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Mitogenic Activity of Growth Factors in the Human Endometrial Adenocarcinoma Cell Lines HEC-1-A and KLE[1,2]

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Endometrial adenocarcinoma is the most common gynecologic malignancy occurring in the United States. Evidence is accumulating that links peptide growth factors with malignant proliferation. Epidermal growth factor (EGF) and insulin-like growth factor I (IGF-I) are known mitogens for endometrial adenocarcinoma *in vitro*. However, the biological activity of other growth factors in this malignancy is unclear. This study was undertaken to determine the influence of growth factors on the mitogenic activity of the human endometrial adenocarcinoma cell lines HEC-1-A and KLE. Incubation with EGF, IGF-I, insulin-like growth factor II (IGF-II), or insulin stimulated a time-dependent mitogenic response in both cell lines, with the peak response occurring at 24 hr for HEC-1-A and 48 hr for KLE. After two doubling intervals, the number of HEC-1-A cells was increased 3.5-fold by EGF (100 ng/ml), 2.7-fold by IGF-I (100 ng/ml), 2.3-fold by IGF-II (100 ng/ml), and 2.2-fold by insulin (1000 ng/ml) when compared to untreated controls ($P < 0.05$). The number of KLE cells was increased 2.6-fold by EGF (100 ng/ml), 2.3-fold by IGF-I (100 ng/ml), 2.1-fold by IGF-II (100 ng/ml), and 2.0-fold by insulin (1000 ng/ml) when compared to untreated controls ($P < 0.05$). Similar results were obtained when DNA content was measured. PDGF failed to stimulate any mitogenic response in either cell line at all concentrations tested (0.1–100 ng/ml). These findings suggest that EGF, IGF-I, IGF-II, and insulin may play a regulatory role in the proliferation of endometrial adenocarcinoma.

INTRODUCTION

Adenocarcinoma of the endometrium is the most common gynecologic malignancy occurring in the United States, with an estimated 33,000 new cases and 4000 deaths in 1990 [1]. Although an association between unopposed estrogen stimulation and the development of endometrial hyperplasia is well-established [2], the stimulus for malignant transformation is currently unknown. Evidence is accumulating to link peptide growth factors with the development and proliferation of human malignancies. Among the many growth factors characterized to date, several have properties that indicate that they may play a role in the growth regulation of endometrial adenocarcinoma.

Epidermal growth factor (EGF) and insulin-like growth factor I (IGF-I) are mitogenic for a variety of human adenocarcinoma cell lines, including breast [3,4] and endometrium [5]. A recent study demonstrated that EGF binding to its receptor (EGF-R) undergoes cyclic alterations during the menstrual cycle, with increased binding during the proliferative phase [6]. Our laboratory demonstrated the presence of EGF-R in endometrial adenocarcinoma, with an inverse correlation between receptor content and histologic grade [7]. In addition, we demonstrated a positive correlation between IGF-I receptor (IGF-IR) content and histologic grade [8].

However, the biological role of other growth factors, including insulin-like growth factor II (IGF-II), insulin, and platelet-derived growth factor (PDGF) in endometrial adenocarcinoma is unclear. Insulin is mitogenic for carcinoma cell lines, many of which are dependent upon

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insulin for proliferation. Recent data have demonstrated the presence of specific, high-affinity binding sites for insulin in endometrial adenocarcinoma [9]. Furthermore, at high concentrations insulin binds to the IGF-IR and may function in a fashion similar to that of IGF-I [10].

IGF-II is closely related to IGF-I and insulin. It appears to be important in fetal development [11], but with uncertain function in the adult. IGF-II messenger RNA (mRNA) is expressed by several human carcinomas, most notably breast [12], but also colon [13], liver [14], and Wilm's tumor [15]. In addition, IGF-II is mitogenic for breast adenocarcinoma cells in culture [3,12].

PDGF is felt to have a central role in the regulation of normal cell growth, as well as mediating proliferation of neoplastic cells. Numerous human carcinoma cell lines produce PDGF, including glioblastoma [16] and breast [17]. Recent evidence indicates that human endometrium contains PDGF and the PDGF receptor [18].

These findings raise the possibility that EGF, IGF-I, IGF-II, insulin, and PDGF may play a regulatory role in the proliferation of endometrial adenocarcinoma. The present study was undertaken to determine the influence of these growth factors on the mitogenic activity of two established human endometrial adenocarcinoma cell lines.

MATERIALS AND METHODS

Growth Factors

Human recombinant EGF and platelet-derived growth factor were purchased from Biosource International (Camarillo, CA). Human recombinant insulin-like growth factor I and II (IGF-I and IGF-II) were a generous gift from Eli Lilly Research Laboratories (Indianapolis, IN). Human recombinant insulin was purchased from Sigma Chemical Co. (St. Louis, MO).

Reagents and Media

Cell culture media, fatty acid-free bovine serum albumin (BSA), fetal bovine serum (FBS), and trypsin were purchased from Sigma Chemical Co.

