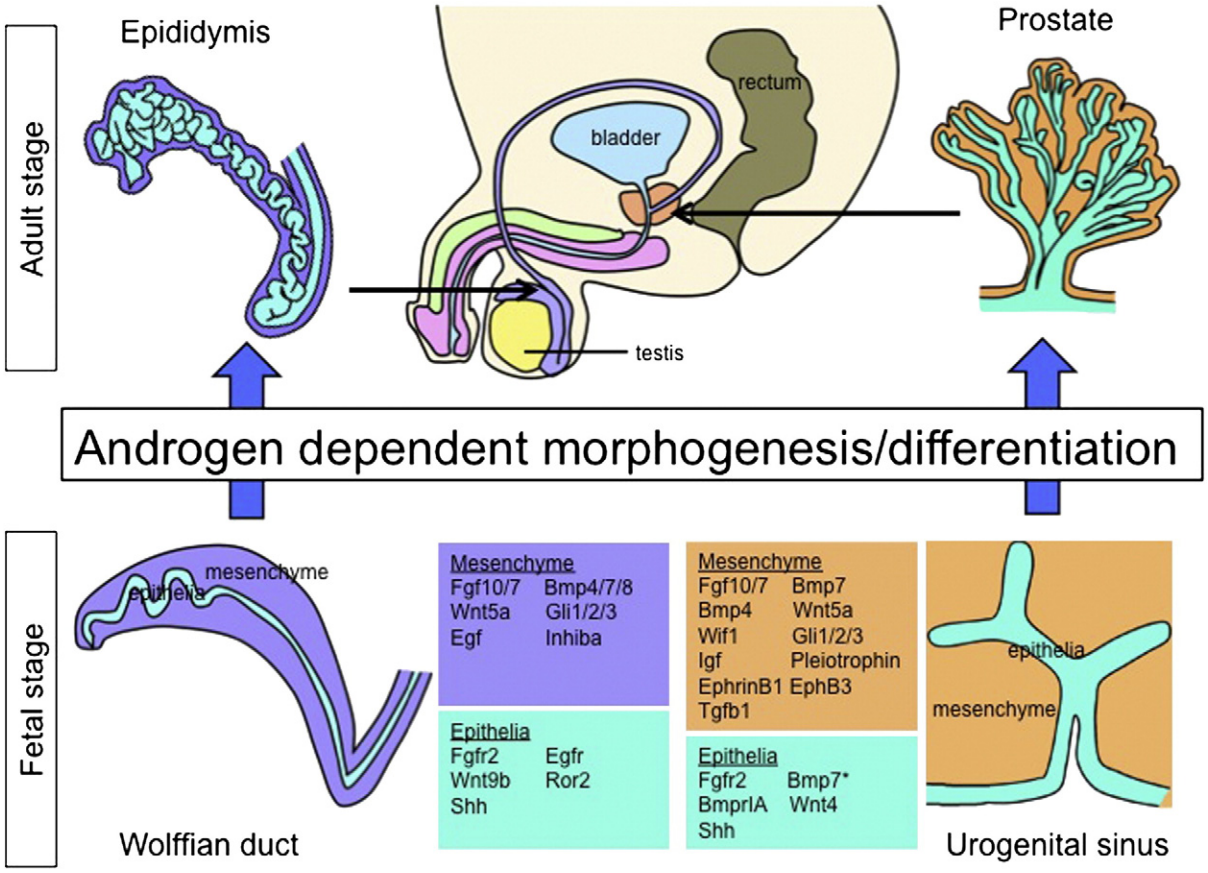

Fig. 1. A schematic diagram of male genital tract masculinization. WD epithelium is stabilized by androgen exposure, and the epididymis elongates and coils three-dimensional manner (left low and upper illustration). Prostate develops from the UGS. During sexual differentiation, solid buds from the urogenital sinus epithelium invade into the urogenital sinus mesenchyme where subsequent branching occurs (right low and upper illustration). Circulating or testis-derived androgens initiate these developmental processes through the mesenchymally expressed AR which regulates paracrine signaling to epithelia. Growth factor related genes which show tissue specific expression patterns are summarized in the scheme. *lobe-specific expression.

molecules produced in the mesenchyme in response to androgens and AR that regulate epithelial development, including cell proliferation, survival and cytodifferentitaion. In contrast, AR function within epithelia was to regulate androgen-dependent secretory proteins expression and secretion. This suggests that the epithelial AR is required for physiological functions of epithelial cells in the male reproductive tract [22].

Growth factors have been identified as paracrine mediators of epithelial–mesenchymal interactions during the development of a variety of organs. Analysis of male sex accessory organs treated with growth factors or their inhibitors demonstrated possible roles for growth factors, such as Insulin-like growth factors (IGF), platelet-derived growth factor (PDGF), nerve growth factor (NGF), epidermal growth factor (EGF), TGF, FGF and others (Fig. 1, [18,23,24]). Some members of the FGF family show mesenchyme restricted expression with reciprocal expression of specific receptors in epithelia (Fig. 1) [25,26]. Addition of FGF7, FGF10, or their inhibitors promoted or inhibited the growth of the ventral prostate and SV respectively, indicating that FGFs may be required for growth [27,28]. Deletion of *Fgf10* by gene knockout (KO) resulted in a loss of prostate and SV development, and cyst formation in epididymis [29]. Although *Fgf10* mRNA increased in response to testosterone *in vitro* [30], it did not appear to be regulated by androgens *in vivo* [28,31]. EGF is another candidate for a mesenchymally produced paracrine growth factor [32–35]. Treatment with Anti-EGF serum led to a disintegration of the WD even in the presence of androgen, and EGF was able to increase anogenital distance (AGD) and to induce epididymal formation in female embryos [32]. EGF expression is not induced by androgen *in vivo*, similar to the lack of regulation of FGFs. Knockout mouse studies on Bone morphogenetic protein (BMP) and SHH signaling also showed the defects in the prostate epithelial cell growth and differentiation indicating them as mediating factor of epithelial–mesenchymal interaction [36–38]. Inhibin-beta A, a subunit of both activin and inhibin, was demonstrated as a mesenchyme-specific gene, acts with testosterone to facilitate epididymal coiling by stimulating epithelial proliferation [39]. Overall, the initial regulations of these gene expressions do not appear to be dependent on the androgen. Other studies have identified Pleiotrophin as expressed in a mesenchyme specific pattern and have shown effects of Pleiotriphin upon both mesenchymal and epithelial growth [40].

In summary, androgens primarily act via AR in mesenchymal tissue and regulate epithelial growth and ductal morphogenesis, perhaps through secreted factors. In general, all of the molecules that appear to be expressed in mesenchyme and which show partial response to AR appear to show modest enrichment in male versus female tissues which challenges their proposed function in male specific development. Furthermore, gene profiling studies to identify AR target transcripts have not detected those molecules suggested to be androgen regulated. Recent efforts to understand possible AR-mediated mechanisms are being investigated using comprehensive gene profiling methods in the developing male reproductive tissues (described below).

4. Tissue and cell specific ablation of AR

Mouse models with various tissue- or cell-specific AR KO have been developed recently (reviewed in [41,42]). Most of these used the Cre-loxP gene recombination system, which attenuated AR expression in specific cells during development or under different physiological contexts. Although human and mouse male reproductive tracts often differ in their anatomy, such mutant mice are valuable models to understand the AR function *in vivo*.

