Immunocytochemical localization of growth factors and their receptors in human pre-embryos and Fallopian tubes

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We utilized indirect immunocytochemistry to demonstrate the presence of growth factors and their receptors in human pre-embryos and Fallopian tubes. In pre-embryos, only transforming growth factor-α (TGF-α) and the intracellular domain of epidermal growth factor receptor (EGFR) were found at the 4-cell stage. In 8- to 14-cell pre-embryos, TGF-α, the intracellular and extracellular domains of EGFR, and insulin-like growth factor-I and its receptor were found. Antibodies against TGF-α stained all Fallopian tube specimens, while the extracellular domain of EGFR was only found in specimens from patients with either blood type A or AB. These results suggest a cross-reactivity between the extracellular domain of the EGFR and blood group antigens. Our novel demonstration of growth factor receptor staining in human pre-embryos shows that growth factor receptor localization is dependent on the developmental stage of human pre-embryos. We have also established a potentially important link between the Fallopian tube which secretes growth factors and the localization of growth factor receptors in pre-embryos. These findings are compatible with the hypothesis that tubal secretions are embryotrophic for the early development of the pre-embryo.

Key words: Fallopian tubes/growth factor receptors/human pre-embryos/immunocytochemistry

Introduction

The Fallopian tube is widely believed to play an integral role in normal fertility. For example, growth factors secreted by tubal epithelium may be embryotrophic or they may have autocrine/paracrine functions. In any event, this muscular tube and its mucosal lining provide the complex yet poorly understood environment required for gamete transport, maturation, fertilization, early pre-embryo development and transport to the uterine cavity (Harper, 1988). The dynamic cyclical changes in epithelial structure respond to variations in ovarian steroid hormones, and numerous investigators have suggested that the mitogenic effects of oestradiol on the tubal epithelium are mediated by growth factors (Verhage et al., 1979; Donnez et al., 1985; Giudice et al., 1992; Lei and Rao, 1992; Carlsson et al., 1993; El-Danasouri et al., 1993; Morishige et al., 1993; Pfeifer and Chegini, 1994).

Epidermal growth factor (EGF) and transforming growth factor-α (TGF-α) share considerable homology (Burgess, 1989). Both compete for binding to the same receptor, the EGF receptor (EGFR). The EGFR consists of an extracellular ligand binding domain connected by a short transmembrane region to a cytoplasmic domain with protein kinase activity (Carpenter, 1987; Carpenter and Cohen, 1990).

TGF-α, EGF and their receptors have been found in human tubal epithelium, endometrium and early gestational extraembryonic membranes. EGF has no effect on oocyte maturation in vitro (Gómez et al., 1993). EGF is present in the endometrial glands unrelated to the phase of the menstrual cycle, although oestradiol and EGF appear to have a synergistic effect on the stimulation of glandular proliferation (Haining et al., 1991). Furthermore, EGF appears to have an inhibitory effect on development from the 4-cell to the blastocyst stage in mouse embryos and on spreading of the trophectoderm (Goldman et al., 1993). This effect may be caused by competition with TGF-α for the EGFR. The EGFR is weakly expressed on mouse pre-embryonic blastomeres prior to the 4-cell stage, but then shows a substantial increase in concentration after the 4-cell stage, especially on the apical cell surface membranes of blastomeres (Wiley et al., 1992). TGF-α promotes blastocoel expansion in the mouse embryo (Dardik and Schultz, 1991) by binding to a functional EGFR in the mouse blastocyst (Dardik et al., 1992). Other work has suggested that maternal growth factors may regulate embryonic proliferation and differentiation (Kommoss et al., 1990; Hofmann et al., 1991; Lei and Rao, 1992; Xynos et al., 1992; El-Danasouri et al., 1993; Horowitz et al., 1993; reviewed by Schultz and Heyner, 1993; Harvey et al., 1995).