Cell Cultures

Human endometrial adenocarcinoma cell lines HEC-1-A (HTB-112) and KLE (CRL-1622) were purchased from the American Type Culture Collection (Rockville, MD). HEC-1-A and KLE were derived from a moderately differentiated and poorly differentiated endometrial adenocarcinoma, respectively. The cells were cultured in phenol red-free Dulbecco's modified Eagle/Ham's nutrient mixture F12 (DME/F12) supplemented with 10% FBS, 50 μg/ml gentamycin, and 2 u/ml nystatin. The cells were maintained in 75-cm^2 tissue culture flasks (Corning Glass Works, Corning, NY) under sterile conditions at 37°C in water-saturated air containing 5% CO_2. For subculturing, the medium was removed and replaced with fresh trypsin (0.02%)/ethylenediaminetetraacetic acid (EDTA) (0.25%) solution. After 2 min the solution was removed and the flasks maintained at 37°C for approximately 10 min to allow cells to detach. Fresh medium was added and the cell suspension centrifuged at 150*g* for 10 min. The supernatant was discarded and the cell pellet resuspended in fresh medium. The cells were either dispensed into new flasks (subculture ratio 1:2) or plated onto 24-well Falcon–Primaria-coated plates (Becton–Dickinson Co., Lincoln Park, NJ) for studies. If the cells were to be used for studies, the flasks were rinsed with PBS twice and incubated for 48 hr prior to plating in phenol red-free DMEM or DME/F-12 supplemented with 0.3% BSA. The cell cultures were tested regularly for mycoplasma contamination.

Thymidine Incorporation Assay

[^{3}H]-thymidine incorporation studies were performed in 24-well coated plates using a modification of the technique described previously [18]. Cells were plated at a density of 5×10^4 cells (HEC-1-A) or 25×10^4 cells (KLE) in 500 μl phenol red-free Dulbecco's modified Eagle medium (DMEM) supplemented with 0.3% BSA. These densities were based on initial experiments performed by our laboratory to determine the optimal plating density for the cell lines (data not shown) and were subsequently used for all studies. After 24 hr for attachment, the cells were washed twice with phosphate-buffered saline 0.05 *M* (PBS). The medium was replaced with fresh DMEM supplemented with 0.3% BSA. The growth factors were added directly to the wells and the cells incubated for an additional 24 (HEC-1-A) or 48 (KLE) hr. Four hours prior to completion, 1 μCi [methyl-^{3}H]thymidine (ICN Radiochemicals, Irvine, CA) was added to each well. The incubation was terminated by washing the cells with ice-cold PBS. Ice-cold 5% trichloracetic acid was added to remove the acid-soluble[^{3}H]-thymidine pool, and the cells were washed again with PBS. To dissolve the remaining incorporated [^{3}H]-thymidine, the cells were treated with 0.2 *N* sodium hydroxide and transferred to scintillation vials. The wells were washed with PBS, which was added to the vials. After neutralization with 0.2 *N* hydrochloric acid, 10 ml of Biosafe II scintillation fluid was added to the vials, and the incorporated counts were quantified using a scintillation counter.

Cell Growth Assay

HEC-1-A and KLE cells were plated in quadruplicate onto 24-well plates containing 500 μl phenol red-free

DME/F12 medium supplemented with 0.3 % BSA, without (control) or with various growth factors. The medium and supplements were replaced every other day. At the indicated time, the medium was removed and the cells were washed with PBS. The cells were harvested by treatment with 500 μl trypsin/EDTA as described above. The trypsin was neutralized with 500 μl fresh medium. The cell suspension was aspirated into a vial and centrifuged at 150*g* for 5 min. The supernatant was discarded and the cell pellet resuspended in 1 ml fresh medium. Four aliquots from each replicate were counted on a Coulter Counter.

DNA Assay

HEC-1-A and KLE cells were plated in triplicate onto 24-well plates containing 500 μl phenol red-free DME/F12 medium supplemented with 0.3 % BSA, without (control) or with various growth factors. The medium and supplements were replaced every other day. The DNA assay was performed by a modification of the method previously described [20]. Briefly, at the indicated time, the medium was removed and the wells washed twice with PBS. The cells were dissolved by adding 0.5 *N* sodium hydroxide and incubated at 37°C for 1 hr. High-salt buffer (2 *M* sodium chloride, 0.5 *M* sodium hypophosphate, pH 7.4) and bisbenzimidazole (32 μg/ml) (Aldrich, Milwaukee, WI) were added. The pH was adjusted to 7.2–7.4 by the addition of 0.5 *N* hydrochloric acid. DNA standards were prepared using calf thymus DNA and subjected to similar treatment. Fluorescence was measured at 356 nm (excitation) and 458 nm (emission) wavelengths.

Analysis

The data were analyzed by the Student *t* test or one-way ANOVA as appropriate. Each experiment was performed at least twice, with a minimum of three replicates per experiment. The data are expressed as means ± SD. All tests were two-tailed, and $P < 0.05$ was considered significant.

RESULTS

Time Course of Growth Factor Stimulation of Mitogenesis

HEC-1-A and KLE cells were cultured in serum-free medium with 100 ng/ml of EGF, IGF-I, IGF-II, or PDGF, or 1000 ng/ml of insulin for 12–72 hr, and [^{3}H]thymidine incorporation quantified. The results of a representative experiment are shown in Fig. 1. EGF, IGF-I, IGF-II, and insulin stimulated incorporation with a peak at 24 hr for HEC-1-A and 48 hr for KLE. PDGF failed to stimulate incorporation in either HEC-1-A or KLE.

