Role of foxj1 and estrogen receptor alpha in ciliated epithelial cell differentiation of the neonatal oviduct

A Okada1,2, Y Ohta2,6, S L Brody3, H Watanabe5,6, A Krust4, P Chambon4 and T Iguchi5,6

1Safety Research Laboratories, Yamanouchi Pharmaceutical Co., Ltd, Tokyo 174-8511, Japan
2Department of Veterinary Science, Faculty of Agriculture, Tottori University, Tottori 680-8553, Japan
3Department of Internal Medicine, Washington University School of Medicine, St Louis, Missouri 63110-1093, USA
4Institut de Génétique et de Biologie Moléculaire et Cellulaire, CNRS/INSERM/ULP, Collège de France, BP 163, 67404 Illkirch Cedex, France
5Center for Integrative Bioscience, Okazaki National Research Institutes, and Department of Molecular Biomechanics, School of Life Science, The Graduate University for Advanced Studies, Okazaki 444-8585, Japan
6CREST, Japan Science and Technology, Kawaguchi 332-0012, Japan

(Requests for offprints should be addressed to T Iguchi, 5-1 Higashiyama, Myodaiji, Okazaki 444-8585, Japan; Email: taisen@nibb.ac.jp)

Abstract

Estrogen regulates proliferation and differentiation of epithelial cells in the mammalian oviduct, but pathways for cell-specific differentiation are not well understood. In the epithelial cells of the developing rat oviduct, we found estrogen receptor (ER)α is expressed at birth and persists in all cells through neonatal day (ND) 7 when ciliated cells appear. To determine a specific function of ER and foxj1, a transcription factor known to have fundamental roles in ciliogenesis in the lung, in differentiation of the ciliated epithelial cells, we treated newborn rats from ND 0 to 5 with estradiol-17β (E2) with and without a selective ER antagonist. E2 enhanced the number of proliferating cells and accelerated the process of epithelial cell differentiation resulting in ciliogenesis by ND 5, and co-treatment with an ER antagonist inhibited these changes. Foxj1 was expressed only in the infundibulum and ampulla (INF/AMP). That expression preceded the appearance of cilia and was induced by E2. Cilia were absent in oviducts of foxj1-deficient mice, indicating that foxj1 plays a critical role in oviductal ciliogenesis. However, we found the presence of cilia in the ERα-deficient mouse oviduct. The widespread expression of ERα in oviductal epithelium, but restriction of cilia to the INF/AMP regions, and importantly, the presence of cilia in the ERα-deficient mice, suggested ER signaling is not essential for ciliated epithelial cell differentiation. These observations demonstrate that, although E2 stimulates the differentiation process of ciliated epithelial cells, foxj1 is directly required for epithelial cell ciliogenesis of the neonatal oviduct.

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Introduction

The mammalian oviduct or fallopian tube has fundamental roles in gamete transport, fertilization, and early embryo development that are facilitated by ciliated and secretory epithelial cells lining the lumen. Several experimental models have shown that oviductal development is regulated by the ovarian sex steroid hormones estrogen and progesterone (Jansen 1984). In the chick and mouse oviduct, estradiol-17β (E2) induces epithelial cell proliferation and differentiation (Anderson & Hein 1976). In this regard, the differentiation of the secretory epithelial cell phenotype to the ciliated epithelial cell phenotype can be induced by E2 in primary cultured human fallopian tube epithelial cells (Comer et al. 1998). In addition, the appearance of oviductal epithelial cilia, typically observed at day 5 after birth (Komatsu & Fujita 1978), can be accelerated by treatment with E2 in neonatal mouse and hamster oviducts (Eroschenko 1982, Abe & Oikawa 1993). Thus E2 must activate critical pathways required for ciliated epithelial cell differentiation in the oviduct.

