Epidermal Growth Factor Stimulates Proliferation of Mouse Uterine Epithelial Cells in Primary Culture

Authors: Shiraga, Masahiro, Komatsu, Noriko, Teshigawara, Kiyoshi, Okada, Akinobu, Takeuchi, Sakae, et al.

Source: Zoological Science, 17(5) : 661-666

Published By: Zoological Society of Japan

URL: https://doi.org/10.2108/zsj.17.661

ZOOLOGICAL SCIENCE **17**: 661–666 (2000)

Epidermal Growth Factor Stimulates Proliferation of Mouse Uterine Epithelial Cells in Primary Culture

Masahiro Shiraga[1], Noriko Komatsu[1], Kiyoshi Teshigawara[1], Akinobu Okada[1], Sakae Takeuchi[1], Hiroshi Fukamachi[2], and Sumio Takahashi[1]*

[1]*Department of Biology, Faculty of Science, Okayama University, Tsushima, Okayama 700-8530, and*
[2]*Department of Biological Sciences, Graduate School of Science, University of Tokyo, Hongo, Bunkyo-ku, Tokyo 113-0033, Japan*

ABSTRACT—Epidermal growth factor (EGF) is one of growth factors that are thought to mediate the stimulatory effects of estrogen on the proliferation of uterine epithelial cells. The present study was attempted to obtain direct evidence for the mitogenic effects of EGF on uterine epithelial cells, and to prove that EGF and EGF receptors are expressed in these cells. Mouse uterine epithelial cells were isolated from immature female mice and cultured with or without EGF for 5 days. EGF (1 to 100 ng/ml) significantly increased the number of uterine epithelial cells, and the maximal growth (141.9±8.3% of controls) was obtained at a dose of 10 ng/ml. In addition, EGF (0.1 to 100 ng/ml) increased the number of DNA-synthesizing cells immunocytochemically detected by bromodeoxyuridine uptake to the nucleus. Northern blot analysis revealed that the uterine epithelial cells expressed both EGF mRNA (4.7 kb) and EGF receptor mRNAs (10.5, 6.6, and 2.7 kb) These results suggest that the proliferation of uterine epithelial cells is regulated by the paracrine and/ or autocrine action of EGF. Our previous study demonstrated the mitogenic effect of IGF-I on uterine epithelial cells. To examine whether the EGF- and IGF-I signaling act at the same level in the regulation of the proliferation of uterine epithelial cells, the cultured cells were simultaneously treated with IGF-I and EGF. IGF-I was found to additively stimulate the mitogenic effects of EGF, suggesting that the EGF-induced growth of uterine epithelial cells is distinct from IGF-I-induced growth.

INTRODUCTION

Estrogens and progestin regulate the growth of uterine endometrial cells (Huet-Hudson *et al.,* 1989). Estrogen stimulates the growth of uterine epithelial cells *in vivo* but not *in vitro* (Fukamachi and McLachlan, 1991; Astrahantseff and Morris, 1994). It has been proposed that the estrogen-induced proliferation of uterine epithelial cells is mediated by several growth factors in an autocrine and/or paracrine manner (DiAugustine *et al.,* 1988; Brigstock, 1991; Nelson *et al.,* 1991; Borgundvaag *et al.,* 1992; Das *et al.,* 1994; Wang *et al.,* 1994; Zhang *et al.,* 1994).

EGF is thought to be a mediator of estrogen action, as EGF stimulates DNA synthesis in mouse uterine epithelial cells both *in vitro* and *in vivo* (Tomooka *et al.,* 1986; Ghosh *et al.,* 1991; Nelson *et al.,* 1991), and EGF-specific antibody blocks the estrogen-induced mitogenesis of uterine epithelial cells (Nelson *et al.,* 1991). Moreover, EGF is detected by immunocytochemistry in the borders of uterine luminal and glandular epithelial cells in immature mice, and estrogen treatment increases the amount of prepro-EGF mRNA levels in mouse uteri (DiAugustine *et al*., 1988).