4.1. The function of mesenchymally expressed AR: the prostate and seminal vesicle

Mesenchymal cell specific AR KO have been reported in prostate and SV. Smooth muscle myosin heavy chain (SMMHC)-Cre was used to establish smooth muscle AR KO in prostate and SV [43,44]. Smooth muscle cells are the major stromal component of adult male reproductive tract. Prostate and SV weight were reduced with various histological abnormalities such as hyperplasia and impaired epithelial cell functions in such models. These studies also suggested that mesenchymal AR limits the epithelial proliferative responses to exogenous estrogen, possibly by attenuating the production of growth factors [45]. In the prostate, AR gene deletion also induces inflammation, fibrosis, and reduced expression of epithelial, smooth muscle, and stem cell markers. Yu et al. developed two types of prostate stromal AR KO mouse models; fibroblast-specific protein 1 and smooth muscle 22α promoter driven Cre-mediated AR KO (FSP-AR KO, SM-AR KO) mice [46,47]. FSP1 driven AR deletion is prominently observed in the ventral prostate (VP). On the other hand, the AR is maximally reduced in the anterior prostate (AP) in the SM-AR KO model. Comparison of these models revealed different roles for AR. Epithelial cells in both models showed decreased cell proliferation. In addition, FSP-AR KO VP showed cuboidal and flattened epithelial cells, whereas SM-AR KO AP showed fewer epithelial luminal infoldings. Male FSP-AR/SM-AR double KO mice showed reduced prostate size with impaired branching and partial loss of the infolded glandular structure [48]. Epithelial cell proliferation was reduced and luminal epithelial cell death was increased in the double AR KO mouse than that in the FSP-AR KO or SM-AR KO. The molecular pathways of epithelial development were mediated, at least in part, by stromal growth factors. In FSP-AR KO mouse prostate, IGF1, FGF7, FGF10 and Hepatocyte growth factor (HGF) were decreased, whereas in the SM-AR KO model, only IGF1 expression was decreased. These alterations of growth factors might contribute to the altered prostate epithelial structures and reduced epithelial proliferation [46,47]. Altogether, stromal AR has been confirmed as a positive regulator of epithelial cell proliferation and survival, and these effects may be mediated by stromally expressed growth factors.

4.2. The function of epithelially expressed AR: the epididymis and prostate

Tissue recombination experiments indicated that epithelially expressed AR was important for epithelial function in regard to secretory protein expression. These studies have been extended using an epithelial-specific AR KO mouse, and characterization of effects in the epididymis and prostate. In the epididymis, where AR expression is highly abundant, AR regulates the secretion of seminal fluid proteins in the epithelia. Male embryos of WD epithelium-specific AR KO mice were generated using activating enhancer-binding protein 2-alpha promoter-driven Cre (AP2α-Cre) [49]. In this epididymal epithelia-specific AR KO mouse, almost complete AR deletion was observed throughout the WD. In the absence of epithelial AR, the WD formed a highly coiled structure consistent with the previous results showing the importance of mesenchymal AR for the WD morphogenesis. However, postnatal analysis revealed that principal and basal cell differentiation was specifically perturbed in epithelia-specific AR KO mice. A conditional mutant with a mosaic pattern of AR loss demonstrated cell-autonomously disrupted expression of p63 — a regulator of basal cell differentiation. This shows the potential of AR regulation of p63 in epididymis in contrast to other epithelial structures such as skin. Other epididymal epithelia-specific AR KO models using ribonuclease 10 promoter-driven Cre, forkhead box G1 promoter-driven Cre and probasin-Cre have been also reported [50–52]. Most of these mice show AR deletion in the postnatal principal cells of the epididymal epithelia, but not in basal and other cell types. In these models, epithelial hypoplasia was observed in the caput region of the epididymis indicating that the epithelial AR in the epididymal principal cell is required for proper development and function of the proximal epididymis.

Probasin-Cre has been used for AR ablation in prostate epithelia [53–55]. These mutant mice showed increased apoptosis in epithelial luminal cells and increased proliferation in epithelial basal cells resulting in impaired epithelial infoldings. A basal cell-specific AR KO was generated by crossing cytokeratin 5-Cre mice with AR floxed mice

[56]. Although AR expression is low in basal cells, it plays a suppressive role for their proliferation and induced differentiation into luminal epithelial cells.

Intriguingly, epithelial AR ablation in reproductive organs showed stromal thickening [51,52] or hypoplasia [54,55]. These data suggest that epithelial AR also played an important role in the maturation of male reproductive tract stroma. It is well established that epithelia regulate stromal differentiation in various organ, and these studies indicate that AR action in epithelia contributes to the stromal architecture. Using gene expression analysis of epithelial AR KO prostate, TGF-β1 has been suggested to play an important role as an epithelial regulator of stromal differentiation [55]. In summary, AR in epithelia regulates epithelial cell differentiation as well as stromal differentiation.

5. The development of external genitalia

Important insight into AR action has been gained from the study of the effects of AR upon the development of male sex accessory organs such as prostate, SV and epididymis. Further insight may result from the study of androgen action upon the external genitalia as these are an androgen target organ with a unique structure and developmental program. In addition, the external genitalia are the most common site of human birth defects, and impaired urethral closure in males (hypospadias) is the most common among them. It has been speculated that defects in androgen action contribute to hypospadias. Elucidation of the mechanism of androgen actions in external genitalia development will provide new insight into the pathogenic mechanisms of such disease and is clinically relevant to pediatrics and urology. Here, the background of the external genitalia masculinization and recent progress of the research are summarized.

5.1. Role of androgen signaling during genital tubercle masculinization

External genitalia are morphologically diverse among animals with internal fertilization. These different morphologies are important for efficient copulation and successful reproduction. There is considerable crosstalk between growth factor signaling and androgen signaling during external genitalia development. Growth factor functions in epithelial–mesenchymal interactions have been well characterized during genital tubercle (GT) development using genetically modified mouse models (reviewed in [57,58]).

The GT is the main anlage for external genitalia and it undergoes masculinization in response to androgens which is well characterized morphologically in the mouse. Male GT masculinization involves the formation of a tubular urethra with well-developed prepuce, and the condensation of a bilaterally segmented prospective corporal body (Fig. 2). In contrast to the male, female GTs do not form a tubular urethra with an unclosed prepuce at the lower (ventral) midline of the GT. Male specific GT differentiation occurs at E16.5 in mice and 12 weeks in humans [59].

Sexually dimorphic expression of AR is observed in GT mesenchyme adjacent to the urethra (bilateral mesenchyme shown in Fig. 2) at E15.5 in the case of mouse embryos [60]. In human, AR expression is observed in male fetuses between 12 and 14 weeks of gestation. However, comparative analysis of the early phase of human male and female embryos is required to address the initiation and dimorphic expression of AR expression [61,62]. During later stages of GT development in both mouse and human, there is expression of AR in the lateral and upper GT mesenchyme in both sexes in a non-dimorphic pattern. Consequently, different tissue lineages are expected to emerge from these subdivisions of the GT mesenchyme including the corporal tissues, prepuce and penile bones in case of mice. The temporal- and region-dependent AR

