Developmental origin of vaginal epithelium

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ABSTRACT

The developmental origin of vaginal epithelium has been controversial for nearly a century, with speculation that vaginal epithelium originates from the Müllerian duct, Wolffian duct, and/or urogenital sinus. None of these possibilities have been definitively proven or disproven by direct scientific data. To define precisely the origin of vaginal epithelium, epithelial cells of the Müllerian duct, Wolffian duct, or urogenital sinus were fluorescently labeled in mouse embryos by crossing tdTomato-EGFP dual-reporter transgenic mice with transgenic mouse lines that express Cre-recombinase in each type of epithelium. In embryos and newborn mice, the vagina consisted of fused Müllerian ducts plus the sinus vagina of urogenital sinus origin. However, the proportion of the sinus vagina was significantly reduced as the Müllerian vagina grew caudally. By postpartum day 7, the Müllerian vagina extended to the caudal end of the body, whereas the sinus vagina remained only at the junction between the vagina and perineal skin. As the vagina opened in puberty, urogenital sinus epithelium was detected only in the vulva, but not in the vagina. Additionally, from embryo to adult stages, residual Wolffian duct epithelium was present in the dorsolateral stromal wall of the vagina, but not within vaginal or vulvar epithelium. In conclusion, adult mouse vaginal epithelium is derived solely from Müllerian duct epithelium.

1. Introduction

The majority of the mammalian female reproductive tract develops from common embryonic structures, the paramesonephric or Müllerian ducts (MDs) (reviewed in Kobayashi and Behringer, 2003; Kurita and Nakamura, 2008; O’Rahilly, 1973; Witschi, 1959; Yin and Ma, 2005). The MDs form as invaginations of coelomic epithelium into the urogenital ridge mesenchyme, and the formed ducts then grow caudally through urogenital ridge mesenchyme in close apposition to the mesonephric or Wolffian ducts (WDs) (Koff, 1933; Witschi, 1959; Guioli et al., 2007; Orvis and Behringer, 2007; Kobayashi et al., 2005, 2004). In females, the caudal tip of the MDs reaches the urogenital sinus (UGS) and fuses with the vaginal bulbs, which are solid epithelial cords on the caudal tip of the MDs, and the lower portion (sinus vagina) develops from the UGS (Koff, 1933; Bloomfield and Frazer, 1927). Residual portions of the WDs are also present near the MD/UGS junction (Hart, 1901; Bloomfield and Frazer, 1927; Koff, 1933; Mauch et al., 1985; Grünwald, 1941). The union of these structures forms a flat epithelial cord called the vaginal plate (Koff, 1933). It has been proposed that a significant portion of the vagina forms by simultaneous growth and canalization of the vaginal plate. However, the degree of contribution of the MDs, WDs, and UGS to formation of the vaginal bulb and plate, as well as the adult vagina continues to be debated.

In order to understand the mechanisms underlying normal and abnormal development of lower female urogenital tracts, it is essential that the cellular origin of vaginal epithelium be definitively resolved. There are four major models for the developmental origin of vaginal epithelium. The most widely accepted of these is the “UGS+MD origin” model, in which the upper two-thirds of the vagina (Müllerian vagina) develops from the caudal portion of the MDs, and the lower portion (sinus vagina) develops from the UGS (Moore and Persaud, 2002; Sadler, 2004; Koff, 1933; Forsberg, 1963, 1973; Kobayashi and Behringer, 2003; Yin and Ma, 2005; Shapiro et al., 2000; Gilbert, 2003; Del Vecchio, 1982; Cunha, 1975). In this model, the vaginal bulb consists solely of epithelium of UGS origin, and is thus referred to as the “sinovaginal bulb”. Therefore, the lower vagina develops through growth and canalization of UGS epithelium (UGE) (Koff, 1933; Forsberg, 1963). However, according to the alternative “MD origin” and “MD+WD origin” models, the vaginal bulb/plate is derived from the MDs (Bloomfield and Frazer, 1927; Cai, 2009) or WDs (Forsberg, 1963; Witschi, 1970; Drews, 2007), and thus the vagina develops from either the MDs alone or MDs plus WDs (Hart, 1901; Bloomfield and Frazer, 1927; Mauch et al., 1985; Sánchez-Ferrer et al., 2006). Finally, the UGS origin model...
suggests that the entire squamous epithelium of the cervix and vagina are derived solely from UGE (Arey, 1954; Bulmer, 1957, 1959; Ferris, 2004; Fliegner, 1994; Zuckerman, 1940). In this view, squamous epithelium derived from the UGS grows upward and replaces the original columnar epithelium of MD origin.

