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An Evidence of Stromal Cell Populations Functionally Linked with Epithelial Cell Populations in the Mouse Oviduct

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ABSTRACT—The oviductal epithelium consists of two major cell populations, secretory cells and cilial cells. In a previous report, we established clonal cell lines from the epithelium and stroma of an oviduct which allowed us to analyze stromal contribution to epithelial functions. Three stromal cell lines were co-cultured in separated apparatus with 3 epithelial cell lines, respectively. Two stromal cell lines preferentially stimulated *mogp-1* expression on secretory cells and the stimulation was additive with estrogen. The lines had no effect on cilial cells. One stromal cell line preferentially stimulated *foxj1* expression on cilial cells and the stimulation relieved suppression by estrogen. The line had no effect on secretory cells. Experiments with conditioned media of the stromal cells confirmed the results of co-culture experiments, suggesting that the oviductal stroma contains multiple cell populations preferentially regulating or modulating specific cell populations of the epithelium via diffusible factors.

Key words: oviductal cell lines, secretory cell, cilial cell, epithelial-stromal interaction, diffusible factors

INTRODUCTION

The stroma is generally thought to support and maintain its epithelial function and proliferation. During developmental stages, the stroma is inductive and determines epithelial function. This phenomenon is well documented in stomach (Yasugi, 1994; Yasugi and Fukuda, 2000), intestine (Kedinger *et al.*, 1996) and reproductive tract (Donjacour and Cunha, 1991). In adult tissues and organs, the stroma is permissive or conditional, allowing its epithelium to proliferate and/or express specific genes. In female reproductive tract, for instance, epithelia (estrogen receptor $\alpha^{+/+}$) recombined with stroma (estrogen receptor $\alpha^{-/-}$) lost responsiveness to estrogen (Cooke *et al.*, 1997; Kurita *et al.*, 1998). In the inductive and permissive effects, the stroma plays critical roles for its epithelium. However, the fashion is quite distinctive; it is irreversible during developmental stages and is reversible in adult tissues and organs, suggesting that the mechanisms underlying the two phenomena are quite unique. The irreversible phenomenon has attracted many investigators and has been extensively studied for many decades, although the reversible phenomenon had less attention.

The stroma is a complex tissue. It is vascularized and innervated and contains many unidentified cells. Therefore, "stromal effect" is a complex outcome from activities of various cells. When enzymatically digested tissues are observed in primary cultures, epithelial cells are recognized as "polygonal cells" and other non-epithelial cells are collectively called "fibroblasts" or stromal cells. No further observations are usually conducted on stromal cells, although they must contain various cell populations. It is worthwhile that "stromal effects" are analytically reconsidered at cellular level not as whole tissues.

The mouse oviduct differentiates from the Müllerian duct and has a fundamental role in gamete transport, fertilization and subsequent early embryo development. The epithelium is simple columnar and contains ciliated cells and secretory cells (Odor and Blandau, 1973; Oliphant, 1986). Ciliated cells are recognized around 5 days after birth and secretory cells with mucous secretory materials appear approximately 23 days after birth (Komatsu and Fujita, 1978). In an adult oviductal epithelium, both ciliated cells and secretory cells are always present, and changes in relative numbers of the two types of cells are associated with estrous cycle in rodents or menstrual cycle in human (Abe, 1996). We have previously reported that clonal epithelial and stromal cell lines have been established from an oviduct of a p53 deficient mouse at 20-day-old (Umezu *et al.*, 2003). In addition, epithelial cell lines can be classified into 1) a cilial cell type, 2) a secretory cell type and 3) an undifferenti-

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ated (or defected) cell type. Stromal cell lines with a distinct morphology were established, but their functions were undetermined.

In the present study, we attempted to investigate how stromal cells contribute to epithelial functions. We report herein an evidence that the stroma contains multiple cell populations and each population might be linked with an epithelial cell population.

MATERIALS AND METHODS

Animals

CD-1 mice (Charles River Japan, Yokohama, Japan) and $p53^{-/-}$ mice (hybrid between C57BL/6 and CBA) (Tsukada *et al.*, 1993) were maintained at the experimental animal facility, Tokyo University of Science. They were kept under a 12:12 h light: dark cycle at 22–24°C. Standard laboratory feed (MR standard, Nousan LTD, Yokohama, Japan) and tap water were given *ad libitum*. Mice care and handling conformed to the NIH guidelines for animal research. The experimental protocols were approved by the Institutional Animal Care and Use Committee.

Cell culture

We have previously established clonal epithelial and stromal cell lines from a $p53^{-/-}$ mouse oviduct (Umezu *et al.*, 2003). In the present study, the following 3 epithelial and 3 stromal lines were chosen among them. OA-ab line is epithelial and expresses *mogp-1*, a marker of secretory cells. OF-1a line is epithelial and expresses *foxj1*, a marker of cilial cells. OA-2c line expresses none of them. OA-5ca, OA-5cg and OA-5ch are stromal lines and have a distinct morphology. Cells were maintained in medium (1:1 mixture of DMEM and Ham's F-12 without phenol red: DMEM/F12, Sigma, St. Louis, MO, USA) containing 10% heat-inactivated fetal calf serum (Commonwealth Serum Laboratories, Melbourne, Australia) supplemented with penicillin (31 µg/ml, Sigma), streptomycin (50 µg/ml, Sigma) and estradiol-17β (E_2: 10^{-8} M, Sigma) (10%FCS + E_2). Cells were passaged at 1/10. To examine effects of E_2, cells were pre-cultured in 10% dextran-coated charcoal-treated FCS (10% DCCFCS) to remove steroids (Hanazono *et al.*, 1998) for 5 days, then they were cultured in serum-free medium supplemented with or without E_2.