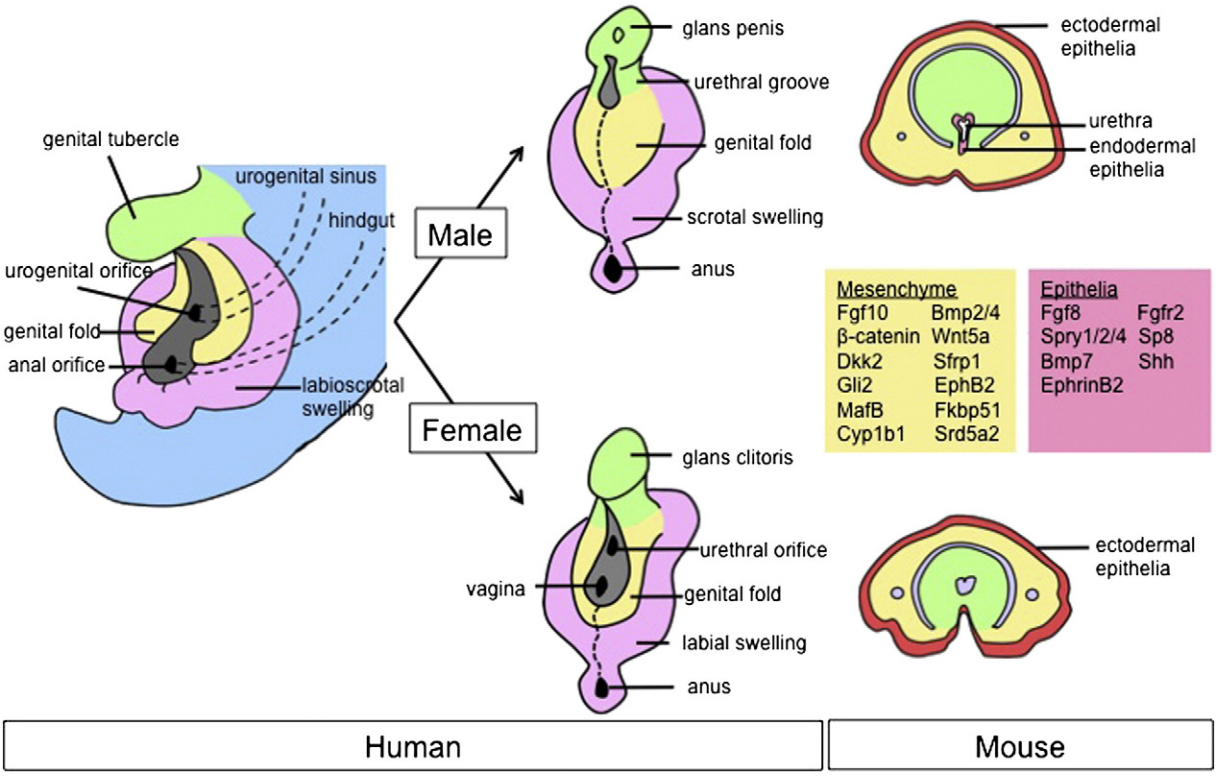

Fig. 2. A schematic diagram of sexual differentiation of external genitalia. In the mouse, the first morphological differences between the male and female GT are evident from E16.5. The fusion of the urethral folds in the ventral midline leads to the formation of the urethral groove, which subsequently becomes the mature urethra (human). This fold is prominent in the male GT after E16.5 (mouse). The canalization of the urethral plate proceeds proximo-distally in the male GT. Genes expressed in the mesenchyme and epithelia are shown. The bilateral mesenchyme is the ventral (lower) side of embryonic GT mesenchyme adjacent to the endodermal epithelia which develops to the urethra (mouse).

gene expression may enable the mesenchyme to regulate the differentiation of various tissues.

Tissue recombination experiments demonstrated the inductive effects of the epithelium on mesenchymal growth and differentiation of GTs [63,64]. In a recent conditional AR KO mice study, the epithelial or mesenchymal specific functions of AR were genetically examined during GT development [60]. AR was highly expressed in the mesenchyme adjacent to the urethral plate epithelium (UPE). Mesenchymal AR KO male GT showed female type development, smaller in size with defective urethral fusion and preputial closure, while the GTs of the epithelium-specific AR KO male mice were morphologically indistinguishable from those of the control males. Such studies demonstrate that the proper mesenchymal AR expression is necessary for GT masculinization [60,64]. This also confirms the general paradigm that AR function in masculinization of reproductive tissues requires paracrine signaling from the mesenchyme to the epithelium.

An important element of androgen action relates to the timing of androgen production and local metabolism of androgens such as conversion to more potent derivatives. The interstitial cells in the testis differentiate into Leydig cells between E12.5 and E13.5 in mouse and synthesize testosterone [65]. A recent study demonstrated that the production of testosterone is not only dependent on the Leydig cell but also the cells within the seminiferous tubule [66]. Testosterone produced in the testis is converted into more potent androgen, 5α-dihydrotestosterone (DHT) in target tissues. DHT possesses a higher binding affinity for the AR, and is more potent in inducing AR signaling than testosterone [67,68]. The conversion of testosterone to DHT is mainly catalysed by the enzyme 5α-reductase 2, which is encoded by the *SRD5A2* gene. SRD5A2 expression is observed in GT mesenchyme [69]. Comparison of patients with CAIS and *SRD5A2* deficiency provides important clinical implications for understanding of testosterone and DHT functions. Patients with CAIS show a similar phenotype to the AR KO mouse, and males exhibit female external genitalia at birth demonstrating that androgen is essential to induce male sexual characteristics [70]. Patients with *SRD5A2* deficiency, display a small penis that resembles an enlarged clitoris, labioscrotal fusion, and urogenital sinus in which there are two separate urethral and vaginal openings [71]. This indicates that testosterone is not sufficient for complete masculinization of the external genitalia during fetal life as well as male sexual maturation during puberty. There is likely a different requirement of androgen among different target organs or tissues. External genitalia and the prostate require DHT for proper masculinization while testosterone is sufficient to establish a functional epididymis and descended testes. This suggests that investigation into the regulation of SRD expression and early distribution of fetal androgen (testosterone) is required. Thus, to understand AR action in GT mesenchyme will require characterization of several factors including ligand production, distribution and local activation, as well as investigation of AR transcriptional targets.

5.2. Role of growth factors for early phase and androgen dependent GT formation

Wnt/β-catenin signaling has been identified as playing fundamental roles in the early phase of GT formation. Canonical Wnt/β-catenin activity is detected during cloaca formation. There are interactions between canonical Wnt/β-catenin signaling and HH and FGF signaling [72,73]. Wnt/β-catenin activity is also detected in late stages of androgen dependent GT formation at E15 of mouse embryos. Its sexually dimorphic activity is detected in the bilateral mesenchyme as higher in males than females. Augmentation of β-catenin in mesenchyme of female mice induced some characteristics of GT masculinization, such as a well-developed prepuce [60]. These observations suggest that elevated Wnt/β-catenin signaling activity may contribute to GT masculinization. Furthermore, *Dkk2* and *Sfrp1*, Wnt signaling inhibitors, display augmented expression in the bilateral mesenchyme of female mouse GT [60]. Mesenchymal AR KO male mice display an increased level of *Dkk2* expression in the mesenchyme adjacent to the urethral tube. These data indicate that some Wnt inhibitors may be in part of androgen signaling. Alternatively, these Wnt inhibitors may be considered as "markers" for female type of GT mesenchyme. Future analysis will involve generating conditional mutant models for functional analysis of such Wnt inhibitors. Analysis of the regulation of such inhibitor gene expression will be also necessary. The expression of Wnt inhibitors in female GT mesenchyme and their repression by androgens in males are an attractive hypothesis to explore to further elucidate the mechanisms involved in GT sexual dimorphism.

In vitro studies indicate Wnt/β-catenin signaling can interact with several other pathways, and cooperation of β-catenin and AR has been suggested. Nuclear localization of both AR and β-catenin is initiated by the exposure to androgen [74,75]. On the other hand, previous reports using prostate cell lines suggested that ligand-activated AR can inhibit Wnt/β-catenin signaling through the active-recruitment of β-catenin by AR leading to a loss of β-catenin mediated transcription [75,76]. These observations suggest the tissue sensitivity for Wnt/β-catenin signaling may differ depending on the cell and developmental context. Further investigation of the developing GT may identify context dependent signaling that leads to dimorphic patterning.