All of the models described above are based upon anatomical/histological observations, with the boundaries of structures inferred from indirect data. Some studies employed histochemical and immunohistochemical analyses to determine the developmental origin of vaginal epithelium. However, the characteristics of epithelial cells change as they differentiate, and thus gene expression profiles at different time points are not definitive markers for cellular developmental origin. For example, simple columnar epithelium of the neonatal mouse uterus can transdiffer­entiate to express squamous cell markers when induced by vaginal mesenchyme (Cunha, 1976; Boutin et al., 1991, 1989; Kurita et al., 2001, 2004). To determine developmental origin of vaginal epithelium, a cell lineage tracing experiment is essential (Stern and Fraser, 2001). To label a cell population in mouse embryos with a tracer, dual transgenic mouse strategies with a site-specific recombinase [e.g. Cre-recombinase (Cre)] and its reporter transgenes have been developed (Zinyk et al., 1998; Branda and Dymecki, 2004). In this system, a transgenic mouse strain expressing a site-specific recombinase in a particular cell type is intercrossed with a reporter mouse strain harboring a transgene that indicates the recombination event by expression of a reporter gene (e.g. β-galactosidase or fluorescent protein). By this method, the cell fate of recombinase-positive cells can be traced by permanent expression of the reporter. However, since the transgene can be activated in multiple cell lineages at different time points, reporter-expression in two cell types does not necessarily indicate their cell-lineage relationship. Recently, Grieshammer et al. demonstrated that embryonic/neonatal (from E17.5 to postpartum day 1) mouse vaginal bulbs were positive for Osr1-Cre, which is expressed in UGE but not in MDE or WD epithelium (WDE) (Grieshammer et al., 2008). This result strongly suggests that the lower part of vagina is of UGS origin. However, the Osr1-Cre transgene may be activated in the MDE and WDE as they differentiate into vaginal bulbs; thus, the MD and/or WD origin of vaginal bulbs cannot be completely discounted. Since the progenies of reporter-positive cells are always positive for the reporter, any possible contribution of MD and WD to the vaginal bulb can be excluded if the vaginal bulb is negative for the reporter when MDE and WDE are labeled. The precise cellular origin of vaginal epithelium can be determined only by mutually exclusive results from cell lineage tracing experiments for MDE, WDE, and UGE. In this study, mouse epithelial cells of embryonic WD, MD, or UGS origin were permanently labeled to express enhanced green fluorescent protein (EGFP), and their developmental fate was followed from the embryo to the adult stages.

