Human Endometrial Fibroblasts Immortalized by Simian Virus 40 Large T Antigen Differentiate in Response to a Decidualization Stimulus

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ABSTRACT

Human endometrial fibroblasts have been immortalized by infection with simian virus 40 large T antigen and established as a permanent cell line, St-2. Biochemical differentiation of this cell line has been demonstrated by the ability of a decidualizing stimulus, 8-bromo-cAMP plus medroxyprogesterone acetate (MPA), to induce PRL secretion and increase the enzymatic activity of estrone sulfatase. MPA, alone or in combination with estradiol, was unable to elicit this response, but potentiated the effect of 8-bromo-cAMP on PRL production and estrone sulfatase activity. The increase in PRL protein was accompanied by an increase in PRL messenger RNA and increased expression of the insulin-like growth factor-binding protein-1 messenger RNA. The St-2 cell PRL transcript was larger than the pituitary PRL transcript, suggesting its initiation from the distal, nonpituitary, PRL promoter. This was confirmed by reverse transcription-PCR analysis of PRL transcripts using primers specific for the additional sequences present only in the 5'-untranslated region of RNA initiated from the distal promoter. Transient transfection of a reporter construct containing 3000 bp of DNA 5' to the decidual-specific promoter of the human PRL gene demonstrated that cAMP was capable of activating this distal promoter in St-2 cells. In conclusion, this novel cell line provides an interesting new model in which to pursue aspects of biochemical differentiation of human endometrium in vitro. (Endocrinology 137: 2225-2231, 1996)

Human endometrium consists not only of epithelial cells and stromal fibroblasts, but also of vascular endothelial cells, macrophages, and lymphoid cells. Orchestration of responses to ovarian steroid hormones by all of these various cell types are necessary for successful implantation and placentation. Topographical and temporal differences in both the growth and differentiation of endometrial elements suggest that local paracrine and autocrine factors modulate the effects of sex steroids (1). After ovulation, glandular epithelial cells of the functional layer will cease to proliferate and undergo secretory transformation. Endometrial stromal cells (ESC) continue to proliferate until differentiation into decidual cells in the mid- to late secretory phase of the cycle (2). Although its exact biological role is still unresolved, decidual transformation is thought to play a pivotal role in controlling trophoblast invasion and regulating menstruation.

The process of ESC decidualization involves extensive morphological and biochemical changes (3, 4). Some of the biochemical events have been characterized in vitro and include the secretion of PRL and insulin-like growth factor-binding protein-1 (IGFBP-1), enhanced activity of estrone sulfatase, and increased expression of type I plasminogen activator inhibitor and CD13 antigen/aminopeptidase-N (5-9). PRL and IGFBP-1 are widely used biochemical markers for decidualization, and several ligands have been identified as being capable of modulating the secretion of these proteins in vitro. For instance, enriched primary cultures of proliferating ESC have been shown to secrete PRL when treated in the presence of progesterone with PGE2, relaxin, gonadotropins, or cAMP (10-14). On the other hand, PRL production by decidual cell cultures can be inhibited by phorbol esters, certain cytokines produced by decidual immune cells such as interleukin-1 and tumor necrosis factor-α, and lipopolysaccharides (15-18). These in vitro differentiation models of ESC have undoubtedly provided a powerful tool to study the mechanisms that govern decidualization. However, the lack of a homogeneous cell population and the limited life span of endometrial cells in these systems have hampered extensive investigation of the molecular events underlying stromal cell differentiation (19). Here we report on the establishment of a permanent endometrial stromal fibroblast cell line, St-2, by retrovirus-mediated transfer of simian virus 40 (SV40) large T antigen. St-2 cells retain the ability to express biochemical correlates of decidualization and provide, therefore, a potential model in which to investigate the molecular endocrinology of human endometrial differentiation.
Materials and Methods