E2 action is mediated by its cognate receptors, estrogen receptor (ER)α and ERβ (Mangelsdorf
et al. 1995). In developing mice and rats, ERα is expressed in oviductal epithelial and stromal cells, but ERβ is absent in both cell types (Yamashita et al. 1989, Li 1994, Mowa & Iwanaga 2000, Okada et al. 2002a, 2003). Pre- and neo-natal exposure to diethylstilbestrol (DES), a synthetic estrogen, caused abnormal morphology and altered cell proliferation in mouse and rat oviducts (Newbold et al. 1983, Okada et al. 2001). However, the oviduct of mice lacking ERα was resistant to neonatal DES exposure (Couse et al. 2001) indicating an essential role of ERα in mediating the estrogen effect on developing neonatal oviduct. Together, these findings suggest that differentiation and proliferation of epithelial cells in the oviduct may be regulated by E2 through ERα. However, the molecular and cellular mechanisms of these events and factors determining the differentiation of ciliated and non-ciliated cells of the oviduct have not been clarified.

Foxj1 (formerly HFH-4) is a member of the fork head box (fox) family of transcription factors with fundamental roles in embryonic development and differentiation (Carlsson & Mahlapuu 2002). Foxj1 has been cloned from mice, rats and humans, and is shown to have expression restricted to ciliated epithelial cells of conducting airways, choroid plexus, and ependyma of the brain, the testis, and the oviduct (Clevidence et al. 1993, Hackett et al. 1995, Brody et al. 1997, Lim et al. 1997, Murphy et al. 1997, Blatt et al. 1999). In the developing mouse lung, the onset of foxj1 expression has been observed from embryonic day 14.5 to 16.5, prior to the appearance of cilia on epithelial cells (Hackett et al. 1995, Blatt et al. 1999, Tichelaar et al. 1999a). Foxj1 gene interruption in mice (foxj1KO) results in perinatal lethality and a lack of ciliated epithelial cells in tissues including the lung and oviduct (Chen et al. 1998, Brody et al. 2000). Transgenic mice overexpressing foxj1 under control of the surfactant protein C promoter in the lung show induction of ciliogenesis in undifferentiated alveolar epithelial cells (Tichelaar et al. 1999b) as demonstrated by the expression of the foxj1 transgene and β-tubulin IV, a ciliated epithelial cell marker (Renthal et al. 1993). These findings indicate that foxj1 has a distinct role in ciliated epithelial cell differentiation in the embryonic through the adult lung. However, the role of foxj1 in the development and differentiation of oviductal ciliated epithelial cells, which are regulated by sex steroid hormones, has not been established. The restricted expression of foxj1 in ciliated cells of the oviduct and a lack of oviductal cilia exhibited in foxj1KO mice led us to hypothesize that foxj1 has a critical role in the E2-dependent ciliated epithelial cell differentiation of the oviduct.

This study shows for the first time that E2 exposure results in the induction of foxj1 in ciliated cells during ER signaling in the neonatal rat oviduct. Interestingly, ERα is down-regulated in the ciliated cells following E2 exposure and the epithelial cells in the mouse oviduct of the null mutant for ERα (ERαKO) have normal foxj1 expression and cilia, suggesting that ER signaling is not directly required for oviductal epithelial cell ciliogenesis. However, evaluation of the foxj1KO mouse oviduct indicates that foxj1 is required for cell-specific down-regulation of ERα and ciliogenesis in ciliated epithelial cells of the neonatal oviduct. Therefore, foxj1 has a critical role in directing oviductal epithelial cell differentiation.

Materials and methods

Animals and treatments

All animals were maintained in accordance with the NIH and the Institutional Guides for the Care and Use of Laboratory Animals. Male and female Sprague–Dawley rats were obtained from Charles River Japan, Inc. (Kanagawa, Japan). Animals were housed individually in stainless-steel cages with controlled temperature (23 ± 2 °C) and relative humidity (55 ± 10%), and a 13 h light:11 h darkness cycle (0800–2100 h). Pelleted food (CRF-1; Oriental Yeast Co., Ltd, Tokyo, Japan) and municipal tap water were freely available. Females (15 weeks of age) were cohabited overnight with males, and the day on which neonates were born was designated as neonatal day (ND) 0. In ontogenetic rat studies, oviducts were removed from untreated neonatal rats on ND 0, 3, 5, 7, 10 and 20. Foxj1KO (Brody et al. 2000) and ERαKO (Dupont et al. 2000) mice, and their wild-type (WT) counterparts (C57/BL/6) were used at 3 weeks and 2 months of age respectively.