Insulin-like growth factor (IGF-I) and its receptor (IGF-IR) have also been studied extensively. IGF-I was localized in human Fallopian tubes, follicular fluid and endometrium, and may be involved in intra-ovarian regulation (Adashi et al., 1985; Froesch et al., 1985; Geisthoevel et al., 1989; Pfeifer and Chegini, 1994; Weigang et al., 1994). Messenger RNA (Zhang et al., 1994) for the IGF-IR was detected in rat oocytes, 1- and 2-cell pre-embryos and blastocysts (but not in 4- and 8-cell embryos). Protein for IGF-IR was detected in the 8- but not the 2-cell preimplantation rat embryo (Carlsson et al., 1993).
Recent experimental manipulations employing sophisticated molecular genetic techniques to produce null mutants with a functional disruption of the genes for growth factors and their receptors have revealed that different growth factors become crucial at different times in development. For example, female mice lacking a functional gene for leukaemia inhibitory factor are fertile but their blastocysts cannot implant, even though these mutant blastocysts can implant and develop normally in wild-type pseudopregnant hosts (Stewart et al., 1992). Mouse embryos with disrupted genes for fibroblast growth factor (FGF-4) can implant and cause deciduization but cannot develop further. These mutant embryos showed defective proliferation of the inner cell mass when cultured in vitro, but this defect could be rescued by exogenous FGF-4 protein added to cultures (Feldman et al., 1995). Liu et al. (1993) showed that newborn mice defective in the IGF-I (lgf-I) or its receptor (lgf-Ir) gene develop to birth but have significant decreases in birth weight; many die soon after birth and often have marked morphological abnormalities in several major organ systems. Recently, Campbell et al. (1995) have reported the presence of cell adhesion molecules such as integrins, E-cadherin and neural cell adhesion molecule (NCAM), on human pre-embryos, detected by immunocytochemistry. No attempt was made to relate these results to the expression of growth factor receptors.

Our study was designed to examine the localization of TGF-α, EGFR, IGF-I and IGF-IR in human pre-embryos and Fallopian tubes, using the simple paradigm that growth factors secreted by tubes are embryotrophic. Our new results (namely that human pre-embryos have extensive expression of EGFR) and our confirmation of previous results (showing TGF-α secretion by Fallopian tubal epithelium), when considered together, are consistent with the hypothesis that tubal growth factor secretions are embryotrophic.

Materials and methods

**Institutional approval and informed consent**

This work was approved by the institutional review board and the ethics committee of the George Washington University, Washington, DC, USA, and conforms to the guidelines established by the ethics committee of the American Society for Reproductive Medicine on human embryo research. Informed consent was obtained from all in-vitro fertilization (IVF) participants who donated pre-embryos to this research and from patients undergoing tubal ligation.

**Clinical IVF protocol**

Pre-embryos were obtained from patients participating in the George Washington University, Washington, DC, USA, IVF programme. Patients underwent stimulation regimens using pituitary suppression with a gonadotrophin-releasing hormone agonist (leuprolide acetate; Lupron; Tap Pharmaceuticals, Chicago, IL, USA) and subsequent ovarian stimulation with human menopausal gonadotrophins (HMG; Pergonal; Serono Laboratories Inc., Norwell, MA, USA), followed by individualized monitoring.

Transvaginal follicular aspiration was performed 35.5 h after human chorionic gonadotrophin (HCG) administration. Oocytes were identified in the follicular aspirates, characterized and placed in Ham’s F-10 nutrient mixtures (Gibco BRL, Gaithersburg, MD, USA; 81200-040) containing 10% plasmanate (Miles Inc., Pharmaceutical Division, Elkhart, IN, USA; 660613-204). All oocytes were cultured at 37°C with 5% CO₂ and 95% air. At 14–18 h after insemination, each oocyte was observed for the presence of pronuclei, indicating that fertilization had occurred. Oocytes with two pronuclei were cultured for an additional 48 h. At 72 h after insemination, all pre-embryos were graded prior to selection for uterine transfer.

**Pre-embryo donation**

Based on pre-embryo quality (morphology and cell number), the patients were counselled about the prognosis for implantation and pregnancy. If available, three or four high-quality pre-embryos were then usually selected for transfer. If additional pre-embryos remained after transfer, patients were given the option of cryopreserving, discarding and/or donating the remaining pre-embryos to this research.

**Fallopian tube collection**

Portions of Fallopian tubes were obtained from 54 women, ranging in age from 26 to 43 years, undergoing elective post-partum tubal ligation by the Pomeroy technique. After removal, the pathology department received a portion of tissue, and the remainder was released for our studies. The medical histories of each post-partum patient were reviewed and three patients were excluded from our study: one patient was positive for human immunodeficiency virus, one patient had insulin-dependent diabetes mellitus and the blood type of the third patient was not available. The pathological analysis of each tubal specimen was normal.