Materials

DMEM-Ham's F-12 mixture (DMEM-F12), collagenase (type 1), deoxyribonuclease (type 1), 8-bromo-cAMP, medroxyprogesterone acetate (MPA), 17β-estradiol (E₂), insulin, apo-transferrin, BSA, antibiotic-antimycotic solution, linoleic acid, polybrene, diaminobenzidine, Hoechst dye 33258 (bisbenzimide), calt thymus DNA, bacitracin, phenylmethylsulfonylfluoride, pepstatin A, leupeptin, and unlabeled estrone sulfate were all obtained from Sigma Chemical Co. (Poole, UK). 6,7-H-Labeled estrone sulfate was purchased from New England Nuclear-DuPont (Stevenage, UK), and 4'-13C-labeled estrone was obtained from Ayes International (Little Cheil, UK). FBS and G-418 sulfate solution (Stevenage, UK), and 4'-13C-labeled estrone was obtained from Amer sham International (Irby Chalfont, UK). Fibroblasts were purchased from Amicon (Stonehouse, UK). RNA markers were purchased from Promega Corp. (Madison, WI), and RNA clean was obtained from AGS (Heidelberg, Germany). Superscript ribonuclease H was purchased from Life Technologies (Eggenstein, Germany). calf thymus DNA, bacitracin, 40 mg/ml phenylmethylsulfonylfluoride, 5 mg/ml pepstatin A, 17β-estradiol (E₂), insulin, apo-transferrin, BSA, antibiotic-antimotic mixture, and 1% ITS. Supernatant was collected from 25 cm² flasks and was centrifuged at 1500 rpm for 5 min, and protein was extracted using a high salt buffer [0.4 M KCl, 20 mg/ml HEPES (pH 7.4), 1 mg/ml NTT, and 20% glycerol] containing a mixture of protease inhibitors (0.5 mg/ml bacitracin, 40 mg/ml phenylmethylsulfonylfluoride, 5 mg/ml pepstatin A, and 5 mg/ml leupeptin). ER and PR were measured by enzyme immunoassay (Abbott Laboratories). The sensitivities of the assay were 1.5 and 1.7 fmol/mg protein for ER and PR, respectively, and the coefficient of variation between assays was 4%. Protein was determined according to the method of Lowry et al. (20).

Primary endometrial cell culture

The ESC from normal proliferative endometrial tissues were isolated from normal cycling women by endometrial suction biopsy at the time of diagnostic laparoscopy. Samples were collected in Earle’s buffered saline containing 100 U/ml penicillin and 100 μg/ml streptomycin. The tissues were washed twice in DMEM-F12, finely minced, and enzymatically digested with collagenase (134 U/ml) and deoxyribonuclease type 1 (156 U/ml) for 1 h at 37 C. After centrifugation at 400 × g for 4 min, the pellet was resuspended in maintenance medium, a mixture of DMEM-F12, 10% FBS, 2% penicillin-streptomycin, and 1% ITS (6.25 μg/ml insulin, 6.25 μg/ml apo-transferrin, 1.25 mg/ml BSA, and 5.35 μg/ml linoleic acid; final concentrations). The cells were strained through a 38-μm sieve to separate stroma from glands, and ESC were further enriched by their rapid adherence.

Transformation of ESC by SV40

The vector ZipSV40–6, a recombinant retrovirus derived from ZipNeoSV(X), carries the SV40 temperature-independent large T gene and the marker gene neomycin acetyl transferase, which confers resistance to the aminoglycoside G418. The retrovirus-packaging cell line (kind gift from Dr. Nick Lemoine, Imperial Cancer Research Fund Oncology Unit, Hammersmith Hospital, London, UK) was plated at 2.5 × 10⁷ cells/150-mm culture dish in medium 199 supplemented with 10% FBS, 2 mM L-glutamine, 100 IU/ml penicillin, and 100 μg/ml streptomycin and grown to 50% confluence. These cells were then inoculated with G-418 sulfate for 24 h and subsequently left in G-418 sulfate-free medium overnight. Medium containing virus particles was prepared by harvesting supernatant from the packaging cells. After centrifugation at 3000 rpm for 10 min, the upper half of the sample was collected and filtered through a 0.45-μm filter. The virus-containing medium was supplemented with polybrene (8 μg/ml) and added to the fibroblast cultures (ESC) for 2 h at 37 C, followed by the addition of an equal volume of virus-free maintenance medium. After 7 days of incubation, ESC were treated with G-418 sulfate (1 mg/ml). Cells surviving G-418 sulfate treatment were harvested, pooled, and passed into continuous culture as St-2 cells.