To investigate the effect of E2, 16–28 female neonates in each group were injected s.c. daily with sesame oil, or 1 or 10 µg of E2 (Sigma, St Louis, MO, USA) for 5 days (ND 0–4). Similarly, sesame oil or 10 µg of ICI 182780 (ICI) (Tocris Cookson玩家来说，您希望在此处显示的自然语言文本是？
Inc., Ballwin, MO, USA) was concomitantly injected for 5 days (ND 0–4). Oviducts were removed 24 h after the last injection (ND 5), and immediately prepared for immunohistochemistry or real-time RT-PCR. All chemicals were dissolved in sesame oil and injected at a dosing volume of 0·05 ml per neonate.

Total RNA preparation and real-time RT-PCR

Procedures for total RNA preparation and real-time RT-PCR have been described previously (Okada et al. 2002b). Total RNA was isolated from oviducts, and reverse-transcribed into cDNA. An aliquot of generated cDNA was amplified with a pair of primers (foxj1, forward 5’TCCAGAATTGG GCAAAAGCA3’ and reverse 5’TGTCTCTGGG TAGGGACCTG3’; and glyceraldehyde-3-phosphate-dehydrogenase (GAPDH), forward 5’TCTACCC ACGGAACTTCAA3’ and reverse 5’ACCCC ATTTGAATGGCGG3’) derived from rat mRNA sequences (GenBank Accession No. L36388 and M17701 respectively). Quantitative real-time PCR was carried out in an ABI Prism 7700 Sequence Detector (Applied Biosystems, Foster City, CA, USA) using SYBR Green PCR Master Mix reagent (Applied Biosystems). PCR cycle parameters were 94°C for 15 s, 60°C for 30 s and 72°C for 60 s, following a hold temperature for 10 min at 95°C. The number of template copies present at the start of the reaction was determined by comparison with a standard scale prepared from rat genomic DNA. The expression level of each target gene was calculated by standardizing the target gene copy number with the GAPDH copy number in a sample. Purity and specificity of the PCR products were confirmed by the omission of the reverse transcriptase, having a single melting temperature, being of the appropriate size and having the proper sequence. The results of analysis is based on duplicate samples from three independent experiments.

Antibodies

A rabbit polyclonal antibody against foxj1 was generated as described previously (Blatt et al. 1999). A mouse monoclonal antibody against ERα (6F11; Novocastra Laboratories Ltd, Newcastle upon Tyne, UK) was used at a dilution of 1:50. Mouse monoclonal antibodies against proliferating cell nuclear antigen (PCNA) (PC10; Dako Corporation, Carpinteria, CA, USA) and β-tubulin IV (ONSA6; BioGenex, San Ramon, CA, USA) were used at a dilution of 1:100 and 1:250 respectively. The binding specificity of these antibodies has been previously established (Bannerjee et al. 1992, Fisher et al. 1997, Blatt et al. 1999, Pelletier et al. 2000).

Tissue preparation and immunohistochemistry

Oviducts were fixed with 4% paraformaldehyde in 0·1 M phosphate buffer overnight at 4°C. Sections cut in paraffin at 4 μm were deparaffinized, rehydrated and autoclaved at 121°C for 15 min in 10 mM citrate buffer, at pH 6·0, for antigen retrieval. Sections were then rinsed in distilled water and incubated with 0·3% hydrogen peroxide for 30 min. After rinsing in 0·01% Triton X-100 in PBS (PBT), sections were treated with normal sheep serum (Dako) for 30 min, and then incubated for 30 min with anti-PCNA antibody, or for 16 h at 4°C with anti-foxj1, -ERα, or -β-tubulin IV antibodies. Sections were rinsed in PBT and treated with Simple Stain Rat PO (Nichirei, Tokyo, Japan) for 30 min. After a final PBT wash, sections were treated with 0·01% 3,3’-diaminobenzidine tetrahydrochloride (Dojindo Laboratories, Kumamoto, Japan) in 0·05 M Tris–HCl at pH 7·6 including 0·068% imidazole (Sigma) and 0·02% hydrogen peroxide for 5 min.

For double immunohistochemistry, sections stained for ERα or PCNA as described above were rinsed in PBT and blocked in normal sheep serum, followed by incubation with the anti-β-tubulin IV antibody overnight at 4°C. Sections were rinsed in PBT and treated with EnVision/AP (Dako) for 30 min. After rinsing in PBT, they were treated with fuchsin (Dako) with levamisole (Dako) for 5 min.