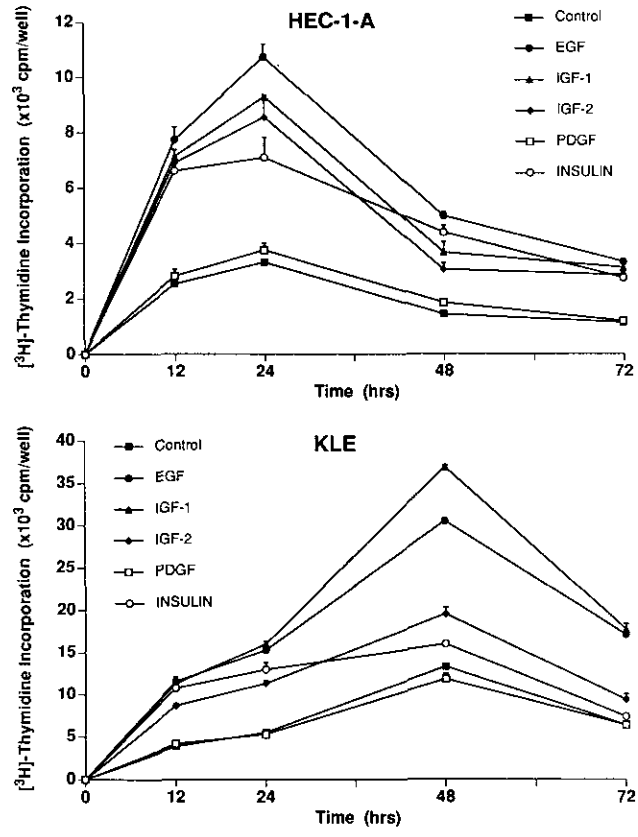

FIG. 1. Time–response curve for the HEC-1-A (top) and KLE (bottom) cell lines treated with medium (control), EGF, IGF-I, IGF-II, insulin, and PDGF.

Dose Response of Growth Factor Stimulation of Mitogenesis

HEC-1-A and KLE cells were cultured in serum-free medium with 0–100 ng/ml of EGF, IGF-I, IGF-II, or PDGF, or 0–1000 ng/ml of insulin for 24 hr (HEC-1-A) or 48 hr (KLE). The results of a representative experiment are shown in Figs. 2 and 3. EGF, IGF-I, IGF-II, and insulin stimulated incorporation in a dose-dependent fashion in both cell lines, while PDGF failed to stimulate incorporation in either cell line at all concentrations tested.

Cell Proliferation Following Growth Factor Stimulation

HEC 1-A and KLE cells were cultured in serum-free medium containing 100 ng/ml of EGF, IGF-I, IGF-II, or PDGF, or 1000 ng/ml of insulin. The cells were counted at the initial plating and then harvested and counted at the indicated times. The results of a representative experiment are shown in Fig. 4. At 2.25 days

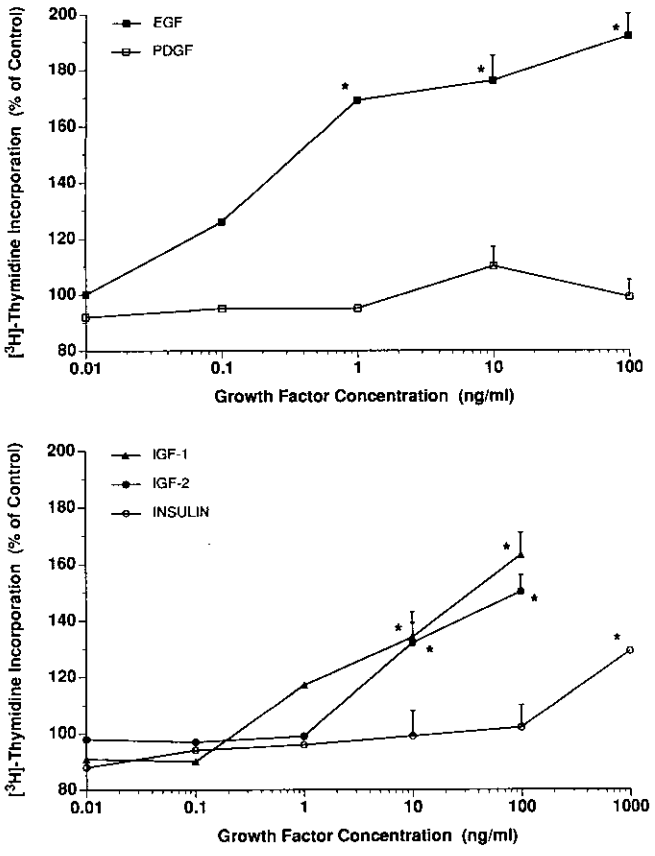

FIG. 2. Dose–response curves for the HEC-1-A cell line treated with EGF and PDGF (top) or IGF-I, IGF-II, and insulin (bottom). ($P < 0.05$ *vs* control.)

(one doubling interval), the number of HEC-1-A cells was increased 1.7-fold by EGF ($P < 0.05$ vs control). At 4.5 days (two doubling intervals), the number of HEC-1-A cells was increased 3.5-fold by EGF, 2.7-fold by IGF-I, 2.3-fold by IGF-II, and 2.2-fold by insulin ($P < 0.05$ vs control). PDGF did not have a significant effect. At 9 days (two doubling intervals), the number of KLE cells was increased 2.6-fold by EGF, 2.3-fold by IGF-I, 2.1-fold by IGF-II, and 2.0-fold by insulin ($P < 0.05$ *vs* control). PDGF did not have a significant effect.

