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Developmental Origin of Vaginal Epithelium

Takeshi Kurita

Division of Reproductive Biology Research, Department of Obstetrics and Gynecology,
Northwestern University Feinberg School of Medicine, Chicago, Illinois 60611

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 has 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.

Keywords

vagina; epithelium; Müllerian duct; urogenital sinus; sinovaginal bulb; vulva

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, 2007 #3299; Kobayashi et al., 2005; Kobayashi et al., 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 dorsal wall of the UGS (Koff, 1933; Bloomfield and Frazer, 1927). Residual portions of the WDs are also present near the MD/UGS junction

To whom correspondence is addressed: Takeshi Kurita, Ph.D., Department of Obstetrics and Gynecology, Division of Reproductive Biology Research, Northwestern University Feinberg School of Medicine, 4th Floor, Suite 4-127, 303 East Superior Street, Chicago, Illinois 60611, Phone: 312-503-0525, Fax: 312-503-0095, t-kurita@northwestern.edu.

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(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; Boutin et al., 1989; Kurita et al., 2001; Kurita et al., 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 day1) 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.

Methods

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).

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 5x and 10x 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 photo-merge function of Adobe Photoshop CS (Adobe, San Jose CA).

Immunofluorescence

Tissues were fixed in 4% paraformaldehyde phosphate-buffered saline (pH 7.4) and processed into paraffin blocks. Tissue sections were cut at 6 μ m, mounted on HistoBond Adhesive Slides (VWR International, 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).

Results

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, EGFP-positive cells were present in the vaginal bulb and urethra in Osr1-Cre reporter embryos, whereas MDE and WDE were EGFP-negative (Fig. 1f). These EGFP expression patterns indicate that the vaginal bulb originates from UGE, and that the embryonic vagina consists of “Müllerian vagina” and “sinus vagina.” Streaks or fragments of EGFP-positive WDE were observed in the dorsolateral stromal wall of the vagina in all female embryos (Fig. 1d and e, asterisks), but not in the epithelium of either Müllerian or sinus vagina.

Dual origin of neonatal mouse vaginal epithelium

At birth (P0), the boundary between the epithelia of the Müllerian and sinus vaginae was clearly defined by areas of red and green fluorescence in Pax2-Cre and Osr1-Cre reporter mice (Fig. 2a and b). Residual WDE was detected in the stromal wall of the vagina of Hoxb7-Cre reporter mice; however, the integrity and size of WDE varied widely, and some mice contained only small fragments of WDE (Fig. 2c). By P3, the proportion of the sinus vagina was significantly reduced as the Müllerian vagina grew caudally. During this period the junction between the vagina and urethra also moved caudally as the UGS split coronally into the sinus vagina and the urethra (Fig. 2d). Epithelial cells of the sinus vagina formed a plate-like epithelial cord (Fig. 2e–g), equivalent to the vaginal plate in the human fetus (Koff, 1933). Whereas the sinus vaginal epithelium-derived vaginal plate never formed a lumen during the caudal growth of the vagina, the Müllerian vaginal epithelium never formed a solid cord. Thus, the lumen of lower vagina formed as the Müllerian vagina grew caudally, displacing the sinus vagina caudally (Fig. 2d and e).

In neonatal mice, the vaginal canal was lined with MDE, which was EGFP-positive in Pax2-Cre reporter mice (Fig. 3d), but EGFP-negative in Osr1-Cre reporter mice (Figs. 2e and 3e–g). Dual immunofluorescence detection of Pax2 and EGFP in Osr1-Cre, Pax2-Cre, and Hoxb7-Cre reporter mice confirmed that Pax2 is expressed in MDE and WDE, but not in the sinus vaginal epithelium throughout neonatal development of the vagina (not shown). In contrast, p63, a master regulator of vaginal epithelial differentiation (Kurita et al., 2001; Kurita et al., 2004), was expressed in both Müllerian and sinus vaginal epithelia, but not in WDE (Fig. 2f–h). MDE and WDE were positively identified by Pax2 immunostaining in the vagina of all reporter strains, which further confirmed that the vaginal canal is lined with MDE (Fig. 2g and h). Residual WDE was in direct contact with sinus vaginal epithelium in some areas, and the intrusion of single WD epithelial cells into the vaginal plate was occasionally observed (Fig. 2f, arrow). However, the distribution of WDE in the sinus vagina was restricted to the site of contact.

