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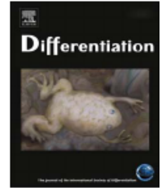

Tissue interactions and estrogenic response during human female fetal reproductive tract development

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ABSTRACT

The role of tissue interactions was explored to determine whether epithelial differentiation within the developing human reproductive tract is induced and specified by mesenchyme in tissue recombinants composed of mouse vaginal mesenchyme + human uterine tubal epithelium (mVgM+hTubE). The tissue recombinants were grown in DES-treated ovariectomized athymic mice. After 2–4 weeks of in vivo growth, several vaginal specific features were expressed in the human tubal epithelium. The mesenchyme-induced effects included morphological change as well as expression of several immunohistochemical markers. Although the mesenchyme-induced shift in vaginal differentiation in the human tubal epithelium was not complete, the partial induction of vaginal markers in human tubal epithelium verifies the importance of mesenchymal-epithelial interactions in development of the human female reproductive tract.

In a separate experiment, DES-induction of uterine epithelial progesterone receptor (PGR) and estrogen receptor 1 (ESR1) was explored in tissue recombinants composed of wild-type or *Esr1*KO mouse uterine mesenchyme + human fetal uterine epithelium (wt UtM+hUtE and *Esr1*KO UtM+hUtE). The rationale of this experiment was to determine whether DES-induction of PGR and ESR1 is mediated directly via epithelial ESR1 or indirectly (paracrine mechanism) via mesenchymal ESR1. DES-induction of uterine epithelial ESR1 and PGR in *Esr1*KO UtM+hUtE tissue recombinants (devoid of mesenchymal ESR1) formally eliminates the paracrine mechanism and demonstrates that DES induction of human uterine epithelial ESR1 and PGR is directly mediated via epithelial ESR1.

1. Introduction

The tacit, but usually unproven, assumption inherent in animal models is that they are reflective of human biology. This approach is generally useful, even though substantial differences exist between human and animal anatomy, development and pathology. One field for which animal/human pathology is particularly congruent is the effects of exogenous estrogens on the developing female reproductive tract. Administration of the potent synthetic estrogen, diethylstilbestrol (DES), to pregnant women from the 1940s to the 1970s resulted in a broad spectrum of estrogen-induced malformations of the uterine tubes, uterine corpus, cervix and vagina that include T-shaped uterotubal junctions, malformed incompetent cervix, abnormally shaped endometrial cavity, vaginal adenosis as well as clear cell vaginal adenocarcinoma (Jefferies et al., 1984; Rennell, 1979; Stillman, 1982; Titus-Ernstoff et al., 2010; Herbst et al., 1971, 1975; Robboy et al., 1977, 1984, 2018; Hoover et al., 2011). An immense animal literature preceded/confirmed the effects of exogenous estrogens on female reproductive tract development. In addition, animal studies have provided a molecular underpinning for the teratogenic effects of exogenous estrogens on urogenital development (Herbst and Bern, 1981; Bern and Talamantes, 1981; Bern et al., 1984; McLachlan et al., 1975, 2001; McLachlan, 1981; Newbold et al., 1983; Newbold and MaLachlan, 1985; Newbold, 1995, 2004, 2008; McLachlan and Newbold, 1996; Kurita et al., 2004; Kurita, 2011; Laronda et al., 2012, 2013). The animal literature on this topic is replete with

Abbreviations: ESR1, Estrogen receptor alpha; DES, diethylstilbestrol; PGR, progesterone receptor

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estrogen-induce vaginal, cervical, uterine and tubal (oviductal) anomalies including cervicovaginal adenosis. While animal models are useful and relevant to many aspects of human biology/pathology, they are frequently the basis of governmental policy designed to protect human health. Accordingly, whenever possible it is important to establish by experimental means the relevance and predictability of animal studies to human biology/pathology.

Even though mouse-human similarities are now appreciated in estrogen-induced anomalies in the female reproductive tract, the molecular mechanisms that lead to human malformations remain enigmatic, despite some clues from animal studies (McLachlan and Newbold, 1996; Kurita et al., 2004; Kurita, 2011; Laronda et al., 2012, 2013; Terakawa et al., 2016). Direct experimentation on xenografts of human fetal female reproductive tracts treated with DES have provided important insights into the genesis of human malformations and have provided essential bio-endpoints of estrogenic endocrine disruptors. In this regard, we pioneered xenograft methods in 1982 in which human fetal female reproductive tracts were grown in athymic mouse hosts treated with DES and other hormonally active agents (Cunha et al., 1987b, 1987a; Robboy et al., 1982; Taguchi et al., 1983). Unfortunately molecular and immunohistochemical advances, not yet discovered, prevented exploration of biological mechanisms in our earlier studies. More recently, we have revisited human female reproductive tract development in a series of three papers that included a compendium of differentiation markers and how DES administration affects them in vivo (Cunha et al., 2017a, 2017b; Robboy et al., 2017).