Epithelial/stromal co-culture

Epithelial and stromal cell lines were co-cultured separately by a culture insert filter (Millicell-PCF filter inserts, tissue culture-treated polyvinyl pyrrolidone membrane, 6-well plate inserts, 0.4-µm pore; Millipore, Bedford, MA, USA). A Millicell filter insert consists of a filter sealed to a cylindrical polystyrene holder. Cells were maintained in 10%FCS with 10^{-8} M E_2. Then epithelial cells were passaged onto the inside of an insert above membrane at a density of 2×10^5 cells/well, and stromal cells were plated onto the bottom of a 6-well plate at a density of 5×10^5 cells/well. Cells became confluent in 24 hr in 10%FCS + E_2, and co-culture was started by setting the insert on the 6-well plate and terminated 24 hr later.

Effect of E_2 on epithelial/stromal co-culture

To investigate the effect of E_2 on co-culture, two epithelial cell lines (OA-ab and OF-1a cells) and two stromal cell lines (OA-5cg and -5ch) were chosen as representatives. They were separately pre-cultured in 10%DCCFCS for 5 days, then epithelial cells were inoculated onto the inside of an insert above membrane at a density of 2×10^5 cells/well, and stromal cells were plated onto the bottom of a 6-well plate at a density of 5×10^5 cells/well. Cells became confluent in 24 hr, and they were washed with phosphate-buffered saline (PBS) and cultured in serum-free medium (DMEM/F12) supplemented with or without E_2 (10^{-8} M). Then co-culture was started by setting the insert on the 6-well plate and terminated 24 hr later.

Conditioned medium of stromal cell lines

Stromal cells were pre-cultured in 10%DCCFCS for 5 days, and they were passaged at a density of 1×10^6 cells onto the bottom of a 60 mm dish. Cells became confluent in 24 hr, and they were washed with PBS, then cultured in serum-free medium supplemented with or without E_2 (10^{-8} M) for 24 hr. The conditioned medium was collected and concentrated with centrifugal filter devices (10,000 MW cut-off, 50% concentrate; Millipore), and mixed with fresh serum-free medium with or without E_2 equivalently. Epithelial cells were inoculated onto the inside of an insert as described in the co-culture section, and the conditioned medium was applied to the inside and outside of the culture insert.

Immunocytochemical staining

Cells in a small volume of suspension were seeded on eight-chamber culture slides (Becton Dickinson Labware, NJ, USA) and incubated in 10%FCS supplemented with 10^{-8} M E_2 for 2 days. For cytoskeleton examination, cytokeratin 18, vimentin and α-actin were employed as a marker for epithelial, fibroblastic and smooth muscle cells, respectively. Cells on slides were fixed in 95% ethanol containing 1% acetic acid at 4°C for 15 min. After rinsing twice with ethanol (99%), the slides were washed twice with cold PBS. For examination of vascular endothelial growth factor (VEGF) receptor 2 (KDR) and cadherin 5 (CD5), cells were incubated in 10%FCS with 10^{-8} M E_2. Cells on slides were fixed in 2% paraformaldehyde for 15 min. The slides were rinsed three times with cold PBS.

The protocol for immunocytochemical staining was described previously (Hanazono *et al.*, 1998). The primary antibodies were anti-cytokeratin 18 antibody (Ks 18.04, 1/10; Progen Biotechnik, Heidelberg, Germany), anti-vimentin monoclonal antibody (VIM 3B4, 1/20; Boeringer Mannheim Biochemica, Mannheim, Germany), anti-KDR polyclonal antibody (91–730, 1/100; IBL, Gunma, Japan) and anti-CD5 polyclonal antibody (LY-1, 1/100; Research
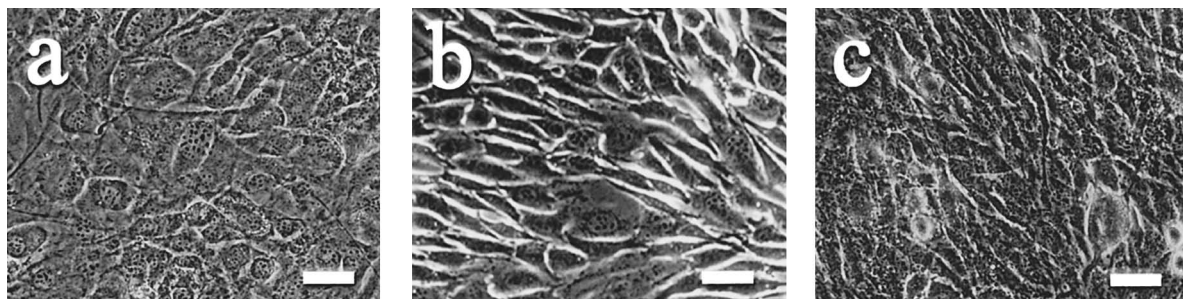

Fig. 1. Morphology of stromal cell lines. (a) OA-5ca cells were flat and had prolonged projections. (b) OA-5cg cells were like wedges or droplets. (c) OA-5ch cells had an unclear edge and became overlapped as cell density increased. Bar=20 µm.