**Immunological reagents**

Five different antisera were used, all at a final concentration of 5 μg/ml in 0.5% bovine serum albumin (BSA)/phosphate buffered saline (PBS). The monoclonal antibody to the extracellular domain of the EGFR was obtained from Sigma (St Louis, MO, USA; E-2760, clone 29.1). It is putatively specific for EGFR in humans and binds to a carbohydrate residue on the external portion of the EGFR molecule. A monoclonal antibody specific for the intracellular domain of the EGFR was obtained from Sigma (E-3138, clone F4). A monoclonal antibody to TGF-α was obtained from Oncogene Science (Uniondale, NY, USA; GF-10, clone 213-4.4). Monoclonal antibodies to IGF-I and IGF-IR were also obtained from Oncogene Science (GF21/GF21L, clone 82-9A for IGF-I and GR11, clone α1R3 for IGF-IR). Our secondary antibody, either goat anti-mouse immunoglobulin (IgG) conjugated with fluorescein isothiocyanate (FITC; Sigma; F-0257) or goat anti-mouse IgG conjugated with Texas Red (Oncogene Science; DC19L), was diluted 1/200 with 0.5% BSA/PBS. We have not tested the specificity of these antibodies with Western blots or immunoprecipitation methods because of the lack of appropriate antigens. In this regard, we have followed the approach of others (Haining et al., 1991; Campbell et al., 1995), relying on the manufacturer’s tests of specificity instead of performing them ourselves.

**Immunocytochemistry of pre-embryos**

Pre-embryos were fixed in 3.7% formaldehyde/PBS and sequentially processed in PBS for 5 min and 3% BSA/PBS for 2 h. They were incubated with primary antibodies overnight in a moist chamber at 4°C, carefully rinsed for 10 min in 0.5% BSA/PBS and incubated in the secondary antibody for 2 h in a moist chamber at 20°C. Specimens were observed in a Nikon epifluorescent microscope as described above. For positive controls, we used human abdominal skin specimens processed in parallel with pre-embryos. Preliminary controls were also performed by eliminating primary antibody staining to control for autofluorescence and by eliminating secondary antibody to control for the nonspecific binding of fluorescent secondary antibody.
the type of primary antibody utilized.

by two different investigators blinded to the patient’s blood type or specific binding of fluorescent secondary antibody respectively.

above without the primary antibody or secondary antibody were used intensity for each section studied, and was evaluated independently as negative controls to rule out tissue autofluorescence and the non-
mens were used as positive controls. Tubal specimens processed as (FITC) or G2A (Texas Red) dichroic mirror. Abdominal skin speci-
were observed in a Nikon epifluorescent microscope with a B2A
in secondary antibody for 2 h in a moist chamber at 20°C. Sections
were carefully rinsed for 10 min in 0.5% BSA/PBS and then incubated
with 300 µl primary monoclonal antibodies overnight in a moist chamber at 4°C. Sections
were processed for routine light microscopy and immunocyto-
chemistry. A total of 43 specimens were from ampullary segments
(3 |µm) were processed for routine light microscopy and immunocyto-
chemistry. A total of 255 tubal sections from 51 patients were stained in

Confocal microscopy

Scanning confocal microscopy of Texas Red-labelled pre-embryos was performed using a Bio-Rad MRC-1000 system coupled to an Olympus Axiovert 35 microscope. Most specimens were observed using the transmission detector in place to capture differential interference contrast (DIC) images and with appropriate filters in place to observe Texas Red fluorescence with a ×20 lens. Fluorescent specimens were observed with a 10–30% maximal intensity of the laser. Slow (F1) scan rates were used, with Kalman averaging for 10 frames. Other than Kalman averaging and contrast manipulation, no image processing enhancements were used on the images presented here. We were also able to gather and merge DIC and fluorescent images, which greatly facilitated our ability to identify labelled cells. Permanent records were obtained by displaying the images on a video monitor and printing them directly using a Mitsubishi colour video printer.