Studies carried out over 40 years ago established that uterine and vaginal mesenchyme induces and specifies epithelial differentiation (Cunha, 1976; Kurita, 2010, 2011; Kurita et al., 2001, 2005). Accordingly, vaginal mesenchyme instructively induces uterine epithelium to undergo vaginal epithelial differentiation (VgM+UtE⇝vaginal differentiation), and uterine mesenchyme instructively induces vaginal epithelium to undergo uterine epithelial differentiation (UtM+VgE⇝uterine differentiation). These inductive effects involve both morphological as well as molecular effects on the target epithelium. During vaginal development in mice, inductive cues from vaginal mesenchyme elicit epithelial expression of ΔN p63 (an isoform of p63) in Müllerian epithelium, which specifies vaginal squamous epithelial differentiation (Kurita and Cunha, 2001; Kurita et al., 2004, 2005; Terakawa et al., 2016). p63 is a member of the p53 family of transcription factors. Likewise, immunohistochemical detection of ΔNp63 in fetal and adult human vaginal epithelium suggests a similar role of ΔNp63 in human vaginal epithelial differentiation (Kurita et al., 2005; Fritsch et al., 2012, 2013; Cunha et al., 2017a). The current paper explores the role of mesenchymal-epithelial interactions in a tissue recombinant model consisting of mouse vaginal mesenchyme + human fetal uterine tube epithelium (mVgM+hTubE). The rationale for this particular experimental model is that expression of several differentiation markers is vastly different in vaginal versus tubal epithelium.

In both human and mouse female reproductive organs, estrogen receptor 1 (ESR1; also known as estrogen receptor α) is the dominant receptor for estrogen (Matsuzaki et al., 1999; Dupont et al., 2000). Earlier, we detailed the ontogeny of ESR1 during human fetal uterine development (Cunha et al., 2017a), demonstrating that ESR1 is first expressed in mesenchymal cells of human uterine corpus. Indeed, ESR1-immunoreactivity in uterine epithelial cells is rarely seen before the 21st gestational week, when endogenous estrogen levels are elevated (Oakey, 1970), thus suggesting that uterine epithelial ESR1 may be estrogen induced. Analysis of human fetal uterine xenografts treated with DES has verified this prediction (Cunha et al., 2017b). However, given that prior to DES treatment, ESR1 was detected in uterine mesenchyme and not epithelium, there are two potential mechanisms of DES induction of uterine epithelial ESR1: (a) DES may induce epithelial ESR1 directly via epithelial ESR1 whose expression is below the sensitivity of immunohistochemistry. (b) Alternatively, DES may induce epithelial ESR1 indirectly via mesenchymal ESR1 (paracrine mechanism). The same question is relevant to DES induction of epithelial progesterone receptor (PGR) in the developing human female reproductive tract (Cunha et al., 2017b).

The goal of the current paper based on our prior work (Cunha et al., 2017a; Robboy et al., 2017) is to use xenograft models (a) to determine the role of mesenchymal-epithelial interactions in epithelial differentiation during human female reproductive tract development, and (b) to determine whether estrogen regulates human uterine epithelial ESR1 and PGR via direct or paracrine mechanisms using tissue recombinants composed of human fetal uterine epithelium combined with mouse uterine mesenchyme derived from wild-type or *Esr1* knockout (*Esr1*KO) mice.

2. Materials and methods

2.1. General comments

The Committee on Human Research at UCSF (IRB# 12–08813) approved the collection of human fetal specimens devoid of patient identifiers after elective termination of pregnancy. Fetal age was estimated using heel-toe length (Drey et al., 2005). Gender was determined by Wolffian and Müllerian duct morphology as previously described (Robboy et al., 2017). Female internal genitalia were identified and isolated from the abortus specimen using a dissecting microscope. For this study 10 human fetal specimens were used at 8, 9, 10, 12, 13, 14, and 18 weeks of gestation.

2.2. Response of human fetal grafts of uterine corpus to DES in vivo

Intact human fetal reproductive tracts containing the uterine tube, uterine corpus, uterine cervix and vagina were grown for 4 weeks under the renal capsule of untreated and DES-treated (20 mg DES subcutaneous pellet) of ovariectomized female athymic mice as described previously (Cunha et al., 2017b). Histology and immunohistochemistry for ESR1 and PGR were performed on tissue sections as described below.