Diagnostic Inc, Flanders, NJ, USA). After washing three times with cold PBS, cells were incubated with fluorescein isothiocyanate (FITC)-conjugated anti-mouse IgG serum (Biosource International, Camarillo, CA, USA) or FITC-conjugated anti-rabbit IgG serum (Biosource International) at room temperature for 2 hr. On control slides, incubation with the primary antibody was omitted.

Northern blot analysis

Isolation of total RNA. Total RNA was isolated from cells with a method as described (Chomzynski and Sacchi, 1987) with a modification of the guanidine isothiocyanate-phenol-chloroform extraction method (TRIZOL, Invitrogen Corp., California, USA).

Probe preparation. A 411-bp probe against mouse oviduct-specific glycoprotein (*mogp-1*) (Sendai *et al.*, 1995; Takahashi *et al.*, 2000) was generated from mouse oviduct cDNA using primers based on the *mogp-1* sequence (GenBank D32137; forward, 5'-GATTTC-TAAGGCCACTGCT-3'; reverse, 5'-AAGAGTCGTATCCATCTCCG-3'). A 259 bp probe against mouse HNF-3/forkhead homolog-4 (*hfh-4/foxj1*) (Hackett *et al.*, 1995; Brody *et al.*, 1997; Murphy *et al.*, 1997; Blatt *et al.*, 1999) was using primers based on the *foxj1* sequence (GenBank L13204; forward, 5'-ACAGGAGAGGGAAG-GCTGGGG-3'; reverse, 5'-GCGGCCATGGACTGTGAGATCCAC-3'). Resultant bands were subcloned into pGEM T-easy vector (Promega, Madison, WI, USA) and sequenced to confirm sequence
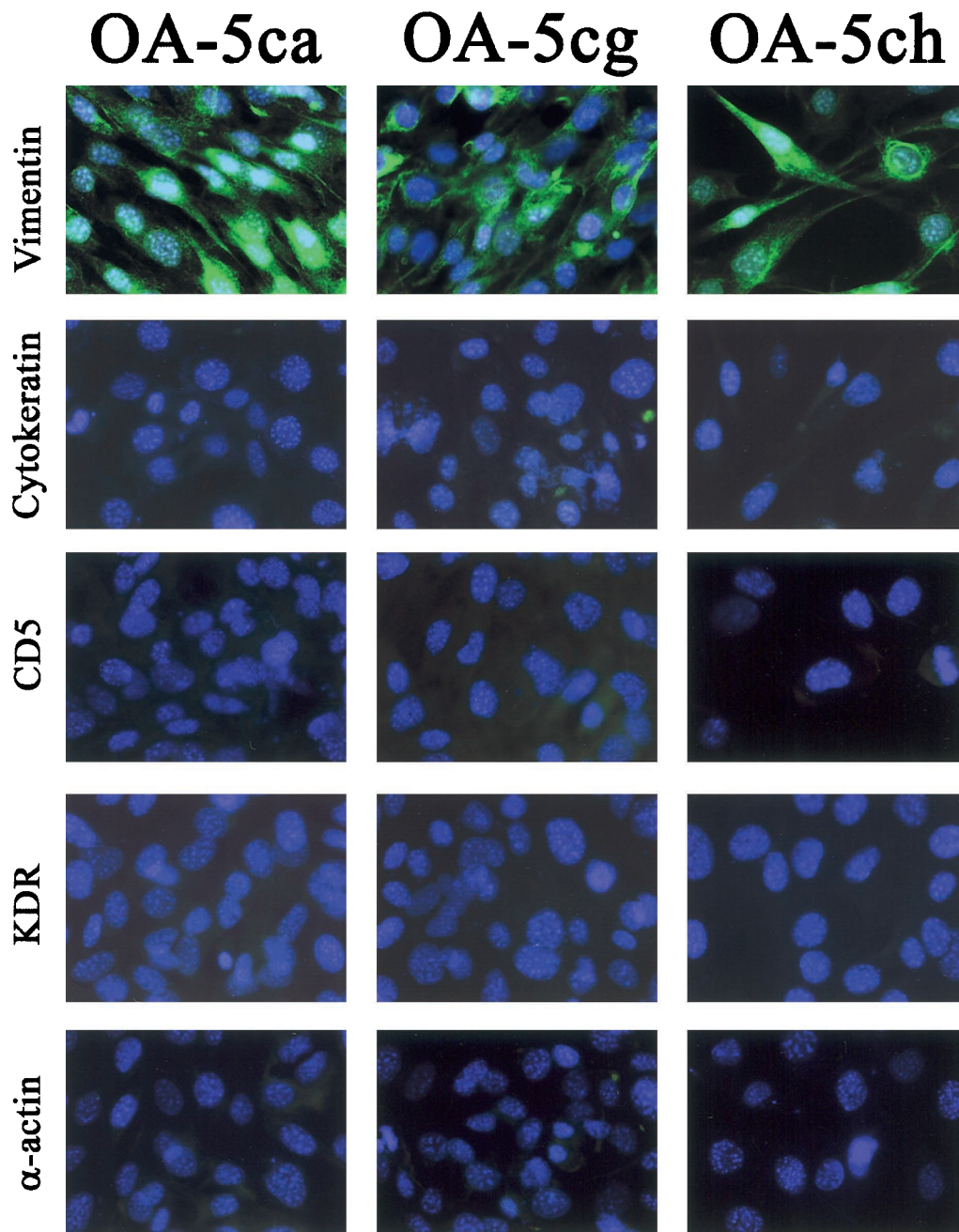

Fig. 2. Immunocytochemistry of stromal cell lines on intermediate filaments and vascular endothelial markers. OA-5ca, -5cg and -5ch cells were plated in 10%FCS supplemented with E$_2$. Cells were stained with antibodies. They were positive for only vimentin, but negative for cytokeratin 18, α-actin, KDR and CD5. Cells stained without the primary antibodies were negative (data not shown). Magnification: $\times$300.