St-2 cell culture

St-2 cells used in this study were cultured in maintenance medium supplemented with E₂ (10⁻¹⁰ M) at 37 C in a humidified atmosphere of 5% CO₂ in air. In experiments cells were plated in 25- or 150-cm² flasks and allowed to reach confluence. Confluent monolayers were treated with 8-bromo-cAMP and MPA, alone or in combination, in DMEM-F12 containing 2% charcoal-treated FBS (DCC), 1% of an antibiotic-antimycotic mixture, and 1% ITS. Supernatant was collected from 25-cm² flasks for measurement of PRL, and cells were retrieved for assay of estrone sulfate. Cells grown in 150-cm² flasks were harvested for preparation and analysis of RNA.

PRL assay, protein extraction, and ER and PR assay

PRL levels in samples concentrated 8-fold by ultrafiltration (Centriprep 10) were measured by microparticle enzyme immunoassay (Axisym System, Abbott Laboratories, North Chicago, IL). The coefficient of variation within assays was 2–3%, and that between assays was 6–8%. DMEM-F12 supplemented with DCC did not show measurable PRL concentrations.

For estrogen receptor (ER) and progesterone receptor (PR) measurements, maintenance cells (four confluent 150-cm² flasks) were washed with unlabeled PBS and removed by gentle scraping. The cell suspensions were centrifuged at 1500 rpm for 5 min, and protein was extracted using a high salt buffer [0.4 M KCl, 20 mM HEPES (pH 7.4), 1 mM DTT, and 20% glycerol] containing a mixture of protease inhibitors (0.5 mg/ml bacitracin, 40 mg/ml phenylmethylsulfonylfluoride, 5 mg/ml pepstatin A, and 5 mg/ml leupeptin). ER and PR were measured by enzyme immunoassay (Abbott Laboratories). The sensitivities of the assay were 1.5 and 1.7 fmol/mg protein for ER and PR, respectively, and the coefficient of variation between assays was 4%. Protein was determined according to the method of Lowry et al. (20).

Estrone sulfate assay

Cells were washed once with serum-free medium and stored at −20 C until assayed. PBS sucrose [50 mM phosphate buffer (pH 7.4), 140 mM NaCl, and 250 mM sucrose] was added to each treatment flask (1.5 ml/T25 flask), and the cells were scraped and sonicated on ice using two 3-sec cycles with 1-min cooling periods (10-micron amplitude, MSE Soniprep 150, Fisher Scientific UK, Loughborough, Leics, UK). The sonicated cells were centrifuged (10,000 rpm for 5 min), and 0.5 ml of the supernatant was assayed, in duplicate, using [6,7-3H]estrone (4 × 10⁶ dpm) adjusted to a final concentration of 20 μM with unlabeled substrate. After incubation of the substrate with sonicated cell supernatants at 37 C for 60 min, the protein formed was isolated from the mixture (1 ml) by extraction with toluene (4 ml). [4,14C]Estrone (7 × 10⁶ dpm) was used to monitor procedural losses. The 3H and 14C contents in the toluene were determined by scintillation spectrometry. Each assay also included tubes without cell supernatants to assess the apparent nonenzymatic hydrolysis of the substrate.