2.3. Preparation of heterotypic tissue recombinants

Tissue recombinant studies included: (a) mouse vaginal mesenchyme + human uterine tubal epithelium (mVgM+hTubE) and (b) wild-type or *Esr1*KO mouse uterine mesenchyme + human fetal uterine epithelium (wt UtM+hUtE and *Esr1*KO UtM+hUtE). For mVgM+hTubE tissue recombinants, mouse vaginal mesenchyme was isolated from 3-day-old neonatal mice and the tube epithelium was derived from 12 to 13 week specimens. To explore regulation of human uterine epithelial ESR1 and PGR, uterine mesenchyme was isolated from 5-day-old wild-type and *Esr1*KO neonatal mice and the human uterine epithelium was derived from 10 to 12 week specimens. Heterozygous male and female *Esr1*KO mice, a gift from Drs. Pierre Chambon and Andrée Krust, were bred to produce the *Esr1*KO neonatal mice, which were genotyped as described previously (Dupont et al., 2000). Tissue recombinant and xenografting methods have been described previously (Cunha, 1976; Cunha and Baskin, 2016). For mVgM+hTubE, wt UtM+hUtE and *Esr1*KO UtM+hUtE tissue recombinants, all hosts were ovariectomized at the time of grafting and were treated with a 20 mg DES pellet or were untreated (sham). For hosts bearing mVgM+hTubE tissue recombinants, the rationale for treating the hosts with DES was to promote stratified squamous vaginal differentiation. In response to DES grafts of human fetal uterine tube remain simple columnar, while grafts of human fetal vagina differentiate a thick glycogenated stratified epithelium (Cunha et al., 2017b). Thus, should vaginal differentiation be elicited in human tubal epithelium by mouse vaginal mesenchyme, epithelial differentiation should be distinctive. After 2 or 4 weeks of growth under the renal capsules, the tissue recombinants were

Table 1
Antibodies used in this study.

Antibody #	Source	Catalogue #	Dilution
AR (Androgen receptor)	GeneTax	EPR1535(2)	1/100
ESR1 (Estrogen receptor 1)	Dako	Ab16660	1/100
KRT14 (Keratin 14)	BioGenex	LL002	1/100
KRT19 (Keratin 19)	EB Lane	LP2K	1/100
PGR (Progesterone receptor)	Abcam	Ab16661	1/100
RUNX1 (runt-related transcription factor 1)	Abcam	Ab92336	1/100
TP63 (Tumor protein 63)	Santa Cruz Biotechnology	sc-8343	1/100

harvested, fixed in 10% buffered formalin, embedded in paraffin and serially sectioned at 7 µm. Every twentieth section was stained with hematoxylin and eosin. Remaining paraffin sections were utilized for immunohistochemical staining with the antibodies indicated (1) (Table 1).

Immunostaining was detected using horseradish peroxidase-based Vectastain kits (Vector Laboratories, Burlingame, CA). Negative controls lacked the primary antibody. Tissue recombinants composed of mouse mesenchyme and human epithelium were stained with Hoechst dye 33,258 to verify the species origin of the tissues as described (Cunha and Vanderslice, 1984). This study is based upon the analysis of 18 mVgM+hTubE, 6 wt UtM+hUtE and 5 *Esr1*KO UtM+hUtE tissue recombinants (Table 2).

3. Results

3.1. Epithelial and mesenchymal differentiation in grafts of human fetal female reproductive tracts grown in DES-treated ovariectomized female hosts

3.1.1. General comments

Age is an important factor affecting expression of epithelial differentiation markers (Cunha et al., 2017a). All human fetal specimens used for tissue recombinant studies were 12–13 weeks of gestation old at which time epithelial differentiation markers are beginning to be expressed, well before terminal epithelial differentiation.