Table 1. Summary of immunocytochemical staining

Cell lines	CK	VT	ERα	PR	KDR	CD5	α-actin
OF-1a	+*	−	+*	+*	−	−	−
OA-ab	+*	−	+*	+*	−	−	−
OA-2c	+*	−	+*	+*	−	−	−
OA-5ca	−*	+	+*	+*	−	−	−
OA-5cg	−*	+	+*	+*	−	−	−
OA-5ch	−*	+	+*	+*	−	−	−

CK; cytokeratin 18, VT; vimentin, ERα; estrogen receptor α, PR; progesterone receptor
KDR; vascular endothelial growth factor (VEGF) receptor 2, CD5; cadherin 5
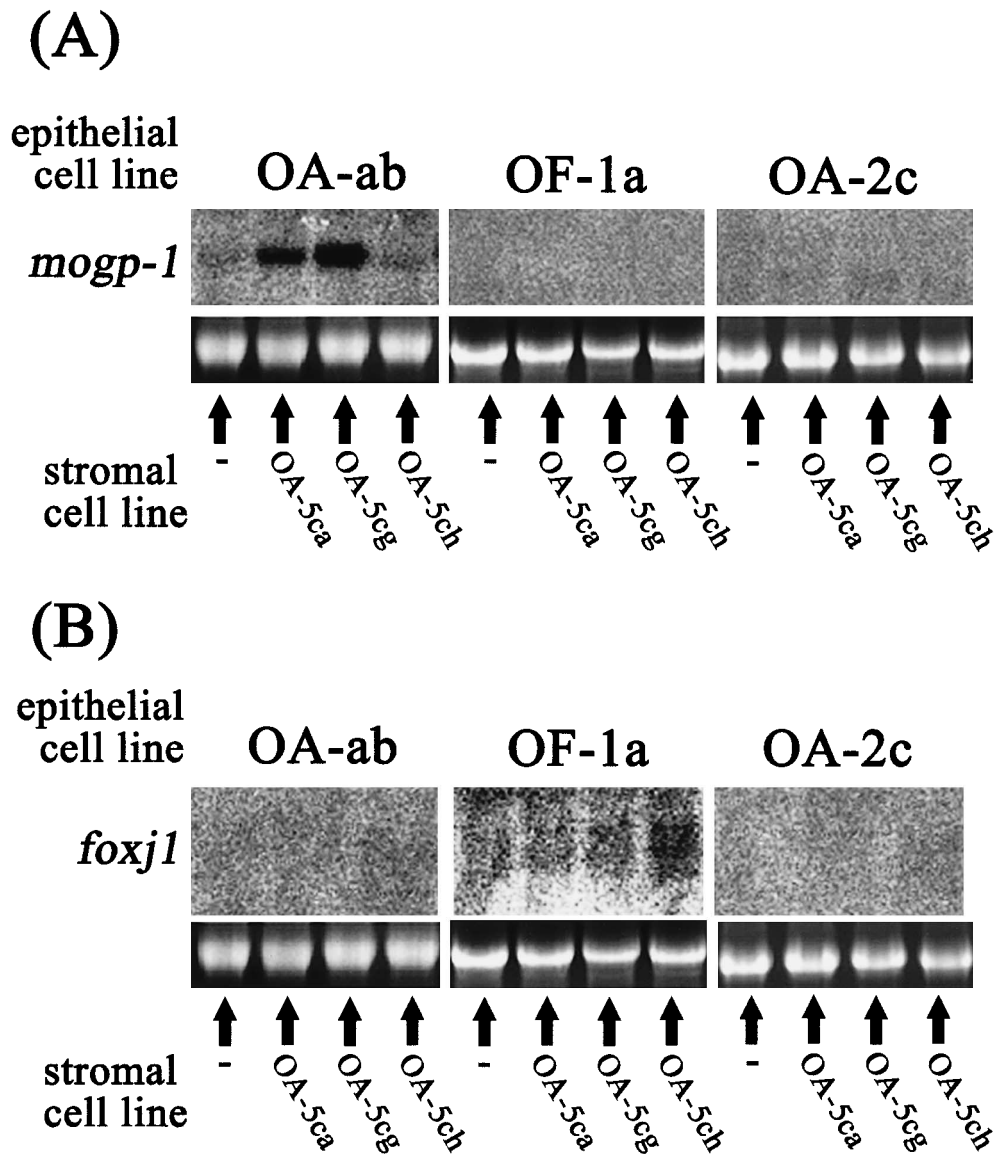+; detected, −; undetected
*; cf. Umezu *et al.*, 2003

