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Review article

Development of human male and female urogenital tracts

Gerald R. Cunha*, Laurence Baskin

University of California, San Francisco, Department of Urology, San Francisco, CA 94143, United States

1. Introduction

Animal models, principally mouse, have been used with the tacit, but unproven, assumption that morphogenetic and molecular mechanisms in animal models are relevant to human ontogeny and pathology. For many developing organs the morphogenetic processes appear to be fairly similar in humans and mice, which has led to the reasonable speculation that the underlying molecular mechanisms may also be similar if not identical in these species. However, proof of similarity of morphogenetic mechanisms requires detailed descriptions of human organogenesis so that valid human/mouse comparisons can be made. While mouse organogenesis is known in great detail, this is definitely not the case for human organogenesis, which for the most part is based upon dated studies employing histology. Moreover, many differences in human versus mouse organogenesis have been reported, and adult anatomical differences in the mouse versus human are manifestations of differences in morphogenetic processes. It is notable that many morphogenetic and anatomic differences in mice and humans exist for several urogenital organs as described below for prostate, female reproductive tract, penis, and clitoris.

2. Prostate

The verumontanum is present in both human and mouse prostates, yet their adult morphologies are radically different (Cunha et al., 2018c). The especially prominent rhabdosphincter seen in association with the verumontanum in mice does not exist in humans. The mouse prostate has a ventral lobe, which is absent in humans, and morphology and zonal/lobar organization of the prostate are substantially different in mice versus humans (Cunha et al., 2018c; Shappell et al., 2004). The human prostate is surrounded by a thick connective tissue capsule, which does not exist in the mouse.

3. Female reproductive tract

Derivation of vaginal epithelium from Müllerian versus urogenital sinus epithelium is entirely different in mouse (in which adult vaginal epithelium is exclusively derived from Müllerian epithelium (Kurita, 2010)) versus human (in which evidence demonstrates that adult human vaginal epithelium is derived from urogenital sinus epithelium as suggested previously by Bulmer (Bulmer, 1957; Robboy et al., 2017; Cunha et al., 2018c). The degree of fusion of the embryonic Müllerian ducts is extensive in humans, and in mice only involves the extreme caudal portions of the Müllerian ducts, resulting in a bicornuate uterus. Regulation of the uterine epithelial progesterone receptor is entirely different between the mouse versus human. In the mouse, the uterine epithelial progesterone receptor (PGR) is strongly expressed following ovariectomy and is down regulated upon estrogen administration, an effect mediated indirectly via the stromal estrogen receptor 1 (ESR1) (paracrine mechanism) (Kurita et al., 2000). In contrast, in humans, PGR is regulated directly via ESR1 in the uterine epithelium (Janne et al., 1975; Horwitz and McGuire, 1979; Kurita et al., 2005; Cunha et al., 2018a).

4. Penis

Development of the penile urethra is dramatically different between the mouse and human. In the mouse the proximal portion of the penile urethra forms via by direct canalization of the urethral plate (Hynes and Fraher, 2004a, 2004b; Seifert et al., 2008), while the distal aspect of the mouse penile urethra (including the urethral meatus) forms via epithelial fusion events (G. Liu et al., 2018). In contrast, in humans that portion of the urethra within the penile shaft occurs via canalization of the urethral plate to form a wide diamond-shaped urethral groove whose edges (urethral folds) fuse to form the urethra within the penile shaft (Baskin et al., 2018). Formation of the human penile urethra within the glans occurs via direct canalization of the urethral plate (G. Liu et al., 2018; X. Liu et al., 2018). Thus, while epithelial fusion events and direct canalization of the urethral plate are morphogenetic mechanisms utilized in both human and mouse penile urethral development, these two disparate mechanisms occur in completely different regions within the developing penis (G. Liu et al., 2018; X. Liu et al., 2018). Another mouse/human difference is that the mouse penis contains bone and cartilage, which are absent from the human penis. The mouse penis has a substantial anatomical process (the male urogenital mating protuberance), which extends distal to the urethral meatus, while such a protuberance does not exist in the human penis. Finally, mice have two prepuces (internal and external), while humans have one

* Corresponding author.
E-mail address: Gerald.Cunha@ucsf.edu (G.R. Cunha).