FGF signaling has been shown to regulate the initial androgen independent phase of GT development. Regulation of GT outgrowth and its initial lower (ventral) GT development involves signal crosstalk between FGF, HH, and BMP signaling [77–79]. In addition, FGF signaling may be involved in late-stage androgen dependent GT morphogenesis. FGF signaling has also been correlated with hypospadias-like phenotypes [80,81]. *Fgfr2-IIIb* is expressed in the urethral epithelium while *Fgf10* is expressed in the GT bilateral mesenchyme [78] (Fig. 2). *Fgfr2-IIIb* KO mouse embryos exhibited severe hypospadias with disorganized urethral plate epithelium and decreased cell proliferation [80,81]. Despite the relatively strong phenotypes of null mutant of *Fgf10* and *Fgfr2* genes, these genes were found as "moderately" mutated in some human cases of hypospadias [82]. However, initial analysis of sexually dimorphic gene expression did not reveal FGF signaling as differentially expressed between males and females [83]. A recent study revealed that *Spry* genes, which have been identified as FGF signaling inhibitors [84,85], regulate various elements of organogenesis. Double KO of *Spry1* and *Spry2* effects upon urethra formation [86]. In addition to affecting the early phase of GT outgrowth, the double KO mice show some sex-dependent phenotypes in hormone dependent GT differentiation. Further mechanistic analyses of FGF signaling and its regulators will determine which elements are hormone dependent or independent.

HH signaling has been identified as one of the critical early regulators of GT development. *Shh* is expressed in the endodermal urethral epithelia while its signal effector, *Gli2*, is expressed in the bilateral GT mesenchyme [77,87]. Introduction of a null mutation in *Shh* led to GT agenesis [78]. *Shh* knockdown experiments in organ culture system showed a reduction of *Fgf10* expression indicating their crosstalk [78]. In addition, late-staged Shh functional analyses have been recently performed using conditional mutants of *Shh*. It was shown that SHH signaling may also be involved in modulating androgen dependent (late stage) GT development [87].

EphB2 (Ephrin receptor B2) expression is temporally and spatially different between male and female GT [88]. The expression of *EphB2* in male is detected in the proximal GT extending to the urethral plate. This pattern can be ectopically induced by DHT treatment in female [88], suggesting that the regulation of *EphB2* gene by AR needs to be further investigated. *EphrinB2* mutants also exhibit severe hypospadias with a reduced anogenital distance (AGD), an open perineum [89]. The musculoskeletal system is sensitive to the action of androgen as documented in the effects of androgen exposure on muscle mass [90]. Thus, AGD and perineum muscle formation are also effective indicators of masculinization [91].

Growth factors are involved in the development of the male internal reproductive tissue as well as the hormone dependent and independent growth of the GT. Identification of mechanisms of androgen and AR signaling in these systems will determine if similar or different target molecules are effectors of androgen action in different male reproductive tissues. This can be addressed by gene profiling methods to define AR regulated transcripts, and subsequent analysis of their distribution and functions.

5.3. Novel GT masculinization regulator candidates showing sexually dimorphic expression

In addition to well-known growth factor related genes, recent molecular analyses identified several other types of regulators expressed prior to GT morphological dimorphism, such as MAFB, CYP1B1 and FKBP51 [83]. *Cyp1b1* expression is higher in the male as compared to female. CYP1B1, one of the cytochrome P450 enzymes and a possible target of aryl hydrocarbon receptor (AhR), can metabolize testosterone [83,92]. AhR mediates teratogenic responses in embryos and hypospadias may be one of the results of exposure to a teratogenic agent [93,94]. There is controversy regarding possible increase of hypospadias in some polluted residential regions. Potentially, there may be crosstalk between AhR and androgen signaling, regulating CYP1B1 expression in male GT. *Fkbp51* expression is higher in male GT compared to female [83]. FKBP51 is a chaperone for steroid receptors, and interacts with AR in a prostate cancer cell line [95]. Its forced expression stimulates AR-mediated transcription in prostate cancer cells. Similarly, *Fkbp52* KO mice exhibit varied phenotypes reminiscent of AR mutations with hypospadias, ambiguous genitalia, undescended testis [96]. MAFB is a basic leucine zipper (bZIP) transcription factor that plays an important role in hindbrain and otic development [97]. The possibility of its sex dependent expression and function in GT development merits investigation. Whether such factors also function in late-staged hormone dependent GT dimorphisms will require investigation. Recent GFP knock-in mouse strains that label bilateral mesenchyme may allow isolation of purified mesenchymal cells to enable gene profiling or analysis of distinct mesenchymal subsets (Suzuki et al. unpublished). Thus, GT may offer unique research advantages as it displays morphological dimorphism from the common GT early anlage which diverges into male and female GT patterns. Analysis of dimorphic GT development and patterning is an area of research that has only recently started. Molecular analysis of the mechanisms of GT formation involves several interdisciplinary research fields including molecular genetics, mouse mutant analysis, androgen-mediated hormonal control of gene regulation, cellular and tissue morphogenesis. Comparison of GT organogenesis with other hormone dependent tissues will help to define common mechanisms involved in hormonal patterning of reproductive tissue.

6. Molecular regulation of AR functions: comprehensive expression analysis and genome-wide mapping of androgen receptor binding sites

Analyses utilizing tissue recombination or tissue specific AR KO mouse have revealed the importance of epithelial–mesenchymal signalling and identified some of the molecules involved in male specific organ development. However, these analyses lack comprehensive information on AR downstream signaling pathways such as growth factors, and has not fully identified the molecular mechanisms that connect these growth factors and AR. Recent advances in comprehensive methods targetting the whole-genome for the identification of AR targets or cofactors have enabled new approaches to understanding AR actions. Applying these methods *in vivo* will reveal the developmental context-dependent AR downstream gene networks.

To understand the downstream gene networks of androgen signaling during sexual differentiation, comprehensive gene expression analyses using microarray or next generation sequencing have been performed with developing epididymis, prostate and GT [83,98–103]. These have correlated poorly with the candidates such as FGF ligands and BMP ligands as androgen-regulated genes [98,100]. More intriguingly, these analyses revealed that most of the gene expression regulated by androgen is tissue-, cell- and stage-specific involved in several biological pathways. For example, castration and/or DHT injection induces gene expression changes in the epididymis and prostate [99,103]. Such up- or down-regulated gene profiles are poorly identical with the profiles between caput, corpus, and cauda of the epididymis, and ventral, dorsal and lateral prostate. Thus, the presence of developmental and environmental context dependent transcriptional regulation by androgen has been suggested. Additionally, it may be that the complexity of these tissues is too high in order for a common AR signature to be deconvoluted from such different tissue types.

Recent techniques to identify genomic binding sites of transcription factors have been developed that include chromatin immunoprecipitation followed by hybridization of the immunoprecipitated DNA pool to a tiling array (ChIP-chip) or end sequencing of immunoprecipitated DNA fragments (ChIP-seq). *In vivo* global mapping of AR binding sites in normal androgen target tissue was initially reported in the epididymis [104]. These results showed that most AR binding regions harbored consensus androgen response elements (ARE) combined with multiple binding motifs of other transcription factors. Further analyses suggested that tissue specific collaborating transcription factors are required to guide AR to the appropriate chromosomal location; Foxa1 in prostate; Hnf4a in kidney; AP-2α in epididymis [105]. Such collaborating factors enable AR to achieve its tissue or stage specific functions. Elucidation of these genome-wide mappings of AR binding site and collaborating factors *in vivo* would provide more understanding for context-dependency of androgen signaling.