2. Methods

2.1. Animals

All procedures involving animals were approved by the Animal Care and Use Committee of Northwestern University. Dual-reporter tdTomato-EGFP mice (Muzumdar et al., 2007) and Hoxb7-Cre transgenic [Tg(Hoxb7-cre)13Amc/J] mice (Yu et al., 2002) were purchased from the Jackson Laboratory (Bar Harbor, ME). Pax2-Cre transgenic [Tg(Pax2-cre)1Akg] (Ohyama and Groves, 2004) and Osr1-Cre transgenic [FVB/N-Tg(Osr1-cre)4Mrt/Mmmh] (Grieshammer et al., 2008) mice were purchased from Mutant Mouse Regional Resource Centers (http://www.mmrrc.org/index.html). Mice were housed and bred in a controlled barrier facility within Northwestern University’s Center of Comparative Medicine. Temperature, humidity, and photoperiod (12 h light, 12 h dark) were kept constant. Animals were allowed access to food and water ad libitum. Most, if not all, cells in the dual-reporter tdTomato-EGFP mice fluoresce red due to the expression of a red fluorescent protein, tdTomato (Shaner et al., 2004) driven by the chicken β-actin promoter. Excision of the floxed tdTomato sequence by Cre-recombinase allows expression of EGFP, and cells that were once red fluoresce green. Dual-reporter tdTomato-EGFP mice were intercrossed with the three transgenic mouse lines expressing Cre-recombinase in MDE and WDE (Pax2-Cre) (Ohyama and Groves, 2004), UGE (Osr1-Cre) (Grieshammer et al., 2008), or WDE (Hoxb7-Cre) (Yu et al., 2002; Kobayashi et al., 2005). Mouse genotype was determined by examination of EGFP expression in the kidney/ureter (Pax2-Cre and Hoxb7-Cre), or bladder (Osr1-Cre) at the time of tissue collection, and then confirmed by PCR. At least 8 mice from each strain were analyzed at embryonic day (E) 15–16, E17–18, postpartum day (P) 0–1, P2–3, P4–5, P6–7, P8–9, P10–12, P19–22, P28–30 (4 weeks), and P60–63 (2 months).

2.2. Fluorescence microscopy

To identify EGFP-positive cells, the vagina was dissected, and the tissue was flattened between two histology slides and scanned for the presence of green fluorescence under an inverted fluorescence microscope using 5× and 10× objectives. Fluorescence and phase-contrast microscopy images were captured using a SteREO Discovery V12 microscope with a fluorescence module (Carl Zeiss, Chicago, IL) and an Axio Observer microscope (Carl Zeiss). Captured images were merged using the automated photomerge function of Adobe Photoshop CS (Adobe, San Jose, CA).

2.3. Immunofluorescence

Tissues were fixed in 4% paraformaldehyde-phosphate-buffered saline (pH 7.4) and processed into paraffin blocks. Tissue sections were cut at 6 μm, mounted on HistoBond Adhesive Slides (VWR Internations, West Chester, PA), deparaffinized, and rehydrated through a series of xylene and ethanol. The sections were heated at 95 °C for 40 min in citrate buffer pH 6.0 (Shi et al., 1993) prior to addition of anti-GFP (Abcam, Cambridge, MA), anti-p63 (Santa Cruz Biotechnology, Santa Cruz, CA), and/or anti-Pax2 (COVANCE, Princeton, NJ) antibodies. Secondary antibodies conjugated with DyLight 488 or 549 were purchased from Jackson ImmunoResearch Laboratory (West Grove, PA).

3. Results

3.1. The vaginal bulb is of UGS origin

Dual-reporter tdTomato-EGFP mice were intercrossed with the three transgenic mouse lines expressing Cre-recombinase in MDE and WDE (Pax2-Cre) (Ohyama and Groves, 2004), UGE (Osr1-Cre) (Grieshammer et al., 2008), or WDE (Hoxb7-Cre) (Yu et al., 2002; Kobayashi et al., 2005). The expression of EGFP in the respective tissues was confirmed at E15 (not shown).

At E17, the MDs, WDs, and vaginal bulb were identifiable by phase-contrast microscopy (Fig. 1a–c). In female Pax2-Cre/tdTomato-EGFP reporter embryos, MDE and WDE fluoresced green, but EGFP did not localize to the vaginal bulb epithelium (Fig. 1d), indicating that the vaginal bulb is not derived from the MDs or WDs. The absence of WDE in vaginal bulb epithelium was confirmed in Hoxb7-Cre reporter mice (Fig. 1e). In contrast,