All sections, except those for the ontogeny study, were lightly counter-stained with hematoxylin (Dako A/S, Glostrup, Denmark). Normal mouse IgG and normal rabbit immunoglobulin fraction (both Dako A/S) were used as negative controls in place of primary antibodies for ERα, PCNA, β-tubulin IV and foxj1 immunostaining respectively, showing no specific immunoreactivity.

Evaluation and statistical analysis

Sections were examined and photographed using a light microscope attached to a digital camera.
(BX60 and DP50; Olympus Optical Co., Ltd, Tokyo, Japan). Cell proliferation was calculated as a percentage of the total PCNA-labeled cells counted; all epithelial cells in each section evaluated were counted. For stroma, all cells in the five layers most adjacent to the epithelium in each section were counted. Ciliated cell differentiation was evaluated as a percentage of the total ducts including \( \beta \)-tubulin IV-labeled cells in a section. At least seven specimens from each of five animals were examined for all investigations. Although, following treatment with E2, regional differentiation occurred in the oviduct, PCNA and \( \beta \)-tubulin IV expressions were evaluated throughout oviductal regions.

Statistical analysis was carried out, using Duncan’s multiple comparison test, for the effects of hormones on \( \beta \)-tubulin IV, foxj1 and PCNA expressions. Data are represented as means ± s.d. and considered significantly different at \( P < 0.05 \).

## Results

### Differentiation of ciliated epithelial cells in the untreated-neonatal rat oviduct

To evaluate the differentiation of oviductal epithelial cells, the expression of cilia protein \( \beta \)-tubulin IV was used as a marker of ciliated cells and evaluated by immunohistochemistry during postnatal oviductal development (Fig. 1). The neonatal oviduct is a simple tube structure from ND 0 to ND 5, and subsequently differentiates morphologically into the infundibulum (INF), ampulla (AMP), isthmus (IST) and uterotubal junction (UTJ) after ND 7. No expression of \( \beta \)-tubulin IV was found in the oviduct from ND 0 to 5. However, on ND 7, \( \beta \)-tubulin IV appeared on the luminal surface of some epithelial cells. Following morphological differentiation of the oviduct into different regions, the number of cells expressing \( \beta \)-tubulin IV increased. Ciliated cells were numerous in the INF/AMP on ND 20 and at diestrus of the cycling oviduct. Only rare epithelial \( \beta \)-tubulin IV-positive cells were detected in the IST/UTJ. Thus, consistent with a previous report that used electron microscopy to detect ciliated cells, the appearance of cilia in the oviduct was after ND 5, following morphological development of the oviduct (Komatsu & Fujita 1978), and was restricted to specific regions.

### Ontogeny of foxj1 in the untreated neonatal rat oviduct

Transcription factor foxj1 is expressed in ciliated cells prior to the appearance of cilia in the airway (Blatt et al. 1999). To evaluate changes in expression of foxj1 mRNA in the developing rat oviduct, quantitative real-time RT-PCR was performed (Fig. 2A). A low level of foxj1 mRNA was detected in the neonatal oviduct on ND 0 and 3. Oviductal foxj1 mRNA increased dramatically from ND 5 and reached a high level similar to that in the diestrus oviduct of cycling rats by ND 20.

At the protein level, no expression of foxj1 was found in oviductal tissue from ND 0 to 5 (Fig. 2B). The onset of foxj1 expression was on ND 7, at which time protein was detected in the nuclei of epithelial cells. The expression of foxj1 increased on ND 10 (not shown) and ND 20 in the INF/AMP, and was most striking in the INF/AMP.
at the time of diestrus in the cycling oviduct. No foxj1 expression was detected in the IST/UTJ after morphological differentiation on ND 20 and adult diestrus. The protein level of foxj1 may be low and below the limit of immunohistochemical detection from ND 0 to 5, since its mRNA expression was evident at a low level. Thus, as in the airway of the lung, the expression of foxj1 preceded the appearance of cilia, suggesting that foxj1 similarly regulated ciliogenesis in the oviduct.