DNA Content Following Growth Factor Stimulation

HEC-1-A and KLE cells were cultured in 24-well plates in parallel with the cultures for cell proliferation in serum-free medium containing 100 ng/ml of EGF, IGF-I, IGF-II, or PDGF, or 1000 ng/ml of insulin. The results of a representative experiment are shown in Fig. 5. The DNA content per well was measured at the initial plating and at the indicated times. At 2.25 days (one doubling interval), the DNA content of HEC-1-A wells was increased 1.8-fold by EGF ($P < 0.05$ vs control). At 4.5 days (two doubling intervals), the DNA content of HEC-1-A wells was increased 3.4-fold by EGF, 2.6-fold by IGF-I, 2.0-fold by IGF-II, and 2.1-fold by insulin ($P < 0.05$ vs control). PDGF did not have a significant effect. At 9 days (two doubling intervals), the DNA content of KLE wells was increased 2.5-fold by EGF, 2.3-fold by IGF-I, 2.0-fold by IGF-II, and 1.8-fold by insulin ($P < 0.05$ *vs* control). PDGF did not have a significant effect.

DISCUSSION

The present study demonstrates the influence of various growth factors on the mitogenic activity of human endometrial adenocarcinoma cells in serum-free medium. Under these conditions, the mitogenic activity of HEC-1-A and KLE was stimulated by EGF, less effectively stimulated by IGF-I, IGF-II, and insulin, and not stimulated by PDGF.

The interval between treatment and mitogenic response differed significantly for HEC-1-A and KLE. The peak mitogenic response occurred at 24 hr for HEC-1-A and 48 hr for KLE. These peaks corresponded to the growth rate of the individual cell line. HEC-1-A grows rapidly,

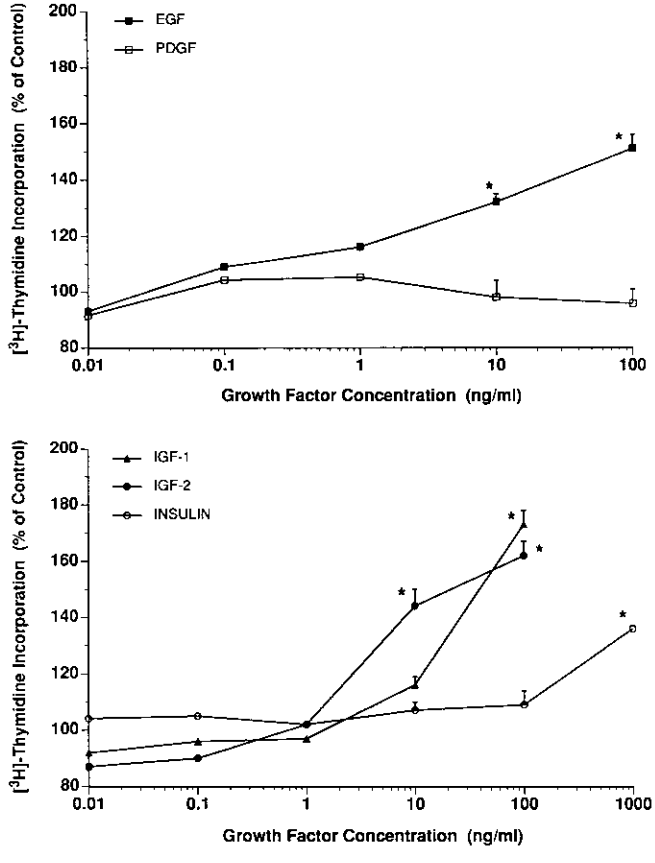

FIG. 3. Dose–response curves for the KLE cell line treated with EGF and PDGF (top) or IGF-I, IGF-II, and insulin (bottom). ($P < 0.05$ *vs* control.)

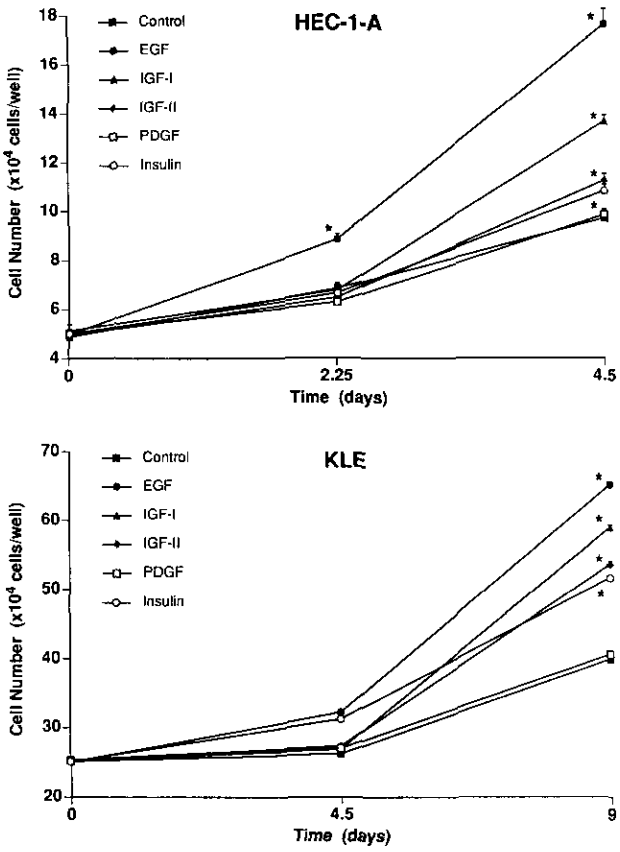

FIG. 4. Cell number for the HEC-1-A (top) and KLE (bottom) cell lines treated with medium (control), EGF, IGF-I, IGF-II, insulin, and PDGF. ($P < 0.05$ *vs* control.)

with a doubling interval of 51.6 hr [21]. In contrast, KLE is more sedate, with a doubling interval of 4.66 days [22].