In adult mice, EGF mRNA is detected only during late proestrus and estrus, and early on day 1 of pregnancy (Huet-Hudson *et al*., 1990), whereas immunoreactive EGF has not been detected in the luminal epithelium in estrogen-treated ovariectomized mice (Falck and Forsberg, 1996). Thus, the localization of EGF-synthesizing cells within the endometrium is still not clear. EGF receptors and EGF receptor mRNA have been detected in rat and mouse uteri (Mukku and Stancel, 1985; Iguchi *et al*., 1993), and EGF-high affinity binding sites are localized in the uterine cells (Mukku and Stancel, 1985; Tomooka *et al*., 1986; Nelson *et al*., 1991; Iguchi *et al*., 1993). However, Das *et al*. (1994) and Tong *et al*.(1996) have reported that EGF receptors are not found in the luminal and glandular epithelium in the adult mouse uteri. Thus, it is still unclear whether EGF is synthesized and EGF receptors are expressed in the uterine epithelial cells. Therefore, the aim of the present study of mouse uterine epithelial cells was to examine the expression of EGF mRNA and EGF receptor

* Corresponding author: Tel. +81-86-251-7866;
FAX. +81-86-251-7876.
E-mail: stakaha@cc.okayama-u.ac.jp

mRNA, and to clarify the effects of EGF on DNA replication in these cells in primary serum-free culture. Because we have found evidence of the mitogenic action of IGF-I on uterine epithelial cells (Shiraga *et al.*, 1997), the effects of IGF-I on EGF action with respect to DNA replication in uterine epithelial cells were also studied.

MATERIALS AND METHODS

Animals

Immature female mice (21–23 days of age) of the ICR strain (CLEA Japan Inc., Osaka, Japan) were used in the present study. They were maintained in a temperature-controlled room and were fed with a commercial diet and tap water *ad libitum*. All animal experiments were performed in accordance with the Guidelines for Animal Experimentation, Faculty of Science, Okayama University, Japan, and the NIH Guide for the Care and Use of Laboratory Animals.

Isolation of uterine epithelial cells

The method for the isolation and culture of mouse uterine epithelial cells has been previously described (Shiraga *et al.*, 1997). Briefly, uteri were dissected out, and the uterine horn was longitudinally cut to expose the endometrial surface. The tissue fragments were kept in 0.1% trypsin (Sigma, MO, USA) in Hanks' solution containing 20 mM HEPES and 0.3% bovine serum albumin at 4°C for 1 hr, and then at 37°C for 50 min. Trypsin was inactivated by soybean trypsin inhibitor (Sigma) at 37°C for 10 min. The epithelial tissues were separated from stromal tissues with forceps under a stereoscopic microscope. The epithelial tissue fragments were collected using a self-generated Percoll gradient (Pharmacia, Uppsala, Sweden). The collected epithelial fragments were further divided into smaller fragments by gentle pipetting. The cell viability was assessed by the trypan blue exclusion test, and was found to be usually more than 90% in each assay.

Preparation of collagen gel substratum for culture

Type I collagen solution was prepared from rat tail collagen fibers (Imagawa *et al.*, 1984). Neutralized and osmolarity-adjusted collagen solution (0.16%) was added to a 12-well culture plate (Falcon, NJ, USA) and allowed to form a gel at 37°C.

Serum-free culture of isolated uterine epithelial cells

A 1:1 mixture of Dulbecco's modified Eagle's medium (DMEM) and Ham's F12 medium without phenol red and calcium was prepared and then supplemented with $CaCl_2$ (0.1 mM), bovine serum albumin (fraction V, 1,000 mg/l, Sigma), hydrocortisone (100 μg/l), triiodothyronine (400 ng/l), transferrin (10 mg/l), glucagon (10 ng/l), parathormone (200 ng/l), and sodium selenite (5 μg/l).

The isolated epithelial cells were seeded at a cell density of 2.5×10^4 cells/cm^2 in collagen gel-coated 12-well culture plates or 9 cm-well plates for RNA extraction, and incubated in a humidified atmosphere of 5% CO_2 in air at 37°C. After a 1-day preculture, EGF (Sigma) and IGF-I (Amersham, Buckinghamshire, UK) were added to the culture media at various concentrations. The culture medium was changed every 2 days.

Determination of the number of uterine epithelial cells

The number of cultured epithelial cells was determined by the tetrazolium assay using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) as a substrate (MTT assay; Alley *et al.*, 1988). The amount of formazan after 4-hr incubation at 37°C was spectrophotometrically determined by measuring the absorbance at 540 nm. The amount of formazan produced correlated well with the number of cells in culture (Shiraga *et al.*, 1997); hence, the MTT method was used to estimate cell numbers in the culture wells. The cell number was expressed as a percentage, with the cell number for the controls regarded to be 100%.

Determination of the proliferating cells

DNA-synthesizing cells were immunocytochemically detected using a Cell proliferation kit (Amersham). Epithelial cells were seeded on collagen-coated slide glass. The collagen-coated slide glass was prepared as follows: collagen solution (0.07%) was placed on slide glass at a concentration of 100 μg/mm^2 and dried under a laminar in sterile air. Bromodeoxyuridine (BrdU) was added to the culture medium (3 mg/ml), and the cultures were continued for 5 hr. The percentage of BrdU-labeled cells to total cells was determined.