3.1.2. Tissue recombinants composed of mouse vaginal mesenchyme plus human uterine tube epithelium (mVgM+hTubE)

Previous studies in mice have shown that differentiation of

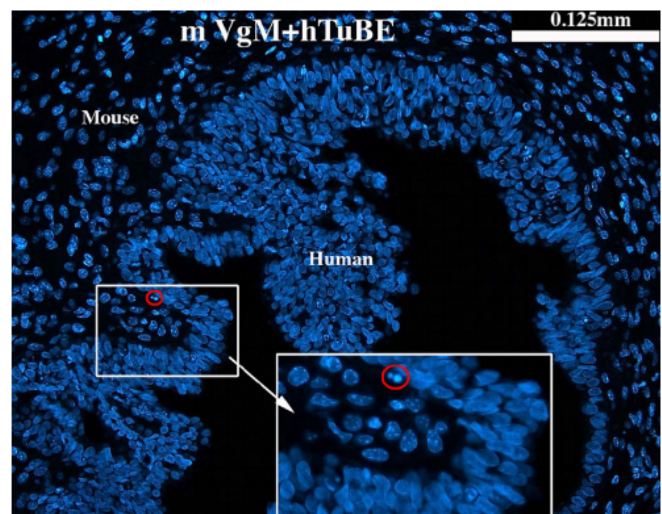

Fig. 1. Section of a tissue recombinant composed of neonatal mouse vaginal mesenchyme plus 13 week human fetal uterine tube epithelium (mVgM+hTubE) stained with Hoechst dye 33258 to verify the species origin of the mesenchyme and epithelium. Mouse nuclei contain many bright chromatin bodies, whereas human nuclei lack such intranuclear bodies (Cunha and Vanderslice, 1984). The red-circled pycnotic nucleus in the lower magnification images can be seen at higher magnification.

Müllerian epithelium within the female reproductive tract of the mouse is specified by inductive cues from the mesenchyme (Cunha, 1976; Kurita et al., 2001; Kurita, 2011). To determine whether mesenchymal induction plays a role in differentiation of human Müllerian epithelium, tissue recombinants were prepared with 3-day postnatal mouse vaginal mesenchyme (known to be a vaginal inductor) and human uterine tubal epithelium from 12- to 13-week fetuses. Sections of all six mVgM+hTubE tissue recombinants were screened with Hoechst dye staining to verify that the mesenchyme was mouse and the epithelium was human (Fig. 1), thus eliminating the potential artifact of mouse vaginal mesenchyme contaminated with its own homotypic mouse epithelium.

Expression of several epithelial differentiation markers (AR, KRT6, TP63 and RUNX1) is distinctly different in the human tubal versus vaginal epithelium (Table 2). Fig. 2 depicts mVgM+hTubE recombinants grown in DES-treated female athymic mouse hosts and indicates that mouse vaginal mesenchyme elicited a partial shift in epithelial histo-differentiation and differentiation marker expression from uterine tubal epithelial differentiation to vaginal epithelial differentiation (Table 2). Expression of some tubal epithelial markers (KRT14 and

Table 2
Epithelial differentiation of human uterine tube, human vagina and mVgM+hTubE tissue recombinants.

Feature	Uterine tube	Vagina	mVgM+hTubE
Simple columnar epithelium	Yes	No	No
Stratified squamous epithelium	No	Yes	Yes
KRT6	No	Yes	Yes
TP63	No	Yes	Yes
Androgen receptor	Yes	No	No
RUNX1	No	Yes	Yes
KRT14	No	Yes	No
KRT19	Yes	No	Yes

Uterine tube epithelial features are shaded red, vaginal epithelial feature are shaded green.