The mitogenic response to each growth factor varied. EGF consistently stimulated mitogenic activity in both HEC-1-A and KLE at a concentration of 10 ng/ml. These results are comparable to data indicating that 10–30 ng/ml of EGF was required to effectively stimulate proliferation of a variety of cultured human cell lines [5,23,24]. As the normal human plasma EGF concentration is 1 ng/ml [25], the requirement for higher concentrations in our study suggests that sufficient EGF may obviate the need for additional plasma factors, such as hormones or other growth factors, that interact with EGF to stimulate proliferation *in vivo* [26,27]. In particular, these results appear to indicate that EGF-induced proliferation in HEC-1-A and KLE is not dependent upon activation by a competence factor, an observation consistent with previous studies of epithelial cells [28,29]. An alternative explanation is that autocrine production in tumor cells may lead to higher local concentrations of EGF. The mRNA for EGF has been detected in normal [30,31] and malignant human endometrium [32], and immunoreactive EGF has been demonstrated in normal human endometrium [18]. Our results, similar to data from normal endometrium [18], reveal a low but consistent degree of [^{3}H]thymidine incorporation in untreated cells. Taken together, these observations suggest that autocrine production of growth factors may indeed occur in endometrial cells. A third possible explanation is that the requirement for higher EGF concentrations may be the consequence of variation in EGF receptor number. The EGF receptor is present in malignant [7,32] and normal endometrium [7,33]. Interestingly, the number of receptors is inversely correlated with the histologic grade of the endometrium [7]. Although a recent study demonstrated cyclic EGF binding in normal endometrium, with increased binding during the proliferative phase [6], such a relationship has not been confirmed by subsequent studies [18,33,34].

IGF-I was less effective than EGF at stimulating the mitogenic activity of each cell line, requiring a concentration of 100 ng/ml for consistent stimulation. Similar concentrations have been reported in human plasma [9], as well as in other human carcinoma cell lines [23]. There was no significant difference in IGF-I levels among women with endometrial cancer and normal controls [9].

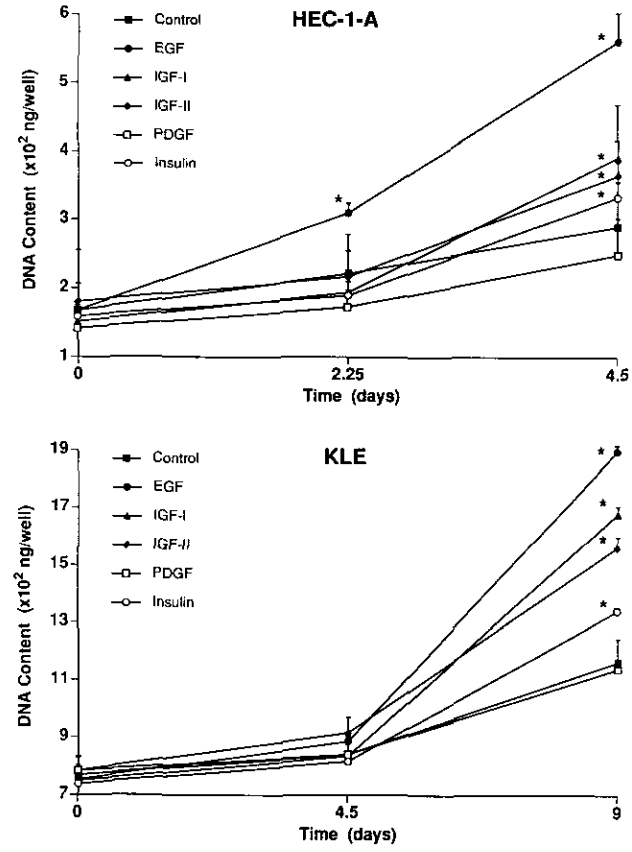

FIG. 5. DNA content for the HEC-1-A (top) and KLE (bottom) cell lines treated with medium (control), EGF, IGF-I, IGF-II, insulin, and PDGF. ($P < 0.05$ *vs* control.)

IGF-I mRNA is detectable in human endometrium, with no apparent difference in expression during the phases of the menstrual cycle [30]. Similarly, IGF-I is secreted by breast cancer cells [35] and uterine tissue [30] in response to 17β-estradiol. In uterine tissue, IGF-I increases estrogen-stimulated DNA synthesis [30]. These observations suggest that IGF-I may mediate estrogen-induced proliferation of endometrium in an autocrine or paracrine fashion.