Northern blot hybridization

Total RNA was extracted from the uterine epithelial cells and the submaxillary glands of adult male mice using the single-step method (Chomczynski and Sacchi, 1987). The samples were electrophoresed on 1% formaldehyde-agarose gel and transferred to nylon membranes (Hybond N$^+$, Amersham, UK). Hybridization was carried out with a ^{32}P-labeled cDNA probe for 16 hr at 42°C. After hybridization, the membranes were washed under stringent conditions for 15 min at 42°C in 5 x SSPE (20 x SSPE=3 M NaCl, 0.1 M $NaH_2PO_4 \cdot H_2O$ and 0.02 M EDTA), for 30 min at 42°C in 1 x SSPE with 0.1% sodium dodecyl sulfate (SDS), and finally for 30 min at 42°C in 0.5 x SSPE with 0.1% SDS. Hybridized signals were detected by autoradiography.

Probes for Northern blot hybridization

A 960-bp mouse EGF cDNA clone (pmEGF-26F12) obtained from the American Type culture Collection (ATCC, MD, USA) and a 452-bp mouse EGF receptor cDNA clone (pcrmEGFR-NK1) prepared and verified by sequencing in our laboratory were used. For the Northern blot hybridization, the cDNAs were labeled with deoxycytidine-5'-[α-^{32}P] triphosphate by the random-primed labeling method using the Random Primer DNA Labeling Kit (Takara, Otsu, Japan).

Immunocytochemical analysis of uterine epithelial cells

Uterine epithelial cells were cultured on the collagen-coated glass slides in medium containing insulin (100 ng/ml) and EGF (10 ng/ml). The cells were fixed by pre-chilled methanol (–20°C) for 30 min and dried overnight at room temperature. For the detection of cytokeratin, a specific marker for epithelial cells, the cells were treated with 0.05% trypsin in PBS for 15 min at 37°C, then incubated with pkk-1 mouse anti-cytokeratin antibody (Labsystems, Finland) for 72 hr at 4°C. Cytokeratin immunoreactivity was visualized using an ABC kit (Vector Laboratories, CA, USA).

Statistics

Statistical analysis was carried out by analysis of variance and Duncan's multiple range test. Each group consisted of at least three wells, and independent studies were performed three times.

RESULTS

Observation of cultured uterine epithelial cells

Uterine endometrial epithelial cells were easily separated from endometrial stromal cells by treatment with trypsin and gentle pipetting. Most epithelial cells attached to a collagen gel substratum within a few hours after seeding. Phase-contrast microscopy demonstrated that the cells were flattened, forming polygonal sheets, a characteristic of epithelial cells in culture. Immunocytochemical analysis showed that the cultured cells expressed cytokeratin (Fig. 1). These observations indicate that the cultured cells were derived from the uterine endometrial epithelial cells alone.

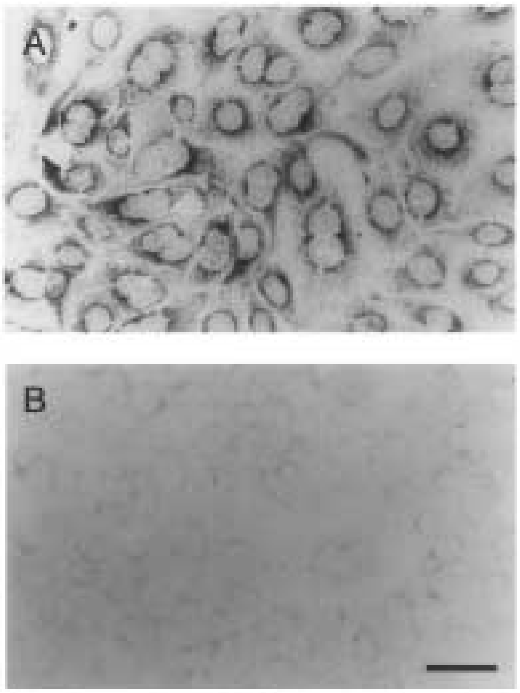

Fig. 1. Immunocytochemical detection of cytokeratin in uterine epithelial cells on day 5 of culture stained with anti-cytokeratin antibody (A), and control (without antibodies, B). A, cytoplasm of the epithelial cells was strongly stained, but not in the nucleus. Bar=50 μm.

