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Contrasting mechanisms of penile urethral formation in mouse and human

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ABSTRACT

This paper addresses the developmental mechanisms of formation of the mouse and human penile urethra and the possibility that two disparate mechanisms are at play. It has been suggested that the entire penile urethra of the mouse forms via direct canalization of the endodermal urethral plate. While this mechanism surely accounts for development of the proximal portion of the mouse penile urethra, we suggest that the distal portion of the mouse penile urethra forms via a series of epithelial fusion events. Through review of the recent literature in combination with new data, it is unlikely that the entire mouse urethra is formed from the endodermal urethral plate due in part to the fact that from E14 onward the urethral plate is not present in the distal aspect of the genital tubercle. Formation of the distal portion of the mouse urethra receives substantial contribution from the preputial swellings that form the preputial-urethral groove and subsequently the preputial-urethral canal, the later of which is subdivided by a fusion event to form the distal portion of the mouse penile urethra. Examination of human penile development also reveals comparable dual morphogenetic mechanisms. However, in the case of human, direct canalization of the urethral plate occurs in the glans, while fusion events are involved in formation of the urethra within the penile shaft, a pattern exactly opposite to that of the mouse. The highest incidence of hypospadias in humans occurs at the junction of these two different developmental mechanisms. The relevance of the mouse as a model of human hypospadias is discussed.

1. Introduction

Formation of the penile urethra has been studied in rodents with the tacit, but unproven, assumption that developmental processes in rodent models are relevant to human penile development. Clearly there are some developmental events shared between species such as the formation of a solid urethra plate within the embryonic genital tubercle (GT), which is observed in mouse, rat, rabbit, tamar wallaby, spotted hyena and human (Armfield et al., 2016; Li et al., 2015; Hynes and Fraher, 2004a; Kluth et al., 2011; Agras et al., 2006, 2007b, 2007a; Cunha et al., 2014; Butler et al., 1999; Kurzrock et al., 2000). Most of the mouse penile urethra forms by direct canalization of the urethral plate (Hynes and Fraher, 2004a, 2004b; Seifert et al., 2008). We have suggested that the distal portion of the mouse penile urethra, including the urethral meatus, develops via multiple fusion events (Baskin et al., 2001; Sinclair et al., 2016a). This idea regarding formation of the distal portion of the mouse penile urethra has emerged through a focused anatomical analysis of the developing and adult mouse penile urethra and has been dealt with superficially in a series of recent papers (Sinclair et al., 2016b, 2016a; Yang et al., 2010; Mahawong et al., 2014a, 2014b; Blaschko et al., 2013). In this paper this idea is pursued through review of previous studies augmented with additional new observations.

Human penile urethral development occurs via a radically different process that starts with the formation of the solid urethral plate, which extends into the developing glans. The urethral plate within the developing penile shaft canalizes to form an open diamond-shaped urethral groove whose edges (urethral folds) subsequently fuse in the midline in a proximal to distal direction to form the penile urethra (Li et al., 2015; Shen et al., 2016). This process of urethral groove formation and subsequent urethral fold fusion forms the human penile urethra within the penile shaft. However, scanning electron micrographs demonstrate that the urethral groove does not extend into the glans suggesting that formation of the human glandar urethra might occur via a substantially different developmental process (Li et al., 2015; Shen et al., 2016).

The goal of this paper is to explore the morphogenetic mechanisms of penile urethral development in mice and humans from the perspec-

Abbreviations: MUMP, male urogenital mating protuberance; E, embryonic; P, postnatal; K, keratin; GT, genital tubercle; OPT, optical projection tomography; PUG, preputial-urethral groove; PUC, preputial-urethral canal; VPG, ventral penile groove; AR, androgen receptor

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Table 1
Antibodies used in this study.

Antibody	Source	Catalogue #	Concentration
Keratin 6	Acris Antibodies	AM21068PU-S	1/200
Keratin 7	E.B. Lane[a]	LP1K	1/10
Keratin 8	E.B. Lane[a]	LE41	1/10
Keratin 10	Dako	M7002	1/50
Keratin 14	BioGenex	LL002	1/100
Keratin 19	E.B. Lane[a]	LP2K	1/10
Uroplakin1	T. T. Sun[b]		1/100
Foxa1	Atlas Antibodies	HPA050505	1/500
Androgen receptor	Genetex	GTX62599	1/100

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Table 2
List of human specimens.

Code	Heel-Toe (mm)	Age (weeks)
AC324	13.5	13
AC560	14	14
AC302	15.3	15
AC280	16.0	16
AC416	17.0	17

tive of: (a) direct canalization of the urethral plate and (b) epithelial fusion events. Our studies suggest but that in these two species both morphogenetic mechanisms occur, but in different proximal-distal regions of the developing penis. Accordingly, the relevance of mouse penile development as a model relevant to human penile development will be discussed.

