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Molecular mechanisms of development of the human fetal female reproductive tract

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ABSTRACT

Human female reproductive tract development rests mostly upon hematoxilyn and eosin stained sections despite recent advances on molecular mechanisms in mouse studies. We report application of immunohistochemical methods to explore the ontogeny of epithelial and mesenchymal differentiation markers (keratins, homobox proteins, steroid receptors), transcription factors and signaling molecules (TP63 and RUNX1) during human female reproductive tract development. Keratins 6, 7, 8, 10, 14 and 19 (KRT6, KRT7, KRT8, KRT10, KRT14, KRT19) were expressed in a temporally and spatially dynamic fashion. The undifferentiated Müllerian duct and uterovaginal canal, lined by simple columnar epithelia, expressed KRT7, KRT8 and KRT19. Glandular derivatives of the Müllerian duct (uterine tube, uterine corpus and endocervix) maintained expression of these keratins, while tissues that undergo stratified squamous differentiation (exocervix and vagina) expressed KRT6, KRT14 and KRT10 during development in an age-dependent fashion. TP63 and RUNX1 were expressed prior to KRT14, as these two transcription factors are known to be upstream from KRT14 in developing Müllerian epithelium. In the vagina, KRT10, a marker of terminal differentiation, appeared after endogenous estrogens transformed the epithelium to a thick glycogenated squamous epithelium. Uroplakin, a protein unique to urothelium, was expressed only in the bladder, urethra and vaginal introitus, but not in the female reproductive tract itself. Mesenchymal differentiation was examined through immunostaining for HOXA11 (expressed in uterine mesenchyme) and ISL1 (expressed in vaginal mesenchyme). A detailed ontogeny of estrogen receptor alpha (ESR1), progesterone receptor (PGR) and the androgen receptor (AR) provides the mechanistic underpinning for the teratogenicity of estrogens, progestins and androgens on female reproductive tract development. Immunohistochemical analysis of differentiation markers and signaling molecules advance our understanding of normal development of the human female reproductive tract. These observations demonstrate remarkable similarities in mouse and human female reproductive tract development, but also highlight some key differences.

1. Introduction

Parenchyma of the female reproductive tract develops from epithelia of the Müllerian duct and urogenital sinus (Orvis and Behringer, 2007; Guioli et al., 2007; Koff, 1933; Kurita and Nakamura, 2008; Bulmer, 1957; Robboy et al., 2017; Robboy and Mutter, 2014; Mutter and Robboy, 2014). The bilateral Müllerian ducts develop as invaginations of coelomic epithelium forming ductal structures that grow caudally down the urogenital ridges to join the endodermal urogenital sinus (UGS) (Kobayashi and Behringer, 2003; Jaubert et al., 2009; Robboy et al., 2017). As the right and left Müllerian ducts approach the UGS, they fuse in the midline to form the uterovaginal canal. The degree of midline fusion of the Müllerian ducts varies with the species. In rats and mice only the caudal portions of the Müllerian ducts fuse in the midline to form the cervix and the Müllerian vagina. Unfused Müllerian ducts in rats and mice form bilateral oviducts, bilateral uterine horns and cervical canals. In humans, the Müllerian ducts undergo extensive fusion to form the midline uterovaginal canal

Abbreviations: MD, Müllerian duct; WD, Wolffian duct; UGS, urogenital sinus; UGE, urogenital sinus epithelium; MDE, Müllerian epithelium; ESR1, estrogen receptor alpha; PGR, progesterone receptor; AR, androgen receptor; KRT, keratin; H & E, hematoxylin and eosin

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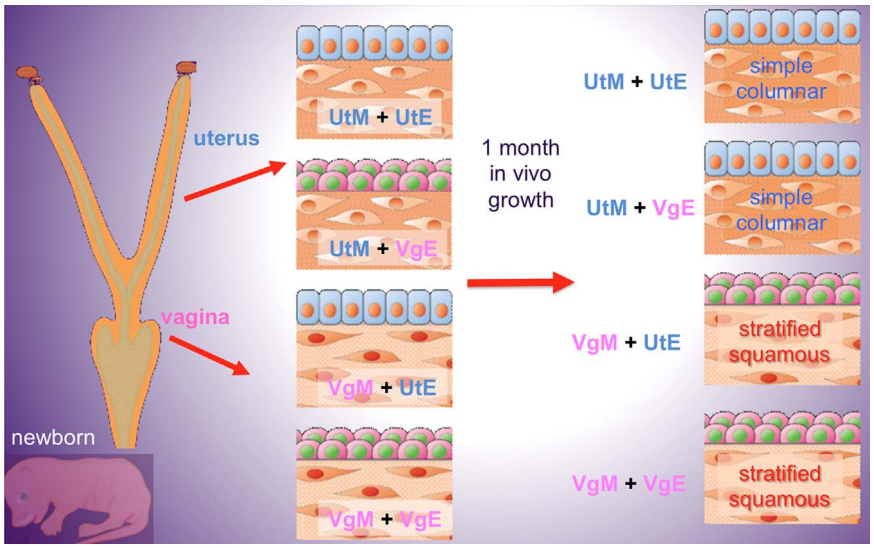

Fig. 1. Schematic of tissue recombinants between epithelium and mesenchyme of the neonatal mouse uterus and vagina. UtM = uterine mesenchyme, VgM = vaginal mesenchyme, UtE = uterine epithelium. VgE = vaginal epithelium.

destined to form the uterine fundus, uterine corpus, uterine cervix and vagina (Kobayashi and Behringer, 2003; Koff, 1933; Kurita and Nakamura, 2008; Robboy et al., 2017; Moore and Persaud, 2003). Relatively short bilateral uterine tubes are all that remain of the individual right and left human Müllerian ducts.