Northern blot analysis of EGF mRNA and EGF receptor mRNA

To determine whether the endometrial epithelial cells synthesize EGF and EGF receptors, Northern blot analysis was performed with the labeled EGF- and EGF receptor-cDNA probes. Male submaxillary glands contained high levels of approximately 4.7-kb EGF mRNA transcripts, and the uterine epithelial cells cultured for 6 days contained EGF mRNA transcripts of the same size (Fig. 2). The size of these transcripts is consistent with the sizes previously observed in mouse uteri (DiAugustine *et al*., 1988).

EGF receptor mRNA transcripts (10.5, 6.6 and 2.7 kb in size) were detected in the cultured uterine epithelial cells. The major transcripts of 2.7 and 6.6 kb correspond to the truncated, and full-length receptor mRNAs, respectively, as reported by Das *et al*. (1994).

Effect of EGF on the growth of uterine epithelial cells

The number of uterine epithelial cells was determined by the MTT method. EGF increased the epithelial cell number in a dose-dependent manner (1 to 100 ng/ml) on day 5 of culture (Fig. 3). Maximal growth was obtained at a dose of 10 ng/ml (141.9±8.3% compared with controls). The epithelial cells continued to grow until day 5 of culture with EGF (10 ng/ml) treatment (Fig. 4). DNA-replicating cells were detected by the immunocytochemical observation of BrdU uptake to the cell nucleus. EGF (10 ng/ml) increased the percentage of BrdU-labeled cells in a dose-dependent manner (Fig. 5).

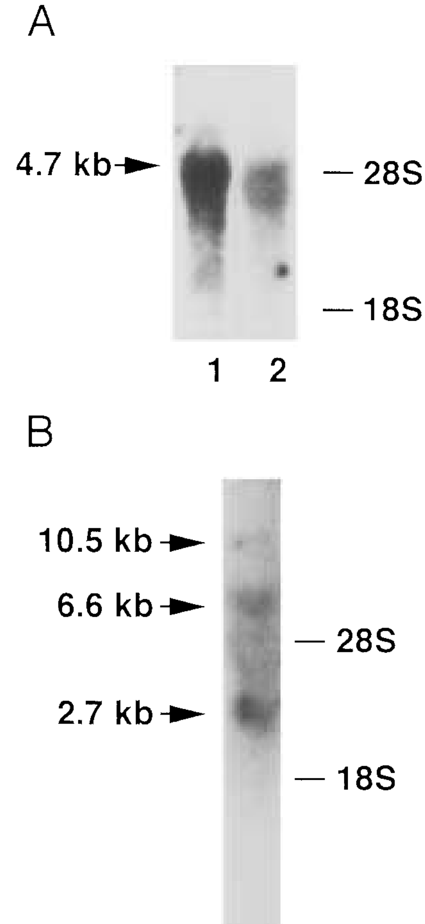

Fig. 2. Northern blot analysis of EGF mRNA (A) and EGF receptor mRNA (B) in mouse uterine epithelial cells. The positions of 18S and 28S ribosomal RNAs are shown. A: EGF mRNA was detected in mouse submaxillary glands (lane 1) as the reference, and in the uterine epithelial cells (lane 2). B: three signals of EGF receptor mRNA are detected in the uterine epithelial cells (10.5, 6.6 and 2.7 kb).

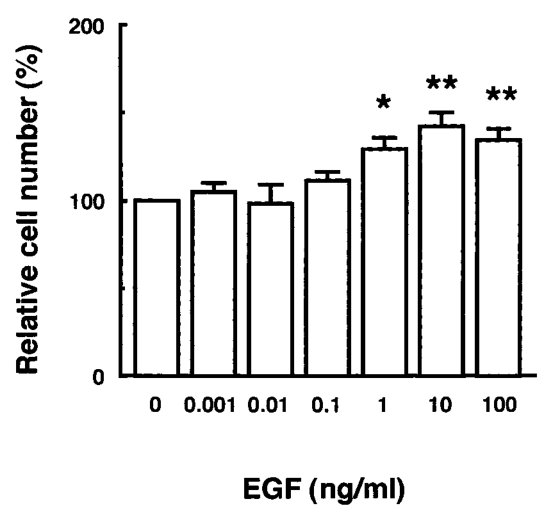

Fig. 3. Effect of EGF on the number of mouse uterine epithelial cells on day 5 of culture. Each column shows mean of three independent experiments. Bars show standard errors of mean. *, P<0.05, **, P<0.01 vs. control (EGF 0 ng/ml).