By P8, the Müllerian vagina extended to the caudal (perineal) end of the body (future site of vaginal orifice), and the entire vaginal epithelium was positive for EGFP in Pax2-Cre reporter mice (Fig. 3a). Epithelium of UGS origin targeted by Osr1-Cre, in contrast, was detected only

at the junction between the vagina and perineal skin (Fig. 3b). In *Hoxb7*-Cre reporter mice, streaks or fragments of WDE were still detectable around the boundary between the sinus and Müllerian vaginae, but not in vaginal epithelium (Fig. 3c). In prepubertal mice (P12), the vaginal canal was lined with MDE expressing p63 and Pax2, which was EGFP-positive in Pax2-Cre reporter mice (Fig. 3d) but EGFP-negative in *Osr1*-Cre reporter mice (Fig. 3e–g). The epithelium of the sinus vagina, which was positive for p63 but negative for Pax2, remained solid at the junction between the perineal body surface and vagina (Fig. 3d–f).

Adult vaginal epithelium is derived solely from MDE

Presence/absence of EGFP positive cells were examined at the cellular level in the lower genital tract of pubertal mice (4-week-old) when the vagina opened. In Pax2-Cre reporter mice, the entire vaginal epithelium was strongly positive for EGFP indicative of MDE derivation, and the expression of EGFP abruptly stopped at the vulva (Fig. 4a and c). *Osr1*-Cre reporter mice showed a complementary pattern of EGFP expression in the vulvar epithelium but not the vagina (Fig. 4b and d). In *Hoxb7*-Cre reporter mice, vaginal and vulvar epithelia were negative for EGFP (not shown). In all three reporter-strains, the expression patterns of EGFP in the vaginal epithelium at puberty were maintained in mature females (2-months-old). These data indicate that the vaginal epithelium of the adult mouse is solely derived from MDE.

Discussion

The emergence of gene-targeted mutagenesis studies of mice has unquestionably expanded our knowledge of the developmental biology of the female reproductive tract (Kobayashi and Behringer, 2003; Yin and Ma, 2005; Kurita and Nakamura, 2008). Nevertheless, the developmental origin of vaginal epithelium has been debated for decades without definitive proof. While many embryology textbooks advocate the MD+UGS model (Russell, 1989; Moore and Persaud, 2002; Sadler, 2004; Gilbert, 2003; Carlson, 1999; Forsberg, 1978), the present cell lineage tracing experiment precisely determined that the entire epithelium of adult mouse vagina is derived solely from MDE. Mutually exclusive expression patterns of EGFP in *Osr1*-Cre and Pax2-Cre reporter mice confirmed this conclusion. Although UGE does not contribute to the adult vagina, the sinus vagina connects the MDE and urethral epithelia and guides the caudal growth of Müllerian vagina to the perineal surface of the body. Thus, the UGS plays a critical role in the development of the vagina by providing the path for caudal growth of MD. In the conventional MD and MD+WD origin models, the vaginal bulbs/plate were proposed to arise from the MDs or WDs (Bloomfield and Frazer, 1927; Cai, 2009; Drews et al., 2002). However, this study found that the MDs and WDs do not contribute epithelial cells to the vaginal bulb and plate. Although the WDs play a critical role in the caudal growth of the MDs within the urogenital ridge (Grünwald, 1941; Kobayashi et al., 2005), their role in subsequent vaginal development is unlikely.