The process of vaginal development in mice differs somewhat from that in humans. At birth the so-called Müllerian vagina (fused Müllerian ducts) is in contact with a dorsal projection of the UGS, the called the sinus vagina (Kurita, 2011). The sinus vagina is a transient epithelial structure that maintains a connection with the Müllerian vaginal epithelium throughout development. Cell lineage tracing studies carried out in mice (Kurita, 2010) have shown that as organogenesis progresses, the sinus vagina is gradually reduced in size relative to the Müllerian vagina. Indeed, at puberty when the vagina opens to the exterior, urogenital sinus epithelium (UGE) was detected only in the vulva, and not in the vagina. Thus, in mice adult vaginal epithelium is derived solely from Müllerian duct epithelium (MDE) (Kurita, 2010).

The contribution of Müllerian versus urogenital sinus epithelium (UGE) to human vaginal epithelium has been debated for over half a century. The two most popular theories of derivation of human vaginal epithelium are based upon the analysis of H & E-stained histological sections (Bulmer, 1957; Koff, 1933). Koff concluded that epithelium of the upper 4/5^{ths} of the vagina is lined with Müllerian-derived epithelium, while the lower 1/5th of the vagina is derived from UGE (Koff, 1933). Bulmer asserts that UGE replaces Müllerian epithelium (MDE) of the lower uterovaginal canal, and thus concluded that human vaginal epithelium is exclusively derived from UGE (Bulmer, 1957). Using immunohistochemical markers of Müllerian epithelium (PAX2) and urogenital sinus epithelium (FOXA1), we have shown that during the course of human vaginal development the PAX2/FOXA1 boundary within the vaginal rudiment progressively extends cranially so that at 21 weeks of gestation epithelial FOXA1 staining was observed from the vaginal introitus to the cervix. Thus, our immunohistochemical studies reported in a companion article (Robboy et al., 2017) support Bulmer's proposal (1957) that human vaginal epithelium derives solely from UGE.

The cellular and molecular mechanisms of epithelial differentiation in Müllerian duct-derived organs have been extensively studied utilizing animal models. In mouse, the epithelium of the midline fused Müllerian ducts (the anlagen of the Müllerian vagina) is undifferentiated and simple columnar at 16.5 days of gestation, whereas the sinus vagina consists of a solid dorsal epithelial cord expressing TRP63 (specifically the ΔN isoform) and keratin 14 (KRT14), markers of squamous epithelium (Kurita and Cunha, 2001; Kurita et al., 2005, 2004). In the course of development the epithelium of the Müllerian vagina expresses ΔNp63 followed temporally by keratin 14 during conversion of the simple columnar epithelium of the Müllerian vagina into stratified squamous epithelium. This process progresses from caudal to cranial within the Müllerian vagina, and is completed around postnatal day 7 (Kurita and Cunha, 2001; Kurita et al., 2005, 2004). The potential importance of ΔNp63 in human vaginal development comes from immunohistochemical studies demonstrating expression of TP63 during human vaginal development (Kurita et al., 2005; Fritsch et al., 2012, 2013).

Epithelial differentiation in the cervix/vagina versus that of the uterus is induced and specified by the associated mesenchyme. This conclusion is based upon heterotypic tissue recombinants of epithelium and mesenchyme derived from the neonatal mouse uterus and vagina (Cunha, 1976). Homotypic vaginal (VgM + VgE) and uterine (UtM + UtE) tissue recombinants express vaginal or uterine epithelial differentiation as expected (Fig. 1). In heterotypic tissue recombinants vaginal mesenchyme induces uterine epithelium to undergo vaginal epithelial differentiation (VgM + UtE⇨vaginal differentiation), and uterine mesenchyme induces vaginal epithelium to undergo uterine epithelial differentiation (UtM + VgE⇨uterine differentiation). These remarkable mesenchyme-induced transformations in epithelial differentiation involve morphological as well as molecular changes in epithelial differentiation (Table 1). For example, induction of vaginal epithelial differentiation in VgM + UtE tissue recombinants involves epithelial induction of ΔNp63 and KRT14 in uterine epithelium (Kurita et al., 2001, 2004).

Studies from Kurita's lab have documented the molecular mechanisms underlying mesenchymal induction of vaginal epithelial differentiation. Mouse genetic studies established that ΔNp63 is the determining transcription factor of cervicovaginal epithelial cell fate. Thus, in *Trp63* null mice, the epithelium of the Müllerian vagina remains simple columnar epithelium and expresses uterine instead of cervicovaginal markers (Kurita et al., 2004; Laronda et al., 2013). During normal development vaginal mesenchyme induces ΔNp63 expression in MDE and subsequent vaginal epithelial differentiation by activating 3 independent and essential signaling pathways present in MDE: (a) BMP4-SMAD, (b) activin A-RUNX1 (runt-related transcription factor 1) and (c) FGF7/10-MAPK pathways (Kurita et al., 2004; Laronda et al., 2013; Terakawa et al., 2016). When BMP4-SMAD, activin A, RUNX1 or FGF7/10-MAPK pathways were disrupted