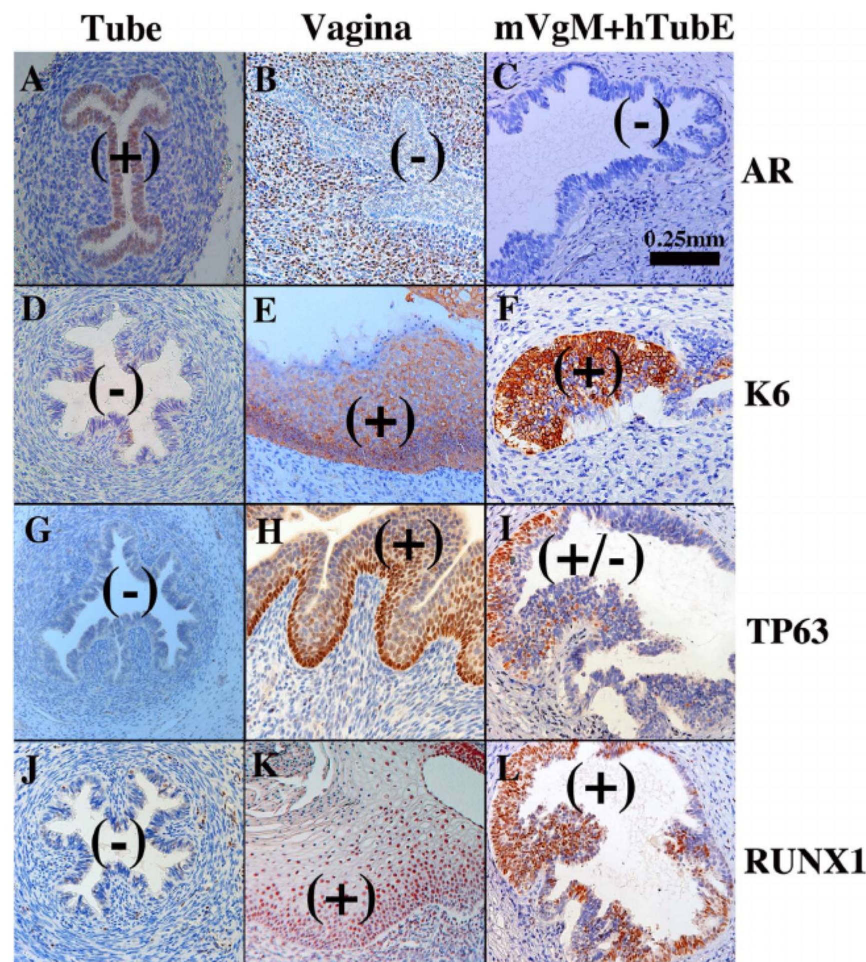

Fig. 2. Tissue recombinants composed of neonatal mouse vaginal mesenchyme plus 13 week human fetal uterine tube epithelium (mVgM+hTubE) grown for 4 weeks in DES-treated hosts and immunostained for various vaginal epithelial markers as indicated. Human uterine tube (A, D, G, J) and vagina (B, E, H, K) at 16–18 weeks of gestation serve as controls. Note induction of KRT6, TP63 and RUNX1 and down regulation of AR in epithelium of the mVgM+hTubE recombinants, indicative of an effect of mouse vaginal mesenchyme on expression of differentiation markers in human tubal epithelium. (+) and (-) indicate epithelial marker expression.

KRT19) were unchanged in mVgM+hTubE recombinants, suggesting that mouse vaginal mesenchyme was able to elicit only a subset of vaginal differentiation markers.

3.1.3. Tissue recombinants composed of epithelium of the human uterine corpus plus wild-type or Esr1KO uterine mesenchyme (wt UtM+hUtE and Esr1KO UtM+hUtE)

The background for this experiment is based upon the ontogeny of ESR1 in the human fetal uterine corpus. From 8 to 13 weeks ESR1 is undetectable in both the epithelium and mesenchyme of the human fetal uterine corpus (Fig. 3A-C). Subsequently, ~14 weeks to 18 weeks the mesenchyme of the human fetal uterine corpus become ESR1-positive (Fig. 3D), while the epithelium remains mostly ESR1-negative with the exception of rare ESR1-positive epithelial cells interspersed within the ESR1-negative uterine epithelial cells (Cunha et al., 2017a). When 10- and 13-week human fetal female reproductive tracts are grown for 4 weeks in DES-treated ovariectomized female hosts, ESR1 was induced in epithelium of the uterine corpus (Fig. 3E & F) (Cunha et al., 2017b). To determine whether DES induction of human fetal uterine epithelial ESR1 is mediated indirectly via mesenchymal ESR1 (paracrine effect) or directly via epithelial ESR1, we prepared tissue recombinants composed of epithelium of the human uterine corpus (hUtE) and uterine mesenchyme from either *Esr1*-positive wild-type mice or *Esr1*-negative *Esr1*KO mice (wt UtM+hUtE and *Esr1*KO UtM +hUtE). For this experiment we used hUtE from 10- and 12-week fetuses, an age before epithelial ESR1 was detectable (see Fig. 3A-C).

wt UtM+hUtE and *Esr1*KO UtM+hUtE tissue recombinants were grown under the renal capsule of female athymic mice that were ovariectomized at the time of grafting and implanted subcutaneously with a 20 mg DES pellet. After 1 month of growth, sections of all tissue recombinants were stained with Hoechst dye 332,598 to verify that the stroma of the harvested grafts was mouse and the epithelium was human (Fig. 4E) as described previously (Cunha and Vanderslice, 1984). ESR1 immunstaining of all tissue recombinants also verified the mesenchymal genotype (Fig. 4A & C): wt UtM+hUtE tissue recombinants contained ESR1-positive mesenchymal cells (Fig. 4A), whereas *Esr1*KO UtM+hUtE tissue recombinants were devoid of ESR1-positive mesenchymal cells (Fig. 4C). wt UtM+hUtE tissue recombinants consistently (6/6) contained an ESR1-positive human uterine epithelium (Fig. 4A). *Esr1*KO UtM+hUtE tissue recombinants also (5/5) contained an ESR1-positive human uterine epithelium even though the surrounding stromal cells were ESR1-negative (Fig. 4C).