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prepuce (Blaschko et al., 2013; Phillips et al., 2015).

5. Clitoris

Human and mouse clitori are entirely different. The human clitoris develops from the ambisexual genital tubercle and exhibits considerable anatomic homology with the penis, each having a glans, a shaft and a corporal body. The mouse clitoris exhibits little anatomic homology with the mouse penis in so far as the mouse clitoris lacks defined erectile bodies and is represented as stroma enclosed within the U-shaped clitoral epithelial lamina whose morphogenesis has yet to be described (Rodriguez et al., 2011, 2012; Sinclair et al., 2016).

6. Human versus mouse development

Uncertainty regarding the congruence between mouse versus human urogenital tract development can only be resolved through detailed examination of morphogenetic processes and molecular mechanisms in these two species. Human developmental research is far behind that of the mouse for the reason that access to unfixed and fixed human embryonic and fetal organs is limited. In some states/countries research on human fetal organ is illegal. However, validation of the relevance of animal models for human development can only be achieved through direct experimentation on developing human organs both from a morphogenetic as well as a molecular perspective.

Until recently, human urogenital development has been largely based upon studies decades old that utilized standard histological methods, which are the basis of text books on human embryology commonly used in medical schools. Only recently have immunohistochemical studies revealed some of the molecules involved in human urogenital development. The understanding of urogenital organogenesis requires a comprehensive analysis of organ rudiments over a time frame from the earliest anlage extending onward to advanced development. Such temporally inclusive information does not exist for the development of any human urogenital organ. Molecular studies of human urogenital development are even more limited.

7. Human development

Over the past few years our colleagues at UCSF have devoted considerable effort in multi-disciplinary studies on development of organs of human male and female fetal urogenital tracts (bladder, prostate, male and female external genitalia, uterus, vagina, and uterine tube) with the goal of describing the ontogeny of human reproductive tracts from the earliest anlage stages possible (6–8 weeks of gestation) to advanced development at about 20 weeks of gestation. For these studies we employed a wide range of techniques (histology, gross morphology, scanning electron microscopy, optical projection tomography, Lightsheet microscopy ™, immunohistochemistry, xenografting and epithelial-mesenchymal tissue recombinations). These studies have advanced our understanding of human reproductive tract development and set the stage for detailed molecular studies.

8. Macroscopic whole-mounts

The paper by Shen et al. entitled “Macroscopic whole-mounts of the developing human fetal urogenital-genital tract: Indifferent stage to male and female differentiation” is an essential primer for those interested in development of male and female human fetal reproductive tracts (Shen et al., 2018a). Surgical termination of pregnancy is typically a disruptive process, which presents challenges in identifying and isolating developing organs whose morphology changes with time. This paper by Shen et al. presents a compilation of wholemount photographs and morphometric measurements of the developing human prostate, female internal genitalia, bladder, kidney, testes, ovaries, and male and female external genitalia that will aid the investigator interested in pursuing human reproductive tract development.

9. Three-dimensional imaging

The paper by Isaacson et al. entitled “Three-dimensional imaging of the developing human fetal urogenital-genital tract: indifferent stage to male and female differentiation“ is a primer on three-dimensional imaging techniques to visualize the developing human fetal urogenital tract: optical projection tomography, scanning electron microscopy and Lightsheet ™ fluorescence microscopy (Isaacson et al., 2018). Three-dimensional (3D) imaging coupled with two-dimensional (2D) histologic and immunohistochemical imaging is particularly informative. In some cases, specimens used to acquire 3D images can be retrieved, embedded in paraffin, sectioned and processed for immunohistochemistry.