Genome wide analysis of AR binding sites also shed light on non-coding RNA as a target of AR. Previous reports demonstrated that AR binding sites were also associated with 63 microRNAs (miRNAs), including miR-29a [104]. These miRNAs are also targets of AR transcription in prostate development and cancer [98,106]. It is known that miRNAs are involved in a variety of cellular processes and organ development regulating large number of genes. They are initially produced as longer precursors that are processed by the RNaseIII enzyme Dicer1 to become fully functional. Conditional KO of *Dicer1* in the two most proximal segments of the mouse epididymis resulted in de-differentiation of the epididymal epithelia with reduction of AR expression [107]. Thus, miRNAs are suggested to modulate the androgen signaling in male reproductive tract development. Conversely, AR expression was suppressed by miR-29a in mouse epididymis [108]. Further analyses based on such genome wide information will enhance our understanding of the gene networks regulated by androgens during male reproductive tract development.

7. Conclusion and future prospects

Recent development of tissue-specific AR KO mouse models has revealed several cellular functions of AR during male reproductive tract masculinization. In addition, there is a growing literature regarding AR targets and signaling in specific organs and tissue compartments. Androgen action during sexually dimorphic development of the reproductive tissues is one of the most promising paradigms for understanding the key mechanisms controlled by steroid receptor signaling.

Acknowledgements

We thank Drs. Robert Kypta, Blanche Capel, Denis Duboule, Gail Prins, Richard Behringer, Barry Hinton and Hiort Olaf for their supports. We also express our appreciation to Drs. Daisuke Matsumaru, Yukiko Ogino, Shinichi Miyagawa Ryuma Haraguchi, Kentaro Suzuki, Akiko Omori and Lerrie anne Ipulan for their assistances. We thank Tomiko I. Iba for technical helps. This work is supported by Grant-in-Aid for

Young Scientists B (25860771), for Scientific Research on Innovative Areas: Molecular mechanisms for establishment of sex differences (22132006).