Fig. 3. Northern blot analysis of *mogp-1* and *foxj1* mRNA on epithelial cells co-cultured with stromal cells. OA-ab, OF-1a and OA-2c epithelial cell lines were co-cultured with OA-5ca, -5cg and -5ch stromal cell lines separately by culture insert filters. Total RNA was prepared from mono-cultured or co-cultured epithelial cells, and RNAs were electrophoresd on agarose gel. Expression levels of *mogp-1* (A) and *foxj1* (B) were analyzed by Northern blot. mRNA of *mogp-1* and *foxj1* were detected as a single band at approximately 2.8 kb and 5.5 kb, respectively. Ethidium bromide staining demonstrated quality and content of total RNA.

identity (GenBank accession no. D32137 and L13204). Probes were generated after *EcoR*I digestion and gel purification. Fifty ng of subcloned PCR products was linearized for use as templates in [^{32}P]dCTP-labeled DNA probes for *mogp-1* and *foxj1*. The labeled DNA probes were synthesized from free cDNA using BcaBEST Labeling Kit according to the instructions supplied by the manufacturer (TAKARA, Kyoto, Japan) and used to detect *mogp-1* and *foxj1* messages for Northern blot.

Hybridization. Twenty μg of total RNA from cells was separated on 1% formaldehyde-agarose gels and transferred to nylon membranes (HybondTM-N+; Amersham Bioscience, Bucks, UK) and immobilized by UV crosslinking. Hybridization was done with ^{32}P-labeled probes at 42°C in 5× SSPE buffer (50 mM NaH$_2$PO$_4$, 4 mM EDTA, 0.75 M NaCl, pH 7.4), 50% formamid, 5× Denhaedt's reagent, 0.5% SDS and 100 μg/ml denatured salmon sperm DNA. Nylon membranes were washed at 42°C in 0.2× SSC, 0.1% SDS. They were exposed to Fuji imaging plate (Fuji Photo Film, Tokyo, Japan) overnight, and visualized using a Fujix BAS 2000 Phoshorimager (Fuji Photo Film).

Mogp-1 and *foxj1* mRNA signals on the blots were normalized to the respective ethidium bromide staining of 18S rRNA using the NIH Image program version 1.61.

Statistical analysis

The data were analyzed by unpaired student t-test used to examine differences between two groups, and variance (ANOVA) and Duncan's multiple range test used to examine differences among the various groups.

RESULTS

Characterization of stromal cell lines

OA-5ca, -5cg and -5ch cell lines had a distinct morphology. OA-5ca cells were flat, overlapped and had prolonged projections (Fig. 1a). OA-5cg cells were like wedges and had prolonged projections (Fig. 1b). OA-5ch cells had an unclear edge and overlapped each other (Fig. 1c). OA-5ca, -5cg and -5ch cell lines were positive for vimentin, but negative for cytokeratin 18 (Fig. 2). All 3 cell lines expressed both estrogen receptor (ERα) and progesterone receptor (PR) (cf. Table 1). The cell lines were further characterized with VEGF Receptor 2 (KDR), cadherin 5 (CD5) and α-actin. None of them was positive for KDR or CD5 (Fig. 2). The results of the present study and our previous study (Umezu *et al.*, 2003) on epithelial cell lines were summarized in Table 1.

Effect of co-culture with stromal cell lines on *mogp-1* and *foxj1* expression

Northern blot analyses were performed on OA-ab, OA-2c and OF-1a epithelial cell lines co-cultured in 10%FCS + E$_2$ with 3 stromal cell lines (OA-5ca, -5cg and -5ch), respectively.

mRNA for *mogp-1* was detected as a single band at approximately 2.8 kb in OA-ab cells, and *mogp-1* expression was clearly increased by co-culture with stromal OA-5ca and OA-5cg cells, but not with OA-5ch cells (Fig. 3A). *Foxj1* expression was not detected in OA-ab cells co-cultured with any stromal cell lines (Fig. 3B).

mRNA for *foxj1* was detected as a single band at approximately 5.5 kb in OF-1a cells, and *foxj1* expression

was increased by co-culture with OA-5ch cells, but not with OA-5ca or -5cg cells (Fig. 3B). *Mogp-1* expression was not detected in OF-1a cells co-cultured with any stromal cell lines (Fig. 3A).

Neither *mogp-1* nor *foxj1* was detected in OA-2c cells. Co-culture with any stromal cells had no effect on OA-2c cells in induction of *mogp-1* and *foxj1* expression (Fig. 3A, 3B).

Besides the gene induction, co-culture with stromal cells caused a morphological change; cilial cells (OF-1a) became significantly thickened when cultured with stromal cells (OA-5ch). No prominent morphological change was observed on secretory cells (OA-ab) when cultured with stromal cells (data not shown).