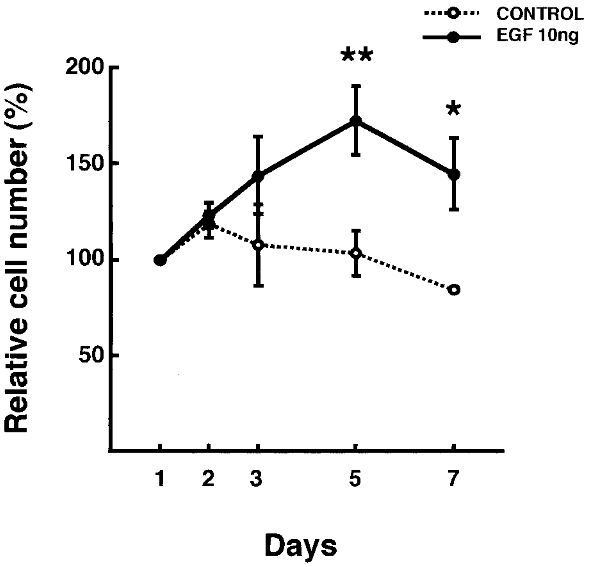

Fig. 4. The growth curve of mouse uterine epithelial cells in presence of EGF (10 ng/ml, closed circle) or none (open circle). Each point shows mean of three independent experiments. Bars show standard errors of mean. *, P<0.05, **, P<0.01 vs. each day of control.

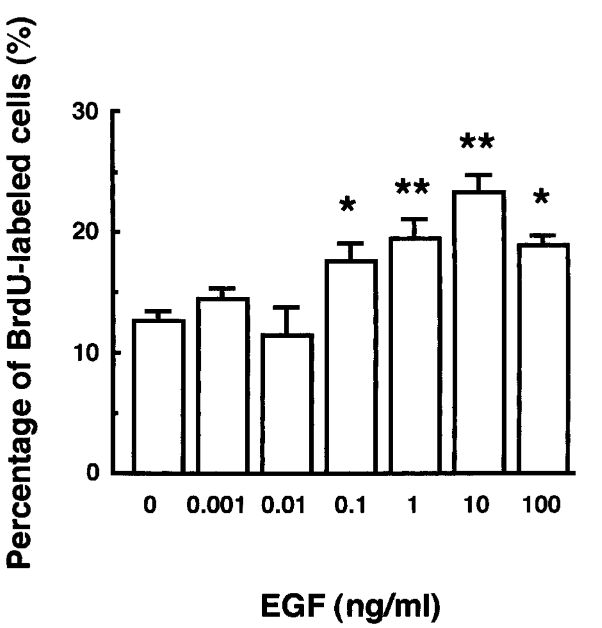

Fig. 5. Effect of EGF on the percentage of BrdU-labeled mouse uterine epithelial cells. The cells were cultured for 3 days. Each column shows mean of three independent experiments. Bars show standard errors of mean. *, P<0.05, **, P<0.01, vs. control (IGF-I 0 ng/ml).

Interaction between EGF- and IGF-I induced proliferation of mouse uterine epithelial cells

We found in our previous study that IGF-I stimulates the proliferation of uterine epithelial cells in a dose-dependent manner (Shiraga *et al.*, 1997). In the present study, the uterine epithelial cells were cultured for 5 days with EGF (0.1 ng/ml, low), EGF (10 ng/ml, high), IGF-I (0.1 ng/ml, low), IGF-I (10 ng/ml, high), and combinations of these doses (Fig. 6).

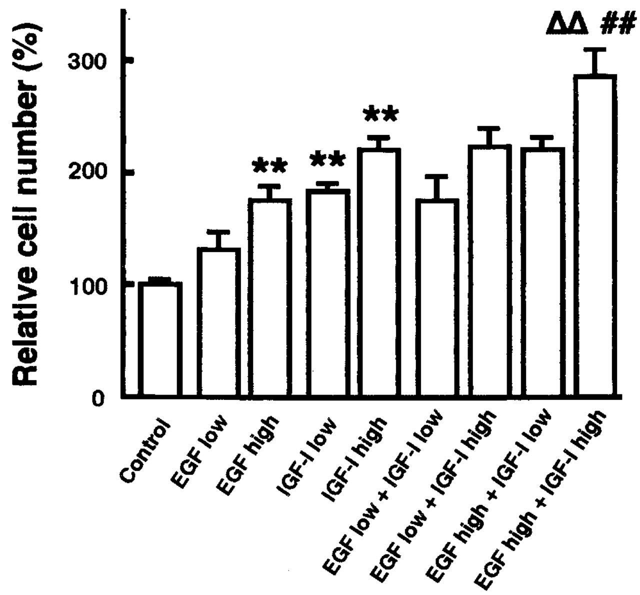

Fig. 6. Interaction between EGF and IGF-I on the number of mouse uterine epithelial cells. The cells were cultured for 5 days with EGF 0.1 ng/ml (EGF low), 10 ng/ml (EGF high) or IGF-I 0.1 ng/ml (IGF-I low), IGF-I 10 ng/ml (IGF-I high) or combination of these doses. Each column shows mean of four culture wells. Bars show standard errors of mean. **, P<0.01 vs. control (IGF-I 0 ng/ml), △△, P<0.01 vs. EGF high, ##, P<0.01 vs. IGF-I high. Three independent experiments were performed, and similar results were obtained.