References

[1] A. Jost, Recherches sur la différenciation sexuelle de l'embryon de Lapin, Arch. Anat. Microsc. Morphol. Exp. 36 (1947) 151–315.
[2] S.E. Hannema, I.A. Hughes, Regulation of Wolffian duct development, Horm. Res. 67 (2007) 142–151.
[3] S.Y. Tong, J.M. Hutson, L.M. Watts, Does testosterone diffuse down the wolffian duct during sexual differentiation? J. Urol. 155 (1996) 2057–2059.
[4] M.B. Renfree, J. Fenelon, G. Wijiyanti, J.D. Wilson, G. Shaw, Wolffian duct differentiation by physiological concentrations of androgen delivered systemically, Dev. Biol. 334 (2009) 429–436.
[5] C.S. Chang, J. Kokontis, S.T. Liao, Molecular cloning of human and rat complementary DNA encoding androgen receptors, Science 240 (1988) 324–326.
[6] D.B. Lubahn, D.R. Joseph, P.M. Sullivan, H.F. Willard, F.S. French, E.M. Wilson, Cloning of human androgen receptor complementary DNA and localization to the X chromosome, Science 240 (1988) 327–330.
[7] H. Escriva, F. Delaunay, V. Laudet, Ligand binding and nuclear receptor evolution, Bioessays 22 (2000) 717–727.
[8] Y. Ogino, H. Katoh, S. Kuraku, G. Yamada, Evolutionary history and functional characterization of androgen receptor genes in jawed vertebrates, Endocrinology 150 (2009) 5415–5427.
[9] J. Li, F. Al-Azzawi, Mechanism of androgen receptor action, Maturitas 63 (2009) 142–148.
[10] H. Gronemeyer, V. Laudet, Transcription factors 3: nuclear receptors, Protein Profile 2 (1995) 1173–1308.
[11] O. Susumu, Major sex-determining genes, Springer, Berlin, 1979. 140.
[12] M.F. Lyon, S.G. Hawkes, X-linked gene for testicular feminization in the mouse, Nature 227 (1970) 1217–1219.
[13] W.G. Yarbrough, V.E. Quarmby, J.A. Simental, D.R. Joseph, M. Sar, D.B. Lubahn, K.L. Olsen, F.S. French, E.M. Wilson, A single base mutation in the androgen receptor gene causes androgen insensitivity in the testicular feminized rat, J. Biol. Chem. 265 (1990) 8893–8900.
[14] N.J. Charest, Z.X. Zhou, D.B. Lubahn, K.L. Olsen, E.M. Wilson, F.S. French, A frameshift mutation destabilizes androgen receptor messenger RNA in the Tfm mouse, Mol. Endocrinol. 5 (1991) 573–581.
[15] B. Gottlieb, L.K. Beitel, A. Nadarajah, M. Paliouras, M. Trifiro, The androgen receptor gene mutations database: 2012 update, Hum. Mutat. 33 (2012) 887–894.
[16] P.S. Cooke, P. Young, G.R. Cunha, Androgen receptor expression in developing male reproductive organs, Endocrinology 128 (1991) 2867–2873.
[17] H. Takeda, C. Chang, Immunohistochemical and in-situ hybridization analysis of androgen receptor expression during the development of the mouse prostate gland, J. Endocrinol. 129 (1991) 83–89.
[18] G.R. Cunha, E.T. Alarid, T. Turner, A.A. Donjacour, E.L. Boutin, B.A. Foster, Normal and abnormal development of the male urogenital tract. Role of androgens, mesenchymal–epithelial interactions, and growth factors, J. Androl. 13 (1992) 465–475.
[19] G.R. Cunha, Role of mesenchymal–epithelial interactions in normal and abnormal development of the mammary gland and prostate, Cancer 74 (1994) 1030–1044.
[20] G.R. Cunha, P.S. Cooke, T. Kurita, Role of stromal–epithelial interactions in hormonal responses, Arch. Histol. Cytol. 67 (2004) 417–434.
[21] G.R. Cunha, P. Young, S.J. Higgins, P.S. Cooke, Neonatal seminal vesicle mesenchyme induces a new morphological and functional phenotype in the epithelia of adult ureter and ductus deferens, Development 111 (1991) 145–158.
[22] G.R. Cunha, P. Young, Inability of Tfm (testicular feminization) epithelial cells to express androgen-dependent seminal vesicle secretory proteins in chimeric tissue recombinants, Endocrinology 128 (1991) 3293–3298.
[23] Y. Sugimura, G.R. Cunha, A.A. Donjacour, Morphogenesis of ductal networks in the mouse prostate, Biol. Reprod. 34 (1986) 961–971.
[24] P.C. Marker, A.A. Donjacour, R. Dahiya, G.R. Cunha, Hormonal, cellular, and molecular control of prostatic development, Dev. Biol. 253 (2003) 165–174.
[25] L. Huang, Y. Pu, S. Alam, L. Birch, G.S. Prins, The role of Fgf10 signaling in branching morphogenesis and gene expression of the rat prostate gland: lobe-specific suppression by neonatal estrogens, Dev. Biol. 278 (2005) 396–414.
[26] Y. Pu, L. Huang, L. Birch, G.S. Prins, Androgen regulation of prostate morphoregulatory gene expression: Fgf10-dependent and -independent pathways, Endocrinology 148 (2007) 1697–1706.
[27] Y. Sugimura, B.A. Foster, Y.K. Hom, J.H. Lipschutz, J.S. Rubin, P.W. Finch, S.A. Aaronson, N. Hayashi, J. Kawamura, G.R. Cunha, Keratinocyte growth factor (KGF) can replace testosterone in the ductal branching morphogenesis of the rat ventral prostate, Int. J. Dev. Biol. 40 (1996) 941–951.
[28] A.A. Thomson, G.R. Cunha, Prostatic growth and development are regulated by FGF10, Development 126 (1999) 3693–3701.
[29] A.A. Donjacour, A.A. Thomson, G.R. Cunha, FGF-10 plays an essential role in the growth of the fetal prostate, Dev. Biol. 261 (2003) 39–54.
[30] W. Lu, Y. Luo, M. Kan, W.L. McKeehan, Fibroblast growth factor-10. A second candidate stromal to epithelial cell andromedin in prostate, J. Biol. Chem. 274 (1999) 12827–12834.
[31] A.A. Thomson, Role of androgens and fibroblast growth factors in prostatic development, Reproduction 121 (2001) 187–195.
[32] C. Gupta, S. Siegel, D. Ellis, The role of EGF in testosterone-induced reproductive tract differentiation, Dev. Biol. 146 (1991) 106–116.
[33] C. Gupta, A. Chandorkar, A.P. Nguyen, Activation of androgen receptor in epidermal growth factor modulation of fetal mouse sexual differentiation, Mol. Cell. Endocrinol. 123 (1996) 89–95.
[34] C. Gupta, M. Singh, Stimulation of epidermal growth factor gene expression during the fetal mouse reproductive tract differentiation: role of androgen and its receptor, Endocrinology 137 (1996) 705–711.
[35] C. Gupta, The role of epidermal growth factor receptor (EGFR) in male reproductive tract differentiation: stimulation of EGFR expression and inhibition of Wolffian duct differentiation with anti-EGFR antibody, Endocrinology 137 (1996) 905–910.