Effect of E$_2$ on epithelial/stromal co-culture

To study the effects of E$_2$ on epithelial/stromal co-culture, epithelial and stromal cells were cultured in the co-culture system in medium supplemented with or without E$_2$. In this study, serum-free medium was used to reduce possible effects of serum-born factors.
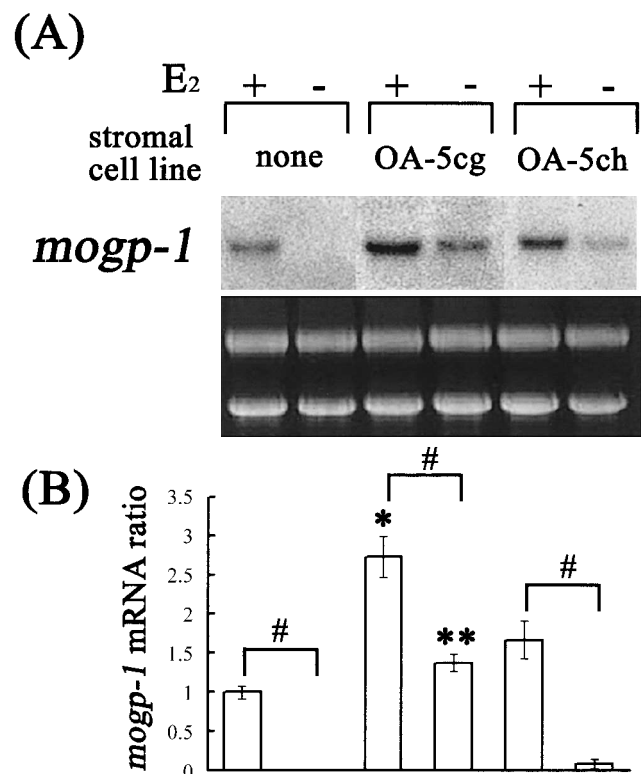

Fig. 4. Effect of E$_2$ on expression of *mogp-1* in epithelial/stromal co-culture. OA-ab cells were plated on cell culture inserts, and co-cultured with OA-5cg and -5ch cells in serum-free medium supplemented with or without 10^{-8} E$_2$. Expression levels of *mogp-1* were analyzed by Northern blot (A). Densitometric quantification of *mogp-1* mRNA in OA-ab cells was shown in (B). The results were normalized to those for 18S rRNA levels. Each column represents the mean (±S. E. M.) of three independent experiments. #, $P<0.05$ (in the presence of E$_2$ *vs.* in the absence of E$_2$). *, $P<0.01$ (*vs.* mono-culture of OA-ab cells in the presence of E$_2$). **, $P<0.01$ (*vs.* mono-culture of OA-ab cells in the absence of E$_2$).

mRNA for *mogp-1* was not detectable in mono-cultured OA-ab cells in serum-free medium. Addition of E_2 caused apparent *mogp-1* expression in OA-ab cells (Fig. 4, $P<0.05$). *Mogp-1* expression was augmented by co-culture with stromal cell lines. Co-culture with OA-5cg cells had significant effect on *mogp1* expression (Fig. 4, $P<0.01$ *vs.* mono-culture of OA-ab cells in presence of E_2, $P<0.01$ *vs.* mono-culture of OA-ab cells in the absence of E_2). E_2 additively increased the *mogp-1* expression in co-culture. On the other hand, co-culture with OA-5ch cells had no significant effect on *mogp-1* expression (Fig. 4).

mRNA for *foxj1* was detectable in mono-cultured OF-1a cells in serum-free medium and E_2 suppressed the expression (Fig. 5, $P<0.05$). *Foxj1* expression was augmented by co-culture with OA-5ch cells. Co-culture with OA-5ch cells had significant effect on *foxj1* expression (Fig. 5, $P<0.05$ *vs.* mono-culture of OF-1a cells in presence of E_2, $P<0.01$ *vs.* mono-culture of OF-1a cells in the absence of E_2). Co-culture with OA-5ch cells released the inhibition caused by E_2, but the releasing effect was not evident in co-culture with

OA-5cg cells (Fig. 5, $P<0.05$ *vs.* mono-culture of OF-1a cells in the absence of E_2).

Effect of conditioned medium of stromal cell lines on *mogp-1* and *foxj1* expression

The results of co-culture experiments described above strongly suggested that the marker gene expression was enhanced by diffusible factors derived from stromal cells. To eliminate direct involvement of stromal cells, epithelial cell lines were plated on filter inserts and cultured in conditioned medium prepared from stromal cell lines (OA-5cg and OA-5ch).

Mogp-1 expression of OA-ab cells in the presence of E_2 was induced by conditioned medium of OA-5cg cells by 2.5-folds over the control (Fig. 6, $P<0.01$ *vs.* none-conditional medium culture of OA-ab cells in presence of E_2.). The expression in the absence of E_2 was induced by conditioned medium of OA-5cg cells by 1.5-folds over the control (Fig. 6, $P<0.01$ *vs.* none-conditional medium culture of OA-ab cells in the absence of E_2). The expression was detectable
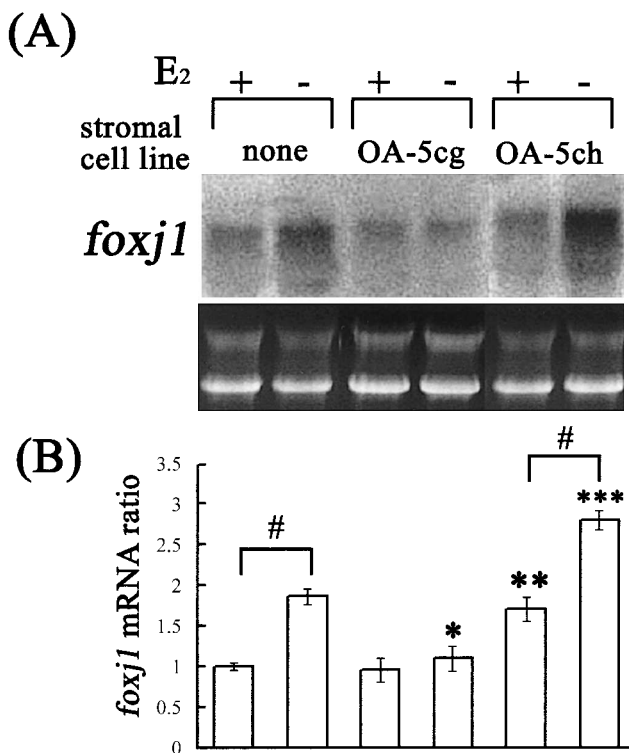