2. Materials and methods

2.1. Mouse studies

The University of California, San Francisco (UCSF) Institutional Animal Care and Use Committee approved all animal protocols. Timed-pregnant CD-1 mice (Charles River Breeding Laboratories, Wilmington, MA, USA) were housed in polycarbonate cages ($20 \times 25 \times 47\ cm^3$) with laboratory-grade pellet bedding in the UCSF Pathogen Specific Barrier housing facility. The mice were given Purina lab diet (#5058) and tap water ad libitum. They were acclimated to 20° to 23 °C and 40–50% humidity on a schedule of 14 h light and 10 h dark. Embryos and neonates were collected at the following ages: embryonic days 14, 15, 16, 17, and 18/birth. At least 3 mice were used for each time point. Additional mice at these time points were used for immunohistochemical studies. Accordingly this paper is based upon analysis of 45 mice.

External genitalia were dissected and photographed using a digital camera, and were then fixed in 10% buffered formalin. Samples were paraffin embedded and serially sectioned at 7 μm for histological staining with hematoxylin and eosin (H & E). Immunohistochemistry (IHC) was carried out as previously described (Rodriguez et al., 2012) on sections of mouse and human external genitalia using the antibodies indicated in Table 1. Signal detection was achieved using either the Vector ABC System (Vector Laboratories, Foster City, CA, USA) followed by exposure to diaminobenzidine (Sigma®). Alternatively, immunoflourescent detection was carried out following incubation with goat anti-rabbit fluorophore-conjugated secondary antibodies (diluted 1:500, Abcam) for 1 h at room temperature. Sections exposed to all steps except the application of the primary antibodies were used as negative controls.

Metrics of pertinent key morphological features were obtained by counting the number of serial transverse sections from the first section of the distal tip of the GT to the solid or canalized urethral plate (urethra). Actual distances were converted to microns from the distal

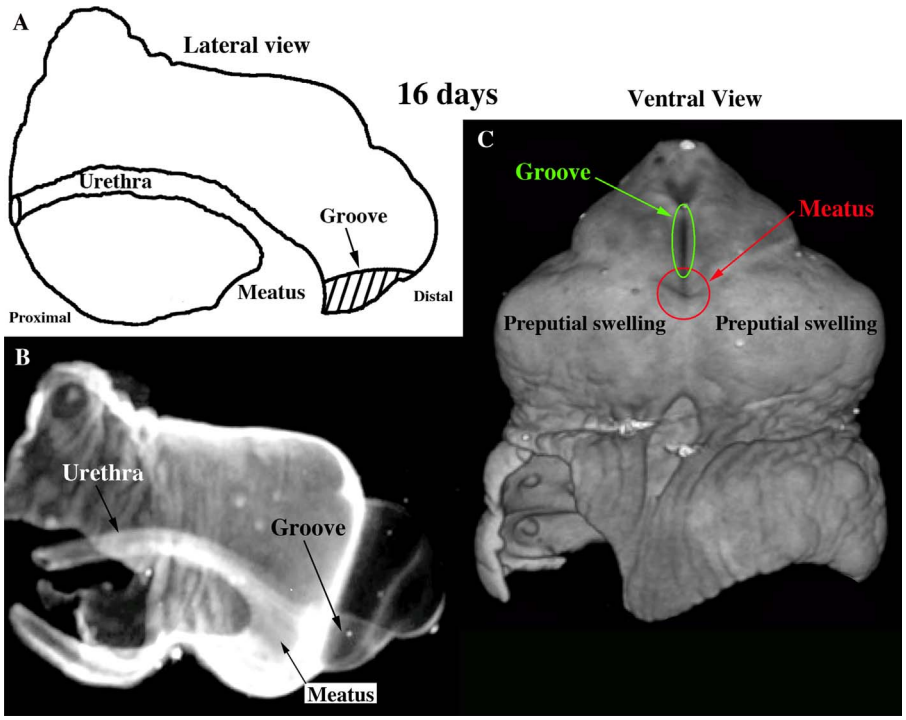

Fig. 1. Diagrammatic (A) and optical projection tomography (OPT) images of the E16-day embryonic genital tubercle. Image (B) is a semi-transparent lateral view of an E16-day external genitalia stained with an antibody to E-cadherin in which the canalized urethral plate (urethra) can be seen opening to the exterior into a groove a considerable distance from the distal tip of the GT. (A) is a diagrammatic representation of (B). (C) is an ventral OPT surface rendering of specimen (B) in which the urethral meatus and ventral penile groove can be seen. Modified from Sinclair et al. (2016b) with permission.