[36] S.H. Freestone, P. Marker, O.C. Grace, D.C. Tomlinson, G.R. Cunha, P. Harnden, A.A. Thomson, Sonic hedgehog regulates prostatic growth and epithelial differentiation, Dev. Biol. 264 (2003) 352–362.
[37] A. Omori, S. Miyagawa, Y. Ogino, M. Harada, K. Ishii, Y. Sugimura, H. Ogino, N. Nakagata, G. Yamada, Essential roles of epithelial bone morphogenetic protein signaling during prostatic development, Endocrinology (2014) en20132054.
[38] M.L. Lamm, C.A. Podlasek, D.H. Barnett, J. Lee, J.Q. Clemens, C.M. Hebner, W. Bushman, Mesenchymal factor bone morphogenetic protein 4 restricts ductal budding and branching morphogenesis in the developing prostate, Dev. Biol. 232 (2001) 301–314.
[39] J. Tomaszewski, A. Joseph, D. Archambeault, H.H. Yao, Essential roles of inhibin beta A in mouse epididymal coiling, Proc. Natl. Acad. Sci. U. S. A. 104 (2007) 11322–11327.
[40] B. Orr, G. Vanpoucke, O.C. Grace, L. Smith, R.A. Anderson, A.C. Riddick, O.E. Franco, S.W. Hayward, A.A. Thomson, Expression of pleiotrophin in the prostate is androgen regulated and it functions as an autocrine regulator of mesenchyme and cancer associated fibroblasts and as a paracrine regulator of epithelia, Prostate 71 (2011) 305–317.
[41] C. Chang, S.O. Lee, R.S. Wang, S. Yeh, T.M. Chang, Androgen receptor (AR) physiological roles in male and female reproductive systems: lessons learned from AR-knockout mice lacking AR in selective cells, Biol. Reprod. 89 (2013) 21.
[42] X. Zhou, Roles of androgen receptor in male and female reproduction: lessons from global and cell-specific androgen receptor knockout (ARKO) mice, J. Androl. 31 (2010) 235–243.
[43] M. Welsh, L. Moffat, L. Jack, A. McNeilly, D. Brownstein, P.T. Saunders, R.M. Sharpe, L.B. Smith, Deletion of androgen receptor in the smooth muscle of the seminal vesicles impairs secretory function and alters its responsiveness to exogenous testosterone and estradiol, Endocrinology 151 (2010) 3374–3385.
[44] M. Welsh, L. Moffat, A. McNeilly, D. Brownstein, P.T. Saunders, R.M. Sharpe, L.B. Smith, Smooth muscle cell-specific knockout of androgen receptor: a new model for prostatic disease, Endocrinology 152 (2011) 3541–3551.
[45] P.A. Berry, N.J. Maitland, A.T. Collins, Androgen receptor signalling in prostate: effects of stromal factors on normal and cancer stem cells, Mol. Cell. Endocrinol. 288 (2008) 30–37.
[46] S. Yu, C.R. Yeh, Y. Niu, H.C. Chang, Y.C. Tsai, H.L. Moses, C.R. Shyr, C. Chang, S. Yeh, Altered prostate epithelial development in mice lacking the androgen receptor in stromal fibroblasts, Prostate 72 (2012) 437–449.
[47] S. Yu, C. Zhang, C.C. Lin, Y. Niu, K.P. Lai, H.C. Chang, S.D. Yeh, C. Chang, S. Yeh, Altered prostate epithelial development and IGF-1 signal in mice lacking the androgen receptor in stromal smooth muscle cells, Prostate 71 (2011) 517–524.
[48] K.P. Lai, S. Yamashita, S. Vitkus, C.R. Shyr, S. Yeh, C. Chang, Suppressed prostate epithelial development with impaired branching morphogenesis in mice lacking stromal fibromuscular androgen receptor, Mol. Endocrinol. 26 (2012) 52–66.
[49] A. Murashima, S. Miyagawa, Y. Ogino, H. Nishida-Fukuda, K. Araki, T. Matsumoto, T. Kaneko, K. Yoshinaga, K. Yamamura, T. Kurita, S. Kato, A.M. Moon, et al., Essential roles of androgen signaling in Wolffian duct stabilization and epididymal cell differentiation, Endocrinology 152 (2011) 1640–1651.
[50] A. Krutskikh, K. De Gendt, V. Sharp, G. Verhoeven, M. Poutanen, I. Huhtaniemi, Targeted inactivation of the androgen receptor gene in murine proximal epididymis causes epithelial hypotrophy and obstructive azoospermia, Endocrinology 152 (2011) 689–696.
[51] L. O'Hara, M. Welsh, P.T. Saunders, L.B. Smith, Androgen receptor expression in the caput epididymal epithelium is essential for development of the initial segment and epididymal spermatozoa transit, Endocrinology 152 (2011) 718–729.
[52] U. Simanainen, K. McNamara, R.A. Davey, J.D. Zajac, D.J. Handelsman, Severe subfertility in mice with androgen receptor inactivation in sex accessory organs but not in testis, Endocrinology 149 (2008) 3330–3338.
[53] C.T. Wu, S. Altuwaijri, W.A. Ricke, S.P. Huang, S. Yeh, C. Zhang, Y. Niu, M.Y. Tsai, C. Chang, Increased prostate cell proliferation and loss of cell differentiation in mice lacking prostate epithelial androgen receptor, Proc. Natl. Acad. Sci. U. S. A. 104 (2007) 12679–12684.
[54] U. Simanainen, C.M. Allan, P. Lim, S. McPherson, M. Jimenez, J.D. Zajac, R.A. Davey, D.J. Handelsman, Disruption of prostate epithelial androgen receptor impedes prostate lobe-specific growth and function, Endocrinology 148 (2007) 2264–2272.
[55] Y. Niu, J. Wang, Z. Shang, S.P. Huang, C.R. Shyr, S. Yeh, C. Chang, Increased CK5/CK8-positive intermediate cells with stromal smooth muscle cell atrophy in the mice lacking prostate epithelial androgen receptor, PLoS One 6 (2011) e20202.
[56] S.O. Lee, J. Tian, C.K. Huang, Z. Ma, K.P. Lai, H. Hsiao, M. Jiang, S. Yeh, C. Chang, Suppressor role of androgen receptor in proliferation of prostate basal epithelial and progenitor cells, J. Endocrinol. 213 (2012) 173–182.
[57] L.A. Ipulan, K. Suzuki, S. Matsushita, H. Suzuki, M. Okazawa, S. Jacinto, S. Hirai, G. Yamada, Development of the external genitalia and their sexual dimorphic regulation in mice, Sex Dev. (2014) (Epub ahead of print).
[58] G. Yamada, Y. Satoh, L.S. Baskin, G.R. Cunha, Cellular and molecular mechanisms of development of the external genitalia, Differentiation 71 (2003) 445–460.
[59] D. Wilhelm, P. Koopman, The makings of maleness: towards an integrated view of male sexual development, Nat. Rev. Genet. 7 (2006) 620–631.
[60] S. Miyagawa, Y. Satoh, R. Haraguchi, K. Suzuki, T. Iguchi, M.M. Taketo, N. Nakagata, T. Matsumoto, K. Takeyama, S. Kato, G. Yamada, Genetic interactions of the androgen