To further explore the cellular mechanism of uterine epithelial steroid receptor expression (direct versus paracrine), we examined regulation of the PGR in wt UtM+hUtE and *Esr1*KO UtM+hUtE tissue recombinants. During the time frame of 8–14 weeks of gestation, PGR is undetectable (Fig. 5A-B) in both epithelium and mesenchyme of all organs of the developing human female reproductive tract (Cunha et al., 2017a). However, when human fetal female reproductive tracts were grown in DES-treated ovariectomized female hosts, PGR was induced globally within epithelial and stromal cells throughout the female reproductive tract, especially in the uterine corpus (Fig. 5C)

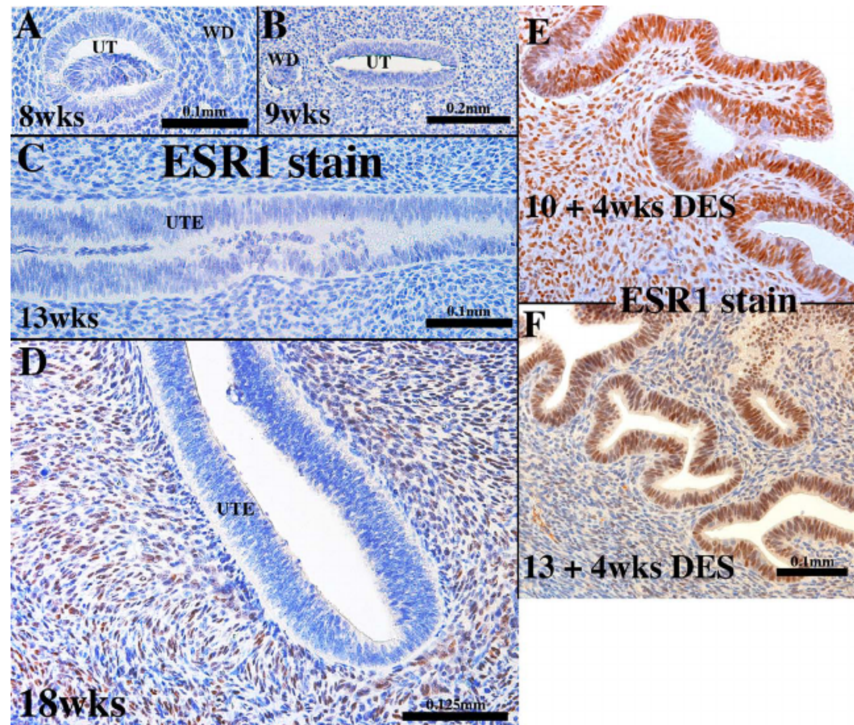

Fig. 3. Sections of developing human fetal uterine corpus immunostained for ESR1 at the ages indicated (A-D). At all stages (8–18 weeks) ESR1 is undetectable in the uterine epithelium. At 18 weeks, the stroma is ESR1-positive. (E & F) are sections of grafts of 10 week (E) and 13 week (F) human fetal uterine corpus grown for 4 weeks in DES-treated ovariectomized hosts and immunostained for ESR1. Note induction of epithelial ESR1.