Neonatal effects of E2 on ciliated epithelial cell differentiation

To investigate the effects of E2 on neonatal development of the oviduct, oil vehicle or 1 or 10 µg E2 were injected into neonatal rats for 5 days from the day of birth. Also, oil vehicle or pure ER antagonist ICI was concomitantly injected with 10 µg E2. Following E2 treatment, changes in the morphology of the primitive oviductal tube into the INF/AMP and IST/UTJ regions were observed on ND 5. Apparent estrogenic effects such as hypertrophy of epithelial and stromal cells, including enlargement of nuclei and dilatation of the lumen in the IST/UTJ were histologically observed (Newbold et al. 1983). In the oil-treated oviduct, no duct with cells immunopositive for /afii9826-tubulin IV was detected on ND 5, but 68% of ducts had differentiated ciliated epithelial cells on ND 7 (Fig. 3). Following treatment with 1 µg and 10 µg E2, 20 and 78% of the ND 5 ducts showed epithelial /afii9826-tubulin IV expression respectively. Treatment with ICI significantly reduced the percentage of /afii9826-tubulin IV-expressing epithelial cells within the duct to 20% (P<0.01) (Fig. 3). From this, it can be deduced that ovarian hormones regulate oviductal morphology and oviductal differentiation, notably affecting differentiation of cells to the ciliated cell phenotype.

Neonatal effects of E2 on foxj1 expression

Next it was determined if the effect of E2 on ciliogenesis was regulated by foxj1. Expression of foxj1 was evaluated in the oil- and hormone-treated oviducts by real-time RT-PCR and immunohistochemistry. Compared with the oil control, E2 treatments of 1 and 10 µg showed 1.2- and 2.2-fold increases respectively (P<0.05) in foxj1 mRNA in the ND 5 oviduct (Fig. 4). Co-treatment with ICI
reduced 10 µg E2-induced foxj1 expression to the oil-treated control level. Although, on ND 5, no foxj1-positive cell was detected in the oil-treated oviduct, E2 induced foxj1 expression in epithelial cells in the INF/AMP, but not in the IST/UTJ region (data not shown). Some of the foxj1-positive cells in the E2-treated INF/AMP had cilia, but others did not. These data suggested that foxj1 expression was regulated by E2.

Neonatal effects of E2 on cell proliferation

E2 treatment is known to be associated with oviductal epithelial cell proliferation as well as differentiation. To determine if E2 directed differentiation of oviductal epithelial cells by altering cell phenotype directly or alternatively by inducing proliferation that was followed by differentiation, the relationship between markers of proliferation and ciliogenesis in the oviductal epithelium was examined. The effect of E2 on cell proliferation was determined by immunohistochemical detection of PCNA (Fig. 5). On ND 5, the control oil-treated oviduct had 16% PCNA-positive epithelial cells, but the 1 and 10 µg E2 treatment significantly increased proliferation to 22% (P<0.05) and 35% (P<0.01) respectively. In the E2-treated tissues, epithelial PCNA was observed in most epithelial cells of the IST/UTJ, but in only some cells of the INF/AMP. Interestingly, PCNA-positive epithelial cells in the INF/AMP region lacked β-tubulin IV expression. Conversely, β-tubulin IV-expressing epithelial cells did not co-express PCNA in the E2-treated oviduct. Concomitant treatment with ICI caused a decrease in the percentage of 10 µg E2-enhanced PCNA-labeled epithelial cells to the control level. These findings might suggest that, in the INF/AMP, E2 induced proliferation of oviductal epithelial cells that subsequently differentiate into ciliated cells.

Neonatal effects of E2 on ER expression

Variation in regional oviductal proliferation and differentiation following E2 treatment suggested that the expression of sex steroid hormone receptors was also cell- and region-specific. We...
therefore investigated the effects of E2 on the expression of ERα and ERβ in oil- and hormone-treated oviducts. In the control oil-treated ND 5 oviduct, there was marked expression of ERα in the nuclei of epithelial and stromal cells (Fig. 6). Following exposure to 1 and 10 µg of E2, the expression of epithelial ERα in both the INF/AMP and IST/UTJ was less detectable at ND 5. Absent receptor expression in ciliated epithelial cells expressing β-tubulin IV of the INF/AMP was noted. Similar epithelial cell expression patterns of ERα were detected in the adult rat oviduct, including the expression in all epithelial cells of the IST, but not in ciliated epithelial cells of the AMP (Fig. 6) as reported (Okada et al. 2003). However, stromal ERα was not changed by E2, and maintained a control level by co-exposure to ICI (not shown). Expression of ERβ was not detected in the oil-treated oviduct as previously reported (Okada et al. 2003), or in the E2-treated oviduct evaluated on ND 5 (data not shown). Taken together, these findings indicate a dynamic change in ERα expression during oviductal growth and differentiation that is cell- and region-specific. The initial presence of ERα in all AMP epithelial cells, followed by loss of expression in ciliated cells, might suggest that E2 was required for a proliferative phase that precedes a ciliogenesis phase.