Although the canalization of the solid vaginal plate has been previously described (Forsberg, 1965; Hunter, 1930; Koff, 1933), this study clearly demonstrates that the epithelium in the sinus vagina remains solid throughout development. Instead, the lower vagina forms via caudal growth of the Müllerian vagina, which is a flat tube. Previous studies by Drews et al. described this process accurately (Drews et al., 2002; Mauch et al., 1985; Drews, 2007). At birth, the mouse Müllerian vagina is lined primarily with columnar epithelium. Thus, the upper part of vaginal epithelium develops via transformation of columnar MDE into squamous vaginal epithelium, as previously described (Kurita and Cunha, 2001; Kurita et al., 2004). In contrast, the epithelium in the lower vagina is squamous by the time of lumen formation (Kurita et al., 2001; Kurita et al., 2004), indicating that squamous differentiation of MDE precedes its caudal growth. Indeed, a small number of cells positive for p63 was present in the Müllerian vagina at its junction with the sinovaginal bulb (Kurita et al., 2004; Kurita et al., 2005). Hence, the

lower part of the vagina forms via proliferation of p63-positive epithelial cells at the caudal end of the Müllerian vagina. In the mouse, the solid epithelial cord in the sinus vagina canalizes only during the formation of the vaginal opening, and the vaginal plate becomes a part of the vulvar epithelium. This process appears to occur parallel to the formation of the hymen in the human fetus, which has been proposed to involve a perforation of the vaginal plate (Hunter, 1930; Bloomfield and Frazer, 1927).

In conclusion, the organogenesis of the vagina revealed in this study provides definitive clarification of the origin of vaginal epithelium. This knowledge regarding normal vaginal development will lead to a better understanding of various congenital abnormalities within the female urogenital tract. For example, complete longitudinal vaginal septum (Haddad et al., 1997; Gearhart et al., 2004) can be explained as an incomplete fusion of two Müllerian ducts. The differential embryonic origin of the vagina (from the MDs) and hymen (from the UGS) may explain the etiology of human congenital abnormalities in which a distinct hymen is present despite the absence of a vagina (Shaw et al., 1983). Finally, the present study calls into question the accuracy of the current theories for the developmental origin of transverse vaginal septum. Transverse vaginal septum has been explained to be a result of the incomplete perforation of vaginal plate, or the improper fusion of sinus and Müllerian vaginae (Levy et al., 1997; Gibson, 2003; Burke, 2005). However, neither the perforation of vaginal plate nor fusion of sinus and Müllerian vaginae occurs in normal development of vagina. The new model of normal vaginal development favors an alternative theory in which the transverse vaginal septum develops via an abnormal proliferation and ingrowth of vaginal stromal wall (Deppisch, 1972; Wenof et al., 1979; Kanagasuntheram and Dassanayake, 1958).

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Figure 1.

Expression of EGFP in embryonic MDE, WDE, and UGE. Phase-contrast (a-c), phase-contrast + fluorescence (d-f) images are shown. The Müllerian vagina (MV), vaginal bulb (VB), and WDs (indicated by *) were present in the E17 vagina (a-c). EGFP signal identifies epithelial cells originating from the WDs and MDs in Pax2-Cre (d), WDs in Hoxb7-Cre (e), and UGS in Osr1-Cre (f) reporter mice. The dotted lines indicate the outline of the VB. While the entire VB epithelium was positive for EGFP in Osr1-Cre reporter mouse embryos (f), EGFP was absent in the VB of Pax2-Cre and Hoxb7-Cre reporter mouse embryos, indicating that the VB arises from the UGS.

Figure 2.

Cellular origin of neonatal vaginal epithelium. EGFP expression was analyzed in female Pax2-Cre (expressed in MDE+WDE, a), Osr1-Cre (expressed in UGE, b, d, e, g and h), and Hoxb7-Cre (expressed in WDE, c and f) reporter mice on P0 (a-c) and P3 (d-h). Longitudinal (e) and transverse (f-h) sections of the female genital tract were stained with DAPI (blue) and the expression of EGFP (green) and p63 (red) in Hoxb7-Cre reporter mice (f) or Pax2 (green) and p63 (red) in Osr1-Cre reporter mice (g-h) were analyzed by dual-immunofluorescence.