The regulation of IGF-I binding in endometrium is complex. At least three different IGF-I binding proteins (IGFBPs) have been detected in uterine tissues [36]. The IGFBPs are thought to modulate the bioavailability of IGF-I by competing with the IGF-I receptor [37]. In normal endometrium, IGF-I receptor and IGFBP-1 levels increase during the late secretory phase [38]. In malignant endometrium, the number of IGF-I receptors varies positively with histologic grade of the tumor [8,9]. Furthermore, endometrial carcinoma cell lines which contain IGF-I receptors, including those used in the present study, also secrete IGFBPs [37]. Thus, variation in IGF-I binding may play an important role in regulating the activity of IGF-I on the proliferation of endometrial carcinoma.

IGF-II was comparable to IGF-I, consistently stimulating the mitogenic activity of each cell line at a concentration of 100 ng/ml. These results agree with those reported for other human carcinoma cell lines, including breast [3] and esophagous [23]. There are no previous reports on the influence of IGF-II on the mitogenic activity of endometrium. The stimulatory activity of IGF-II is interesting since, unlike the other growth factor receptors, IGF-IIR does not possess tyrosine kinase activity [39]. There is, however, considerable cross-reactivity among the ligands and receptors in the insulin/insulin-like growth factor family. Several reports have indicated that the bilogical activity of IGF-II is mediated primarily by the IGF-IR [3,12,23,40]. IGF-II mRNA is expressed by human endometrium [30], as well as by numerous human carcinoma cell lines [12–15]. In human endometrium, IGF-II mRNA levels vary during the menstrual cycle, with the highest levels during the early proliferative phase [41]. Furthermore, IGF-II expression in hormonally responsive cells such as human breast adenocarcinoma and rat uterus is increased by estrogen stimulation [12,30]. Therefore, endometrial IGF-II may function as a local mediator of estrogen-induced proliferation.

Insulin at low concentrations (less than 1000 ng/ml) failed to stimulate a mitogenic response in either cell line. In contrast, at high concentration (1000 ng/ml), there was a moderate increase in mitogenic activity in both cell lines. Several studies have reported that insulin at similar concentration binds to the IGF-I receptor [10,42]. An association between hyperinsulinemia and endometrial adenocarcinoma has been reported [43], possibly as a consequence of increased conversion of androgens to estrogens in endometrial tissue [9]. Recent evidence indicates that normal [44] and malignant [9] endometrium contain specific receptors for insulin. Unlike EGF and IGF-I, the insulin receptor number does not vary significantly with histologic grade [9]. Thus, the mitogenic response to insulin in endometrial carcinoma cells may be mediated by its own receptor or the IGF-I receptor.

Platelet-derived growth factor failed to stimulate mitogenic activity in either cell line at any concentration tested. These findings contrast with previous studies indicating that PDGF produces a mitogenic response in human endometrium at concentrations ranging from 0.5 to 10 ng/ml [18,45]. However, those studies utilized primary endometrial stromal cell cultures derived from endometriosis [45] or benign endometrium [18]. The predominant PDGF receptor in endometrium is the B-type, and it appears that the mitogenic activity of PDGF is mediated primarily via this receptor [18]. The B-type receptor has been localized to stromal cells by immunohistochemical staining in human endometrium [18] and by *in situ* hybridization with complementary DNA in porcine endometrium [46]. Our cell cultures, derived from malignant endometrial glandular tissue, lack significant amounts of stromal cells, possibly explaining their failure to respond to PDGF. Alternatively, malignant transformation or multiple passages may have led to a loss of PDGF receptors.

In conclusion, our results have demonstrated for the first time that IGF-II and insulin stimulate the mitogenic activity of endometrial cancer cells in culture. In addition, these results have confirmed previous reports of the mitogenic activity of EGF and IGF-I, but have failed to demonstrate a mitogenic effect of PDGF.

REFERENCES

1. Silverberg, E., Boring, C. C., and Squires, B. A. CA Statistics, 1990, *CA Cancer J. Clin.* **40,** 9–26, (1990).
2. DiSaia, P. J., and Creasman, W. T. in *Clinical gynecologic oncology* (S. Bircher, Ed.), Mosby, St. Louis, 3rd ed., p. 133 (1989).
3. Cullen, K. J., Yee, D., Sly, W. S., Perdue, J., Hampton, B., Lippman, M. E., and Rosen, N. Insulin-like growth factor receptor expression and function in human breast cancer, *Cancer Res.* **50,** 48–53 (1990).
4. Karey, K. P., and Sirbasku, D. A. Differential responsiveness of human breast cancer cell lines MCF-7 and T47D to growth factors and 17β-estradiol, *Cancer Res.* **48,** 4083–4092 (1988).
5. Korc, M., Haussler, C. A., and Trookman, N. S. Divergent effects of epidermal growth factor and transforming growth factors on a human endometrial carcinoma cell line, *Cancer Res.* **47,** 4909–4914 (1987).
6. Taketani, Y., and Mizuno, M. cyclic changes in epidermal growth factor receptor in human endometrium during menstrual cycle, *Endocrinol. Jpn.* **35,** 19–25 (1988).
7. Reynolds, R. K., Talavera, F., Roberts, J. A., Hopkins, M. P.,

and Menon, K. M. J. Characterization of epidermal growth factor receptor in normal and neoplastic human endometrium, *Cancer* **66,** 1967–1974 (1990).