10. Human prostate development

The paper by Cunha et al. entitled “Development of the human prostate” provides a detailed compilation of human prostatic development that includes human fetal prostatic gross anatomy, histology, and ontogeny of selected epithelial and mesenchymal differentiation markers and signaling molecules throughout the five stages of human prostatic development: (a) pre-bud urogenital sinus (UGS), (b) emergence of solid prostatic epithelial buds from urogenital sinus epithelium (UGE), (c) prostatic bud elongation and branching, (d) canalization of the solid prostatic “ducts”, (e) differentiation of luminal and basal epithelial cells, and (f) secretory cytodifferentiation (Cunha et al., 2018c). Xenograft experiments are described that are designed to assess the actions of androgens and estrogens on human fetal prostatic development. A new model of de novo dihydrotestosterone-induced prostatic development from xenografts of human fetal female urethras emphasizes the utility of the xenograft approach for experimental in vivo initiation of human prostatic development. These studies raise the possibility of molecular mechanistic studies on human prostatic development through the use of tissue recombinants composed of mutant mouse UGM combined with human fetal prostatic epithelium. Advances in the morphogenetic and molecular biology of human prostatic development is surely relevant to the pathogenesis of benign prostatic hyperplasia and prostate cancer as the neoformation of ductal-acinar architecture during development is shared at some level with comparable processes in the pathogenesis of benign prostatic hyperplasia and prostate cancer.

11. Human female development

The paper by Cunha et al. entitled “Development of the human female reproductive tract” reviews the literature of development of the human uterine tube, uterine corpus, uterine cervix and vagina from the ambisexual stage to advanced development at 22 weeks (Cunha et al., 2018b). Historically, this topic has been under represented in the literature, and for the most part is based upon hematoxylin and eosin stained sections. Recent immunohistochemical studies for PAX2 (reactive with Müllerian epithelium) and FOXA1 (reactive with urogenital sinus epithelium) supports Bulmer's interpretation that adult human vaginal epithelium derives solely from urogenital sinus epithelium (Bulmer, 1957), while organs of the upper female reproductive tract (cervix, uterus and uterine tube) are derived from the Müllerian ducts (Robboy et al., 2017). Epithelial and mesenchymal differentiation markers are described during the course of human female reproductive tract development and include keratins, homeobox proteins (HOXA11 and ISL1), steroid receptors (estrogen receptor alpha and progesterone receptor), transcription factors and signaling molecules (TP63 and RUNX1). Most of these differentiation markers are expressed in a temporally and spatially dynamic fashion. The utility of xenografts and epithelial-mesenchymal tissue recombination studies are also reviewed.

12. Human bladder development

Liaw et al. reviewed the development of the human bladder and the ureterovesical junction (Liaw et al., 2018). The bladder and ureterovesical junction form during the fourth to eighth weeks of gestation, and arise from the primitive urogenital sinus following subdivision of the cloaca. The bladder develops through mesenchymal-epithelial interactions between the endoderm of the urogenital sinus and mesenchyme of mesodermal origin. Key molecules in bladder development include Shh, TGF-β, Bmp4, and Fgfr2 that play a key role is the development of smooth muscle of the detrusor. The ureterovesical junction forms from an interaction between the Wolffian duct and the developing bladder. The ureteric bud arises from the Wolffian duct and is incorporated into the developing bladder at the trigone. Following emergence of the ureters from the Wolffian ducts, extensive epithelial remodeling brings the ureters to their final trigonal positions via vitamin A-induced apoptosis. Perturbation of this process is implicated in clinical urinary obstruction or urine reflux. Congenital malformations of the bladder, ureters and the ureterovesical junction are discussed.

13. Human penis and clitoral development

Human penile and clitoral development are described in a paper entitled “Development of the Human Penis and Clitoris“ by (Baskin et al., 2018). In this paper, macroscopic photography, optical projection tomography, light sheet microscopy, scanning electron microscopy, histology and immunohistochemistry were employed to investigate human penile and clitoral development and to emphasize developmental differences between human and mouse (Baskin et al., 2018). The human penis and clitoris develop from the ambisexual genital tubercle. The human genital tubercle differentiates into a penis under the influence of androgens forming a tubular urethra within the penile shaft which develops via canalization of the urethral plate to form a wide diamond-shaped urethral groove (opening zipper) whose edges (urethral folds) fuse in the midline (closing zipper). In contrast, in females, without the influence of androgens, the vestibular plate (homologue of the urethral plate) undergoes canalization to form a wide vestibular groove whose edges (vestibular folds) remain unfused, ultimately forming the Labia minora defining the vaginal vestibule. Immunohistochemistry was used to reveal sex differences of key epithelial and mesenchymal differentiation markers and signaling molecules during human penile versus clitoral development. Development of neurovascular anatomy is similar in both the developing human penis and clitoris and is the key to successful surgical reconstructions.