Fig. 5. Effect of E_2 on expression of *foxj1* in epithelial/stromal co-culture. OF-1a cells were plated on cell culture inserts, and co-cultured with OA-5cg or -5ch cells in serum-free medium supplemented with or without 10^{-8} E_2. Expression levels of *foxj1* were analyzed by Northern blot (A). Densitometric quantification of *foxj1* mRNA in OF-1a cells was shown in (B). The results were normalized to those for 18S rRNA levels. Each column represents the mean ($\pm$S. E. M.) of three independent experiments. #, $P<0.05$ (in the presence of E_2 *vs.* in the absence of E_2). *, $P<0.05$ (*vs.* mono-culture of OF-1a cells in the absence of E_2). **, $P<0.05$ (*vs.* mono-culture of OF-1a cells in the presence of E_2). ***, $P<0.01$ (*vs.* mono-culture of OF-1a cells in the absence of E_2).
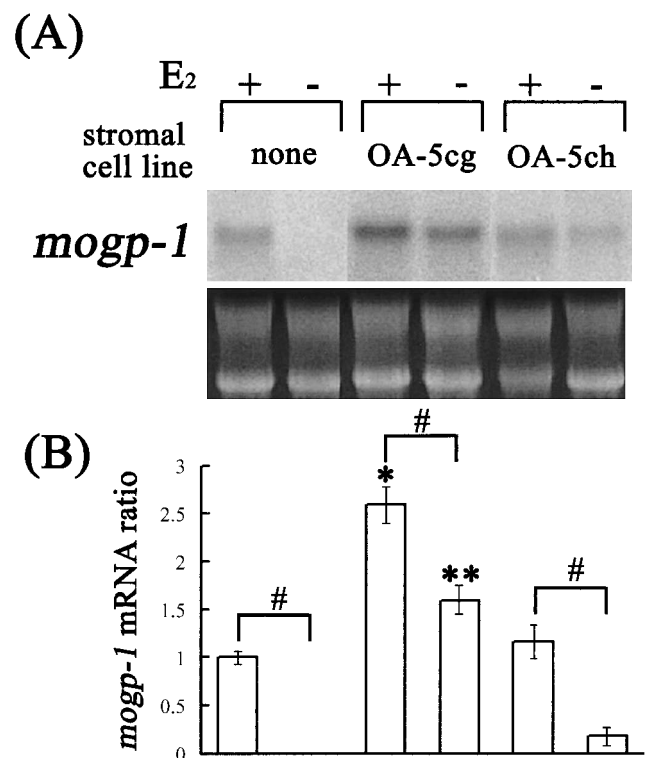
Fig. 6. Effect of conditioned medium of stromal cells on *mogp-1* expression. OA-ab cells were plated on cell culture inserts in conditioned medium with or without 10^{-8} M E_2. Conditioned medium was prepared from OA-5cg or -5ch cells cultured with or without 10^{-8} M E_2. Expression levels of *mogp-1* were analyzed by Northern blot (A). Densitometric quantification of *mogp-1* mRNA in OA-ab cells is shown in (B). The results were normalized to those for 18S rRNA levels. Each column represents the mean ($\pm$S. E. M.) of three independent experiments. #, $P<0.05$ (in the presence of E_2 *vs.* in the absence of E_2). *, $P<0.01$ (*vs.* none-conditional medium culture of OA-ab cells in the presence of E_2). **, $P<0.01$ (*vs.* none-conditional medium culture of OA-ab cells in the absence of E_2).
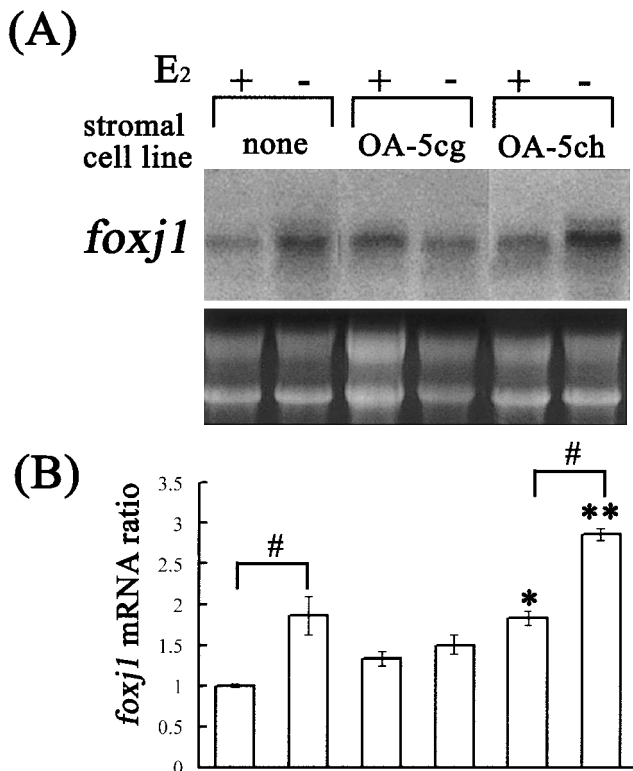
(A)