DNA assay

The DNA content of each culture flask was measured by quantitative fluorometric analysis at room temperature. Cells were solubilized with 0.02% SDS. Aliquots were then mixed with 1 μg/ml Hoechst 33258 in 1 × standard saline citrate (SSC), and fluorescence was measured in a fluorimeter at 341 nm excitation/460 nm emission. Calt thymus DNA was used as standard.

Morphological assessment and immunocytochemistry

Phase contrast microscopy was used to verify phenotypic changes in response to various treatment conditions. Immunocytochemistry was used to confirm the cellular origin of St-2 cells. Cells cultured on coverslips under different experimental conditions were fixed in 95% alcohol for 6 min, rehydrated, and immunocytochemically stained with mouse antihuman antivimentin, anticytokeratin, and antileukocyte common antigen antibodies, biotinylated goat antianimal IgG, and peroxidase-labeled streptavidin. The cells were pretreated with 0.3% H₂O₂ in methanol for 30 min to remove endogenous peroxidase activity and incubated with normal goat serum in PBS for 10 min to block nonspecific binding sites for IgG. After incubation with primary antibodies, the St-2 cells were exposed to biotinylated goat antianimal IgG for 60 min and sub-
sequently exposed to peroxidase-labeled streptavidin in PBS for 1 h. Between each step, cells were washed gently in PBS for 15 min (three times, 5 min each). Diaminobenzidine tetrachloride (0.5 mg/ml in PBS containing 0.01% H2O2) was used to visualize the peroxidase activity. Nuclei were counterstained with hematoxylin.

**Northern blot analysis**

RNA was extracted with RNA-clean, and Northern blot analysis was performed as previously described (21). RNA markers were used for size estimation. Probes were a 500-bp PstI fragment of the human PRL (hPRL) complementary DNA (cDNA) (22), a 380-bp PstI-BamHI fragment of the human IGFBP-1 cDNA (23), and a 200-bp fragment of the human GAPDH cDNA (24). The IGFBP-1 probe was created by reverse transcription-PCR (RT-PCR) from human decidual RNA with the following oligonucleotides: BP-S (positions 506-526, including the internal PstI site) and BP-AS (antisense to positions 864-884, including the internal BamHI site). A blunt-ended PCR product was generated using Pfu-polymerase and inserted into the SphI site of pCR-Script (Stratagene, La Jolla, CA). The glyceraldehyde-3-phosphate dehydrogenase (GAPDH) probe was created by RT-PCR using oligonucleotides GAPDH-5' (positions 363-380) and GAPDH-3' (antisense to positions 543-560).

**RT-PCR**

Total RNA (5 µg) from treated cells was reverse transcribed with Superscript ribonuclease H- and amplified with Pfu-polymerase, as detailed previously (25). Simultaneous amplification of hPRL and GAPDH cDNAs was performed by adding 10 pmol each of the following oligonucleotides to one reaction: 98 (corresponding to positions -136 to -107 in the decidua-specific 5'-untranslated region (UTR) of the hPRL cDNA) (26), 630 (antisense to positions 710-739 in the 3'-UTR of hPRL cDNA), GAPDH-5', and GAPDH-3'. IGFBP-1 and GAPDH cDNAs were amplified simultaneously using 10 pmol each of oligonucleotides BP-S, BP-AS, GAPDH-5', and GAPDH-3'. Southern blots of the PCR products were hybridized with 32P-labeled oligonucleotides 181 (antisense to positions -115 to -96 in the decidua-specific 5'-UTR of the hPRL cDNA) (26), BP-int (positions 818-836 in the human IGFBP-1 cDNA), or GAPDH-int (positions 454-472 in the human GAPDH cDNA). pGEM markers were used for size determination.

**Transient transfections**

St-2 cells were plated at a density of 2.5 x 10^5 cells/well in 12-well plates. Transfections were performed by the calcium phosphate precipitation method in medium supplemented with 10% DCC, 10^-8 M E2, and 0.1% BSA. Triplicate wells received 2 µg construct decidual PRL (dPRL)-300/Luc or the promoterless plasmid pGL2-Basic (25). Cells were washed 16 h later and received fresh medium with the additions indicated in the figure legends. After 48 h, cells extracts were harvested, and luciferase activities were determined, as previously described (25).