Immunocytochemistry of Fallopian tubes

All tissues were fixed in 3.7% formaldehyde/PBS, dehydrated in a graded series of ethanol solutions and paraffin embedded. Sections (3 µm) were processed for routine light microscopy and immunocytochemistry. A total of 43 specimens were from ampullary segments while 11 were from isthmic segments of the Fallopian tube. We found no regional differences in the tubes with respect to the immunocytochemistry. Sections were deparaffinized by sequential processing in toluene, graded ethanol solutions and PBS for 5 min each, then non-specific antibody binding was blocked by incubation for 2 h in 3% BSA/PBS. Sections were incubated with 300 µl primary monoclonal antibodies overnight in a moist chamber at 4°C. Sections were carefully rinsed for 10 min in 0.5% BSA/PBS and then incubated in secondary antibody for 2 h in a moist chamber at 20°C. Sections were observed in a Nikon epifluorescent microscope with a B2A (FTTC) or G2A (Texas Red) dichroic mirror. Abdominal skin specimens were used as positive controls. Tubal sections processed as above without the primary antibody or secondary antibody were used as negative controls to rule out tissue autofluorescence and the non-specific binding of fluorescent secondary antibody respectively.

Immunostaining was graded from the lowest (0) to highest (++++) intensity for each section studied, and was evaluated independently by two different investigators blinded to the patient’s blood type or the type of primary antibody utilized.

<table>
<thead>
<tr>
<th>Specimen</th>
<th>TGF-αb Stain</th>
<th>ng</th>
<th>EGFR extc Stain</th>
<th>n</th>
<th>EGFR intd Stain</th>
<th>n</th>
<th>IGF-Ie Stain</th>
<th>n</th>
<th>IGF-IRf Stain</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Granulosa cells</td>
<td>+++</td>
<td>3</td>
<td>+++</td>
<td>7</td>
<td>+++</td>
<td>16</td>
<td>+++</td>
<td>5</td>
<td>+++</td>
<td>9</td>
</tr>
<tr>
<td>Zona pellucida</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>7</td>
<td>0</td>
<td>16</td>
<td>0</td>
<td>5</td>
<td>0</td>
<td>9</td>
</tr>
<tr>
<td>Spermatozoa</td>
<td>0</td>
<td>3</td>
<td>0</td>
<td>7</td>
<td>0</td>
<td>16</td>
<td>0</td>
<td>5</td>
<td>0</td>
<td>9</td>
</tr>
<tr>
<td>Unfertilized oocyte</td>
<td>++</td>
<td>1</td>
<td>+</td>
<td>3</td>
<td>+</td>
<td>3</td>
<td>0</td>
<td>2</td>
<td>+</td>
<td>3</td>
</tr>
<tr>
<td>Blastomere of 4-cell pre-embryo</td>
<td>++</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>++</td>
<td>4</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Blastomere of 8- to 14-cell pre-embryo</td>
<td>++</td>
<td>1</td>
<td>++</td>
<td>3</td>
<td>++</td>
<td>9</td>
<td>++</td>
<td>1</td>
<td>++</td>
<td>5</td>
</tr>
</tbody>
</table>

EGFR = epithelial growth factor receptor; IGF-I = insulin-like growth factor I; IGF-IR = IGF-I receptor. TGF = transforming growth factor.

Results

Human pre-embryos

Antibodies against TGF-α, the extracellular and intracellular domains of EGFR, IGF-I and IGF-IR all intensely stained the granulosa cells surrounding the human pre-embryos. In all specimens, the most intense staining was invariably observed in granulosa cells (Table I). The zona pellucida and spermatozoa attached to the zona pellucida did not stain with any of the above antibodies. Unfertilized oocytes showed little or no staining of growth factor receptors; however, there was moderate staining of TGF-α. Blastomeres from 4-cell pre-embryos did not stain for IGF-I, IGF-IR and the extracellular domain of the EGFR. Antibodies against TGF-α and the intracellular domain of EGFR stained the blastomeres from 4-cell pre-embryos but less intensely than the granulosa cells (Table I). TGF-α staining was distributed uniformly throughout the cytoplasm of blastomeres, whereas the intracellular domain of EGFR appeared to be differentially localized on the surface of the blastomeres (Figure 1A and B).

Blastomeres from 8- to 14-cell pre-embryos were intensely stained with antibodies against TGF-α and the intracellular domain of EGFR (Figure 1C and D). Antibodies against IGF-I, IGF-IR (Figure 1E and F) and the extracellular domain of EGFR stained these blastomeres moderately (Table I). Once again, TGF-α antibodies stained diffusely. In contrast, IGF-IR and the intracellular and extracellular domains of EGFR appeared to stain the surface of the blastomeres more intensely. The above staining patterns were found regardless of the maternal blood type.

Human post-partum tubes

A total of 255 tubal sections from 51 patients were stained in duplicate; 20 patients had blood type A, 21 had type O, eight had type B and two had type AB.