Oviductal epithelial cell differentiation in the foxj1- and ERα-deficient mice

To understand the role of foxj1 and ERα in the development and differentiation of the mammalian oviduct, the expression of ciliogenesis markers and ERα were evaluated in oviducts from genetically...
deficient foxj1 (foxj1KO) and ERα (ERαKO) mice. In the WT mouse oviduct, β-tubulin IV and foxj1 were expressed in ciliated epithelial cells, as in the untreated rat oviduct (Fig. 7). In the foxj1KO oviduct, no foxj1 or β-tubulin IV was observed as had been previously reported (Chen et al. 1998, Brody et al. 2000). Remarkably, the absence of foxj1 also resulted in persistent expression of ERα in all cells of the AMP, compared with down-regulation of this protein seen in the ciliated cells of WT oviducts. Furthermore, ciliogenesis did not require an intact ERα because in ERαKO mice, epithelial foxj1 and β-tubulin IV were detected in the INF/AMP as in WT mice (Fig. 7). Thus foxj1 is required for ciliogenesis and continued maturation of the oviductal epithelium supporting a critical role for foxj1 in oviductal differentiation.

Discussion

In this report, it has been shown that oviductal epithelial cell differentiation, and particularly ciliogenesis in the developing oviduct, is dependent on fork head transcription factor foxj1. We found that, during normal rat oviductal epithelial cell differentiation, foxj1 mRNA was observed at low levels, even on ND 0, then increased significantly between days 5 and 7. At ND 7 foxj1 protein was first detected immunohistochemically in epithelial cells of the INF/AMP. Coincident with foxj1 expression was the appearance of cilia protein β-tubulin IV, suggesting that epithelial differentiation into ciliated epithelial cells occurred between ND 5 and ND 7. These findings are in agreement with a previous investigation of mouse oviductal epithelial cell differentiation that utilized electron microscopy to demonstrate the onset of ciliogenesis on ND 5 and ongoing differentiation from days 5 to 10 (Komatsu & Fujita 1978). The expression level of foxj1 protein may be so low, from ND 0 to 5, to be below the limit of immunohistochemical detection. Therefore, in the developing oviduct, as well as the developing lung (Hackett et al. 1995, Tichelaar et al. 1999a), foxj1 may be expressed before the appearance of cilia in the oviduct in foxj1 null mice, our findings indicate a key role for foxj1 in the ciliogenesis of the oviduct. Also considered were previous reports of foxj1 function and the absence of cilia in the oviduct in foxj1 null mice, our findings indicate a key role for foxj1 in the ciliogenesis of the oviduct. Furthermore, ciliogenesis did not require an intact ERα because in ERαKO mice, epithelial foxj1 and β-tubulin IV were detected in
Differentiation and E2 treatment was examined. At ND 5, expression of ERα was markedly expressed in both epithelial and stromal cells of the oil-treated oviduct. A pure ER antagonist, ICI, inhibited all E2 effects observed, indicating the dependence of these E2 actions on ER signaling. However, all changes in gene expression associated with E2 treatment were similar to changes observed in the untreated oviduct at ND 7 or later, and several additional observations suggest that E2 is not required for ciliogenesis. First, although E2 affects proliferation throughout the oviduct, ciliogenesis occurs regionally, i.e. only within the INF/AMP region and not in the IST/UTJ, suggesting that foxj1 was regulated through a different pathway. Secondly, cells expressing foxj1 lost ERα and progesterone receptor (A Okada, Y Ohta and T Iguchi unpublished observations) expression. This could be an event that follows ciliogenesis after induction by E2. Thirdly, epithelial differentiation including foxj1 expression and ciliogenesis was normal, however, in the ERαKO oviduct. Therefore, ER signaling is not essential for ciliated epithelial differentiation during the neonatal development of the oviduct. Also consistent with this is the absence of estrogen-responsive elements in the promoter region of the foxj1 gene. Thus, although E2 accelerated differentiation of ciliated epithelial cells, any molecular mechanisms for regulation of foxj1 by E2 remain to be clarified.