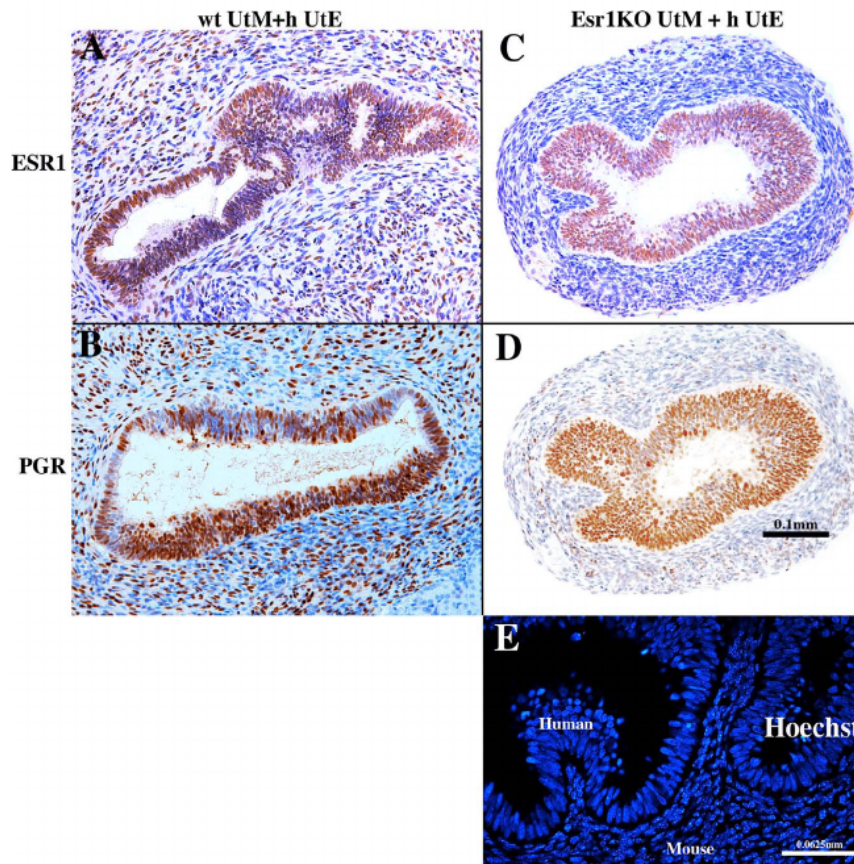

Fig. 4. Tissue recombinants composed of neonatal mouse wild-type uterine mesenchyme plus human fetal uterine tube epithelium (wt UtM+hUtE) (A & B) and αERKO uterine mesenchyme plus human uterine tube epithelium *Esr1*KO UtM+ h UtE) (C & D) grown in DES-treated hosts and immunostained for ESR1 (A & C) and PGR (B & D). DES induced ESR1 and PGR even when the mesenchyme was genetically devoid of ESR1. Sections (C) and (D) are adjacent sections stained for ESR1 (C) and PGR (D). (E) is a section of a graft of *Esr1*KO UtM+hUtE tissue recombinants stained with Hoechst dye 33,258 to verify the tissue origin.

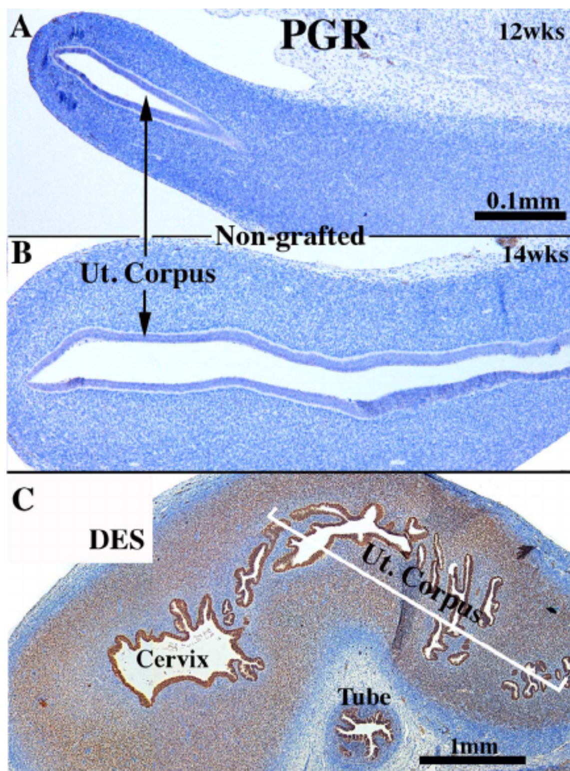

Fig. 5. Sections of non-grafted human female fetal reproductive tracts (A and B) at the ages specified immunostained for PGR. (C) A 13 week human fetal uterine corpus grown for 4 weeks in DES-treated ovariectomized hosts and immunostained for PGR. Note DES induction of epithelial and stromal PGR.

(Cunha et al., 2017b). This observation is in keeping with the established idea that PGR is an estrogen inducible protein (Janne et al., 1975; Horwitz and McGuire, 1979). Epithelial PGR was detected in both wt UtM+ human UtE (Fig. 4B, 6/6) and *Esr1*KO UtM+ human UtE (Fig. 4D, 5/5) tissue recombinants following growth for 1 month in DES-treated hosts, demonstrating the PGR is induced by DES directly via epithelial ESR1.

4. Discussion

Since epithelial differentiation within the female mouse reproductive tract is induced by cues from the mesenchyme (Cunha, 1976), we examined whether this concept also applies to the developing human female fetal reproductive tract through analysis of tissue recombinants composed of mouse vaginal mesenchyme and human tubal epithelium (mVgM+hTubE). The rationale of this experiment was to focus on two organs whose epithelial differentiation is markedly different (uterine tube=simple columnar epithelium versus vagina=stratified squamous epithelium) and whose profile of differentiation markers is also substantially different (see Table 2).