and Wnt/beta-catenin pathways for the masculinization of external genitalia, Mol. Endocrinol. 23 (2009) 871–880.
[61] J. Li, E. Willingham, L.S. Baskin, Gene expression profiles in mouse urethral development, BJU Int. 98 (2006) 880–885.
[62] N.B. Kalloo, J.P. Gearhart, E.R. Barrack, Sexually dimorphic expression of estrogen receptors, but not of androgen receptors in human fetal external genitalia, J. Clin. Endocrinol. Metab. 77 (1993) 692–698.
[63] R. Murakami, T. Mizuno, Proximal–distal sequence of development of the skeletal tissues in the penis of rat and the inductive effect of epithelium, J. Embryol. Exp. Morphol. 92 (1986) 133–143.
[64] E.A. Kurzrock, L.S. Baskin, Y. Li, G.R. Cunha, Epithelial–mesenchymal interactions in development of the mouse fetal genital tubercle, Cells Tissues Organs 164 (1999) 125–130.
[65] A.J. Ross, B. Capel, Signaling at the crossroads of gonad development, Trends Endocrinol. Metab. 16 (2005) 19–25.
[66] Y. Shima, K. Miyabayashi, S. Haraguchi, T. Arakawa, H. Otake, T. Baba, S. Matsuzaki, Y. Shishido, H. Akiyama, T. Tachibana, K. Tsutsui, K. Morohashi, Contribution of Leydig and Sertoli cells to testosterone production in mouse fetal testes, Mol. Endocrinol. 27 (2013) 63–73.
[67] Z.X. Zhou, M.V. Lane, J.A. Kemppainen, F.S. French, E.M. Wilson, Specificity of ligand-dependent androgen receptor stabilization: receptor domain interactions influence ligand dissociation and receptor stability, Mol. Endocrinol. 9 (1995) 208–218.
[68] J.P. Deslypere, M. Young, J.D. Wilson, M.J. McPhaul, Testosterone and 5 alpha-dihydrotestosterone interact differently with the androgen receptor to enhance transcription of the MMTV-CAT reporter gene, Mol. Cell. Endocrinol. 88 (1992) 15–22.
[69] K.S. Kim, W. Liu, G.R. Cunha, D.W. Russell, H. Huang, E. Shapiro, L.S. Baskin, Expression of the androgen receptor and 5 alpha-reductase type 2 in the developing human fetal penis and urethra, Cell Tissue Res. 307 (2002) 145–153.
[70] J.M. MORRIS, The syndrome of testicular feminization in male pseudohermaphrodites, Am. J. Obstet. Gynecol. 65 (1953) 1192–1211.
[71] J. Imperato-McGinley, R.E. Peterson, T. Gautier, E. Sturla, Androgens and the evolution of male-gender identity among male pseudohermaphrodites with 5alpha-reductase deficiency, N. Engl. J. Med. 300 (1979) 1233–1237.
[72] S. Miyagawa, A. Moon, R. Haraguchi, C. Inoue, M. Harada, C. Nakahara, K. Suzuki, D. Matsumaru, T. Kaneko, I. Matsuo, L. Yang, M.M. Taketo, et al., Dosage-dependent hedgehog signals integrated with Wnt/beta-catenin signaling regulate external genitalia formation as an appendicular program, Development 136 (2009) 3969–3978.
[73] C. Lin, Y. Yin, S.M. Bell, G.M. Veith, H. Chen, S.H. Huh, D.M. Ornitz, L. Ma, Delineating a conserved genetic cassette promoting outgrowth of body appendages, PLoS Genet. 9 (2013) e1003231.
[74] D.J. Mulholland, S. Dedhar, G.A. Coetzee, C.C. Nelson, Interaction of nuclear receptors with the Wnt/beta-catenin/Tcf signaling axis: Wnt you like to know? Endocr. Rev. 26 (2005) 898–915.
[75] D.R. Chesire, C.M. Ewing, W.R. Gage, W.B. Isaacs, In vitro evidence for complex modes of nuclear beta-catenin signaling during prostate growth and tumorigenesis, Oncogene 21 (2002) 2679–2694.
[76] D.R. Chesire, W.B. Isaacs, Ligand-dependent inhibition of beta-catenin/TCF signaling by androgen receptor, Oncogene 21 (2002) 8453–8469.
[77] R. Haraguchi, R. Mo, C. Hui, J. Motoyama, S. Makino, T. Shiroishi, W. Gaffield, G. Yamada, Unique functions of Sonic hedgehog signaling during external genitalia development, Development 128 (2001) 4241–4250.
[78] R. Haraguchi, K. Suzuki, R. Murakami, M. Sakai, M. Kamikawa, M. Kengaku, K. Sekine, H. Kawano, S. Kato, N. Ueno, G. Yamada, Molecular analysis of external genitalia formation: the role of fibroblast growth factor (Fgf) genes during genital tubercle formation, Development 127 (2000) 2471–2479.
[79] K. Suzuki, D. Bachiller, Y.P. Chen, M. Kamikawa, H. Ogi, R. Haraguchi, Y. Ogino, Y. Minami, Y. Mishina, K. Ahn, E.B. Crenshaw III, G. Yamada, Regulation of outgrowth and apoptosis for the terminal appendage: external genitalia development by concerted actions of BMP signaling [corrected], Development 130 (2003) 6209–6220.
[80] Y. Satoh, R. Haraguchi, T.J. Wright, S.L. Mansour, J. Partanen, M.K. Hajihosseini, V.P. Eswarakumar, P. Lonai, G. Yamada, Regulation of external genitalia development by concerted actions of FGF ligands and FGF receptors, Anat. Embryol. (Berl) 208 (2004) 479–486.
[81] A. Petiot, C.L. Perriton, C. Dickson, M.J. Cohn, Development of the mammalian urethra is controlled by Fgfr2-IIIb, Development 132 (2005) 2441–2450.
[82] A. Beleza-Meireles, F. Lundberg, K. Lagerstedt, X. Zhou, D. Omrani, L. Frisén, A. Nordenskjöld, FGFR2, FGF8, FGF10 and BMP7 as candidate genes for hypospadias, Eur. J. Hum. Genet. 15 (2007) 405–410.
[83] H. Nishida, S. Miyagawa, D. Matsumaru, Y. Wada, Y. Satoh, Y. Ogino, S. Fukuda, T. Iguchi, G. Yamada, Gene expression analyses on embryonic external genitalia: identification of regulatory genes possibly involved in masculinization processes, Congenit. Anom. (Kyoto) 48 (2008) 63–67.
[84] N. Hacohen, S. Kramer, D. Sutherland, Y. Hiromi, M.A. Krasnow, Sprouty encodes a novel antagonist of FGF signaling that patterns apical branching of the *Drosophila* airways, Cell 92 (1998) 253–263.
[85] O.D. Klein, G. Minowada, R. Peterkova, A. Kangas, B.D. Yu, H. Lesot, M. Peterka, J. Jernvall, G.R. Martin, Sprouty genes control diastema tooth development via bidirectional antagonism of epithelial–mesenchymal FGF signaling, Dev. Cell 11 (2006) 181–190.
[86] S.T. Ching, G.R. Cunha, L.S. Baskin, M.A. Basson, O.D. Klein, Coordinated activity of Spry1 and Spry2 is required for normal development of the external genitalia, Dev. Biol. 386 (2014) 1–11.
[87] S. Miyagawa, D. Matsumaru, A. Murashima, A. Omori, Y. Satoh, R. Haraguchi, J. Motoyama, T. Iguchi, N. Nakagata, C.C. Hui, G. Yamada, The role of sonic hedgehog-Gli2 pathway in the masculinization of external genitalia, Endocrinology 152 (2011) 2894–2903.
[88] A.J. Lorenzo, M.T. Nguyen, S. Sozubir, M. Henkemeyer, L.A. Baker, Dihydrotestesterone induction of EPHB2 expression in the female genital tubercle mimics male pattern of expression during embryogenesis, J. Urol. 170 (2003) 1618–1623 (discussion 1623).
[89] C. Dravis, N. Yokoyama, M.J. Chumley, C.A. Cowan, R.E. Silvany, J. Shay, L.A. Baker, M. Henkemeyer, Bidirectional signaling mediated by ephrin-B2 and EphB2 controls urorectal development, Dev. Biol. 271 (2004) 272–290.
[90] Y. Imai, M.Y. Youn, K. Inoue, I. Takada, A. Kouzmenko, S. Kato, Nuclear receptors in bone physiology and diseases, Physiol. Rev. 93 (2013) 481–523.
[91] L.A. Ipulan, K. Suzuki, Y. Sakamoto, A. Murashima, Y. Imai, A. Omori, N. Nakagata, R. Nishinakamura, P. Valasek, G. Yamada, Non-myocytic androgen receptor regulates the sexually dimorphic development of the embryonic bulbocavernosus muscle, Endocrinology (2014) en20141008.
[92] T.M. Sissung, D.K. Price, A. Sparreboom, W.D. Figg, Pharmacogenetics and regulation of human cytochrome P450 1B1: implications in hormone-mediated tumor metabolism and a novel target for therapeutic intervention, Mol. Cancer Res. 4 (2006) 135–150.
[93] L.E. Gray, J. Ostby, J. Furr, C.J. Wolf, C. Lambright, L. Parks, D.N. Veeramachaneni, V. Wilson, M. Price, A. Hotchkiss, E. Orlando, L. Guillette, Effects of environmental antiandrogens on reproductive development in experimental animals, Hum. Reprod. Update 7 (2001) 248–264.
[94] A. Gupta, N. Ketchum, C.G. Roehrborn, A. Schecter, C.C. Aragaki, J.E. Michalek, Serum dioxin, testosterone, and subsequent risk of benign prostatic hyperplasia: a prospective cohort study of Air Force veterans, Environ. Health Perspect. 114 (2006) 1649–1654.
[95] P.G. Febbo, M. Lowenberg, A.R. Thorner, M. Brown, M. Loda, T.R. Golub, Androgen mediated regulation and functional implications of fkbp51 expression in prostate cancer, J. Urol. 173 (2005) 1772–1777.
[96] J. Cheung-Flynn, V. Prapapanich, M.B. Cox, D.L. Riggs, C. Suarez-Quian, D.F. Smith, Physiological role for the cochaperone FKBP52 in androgen receptor signaling, Mol. Endocrinol. 19 (2005) 1654–1666.
[97] D. Choo, J. Ward, A. Reece, H. Dou, Z. Lin, J. Greinwald, Molecular mechanisms underlying inner ear patterning defects in Kreisler mutants, Dev. Biol. 289 (2006) 308–317.
[98] E.M. Schaeffer, L. Marchionni, Z. Huang, B. Simons, A. Blackman, W. Yu, G. Parmigiani, D.M. Berman, Androgen-induced programs for prostate epithelial growth and invasion arise in embryogenesis and are reactivated in cancer, Oncogene 27 (2008) 7180–7191.
[99] T.R. Chauvin, M.D. Griswold, Androgen-regulated genes in the murine epididymis, Biol. Reprod. 71 (2004) 560–569.
[100] C.J. Bowman, K.J. Turner, M. Sar, N.J. Barlow, K.W. Gaido, P.M. Foster, Altered gene expression during rat Wolffian duct development following di(n-butyl) phthalate exposure, Toxicol. Sci. 86 (2005) 161–174.
[101] K.J. Turner, B.S. McIntyre, S.L. Phillips, N.J. Barlow, C.J. Bowman, P.M. Foster, Altered gene expression during rat Wolffian duct development in response to in utero exposure to the antiandrogen linuron, Toxicol. Sci. 74 (2003) 114–128.
[102] S.E. Hannema, C.G. Print, D.S. Charnock-Jones, N. Coleman, I.A. Hughes, Changes in gene expression during Wolffian duct development, Horm. Res. 65 (2006) 200–209.
[103] F. Jiang, Z. Wang, Identification of androgen-responsive genes in the rat ventral prostate by complementary deoxyribonucleic acid subtraction and microarray, Endocrinology 144 (2003) 1257–1265.
[104] S. Hu, G. Yao, X. Guan, Z. Ni, W. Ma, E.M. Wilson, F.S. French, Q. Liu, Y. Zhang, Research resource: genome-wide mapping of in vivo androgen receptor binding sites in mouse epididymis, Mol. Endocrinol. 24 (2010) 2392–2405.
[105] P. Pihlajamaa, B. Sahu, L. Lyly, V. Aittomäki, S. Hautaniemi, O.A. Jänne, Tissue-specific pioneer factors associate with androgen receptor cistromes and transcription programs, EMBO J. 33 (4) (2014) 312–326.
[106] K.K. Waltering, K.P. Porkka, S.E. Jalava, A. Urbanucci, P.J. Kohonen, L.M. Latonen, O.P. Kallioniemi, G. Jenster, T. Visakorpi, Androgen regulation of micro-RNAs in prostate cancer, Prostate 71 (2011) 604–614.
[107] I. Björkgren, L. Saastamoinen, A. Krutskikh, I. Huhtaniemi, M. Poutanen, P. Sipilä, Dicer1 ablation in the mouse epididymis causes dedifferentiation of the epithelium and imbalance in sex steroid signaling, PLoS One 7 (2012) e38457.
[108] W. Ma, S. Hu, G. Yao, S. Xie, M. Ni, Q. Liu, X. Gao, J. Zhang, X. Huang, Y. Zhang, An androgen receptor-microrna-29a regulatory circuitry in mouse epididymis, J. Biol. Chem. 288 (2013) 29369–29381.