TGF-α

Using antibodies against TGF-α, we found the most intense staining in the cytoplasm of the tubal epithelial cells. All epithelial cells had intense punctate staining distributed uni-
formly throughout their cytoplasm. The nuclei of these cells had no staining (Figure 2A). Some cells showed slightly more staining in their apical portions. Fibroblasts in the lamina propria were also weakly stained. The bands of smooth muscle in the wall of the Fallopian tube were stained diffusely but their intensity was less than that seen in tubal epithelial cells. In the larger blood vessels there was moderate staining of the smooth muscle cells of the tunica media but little or no staining of the tunica adventitia. In addition, the tunica intima, endothelium and red blood cells were unstained. This pattern was unchanged regardless of the tubal segment or the blood type of the patient (Table II).

**EGFR extracellular domain**
Antibodies against the extracellular domain of EGFR intensely stained the apical region of the tubal epithelial cells in a band-like configuration, but only in patients with blood type A or AB (Figure 2B and Table II). In addition, there was weaker peripheral staining surrounding individual tubal epithelial cells. The epithelial cells from patients with blood types O and B failed to stain (Figure 2C). There was only a slight staining in the lamina propria, associated with thin fibres or fibroblast processes. All patients, regardless of blood type, showed an intense staining of the mural smooth muscle especially near the lamina propria. In addition, vascular endothelium and red
Figure 2. Immunocytochemistry of human Fallopian tube epithelium. All photographs were exposed and printed identically so that differences in brightness are reflective of differences in the staining intensity. (A) Transforming growth factor-α; (B) extracellular domain of epidermal growth factor receptor (EGFR), AB patient; (C) extracellular domain of EGFR, O patient; (D) intracellular domain of EGFR, A patient; (E) insulin-like growth factor-I; (F) insulin-like growth factor-I receptor. Bar = 50 μm.

Table II. Tubal epithelial cell localization of antigen*

<table>
<thead>
<tr>
<th>Blood type</th>
<th>TGF-α</th>
<th>EGFR ext</th>
<th>EGFR int</th>
<th>IGF-I</th>
<th>IGF-IR</th>
</tr>
</thead>
<tbody>
<tr>
<td>A (n = 20)</td>
<td>+++</td>
<td>+++</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>AB (n = 2)</td>
<td>+++</td>
<td>+++</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>B (n = 8)</td>
<td>+++</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>O (n = 21)</td>
<td>+++</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

See footnote to Table I for definitions.

*Scoring system: 0 denotes no staining, +++ denotes intense staining.

Antibody to TGF-α.

Antibody to the extracellular domain of EGFR.

Antibody to the intracellular domain of EGFR.

Antibody to IGF-I.

Antibody to IGF-IR.

blood cells were neatly outlined by a thin band of stain on their cell surfaces. Ampullary and isthmic regions stained with equal intensities.

EGFR intracellular domain

The antibodies against the intracellular domain of EGFR did not stain the tubal epithelial cells (Figure 2D). Mural smooth muscle and vascular smooth muscle were also unstained. These antibodies weakly stained the vascular endothelium but not the red blood cells. This staining pattern was found regardless of the tubal segment or the blood type of the patient (Table II).

IGF-I and IGF-IR

Antibodies against IGF-I and IGF-IR failed to stain the tubal epithelium, the vascular endothelium, the red blood cells, the mural smooth muscle and the vascular smooth muscle (Figure
2E and F). Positive controls, run in parallel with these negative slides, showed intense staining. This pattern was observed regardless of the tubal segment or the blood type of the patient.