Our current studies extend prior works indicating that mesenchyme of various female reproductive organs induces and specifies the developmental fate of epithelium in laboratory animals. In our current studies neonatal mouse vaginal mesenchyme was grown in association with human fetal epithelium of uterine tubal origin, and the resulting epithelium expressed vaginal epithelial differentiation both morphologically and with vaginal immunohistochemical markers. Thus, mVgM+hTubE tissue recombinants formed a stratified epithelium that expressed KRT6, TP63, RUNX1, while concurrently abolishing expression of AR, an epithelial differentiation marker normally present in human fetal uterine tube epithelium, but not in human vaginal epithelium (Cunha et al., 2017a). However, the epithelium that formed was not perfectly vaginal as a few tubal pattern markers (absence of KRT14 and presence of KRT19) were retained in the partially induced epithelium. There are several reasons for this partial mesenchyme-induced shift in epithelial differentiation: (a) The mVgM+hTubE tissue recombinants were grown for either 2 or 4 weeks in host mice, and this may be insufficient to achieve full vaginal differentiation. Studies of longer duration, e.g., 1–2 months, may be required to achieve full vaginal epithelial differentiation. (b) Use of uterine tubal epithelium younger in age might result in a more complete vaginal epithelial differentiation as age can affect epithelial responsiveness (Cunha, 1976). Nonetheless, simultaneous induction of partial vaginal differentiation coupled with vaginal mesenchyme-induced loss of at least one tubal differentiation marker formally validates the concept that mesenchymal-epithelial interactions play an important role in differentiation within the human female fetal reproductive tract, perhaps via molecular mechanisms similar to those revealed in mouse studies. In any case, the use of mouse/human heterospecific tissue interaction studies, particularly those employing mutant mouse tissues, could be used in the future to dissect molecular mechanisms of human female (and male) reproductive tract development.

The second experiment in this paper deals with the mechanism of estrogenic induction of ESR1 and PGR in human fetal uterine epithelium, that is, whether DES elicits uterine epithelial ESR1 and PGR (a) directly via epithelial ESR1 or (b) indirectly (paracrine mechanism) via ESR1 in adjacent uterine mesenchyme. This question becomes even more interesting in so far as ESR1 was undetectable by immunohistochemistry in the human fetal uterine corpus at the age of the human uterine epithelium used to prepare the tissue recombinant (Fig. 3).

The background and rationale for this experiment is that ESR1 and PGR, whose expression is normally undetectable in second trimester human fetal uterine epithelium (Cunha et al., 2017a), were induced by DES treatment (Cunha et al., 2017b). Estrogen is known to up-regulate ESR1 mRNA and protein (Read et al., 1989; Saceda et al., 1988) and PGR (Janne et al., 1975; Horwitz and McGuire, 1979) via binding of liganded ESR1 to estrogen-response elements associated in the genes encoding ESR1 and PGR (Liu et al., 2003; Mehta et al., 2016).

We found that uterine epithelial ESR1 and PGR were induced by DES in *Esr1*KO UtM+hUtE tissue recombinants (devoid of mesenchymal ESR1), which formally eliminates the paracrine mechanism and demonstrates that DES induction of human uterine epithelial ESR1 and PGR is directly mediated via epithelial ESR1. With respect to estrogen-induced epithelial PGR in human uterine epithelium, we have previously addressed this question with heterospecific tissue recombinants consisting of adult human uterine epithelium and *Esr1*KO UtM. However, our earlier study was inconclusive as the *Esr1*KO mouse strain utilized for the previous study expressed a truncated form of *Esr1* in UtM (Kurita et al., 2005b). Since the *Esr1*KO mice utilized in the current study are truly *Esr1* null (Dupont et al., 2000), regulation of PGR in human UtE unquestionably does not require mesenchymal *Esr1*. Thus, epithelial PGR was prominently expressed *Esr1*KO UtM+ human UtE tissue recombinants (5/5) having *Esr1*-negative mesenchyme even though *Esr1*was undetectable in the human fetal uterine epithelium used to construct the *Esr1*KO UtM+ human UtE tissue recombinants. DES-induction of ESR1 in human fetal uterine epithelium was also observed in *Esr1*KO UtM+ human UtE tissue recombinants suggesting that estrogenic induction of ESR1 is a direct effect mediated by epithelial *Esr1*. It is perhaps worth noting that the design of this experiment does not exclude a possible role of mesenchymal ESR2.

It is perhaps worth noting that the mechanism of regulating uterine epithelial PGR in mice is vastly different than that in human. In mice uterine epithelial PGR is strongly expressed in untreated ovariectomized mice and is profoundly down regulated upon administration of estrogen, and effect that is mediated indirectly via stromal ESR1 (paracrine mechanism) (Kurita et al., 2000). This finding in mice contrasts strikingly with the direct estrogenic induction of PGR in human cells and tissues.

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