While ERα signaling in the ciliated cell may not be required for the direct induction or maintenance of the ciliated cell phenotype, it is reasonable to assume that E2-mediated ERα signaling functions to activate progenitor cell proliferation and to subsequently generate cells with the capacity to achieve the ciliated cell phenotype. In this regard, E2 treatment was associated with epithelial cell proliferation in a substantial number of epithelial cells of the INF/AMP and all epithelial cells of the IST/UTJ. In addition, we found ciliated cells were not proliferating and did not express PCNA, behavior consistent with a terminally differentiated cell. Thus, while normal cilia formation in the ERαKO oviduct indicates that E2 is not an absolute requirement for epithelial cell differentiation, it may likely be induced via multiple compensatory pathways in the ERαKO or WT mouse.

Differentiation of oviductal epithelial cells is induced by E2, but in later stages it is independent of E2 and is instead dependent on foxj1. In the foxj1KO mouse, a regional difference between the INF/AMP and the IST/UTJ was easily recognized.

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**Figure 7** Effect of foxj1 on oviductal epithelial cell differentiation. Expression of β-tubulin IV, foxj1 and ERα was evaluated by immunohistochemistry in WT, foxj1KO (foxj1−/−), and ERαKO (ERα−/−) oviduct. β-tubulin IV and foxj1 were expressed in similar patterns in WT and ERα−/−oviduct. Epithelial cells in the AMP of the WT oviduct showed marked ERα expression in non-ciliated cells but little expression in ciliated cells. In the foxj1−/−oviduct, ERα protein was highly expressed in all epithelial cells (all non-ciliated cells). Arrowheads indicate ciliated epithelial cells. Bar: 20 µm.
light-microscopically, and ERα and progesterone receptor (A Ōkada, Y Ohta, S L Brody and T Iguchi unpublished observations) were present in all epithelial cells in the INF/AMP region. These findings may indicate that epithelial cells in the foxj1KO oviduct have ceased their differentiation into ciliated epithelial cells due to a lack of foxj1. It is also likely that foxj1 has a much broader role than only the regulation of ciliogenesis and instead is required to switch off the E2 (and other) pathways. In addition to foxj1, other fox genes for sex steroid hormone actions have regulatory roles in reproductive tissues. Foxo1 (FKHR, forkhead homologue of rhabdomyosarcoma), foxo3 (FKHRL1, forkhead-like protein-1), and foxo4 (AFX, a forkhead transcription factor) are expressed in the rodent ovary at specific stages and in specific cells of follicular development and luteinization (Schuur et al. 2001, Zhao et al. 2001), and foxo1 is regulated by E2 in the granulosa cells (Richards et al. 2002). Additionally, foxa1 (HNF-3α) is expressed in the epithelia of the rat prostate and, 7 days following castration, was suppressed in the prostate but restored by testosterone (Kopachik et al. 1998), indicating an important role in development and maintenance of male urogenital tract epithelial cells. Taken together, forkhead transcription factors including foxj1 may regulate sex steroid hormone actions in development, cytodifferentiation and physiological function of reproductive tissues in both males and females.

In summary, this study proposes important mechanisms in the development of the neonatal rat oviduct. Neonatal effects of E2 on epithelial cell differentiation or proliferation were demonstrated to vary inversely in different oviductal regions and cell types. We hypothesize that in early neonatal development, initiation of E2 production from the ovary as well as an increase in oviductal ERα expression occur, and E2 induces foxj1 expression in programmated ciliated cells of the INF/AMP with a loss of ERα that sequentially promotes epithelial ciliogenesis. However, although the stimulation of ER signaling accelerates the differentiation process of ciliated epithelial cells, the presence of cilia in the ERαKO oviduct suggests that it is not fundamentally required for this event. This study suggests that signaling of foxj1 is directly required for oviductal epithelial cell ciliogenesis, and serves as fundamental evidence for understanding molecular and cellular mechanisms of epithelial cell differentiation and proliferation of the neonatal rat oviduct.

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