On P0, the vagina consisted of Müllerian vagina (MV, a) and sinus vagina (SV, b). Fragments of WDE were also present at the junction of MV and SV (c, indicated by *). On P3, EGFP was detected only in the solid epithelium but not in the canalized epithelium in Osr1-Cre reporter mice (d, e). Thus, the vaginal canal in the lower vagina formed by caudal growth of the MV. Also, UGE in the SV (in Osr1-Cre reporter mice) was found only at the caudal end of vagina (the junction between the SV and MV is indicated by the arrows in d and e). The epithelia in the SV and urethra (UR; positive for EGFP in Osr1-Cre reporter mice) were still connected within external genitalia (EG, d).

Transverse images (f-h) represent P3 vaginal tissue sections from caudal (f, connected with urethra) to cranial (h, junction between SV and MV). The solid epithelium of the SV formed a plate-like cord (g, h, p63-positive and Pax2-negative). In contrast, epithelium in the MV formed a flat, tube shape (h, Pax2- and p63-positive [in the basal layer]; the dual-positive cells for pax2 and p63 appear yellow). The WDE (EGFP-positive in Hoxb7-Cre reporter mice, indicated by *) was negative for p63. The WDE was occasionally detected in association with the SV at its caudal end (f, Intrusion of a single WD epithelial cell into the SV is indicated by the arrow). In other sections of the vagina, the WDE was located within the stroma separated from vaginal epithelium (g, Pax2-positive and p63 negative).

Figure 3.

Distribution of MDE, WDE, and UGE in the lower reproductive tract of the female neonatal mouse. Expression of EGFP was analyzed in female Pax2-Cre (a, d), Osr1-Cre (b, e), and Hoxb7-Cre (c) reporter mice at P8 (a-c) and P12 (d-g). In Pax2-Cre reporter mice, the entire epithelium in the vagina (VG), uterus (UT), and cervix (CVX) was positive for EGFP, indicating its MDE origin (a). Solid epithelium of UGS origin was present at the junction between vagina and external genitalia (EG) (b). Streaks/fragments of residual WDE were still present in the dorsal wall of the lower vagina in Pax2-Cre and Hoxb7-Cre reporter mice (a, c, indicated by *).

Expression of EGFP, Pax2, and p63 at the junction of the Müllerian vagina (MV) and sinus vagina (SV) was assessed by dual-immunofluorescence of transverse sections (d-g, dorsal side up, bar = 50 μ m). As observed in earlier stages, the vaginal canal was lined with MDE, which was EGFP-positive in Pax2-Cre reporter mice (d) but EGFP-negative in Osr1-Cre reporter mice (e-g). Throughout the development of the vagina, Pax2 was expressed in epithelial cells of the MDs but was of UGS origin; therefore, the vaginal canal was lined with Pax2-positive epithelium of MD origin (e).

Figure 4.

Distribution of MDE, WDE, and UGE in the vagina and vulva at puberty. The distribution of EGFP-positive cells was examined in the vaginal openings of 4-week-old female Pax2-Cre (a) and Osr1-Cre (b) reporter mice. MDE and UGE were distributed in a complementary pattern; MDE was present in the vagina (vg) but not the vulva (vu), whereas UGE was present in the vulva but not the vagina. The presence of EGFP-positive cells in vaginal epithelium was examined through the surface of the entire vagina in Pax2-Cre (c), Hoxb7-Cre (not shown), and Osr1-Cre (d) reporter mice. Caudal is to the left in panels c and d. EGFP-positive cells were present in the vaginal epithelium of Pax2-Cre, but not Osr1-Cre or Hoxb7-Cre, reporter mice. Bars = 0.5 mm.