8. Talavera, F., Reynolds, R. K., Roberts, J. A., and Menon, K. M. J. Insulin-like growth factor I receptors in normal and neoplastic human endometrium, *Cancer Res.* **50,** 3019–3024 (1990).
9. Nagamani, M., Stuart, C. V., Dunhardt, P. A., and Doherty, M. G. Specific binding sites for insulin and insulin-like growth factor I in human endometrial cancer, *Am. J. Obstet. Gynecol.* **165,** 1865–1871 (1991).
10. Rechler, M. M. The nature and regulation of the receptors for insulin-like growth factors, *Annu. Rev. Physiol.* **47,** 425–442 (1985).
11. Han, V. K. M., D'Ercole, A. T., and Lund, P. K. Cellular localization of somatomedin messenger RNA in the human fetus, *Science* **236,** 193–197 (1987).
12. Yee, D., Cullen, K. J., Paik, S., Perdue, J. F., Hampton, B., Schwartz, A., Lippman, M. E., and Rosen, N. Insulin-like growth factor II mRNA expression in human breast cancer, *Cancer Res.* **48,** 6691–6696 (1988).
13. Tricoli, J. V., Rall, L. B., Karakousis, C. P., Herera, L., Petrelli, N. J., Bell, G. I., and Shows, T. B. Enhanced levels of insulin-like growth factor messenger RNA in human colon carcinomas and liposarcomas, *Cancer Res.* **46,** 6169–6173 (1986).
14. Cariani, E., Lassere, C., Kemeny, F., Franco, D., and Brechot, C. Expression of insulin-like growth factor II, α-fetoprotein and hepatitis B virus transcripts in human primary liver cancer, *Hepatology* **13,** 644–649 (1991).
15. Scott, J., Cowell, J., Robertson, M. E., Priestley, L. M., Wadey, R., Hopkins, B., Pritchard, J., Bell, G. I., Rall, L. B., Graham, D. F., and Knott, T. J. Insulin-like growth factor II gene expression in Wilms' tumour and embryonic tissues, *Nature (London)* **317,** 260–263 (1985).
16. Nister, M., Libermann, T. A., Betsholtz, C., Pettersson, M., Claesson-Welsh, L., Heldin, C. H., Schlessinger, J., and Westermark, B. Expression of messenger RNAs for platelet-derived growth factor and transforming growth factor-alpha and their receptors in human malignant glioma cell lines, *Cancer Res.* **48,** 3910–3918 (1988).
17. Bronzert, D. A., Pantazis, P., Antoniades, H. N., Kasid, A., Davidson, N., Dickson, R. B., and Lippman, M. E. Synthesis and secretion of platelet-derived growth factor by human breast cancer cell lines, *Proc. Natl. Acad. Sci (USA)* **84,** 5763–5767 (1987).
18. Chegini, N., Rossi, M. J., and Masterson, B. J. Platelet-derived growth factor (PDGF), epidermal growth factor (EGF), and EGF and PDGF β-receptors in human endometrial tissue: Localization and *in vitro* action, *Endocrinology* **130,** 2373–2385 (1992).
19. Takahashi, S., Conti, M., and Van Wyk, J. J. Thyrotropin potentiation of insulin-like growth factor I dependent deoxyribonucleic acid synthesis in FRTL-5 cells: Mediation by an autocrine amplification factor(s), *Endocrinology* **126,** 736–745 (1990).
20. Labarca, C., and Paigen, K. A simple, rapid and sensitive DNA assay procedure, *Anal. Biochem.* **102,** 344–352 (1978).
21. Kuramoto, H., Tamura, S., and Natake, Y. Establishment of a cell line of human endometrial adenocarcinoma *in vitro, Am. J. Obstet. Gynecol.* **114,** 1012–1019 (1972).
22. Richardson, G. S., Dickersin, G. R., Atkins, L., Maclaughlin, D. T., Raam, S., Merk, L. P., and Bradley, F. M. KLE: A cell line with defective estrogen receptor derived from undifferentiated endometrial cancer, *Gynecol. Oncol.* **17,** 213–230 (1984).
23. Oku, K., Tanaka, A., Yamanishi, H., Nishizawa, Y., Matsumoto, K., Shiozaki, H., and Mori, T. Effects of various growth factors on growth of a cloned human esophageal squamous cancer cell line in a protein-free medium, *Anticancer Res.* **11,** 1591–1595 (1991).
24. Chen, Y., and Rabinovitch, P. S. Platelet-derived growth factor, epidermal growth factor, and insulin-like growth factor I regulate specific cell-cycle parameters of human diploid fibroblasts in serum-free culture, *J. Cell Physiol.* **140,** 59–67 (1989).
25. Hirata, Y., Moore, G. W., Bertagna, C., and Orth, D. N. Plasma concentrations of immunoreactive human epidermal growth factor (urogastrone) in man, *J. Clin. Endocrinol. Metab.* **50,** 440–444 (1980).
26. Goustin, A. S., Leof, E. B., Shipley, G. D., and Moses, H. L. Growth factors and cancer, *Cancer Res.* **46,** 1015–1029 (1986).
27. Aaronson, A. A. Growth factors and cancer, *Science* **254,** 1146–1153 (1991).
28. Earp, H. S., Hepler, J. R., Petch, L. A., Miller, A., Berry, A. R., Harris, J., Raymond, V. W., McCune, B. K., Lee, L. W., Grisham, J. W., and Harden, T. K. Epidermal growth factor (EGF) and hormones stimulate phosphoinositide hydrolysis and increase EGF receptor protein synthesis and mRNA levels in rat liver epithelial cells: Evidence for protein kinase C dependent and independent pathways, *J. Biol. Chem.* **263,** 13688–13784 (1988).
29. Banks-Shlegel, S. P., and Quintero, J. Human esophageal carcinoma cells have fewer, but higher affinity epidermal growth factor receptors, *J. Biol. Chem.* **261,** 4359–4362 (1986).
30. Murphy, L. J., Gong, Y., Murphy, L. C., and Bhavnani, B. Growth factors in normal and malignant uterine tissue, *Ann. N.Y. Acad. Sci.* **622,** 383–391 (1991).
31. Haining, R. E. S., Schofield, J. P., Jones, D. S. C., Rajput-Williams, J., and Smith, S. K. Identification of mRNA for epidermal growth factor and transforming growth factor-α present in low copy number in human endometrium and decidua using reverse transcriptase–polymerase chain reaction, *J. Mol. Endocrinol.* **6,** 207–214 (1991).
32. Leake, R., Carr, L., and Rinaldi, F. Autocrine and paracrine effects in the endometrium, *Ann. N.Y. Acad. Sci.* **662,** 145–148 (1991).
33. Berchuck, A., Soisson, A. P., Olt, G. J., Clarke-Pearson, D. L., Bast, R. C., Jr., and McCarty, K. S., Jr. Epidermal growth factor receptor expression in normal and malignant endometrium, *Am. J. Obstet. Gynecol.* **161,** 1247–1252 (1989).
34. Bigsby, R. M., Aixin, L., Bomalski, J., Stehmen, F. B., Look, K. Y., and Sutton, G. P. Immunohistochemical study of HER-2/neu, epidermal growth factor receptor, and steroid receptor expression in normal and malignant endometrium, *Obstet. Gynecol.* **79,** 95–100 (1992).
35. Lippman, M. E., Dickson, R. B., Gelmann, E. P., Rosen, N., Krabbe, C., Bates, S., Bronzert, D., Huff, K., and Kalid, A. Growth factor regulation of human breast carcinoma occurs through regulated growth factor secretion, *J. Cell Biol.* **35,** 1–16 (1987).
36. Ballard, J., Baxter, R., Binoux, M., Clemmons, D., Drop, S., Hall, K., Hintz, R. Rechler, M., Rutaten, E., and Schwander, J. On the nomenclature of the IGF binding proteins *Acta. Endocrinol.* **121,** 751–752 (1989). [Letter]
37. Pekonen, K., Nyman, T., and Rutaten, E-M. Human endometrial adenocarcinoma cell lines HEC-1B and KLE secrete insulin-like growth factor binding protein-1 and contain IGF-I receptors, *Mol. Cell. Endocrinol.* **75,** 81–87 (1991).
38. Rutaten, E. M., Pekonen, F., and Makinen, T. Soluble 34K binding protein inhibits the binding of insulin like growth factor I to its cell receptors in human secretory phase endometrium, *J. Clin. Endocrinol. Metab.* **66,** 173–180 (1988).
39. Morgan, D. O., Edman, J. C., Standring, D. N., Fried, V. A.,