**Results**

**Morphological assessment and steroid receptor expression**

Under maintenance conditions, St-2 cells displayed a fibroblastic spindle-shaped morphology (Fig. 1A). Immunostaining was positive for vimentin and negative for cytokeratin and LeuM1, confirming their mesenchymal origin (data not shown).

![Fig. 1. Morphological transformation of St-2 cells. Cells were cultured in DMEM-F12-PBS medium supplemented with E2 (10^-8 M) until confluent. Subsequently, St-2 cells were treated with DMEM-F12 and DCC, as indicated in Materials and Methods, alone (A) or with 8-bromo-cAMP (0.5 mM; B), MPA (10^-8 M; C), or a combination of 8-bromo-cAMP plus MPA (D) for 48 h and then photographed under phase contrast microscopy (×100).](https://academic.oup.com/endo/article-abstract/137/6/2225/3037209)
In the experiments described here, cells were used within 12 passages of removal from liquid nitrogen, as this provided the most consistent results and overcame the slower growth rate of cells observed at later passages. Treatment of confluent monolayers with 8-bromo-cAMP (0.5 mM), alone (Fig. 1B) or in combination with MPA (10^{-6} M; Fig. 1D), transformed the spindle-shaped cells into cells with abundant cytoplasm, resembling decidual cells (2, 12). Morphological changes were apparent after 2 days of treatment. Large intercellular spaces were formed, and the cells displayed multiple long protrusions on the cell surfaces. Treatment of the cells with MPA alone (Fig. 1C) or with a combination of MPA and E_2 (data not shown) was unable to elicit this differentiated phenotype. St-2 cells cultured under maintenance conditions were shown to express ER and PR at concentrations of 17.0 ± 1.85 and 441 ± 46 fmol/mg protein (mean ± SEM), respectively.

Induction of PRL secretion and estrone sulfatase activity in St-2 cells

Immunoreactive PRL secreted from St-2 cells and accumulated in the culture medium was first detected after 48-h incubation with 8-bromo-cAMP, alone or in combination with MPA (Fig. 2A). MPA, alone or in combination with E_2, was insufficient to induce PRL production (data not shown). After 96 h, a synergistic effect between 8-bromo-cAMP and MPA on PRL secretion became apparent. The pure anti-estrogen ZK98.299, partially inhibited the synergistic effect of 8-bromo-cAMP plus MPA on PRL production, suggesting involvement of the PR. In parallel with the increase in PRL production in response to 8-bromo-cAMP plus MPA, a marked increase in a further biochemical correlate of endometrial fibroblast differentiation, estrone sulfatase activity, was observed (Fig. 3).

Effects of 8-bromo-cAMP and MPA on decidual PRL messenger RNA (mRNA) and IGFBP-1 mRNA

Several lines of evidence established that the PRL produced by St-2 cells was transcribed from the upstream dPRL promoter that is also used in ESC. Figure 4 illustrates results from Northern blot analysis of St-2 cell total RNA. The PRL transcript (~1.1 kilobases [kb]) detected in St-2 cells was larger than the PRL transcript from human pituitary. It was dose dependently induced by 8-bromo-cAMP (0.2 and 0.5 mM) in the presence of MPA after 3 days, but was undetectable in control cells or cells treated with MPA alone. IGFBP-1 mRNA expression was very low in untreated or MPA treated cells and was induced by the addition of 0.2 and 0.5 mM 8-bromo-cAMP together with MPA (transcript size, ~1.6 kb).