EGF and IGF-I stimulated the proliferation of uterine epithelial cells. Combination treatment with high doses of EGF (10 ng/ml) and IGF-I (10 ng/ml) increased the cell number more than a single treatment with EGF (10 ng/ml) or IGF-I (10 ng/ml). IGF-I enhanced the EGF-induced growth in an additive manner.

DISCUSSION

We have established a primary culture system for mouse uterine epithelial cells (Shiraga *et al.*, 1997). Most of the cultured cells express cytokeratin-immunoreactivity and show a characteristic morphology of epithelial cells in culture. These observations indicate that the cultured uterine cells in our system consist entirely of epithelial cells. Using this culture system, we have found that EGF treatment increases the total number of cultured cells as well as the number of DNA-replicating cells in a dose-dependent manner, which is consistent with the results reported by Tomooka *et al.* (1986). Therefore, these findings lead us to conclude that EGF directly stimulates the proliferation of epithelial cells through EGF receptors, since uterine epithelial cells alone are cultured under serum-free conditions in our system.

In the present study, Northern blot analysis demonstrated that uterine epithelial cells from the immature mice synthesized a 4.7-kb transcript of EGF mRNA identical in size to the EGF mRNA transcript from the mouse submaxillary gland. In contrast, Huet-Hudson *et al.*(1990) have reported that adult uteri synthesize a 2.4-kb but not a 4.8-kb EGF mRNA transcript, with this 4.8 kb transcript being designated as a 4.7-kb transcript in the present study. The reason for this discrepancy is unclear, but we assume that there may be an age-related difference in the regulatory mechanism of EGF gene expression: for example, a 4.7-kb transcript in immature mouse uteri and a 2.4-kb transcript in adult mouse uteri.

DiAugustine *et al.* (1988) have found immunocytochemically that EGF is located at the border of luminal and glandular epithelial cells in immature and adult mice. Furthermore, in an *in situ* hybridization analysis of adult mouse uteri, EGF mRNA has been detected in the luminal and glandular epithelial cells (Huet-Hudson *et al*., 1990). These findings together with the findings of our Northern blot analysis indicate that EGF is produced in uterine epithelial cells, and hence that the secreted EGF very likely regulates the proliferation of uterine epithelial cells in an autocrine manner.

Estrogen treatment increases the EGF mRNA levels in both immature mouse uteri (DiAugustine *et al*., 1988) and adult ovariectomized mouse uteri (Huet-Hudson *et al*., 1990). EGF expression changes with the estrous cycle in adult uteri (Huet-Hudson *et al*., 1990); estrogen may therefore be required for EGF synthesis in the adult uterus, and changes in uterine EGF expression in adult female mice are considered to be caused by cyclic changes in blood estrogen levels.

Two distinct forms of EGF receptors are found in the mouse uterus, a full-length form (170 kDa), which is a functional receptor, and a truncated form (95 kDa), which is a secreted protein; these forms correspond to the 6.5-kb and 2.7-kb transcripts (Das *et al*., 1994; Tong *et al*., 1996). The truncated EGF receptors are thought to bind to EGF or EGF-related growth factors, or to interact with the full-length EGF receptors located in the cell membrane, resulting in the diminished action of EGF or EGF-related growth factors. We have demonstrated that both forms of EGF receptor transcripts are expressed in the cultured uterine epithelial cells from immature mice. Thus, the present study provides the first evidence at the gene-transcription level that cultured uterine epithelial cells of immature mice express both the full-length and truncated EGF receptors. Because EGF can bind to both full-length and truncated receptors, the growth-promoting activity of EGF in uterine epithelial cells depends upon the balance between the numbers of both types of EGF receptors expressed.

Das *et al.* (1994) have reported that the uterine epithelial cells in the adult mice lack the transcripts of full-length EGF receptors, indicating that functional EGF receptors are not present in the epithelial cells. The reason for the discrepancy with respect to EGF-receptor expression between the previous study and the present study is not clear. Tong *et al.* (1996) hypothesize that in adult mouse uteri, the EGF receptors expressed by blastocysts rather than the uterine epithelial cells are the direct physiological target site for EGF. Thus, there may be age differences in the gene expression of EGF receptors.