Discussion

We have studied the presence and cellular distribution of several growth factors and their receptors in human pre-embryos and the post-partum human Fallopian tube. The tubal environment is thought to be crucial for supporting early development. Our results add some specific candidate growth factors to a list of potentially embryotrophic tubal secretions, and show, for the first time, that growth factor receptors are localized on blastomeres and granulosa cells of human pre-embryos. Although results from human embryos are sparse, numerous studies from animal models suggest that growth factors are embryotrophic (for a review see Schultz and Heyner, 1993). Our results in human pre-embryos are similar to those found for animal models. For example, Corps et al. (1990) found EGFR and IGF-IR in pig preimplantation embryos. Similar results have also been published for mouse embryos (Wiley et al., 1992). We suggest that our results support the hypothesis that TGF-α secreted by the epithelium of the Fallopian tube is embryotrophic. Morishige et al. (1993) cocultured 2-cell mouse embryos with human tubal epithelial cells and found the significant promotion of blastocyst formation. Furthermore, this stimulatory effect was abolished by anti-TGF-α antibodies added to the cocultures. Dardick and Schultz (1991) found that picomolar concentrations of TGF-α can increase the rate of mouse blastocyst development in a concentration-dependent manner because of binding to a functional EGFR (Dardik et al., 1992). In addition, Wiley et al. (1992) have shown that after the 4-cell stage, mouse embryos have a significant expression of EGFR on the apical surface of their blastomeres. Messenger RNA (Zhang et al., 1994) and protein (Carlsson et al., 1993) for the IGF-IR have been detected in preimplantation rat embryos. Human embryos produce TGF-α and IGF-II in culture (Hemmings et al., 1992). Thus, there is a growing body of evidence suggesting that growth factors may have embryotrophic activity prior to implantation.

Although our results are preliminary regarding the staining of pre-embryos, there may be interest in exploring their potential for improving in-vitro embryo development in assisted reproductive technology. For the first time, clear evidence of the localization of growth factors and their respective receptors in human pre-embryos has been reported. Antibodies against TGF-α and its receptor (EGFR) intensely stained granulosa cells and moderately stained the blastomeres of 4- and 8-cell pre-embryos. Antibodies against IGF-I and its receptor intensely stained granulosa cells and moderately stained 8-cell pre-embryos. Our results correlated with the stage of development and support the findings from a previous mouse embryo study (Doherty et al., 1994). The documentation of growth factor secretion by the human Fallopian tubes, and the localization of appropriate receptors in granulosa cells and blastomeres of pre-embryos, raise the possibility that growth factors secreted by tubal epithelial cells are potentially embryotrophic substances. Currently, we are investigating whether pre-embryo development in vitro might be enhanced by the addition of growth factors to growth and transfer media, taking advantage of their putative embryotrophic effects.

In the oestrogen-rich environment of the post-partum Fallopian tube, TGF-α was abundant in the cytoplasm of the tubal epithelial cells, whereas staining of the intracellular domains of EGFR, IGF-I and IGF-IR was not detectable in tubal epithelial cells. In contrast, only those tubal specimens from patients with either blood type A or AB stained positively with antibodies against the extracellular domain of EGFR, while none was detected in specimens from patients with either blood type O or B. These results are most likely caused by cross-reactivity between the monoclonal antibody to the extracellular domain of EGFR and the blood group A antigen, a phenomenon which has been described previously (Freedman et al., 1983; Gooi et al., 1983; Childs et al., 1984; Lei and Rao, 1992). Lei and Rao (1992) raised the theoretical possibility of receptor cross-reactivity between the carbohydrate structures of the EGFR and blood group antigens, but it is not known if these antigens are present in Fallopian tubes. Our study is the first to suggest that this cross-reactivity between the extracellular domain of EGFR (from clone 29.1) and blood group type A antigens exists in human Fallopian tubes. Furthermore, no specimens showed the localization of the intracellular domain of EGFR, suggesting that TGF-α cannot bind to tubal epithelium and therefore does not play an autocrine/paracrine role in the regulation of tubal function.

We failed to demonstrate IGF-I and IGF-IR in tubal epithelial cells, while previous studies reported the immunostaining of both (Pfeifer and Chegini, 1994; Weigang et al., 1994). We utilized the same mouse monoclonal antibodies to IGF-I and IGF-IR as Weigang et al. (1994), but Pfeifer and Chegini (1994) used polyclonal antibodies to IGF-I and a different monoclonal antibody to IGF-IR. Our results may conflict with previous studies because we utilized a different tubal environment, post-partum specimens, rather than different cycle stage hysterectomy specimens, and different primary monoclonal antibodies.

Our TGF-α results were also somewhat different from previous investigations. TGF-α staining was distributed widely in the cytoplasm of the tubal epithelial cells, as opposed to the restricted apical distribution in tubal epithelial cells reported by others (Kommoss et al., 1990; El-Danasouri et al., 1993). Although it is theoretically possible that the tubal TGF-α is synthesized elsewhere and secondarily bound to tubal EGFR, it is more reasonable to propose that the TGF-α synthesized by tubal epithelial cells plays an embryotrophic role for the developing pre-embryo (which has EGFR) rather than an autocrine/paracrine role for the local regulation of tubal function (because tubal epithelium lacks EGFR).

References


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