14. Human glans development

Urethral development within the human penile shaft develops via (1) an “Opening Zipper” that facilitates distal canalization of the solid urethral plate to form a wide urethral groove and (2) a “Closing Zipper” that facilitates fusion of the epithelial surfaces of the urethral folds. Previous examination of hematoxylin and eosin stained sections suggested that urethral development within the human glans penis occurs via an entirely different morphogenetic mechanism than that in the penile shaft (G. Liu et al., 2018). In a paper by Liu et al. presented herein we demonstrate that the urethra within the human penile glans forms via direct canalization of the urethral plate to form a tubular urethra without forming an open urethral groove (X. Liu et al., 2018). The developmental process was revealed by scanning electron microscopy and optical projection tomography as well as examination of serial histologic sections immunostained for epithelial differentiation markers: cytokeratins 6, 7, 10, FoxA1, uroplakin and the androgen receptor. Initially, within the glans the urethral plate is attached ventrally to the epidermis via an epithelial seam, which is remodeled and eliminated, thus establishing midline mesenchymal confluence ventral to the glandular urethra. Epithelial remodeling during this process involves the strategic expression of cytokeratin 7, FoxA1 and uroplakin in endodermal epithelial cells as the tubular glandular urethra forms. The prepuce initially forms on the dorsal aspect of the glans at approximately 12 weeks of gestation with the appearance of a thickening of dorsal penile epidermis called the preputial placode. After sequential proximal to distal remodeling of the urethral plate along the ventral aspect of glans, the preputial folds fuse in the ventral midline.

15. Human mid sagittal immuno-histochemistry

Finally, the paper by Shen et al. entitled, “Immunohistochemical expression analysis of the human fetal lower urogenital tract,” provides a unique perspective of human urogenital development through immunohistochemical studies on midsagittal sections of developing human male and female urogenital tracts from 9 weeks (indifferent stage) to 16 weeks (advanced sex differentiation) of gestation (Shen et al., 2018b). For this purpose immunohistochemistry was performed with antibodies to epithelial, muscle, nerve, proliferation and hormone receptor markers. Key findings are: (1) The corpus cavernosum in males and females extends into the glans penis and clitoris, respectively, during the ambisexual stage (9 weeks) and thus appears to be an androgen-independent event. (2) The entire human male (and female) urethra is endodermal in origin based on expression of FOXA1, keratin 7, uroplakin, and the absence of keratin 10 staining. Endoderm of the urethra interfaces with ectodermal epidermis at the site of the urethral meatus. (3) The surface epithelium of the verumontanum is endodermal in origin (FOXA1-positive) with a possible contribution of PAX2-positive epithelial cells implying additional input from the Wolffian duct epithelium. (4) Prostatic ducts arise from the endodermal (FOXA1-positive) urogenital sinus epithelium near the verumontanum. (5) Immunohistochemical staining of mid-sagittal and para-sagittal sections revealed the external anal sphincter, levator ani, bulbospongiosus muscles and the anatomic relationships between these developing skeletal muscles and organs of the male and female reproductive tracts.

16. Conclusion

Successful analysis of human organogenesis requires 4 essential elements: (1) Legal status for performing research on human fetal material. (2) An adequate source of human material and cooperation with the surgical team. (3) An adequate intellectual background on the basics of developmental biology, ideally on mouse as well as human development. (4) Access to modern analytical techniques. Our group is ideally positioned to perform such work. We have realized that while textbooks of human embryology provide a solid background on human organogenesis, they do not present sufficient detail, particularly on molecular mechanisms. In our opinion, solid descriptions of developmental anatomy are the essential starting point and basis for future molecular studies. Immunohistochemistry, using the most modern of imaging techniques, is an important tool that can elucidate key molecules and molecular pathways in the context of three-dimensional organogenesis. We believe that our recent studies of normal human urogenital developmental lay the foundation for revealing molecular mechanisms of human urogenital development, highlight human/mouse differences, and provide an understanding of the etiology of congenital anomalies of the lower human urogenital tract.

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