The presence of EGF binding sites has been demonstrated in the uterine epithelial cells of immature mice (Tomooka *et al*., 1986; Iguchi *et al*., 1993). A receptor assay has indicated that the dissociation constants (Kd) of EGF receptors in mouse uterine epithelial cells range from 0.06 to 1.8 nM (Mukku and Stancel, 1985; Tomooka *et al*., 1986; Nelson *et al*., 1992). In the present study, the range of Kd in the EGF receptors was within the concentrations of EGF that significantly increased the number of epithelial cells. Hence, the EGF-EGF receptor system in the uterine epithelial cells is thought to be involved in the regulation of uterine epithelial cell function.

It is known that IGF-I stimulates DNA synthesis in epithelial cells (Shiraga *et al*., 1997). In addition, it has previously been demonstrated that uterine epithelial cells synthesize IGF-I (Murphy *et al*., 1987; Ghahary *et al*., 1990; Carlsson and Billig, 1991; Kapur *et al*., 1992; Shiraga *et al*., 1997). The present and these previous findings support the hypothesis that IGF-I synthesized in the epithelial cells regulates the growth and function of these cells. In the present study, IGF-I acted additively with EGF to increase the cell number, suggesting that the signal pathways mediated by these growth factors may be separate, although we cannot eliminate the possibility that EGF-responsive cells and IGF-I-responsive cells are different. The interaction of EGF- and IGF-I-expression has been previously reported in the mouse uterus based on a semiquantitative RT-PCR assay (Hana and Murphy, 1994). It was found that EGF increases IGF-I expression in the stromal cells and, in contrast, that IGF-I increases EGF expression in the epithelial cells. Based on these findings, it was concluded that these paracrine actions between epithelial and stromal cells may facilitate endometrial cell proliferation. Consequently, it appears that the autocrine and paracrine action of EGF and IGF-I produced in the endometrial cells may be important for the regulation of epithelial cell growth. Further study is needed to clarify the interactions between EGF- and IGF-I-system in epithelial cells.

In conclusion, it was found that EGF stimulates the growth of mouse uterine epithelial cells in primary serum-free culture. EGF mRNA and EGF receptor mRNA (full-length and truncated forms) were detected using Northern analysis in the mouse uterine epithelial cells *in vitro*. Our findings suggest that EGF action on epithelial cells is mediated through EGF receptors located in the epithelial cells. In immature mice, uterine epithelial EGF may stimulate the proliferation of epithelial cells. EGF and IGF-I were found to additively stimulate uterine epithelial cell growth, indicating that the EGF-induced growth of uterine epithelial cells may be distinct from IGF-I-induced growth.

ACKNOWLEDGMENTS

This study was supported in part by Grants-in-aid for Scientific Research from the Ministry of Education , Science, Sports and Culture, Japan to S. Takahashi.

REFERENCES

Astrahantseff KN, Morris JE (1994) Estradiol-17β stimulates proliferation of uterine epithelial cells cultured with stromal cells but not cultured separately. In Vitro Cell Dev Biol 30A: 769–776

Borgundvaag B, Kudlow JE, Mueller SG, George SR (1992) Dopamine receptor activation inhibits estrogen-stimulated transforming growth factor-α gene expression and growth in anterior pituitary, but not in uterus. Endocrinology 130: 3453–3458

Brigstock DR (1991) Growth factors in the uterus: steroidal regulation and biological actions. Baillière's Clin Endocrinol Metab 5: 791–808

Carlsson B, Billig H (1991) Insulin-like growth factor-I gene expression during development and estrous cycle in the rat uterus. Mol Cell Endocrinol 77: 175–180

Chomczynski P, Sacchi N (1987) Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. Anal Biochem 162: 156–159

Das SK, Tsukamura H, Paria BC, Andrews GK, Dey SK (1994) Differential expression of epidermal growth factor receptor (EGF-R) gene and regulation of EGF-R bioactivity by progesterone and estrogen in the adult mouse uterus. Endocrinology 134: 971–981

DiAugustine RP, Petrusz P, Bell GI, Brown CF, Korach KS, McLachlan JA, Teng CT (1988) Influence of estrogens on mouse uterine epidermal growth factor precursor protein and messenger ribonucleic acid. Endocrinology 122: 2355–2363

Falck L, Forsberg J-G (1996) Immunohistochemical studies on the expression and estrogen dependency of EGF and its receptor and c-fos proto-oncogene in the uterus and vagina of normal and neonatally estrogen-treated mice. Anat Rec 245: 459–471

