In Vitro Differentiation Of Human Endometrial Stromal Cells: Effect of Transforming Growth Factor B 1 on Prolactin Secretion

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Abstract

Objective: To investigate the role of Transforming Growth Factor ß 1 on differentiation of human endometrial stromal cells.
Study design: A prospective study.
Material and Methods: Human endometrial cells obtained from 9 women were cultured in DMEM-HAMs F12 media to adequate cell confluence and then the flasks were cultured in the presence and absence of TGF h 1. The influence of TGF ß 1 were measured by prolactin production expressed as nanograms of prolactin/mg of total DNA in cells measured by diphenylamine reaction.
Results: The levels of prolactin in the culture medium with and without TGF f3 1 were 0.16±0.27 ng/ug DNA/day and 0.24±0.41 ng/ug DNA/day respectively. There was no significant difference between groups.
Conctusion: There is no direct effect of TGF ß] on differentiation of human endornetrial stromal cells. However it may involve in more complex relationship in this process (JPMA 50:17, 2000).

Introduction

Endometrial stromal cell proliferation and differentiation is under the control of the ovarian steroid hormones[1]. The decidual cells produce bioactive materials such as hormones, growth factors, cytokines and extracellular matrix components. Therefore these cells not only facilitate implantation of the embryo but maintain the function of decidua during pregnancy[2,3].
Over the past decades, in-vivo models have been extensively studied for the characterization of decidualization at molecular and morphological levels. By this way, the role of ovarian hormones in influencing cyclic modifications of endometrial tissue is welL understood. For this purpose an in vitro decidualization model has been developed by progesterone induction of endometrial stromal cells[4].
Prolactine (PRL) and Insulin Like Growth Factor Binding protein I (IGFBP I) are two proteins which are expressed only in differentiated stromal cells, can be used as biochemical marker of differentiation[5,6].
Although much is known about the role of progesterone is decidualization, there may be other factors involved in this process. Growth factors are known to mediate the cyclic hormonally induced changes, in endometrial epithelial and stromal cell proliferation. Members of the transforming growth factor J3 (TGF ß) family have been implicated in regulation of cell proliferation, differentiation, formation of extracellular matrix and cell surface receptors. It has been reported that TGF j3 mRNA is expressed in endometrium and decidua[7]. Its expression in differentiated tissue points out its importance in decidualization. However no direct evidence has been reported for the action of TGF (3 on function differentiation of the endometrial stromal cells during decidual ization.
In this study we have performed experiments using the model of in-vitro decidualization to investigate

the effect of TGF ß on differentiation of human endometrial stromal cells.

Materials and Methods

Tissue collection: Human uterine endometrial tissues were obtained from 9 premenopausal women (age ranging from 35 to 40) undergoing hysterectomy for medical reasons. They were not receiving any hormonal treatment at the time of operation. Tissues were collected in sterile Dulbecco's Modified Eagle's Medium (DMEM: Gibco Grandisland N.Y.) and immediately transferred to the laboratory in cold ice. Culture of Human Endometrial Stromal Cells: The specimen was gently minced into small pieces and washed in fresh medium to remove mucus and debris. Then it was incubated with trypsin EDTA (0.25% Porcine, JRHBiosciences) in a CO, incubator at 37°C for 15 minutes by vigorous shaking. The digested tissue was filtered through a 1 00 m mesh in order to exclude glandular components. The cells were cultured with a complete media containing DMEM-HAMs F 12 (Gibco-BRL) suplemented with 15% Fetal Calf Serum (Gibco-BRL), 2 mML-glutamine, 25 mg/mI antibiotic-antimicotic (Sigma) and I mM Hepes Buffer (JRH-Biosciences). After an initial incubation period of 24 hours at 37.8°C under 7.5% CO2. the spent medium was removed and replaced with fresh medium. Incubation for 7 days was necessary to achieve adequate cell confluence. During this period, media was changed every 72 hours. The morphological gross examination was done by a phase contrast microscope (Nikon)[8]. Viability was established with 0.04% trypan blue exclusion and was always greater than 90%.

Immunocytochemistry: Endometrial stromal cells were identified by using cell specific antigens. The cultured`cells were harvested with trypsin EDTA (0.25% Porcine, JRHBiosciences) for 10 minutes at 37°C incubator. Cell suspension was immediately subjected to centrifugation (1500 x g) and washed with PBS 3 times and fixed with ice-cold methanol for 10 minutes. Light microscopic immunohistochemistry was performed on smears of cultured cells using prediluted monoclonal antisera to desmin. The immunostaining procedure used in this study was an adaptation of Dijkstra's method[9]. The prefixed smears were incubated in cold acetone for 20 minutes and air-dried for 45 minutes and incubation with anti-desmin for 60 minutes at room temperature in hunimidity chamber was followed. A PBS (ph: 7.2-7.4) rinse was followed by secondary antibody rabbit anti-mouse ig peroxidase activity with 3,3 diaminobenzidine-tetrahydrochloride (DAB, Sigma) in 0.5 mg/mI Tris HCL buffer, pH: 7.6, containing 0.01% H2O2 solution. Counterstaining was done with hematoxylin. The control staining was carried out by omitting the primary antibody step10,11 The resulting staining was evaluated on Nikon Microphot FX-A 100 microscope.