(B)

Fig. 7. Effect of conditioned medium of stromal cells on *foxj1* expression. OF-1a cells were plated on cell culture inserts in conditioned medium with or without 10^{-8} M E_2. Conditioned medium was prepared from OA-5cg and -5ch cells cultured with or without 10^{-8} M E_2. Expression levels of *foxj1* were analyzed by Northern blot (A). Densitometric quantification of *foxj1* mRNA in OF-1a cells is shown in (B). The results were normalized to those for 18S rRNA levels. Each column represents the mean (±S. E. M.) of three independent experiments. #, $P<0.05$ (in the presence of E_2 *vs*. in the absence of E_2). *, $P<0.05$ (*vs*. none-conditional medium culture of OF-1a cells in the presence of E_2). **, $P<0.05$ (*vs*. none-conditional medium culture of OF-1a cells in the absence of E_2).

in the absence of E_2. But conditioned medium of OA-5ch cells had no significant effect on *mogp-1* expression (Fig. 6).

Foxj1 expression of OF-1a cells was induced by conditioned medium of OA-5ch cells by 2-folds and 2.7-folds over the control in the presence and absence of E_2, respectively (Fig. 7, $P<0.05$ *vs*. none-conditional medium culture of OF-1a cells in presence of E_2, $P<0.05$ *vs*. none-conditional medium culture of OF-1a cells in the absence of E_2). Conditioned medium of OA-5cg cells had no significant effect on *foxj1* expression (Fig. 7).

DISCUSSION

The oviductal functions are played by the epithelium which consists of two major cell populations, cilial cells and secretory cells. The heterogeneity of the epithelium was solved by establishment of clonal epithelial cell lines. In addition to epithelial cell lines, several stromal cell lines with a distinct morphology were also established (Umezu *et al*., 2003). They were shown not to be endothelial cells or

smooth muscle cells. These epithelial and stromal cell lines made it possible to examine interactions between the epithelium and stroma at cellular level.

Co-culture experiments were conducted between 3 epithelial and 3 stromal cell lines in all combinations. Co-culture clearly enhanced *mogp-1* expression in the combinations between OA-ab/OA-5ca and OA-ab/OA-5cg cells. Such enhancement of *mogp-1* was not evident in other co-culture combinations. Co-culture also enhanced *foxj1* expression in a limited co-culture combination (OF-1a/OA-5ch cells). The results strongly suggest that each stromal cell line has a preference for an epithelial type and supports its function. In our co-culture system, epithelial and stromal cell lines were physically separated by a culture insert filter. Therefore, epithelial/stromal interactions must be mediated by diffusible factors.

Experiments of conditioned medium confirmed the results of co-culture experiments. Conditioned media of OA-5cg and OA-5ch cells increased *mogp-1* expression of OA-ab cells and *foxj1* expression of OF-1a cells, respectively. The diffusible factors must be derived from stromal cells. Growth factors, such as IGF-I, EGF, TGF-α and HGF, were expressed in epithelial and stromal cells in mouse uterus and vagina (Shiraga *et al*., 1997; Brown and Lamartiniere, 2000; Murakami *et al*., 2001). Several secreted signaling molecules such as TGF-β superfamily members and Wnts have been shown to be implicated in epithelial/stromal interaction at developmental stage (Halttunen *et al*., 1996; Hammerschmidt *et al*., 1997; Moon *et al*., 1997; Robert *et al*., 1998). But in the adult oviduct, few information is available on diffusible (or growth) factors involved in epithelial/stromal interaction.

E_2 directly acted on secretory cells and induced *mogp-1* expression. Factors derived from OA-5cg cells (and OA-5ca cells) also induced *mogp-1* expression. *Mogp-1* is one of secretory proteins of the mouse oviduct and contains several motif sequences of transcription factor binding sites including estrogen responsive elements (Takahashi *et al*., 2000). Therefore, both E_2 and stromal factors independently might induce the gene expression. OA-5ch cells might weakly deliver such factors which cause a slight induction of *mogp-1* expression (Fig. 4).

OF-1a cells express mRNA for *foxj1* gene in serum-containing or -free medium (Umezu *et al*., 2003, and Fig. 7). Factors derived from only OA-5ch cells significantly induced *foxj1* expression of OF-1a cells. E_2 directly acts on OF-1a cells and inhibits *foxj1* expression (Umezu *et al*., 2003). Factors derived from OA-5ch cells relieved the suppression of *foxj1* expression caused by E_2. However, it is unknown how E_2 and factors derived from OA-5ch cells compete in *foxj1* expression of OF-1a cells.

OA-2c cells were thought to be undifferentiated (or defected), because they had no expression of *mogp-1* or *foxj1* (Umezu *et al*., 2003). The present study confirmed the observation. However, OA-2c cells expressed *foxj1* mRNA when they were co-cultured with stroaml cell lines estab-

lished from an oviduct of a neonatal p53$^{-/-}$ mouse (unpublished observations), suggesting that OA-2c cells become functional in response to different signals derived from unaged stromal cells.

Finally, clonal cell lines and the co-culture system described in the present study might be useful tools to identify the diffusible stromal factors.

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