Fukamachi H, McLachlan JA (1991) Proliferation and differentiation of mouse uterine epithelial cells in primary serum-free culture: estradiol-17β suppresses uterine epithelial proliferation cultured on a basement membrane-like substratum. In Vitro Cell Dev Biol 27A: 907–913

Ghahary A, Chakrabarti S, Murphy LJ (1990) Localization of the sites of synthesis and action of insulin-like growth factor-I in the rat uterus. Mol Endocrinol 4: 191–195

Ghahary A, Murphy LJ (1989) Uterine insulin-like growth factor-I receptors: regulation by estrogen and variation throughout the estrous cycle. Endocrinology 125: 597–604

Ghosh D, Danielson KG, Alston JT, Heyner S (1991) Functional differentiation of mouse uterine epithelial cells grown on collagen gels or reconstituted basement membrane. In Vitro Cell Dev Biol 27A: 713–719

Hana V, Murphy LJ (1994) Interdependence of epidermal growth factor and insulin-like growth factor-I expression in the mouse uterus. Endocrinology 135: 107–112

Huet-Hudson YM, Andrews GK, Dey SK (1989) Cell type-specific localization of c-*myc* protein in the mouse uterus: modulation by steroid hormones and analysis of the periimplantation period. Endocrinology 125: 1683–1690

Huet-Hudson YM, Chakraborty C, De SK, Suzuki Y, Andrews GK, Dey SK (1990) Estrogen regulates the synthesis of epidermal growth factor in mouse uterine epithelial cells. Mol Endocrinol 4: 510–523

Iguchi T, Edery M, Tsai P-S, Ozawa S, Sato T, Bern HA (1993) Epidermal growth factor receptor levels in reproductive organs of female mice exposed neonatally to diethylstilbestrol. Proc Soc Expl Biol Med 204: 110–116

Imagawa W, Tomooka Y, Yang J, Guzman R, Richards J, Nandi S (1984). Isolation and serum-free cultivation of mammary epithelial cells within a collagen gel matrix. In "Methods for Serum-Free Culture of Cells of the Endocrine System" Ed by DW Barnes, DA Sirbasku and GH Sato, Alan R.Liss, Inc., New York, pp 127–141

Kapur S, Tamada H, Dey SK, Andrews GK (1992) Expression of insulin-like growth factor-I (IGF-I) and its receptor in the peri-implantation mouse uterus, and cell-specific regulation of IGF-I gene expression by estradiol and progesterone. Biol Reprod 46: 208–219

Mukku VR, Stancel GM (1985) Receptors for epidermal growth factor in the rat uterus. Endocrinology 117: 149–154

Murphy LJ, Murphy LC, Friesen HG (1987) Estrogen induces insulin-like growth factor-I expression in the rat uterus. Mol Endocrinol 1: 445–450

Nelson KG, Takahashi T, Bossert NL, Walmer DK, McLachlan JA (1991) Epidermal growth factor replaces estrogen in the stimulation of female genital-tract growth and differentiation. Proc Natl Acad Sci USA 88: 21–25

Nelson KG, Takahashi T, Lee DC, Luetteke NC, Bossert NL, Ross K, Eitzman BE, McLachlan JA (1992) Transforming growth factor-α is a potential mediator of estrogen action in the mouse uterus. Endocrinology 131: 1657–1664

Shiraga M, Takahashi S, Miyake T, Takeuchi S, Fukamachi H (1997) Insulin-like growth factor-I stimulates proliferation of mouse uterine epithelial cells in primary culture. Proc Soc Expl Biol Med 215: 412–417

Tomooka Y, DiAugustine RP, McLachlan JA (1986) Proliferation of mouse uterine epithelial cells *in vitro*. Endocrinology 118: 1011–1018

Tong BJ, Das SK, Threadgill D, Magnuson T, Dey SK (1996) Differential expression of the full-length and truncated forms of the epidermal growth factor receptor in the preimplantation mouse uterus and blastocyst. Endocrinology 137: 1492–1496

Wang X-N, Das SK, Damm D, Klagsbrun M, Abraham JA, Dey SK (1994) Differential regulation of heparin-binding epidermal growth factor-like growth factor in the adult ovariectomized mouse uterus by progesterone and estrogen. Endocrinology 135: 1264–1271

Zhang Z, Funk C, Roy D, Glasser S, Mulholland J (1994) Heparin-binding epidermal growth factor-like growth factor is differentially regulated by progesterone and estradiol in rat uterine epithelial and stromal cells. Endocrinology 134: 1089–1094

(Received November 5, 1999 / Accepted January 23, 2000)