Smith, M. C., Roth, R. A., and Rutter, W. J. Insulin-like growth factor II receptor as a multifunctional binding protein, *Nature (London)* **329,** 301–307 (1987).

40. Casella, S. J., Han, V. K., D'Ercole, A. J., Svoboda, M. E., and Van Wyk, J. J. Insulin-like growth factor II binding to the type I somatomedin receptor: Evidence for two high affinity binding sites, *J. Biol. Chem.* **261,** 9268–9273 (1986).
41. Boehm, K. D., Daimon, M., Gorodeski, I. G., Sheean, L. A., Utian, W. H., and Ilan, J. Expression of the insulin-like and platelet-derived growth factor genes in human uterine tissue, *Mol. Reprod. Dev.* **27,** 93–101 (1990).
42. Van Wyk, J. J., and Underwood, L. E. In *Biochemical actions of hormones,* (J. Litwack, Ed.), Academic Press, New York, Vol. 5, pp. 101–148 (1978).
43. Nagamani, M., Hannigan, E. V., Dihn, T. V., and Stuart, C. V. Hyperinsulinemia and stromal luteinization of the ovaries in women with endometrial cancer, *J. Clin. Endocrinol. Metab.* **67,** 144–148 (1988).
44. Sheets, E. E., Tsibris, J. C., Cook, N. I., Virgin, S. D., DeMay, R. M., and Spellacy, W. N. *In vitro* binding of insulin and epidermal growth factor to human endometrium and endocervix, *Am. J. Obstet. Gynecol.* **153,** 60–65 (1985).
45. Surrey, E. S., and Halme, J. Effect of platelet-derived growth factor on endometrial stromal cell proliferation *in vitro:* A model for endometriosis?, *Fertil. Steril.* **56,** 672–679 (1991).
46. Terracio, L., Ronnstrand, L., Tingstrom, A., Rubin, K., Claesson-Welsh, L., Funa, K., and Heldin, C. H. Induction of platelet-derived growth factor expression in smooth muscle cells and fibroblasts upon tissue culturing, *J. Cell. Biol.* **107,** 1947–1957 (1988).