Experimental Design: The morphologically homogeneous confluent stromal cells were trypsinized and subpassaged in the complete media containing 50 ng/ml progesterone (Sigma). They were permitted to reach confluence 4 days before the start of the experiment. The flasks were cultured in the absence and presence of 1 ng/ml TGF ß for 24 hours. At the end of this period, supernatants were kept for the determination of prolactin levels at -20°C until assayed. In order to exclude the increase in prolactin production caused by cell proliferation the production rate was calculated and expressed as nanograms of prolactin per mg of total DNA in cells measured by diphenylamine reaction[8].

Radioim m unoassay: Pro lactin measurements were made by RIA (Coat a Count Prolactin IRMA kit). The release of prolactin was normalized to DNA content at the end of the experiment as PRL4tg of total DNA. All measurements were done in duplicate.

tatistical analysis: Wilcoxon test was used for this purpose.

Results

Morphology: in order to characterize the endometrial stromal cells, the appearance of cultured cells

was documented with an inverted phase contrast microscope (Nikon). The endometrial strornal cells were observed in culture by their flattened and elongated fibroblast like shape. Moreover stromal cells survived when subcultured as reported previously and their doubling time was 4-5 days. Morphological observations indicated that 98% of the cells were stromal and there was a minimal cross contamination between the two cell types.

Immunocytochemistry: Since a phase contrast microscopy could be subjective and not sufficient to show the purity of cultured cells, indirect immunocytochem ical analysis was done -by a monoclonal antibody for dernin. Diffuse and strong cytoplasmic immunostaining for desrnin was obtained in nearly all (98%) of cultured stromal cells.

Prolactin measurements: The prolactin concentrations in the culture medium with and without TGF f3 were given in Table.

Table. Mean prolactin ng/mg of total DNA amount in flasks.

	PRL (ng/µg DNA/day) Mean±SD
Control flasks	0.16+0.27
TGF β mediated flasks	0.24±0.41

Using Wilcoxon analysis it was observed that there was no significant difference between prolactin secretion of TGF ß mediated flasks and control flasks.

Discussion

This study demonstrated that there is no direct effect of TGF ß on prolactin secretion in vitro endometrial stromal cells.

Earlier studies demonstrated that during luteal phase of menstrual cycle, the differentiated endornetrium secreates prolactin, which can be used as a biochemical marker of decidualisation[12]. The mRNA of PRL released from human decidua and the nucleotide sequence of the cDNA has been shown to be identical of the human pituitary PRL gene. Although they are similar in DNA level, their expression patterns are controlled in different ways.

TGF ß Im RNA was shown in human endometrium[7]. TGFs are expressed by a variety cells in human body. They are responsible for either supression or induction of variety of cell specific activities depending on the cell type. Generally it stimulates mesodermal derived cells while suppresses ectodermal origin. Indeed TGF ßstimulated DNA synthesis in stromal cells in vivo while inhibited DNA synthesis in glandular epithelium[13]. In the same research TGF ß I mRNA was found in much greater levels in stromal cells than endometrial glandular cells during the secretary phase. So they supposed that TGF ßsecreted from the stromal cells may act upon the decidualisation. On the other hand by Tang's study it was reported that TGF and exerted its paracrine effect via the receptors on the stronial cells. They also supposed the same explanation on the decidualisation process, an increase in TGF B expression may lead to growth arrest and transition from cellular proliferation to differentiation because of stimulatory effect on the rate of (3H) thymidine incorporation in culture of endometrial stromal cells without any effect on cell proliferation[4].

We are interested in the relationship between TGF and prolactin secretion. In order to investigate the effect of TGF ß on differentiated cells secretion, an in vitro decidualisation model has been used. The

purity of the endometrial stromal cells were tested both morphologically and with the presence of intermediate filament specific proteins as mentioned in the results. Concentration of the TGF ß(lng/ml) was similar to the previous report[14]. It has a stimulatory effect on the rate of (3H) thymidine incorporation of primary endometrial stromal cells.

Our results indicate that TOF ßdid not have a direct effect on prolactin secretion upon decidualisation. However while examining the net effect of individual growth factors on in vitro systems it should be always kept in mind that in vivo growth appears in carefully orchestrated interaction of the growth factors and other hormones that function in an autocrine and paracrine fashion. This preliminary report shows more complex models and molecular analysis which are still under investigation.

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