A noncoding exon present only in dPRL mRNA (26) was used as the target template for amplification to provide further evidence of expression of the nonpituitary transcript in St-2 cells. Figure 5 shows RT-PCR analysis of St-2 cell RNA, obtained under the indicated treatment conditions, and demonstrates the presence of a 758-bp amplicon consistent with the presence of mRNA transcribed from the distal promoter. The transcript was induced by combination treatment with cAMP and MPA (Fig. 5, lane 4), confirming the induction observed by Northern analysis (Fig. 4). Furthermore, it was possible to demonstrate induction of this transcript after treatment with 8-bromo-cAMP alone (0.5 mM; Fig. 5, upper panel, lane 2), but not by MPA alone (1 μM; Fig. 5, upper panel, lane 3).
FIG. 3. Estrone sulfatase activity in St-2 cells at the end of a 48-h treatment period with 8-bromo-CAMP (0.5 mM), MPA (10−6 M), or the two in combination. Results are the mean ± s.d. of four separate determinations measured in duplicate. St-2 cells displayed a significant increase in estrone sulfatase activity when treated with 8-bromo-CAMP plus MPA (by Student’s t test, P < 0.0001), but not when treated with MPA or 8-bromo-cAMP alone (P > 0.05).

Fig. 4. Northern blot analysis of St-2 cell RNA. A Northern blot with total RNA from St-2 cells (40 μg; lanes 1–4) and human pituitary (0.5 μg; lanes 5 and 6) was successively hybridized to PRL, IGFBP-1, and GAPDH cDNA probes. St-2 cells had been treated for 3 days before RNA extraction (lane 1, control; lane 2, 10−6 M MPA; lane 3, 10−6 M MPA plus 0.2 mM 8-bromo-CAMP; lane 4, 10−6 M MPA plus 0.5 mM 8-bromo-cAMP). As the PRL signal from pituitary was overexposed after 24 h (lane 5), a shorter exposure is shown in lane 6. Hybridization to GAPDH cDNA (transcript size, 1.3 kb) shows even loading of the gel.

Discussion

Progress in understanding the molecular events that regulate ESC decidualization has been limited by the complexity of hormonal and cellular interactions that regulate this differentiation process in vivo. Primary cultures of enriched ESC have been used as model systems that can potentially overcome some of these inherent limitations. However, the limited life span of ESC in vitro and the inevitable contamination of primary cultures with lymphocytes, macrophages, and epithelial cells have precluded extensive investigation of the molecular events underpinning hormone-induced differentiation (19).

Immortalization of rat endometrial cells by retrovirus-mediated transfer of SV40 large T antigen has generated cell lines that express steroid hormone receptors and, hence, the potential to exhibit differentiated function in vitro (27). Here we report that it is possible, using a similar approach, to develop a human fibroblast cell line that displays biochemical features of the parental cells. Endometrial cells enriched for fibroblasts were infected, by retrovirus-mediated transfer, with the SV40 large T antigen gene and selected on the basis of resistance to G418 conferred by the neomycin acetyl transferase gene, also carried by the recombinant retrovirus. A polyclonal immortalized cell line that expressed ER and PR was established and termed St-2.
CAMP. Results are the mean ± SEM of the PRL and IGFBP-1 signals.

Enhanced activity of this enzyme has been observed in response to a decidualization stimulus in vitro and in decidual tissue obtained after term pregnancy (7). Arylsulfatase can also be involved in the conversion of estrone sulfate to estrone, a substrate for E₂ formation. Benedetto et al. (7) suggested that increased enzymatic activity may result in a preferential estrogenic stimulation of decidualized cells, which could explain why stromal cells, in contrast to epithelial cells, maintain their estrogen-dependent PR levels during the late luteal phase of the cycle and pregnancy (7).

Expression of the hPRL gene in extrapituitary sites, such as decidua stromal cells and lymphocytes, is regulated by an alternative dPRL promoter located approximately 6 kb upstream of the pituitary-specific start site (25, 29). Activation of the dPRL promoter does not involve Pit-1, which is essential for pituitary PRL expression, and is differentially controlled in human lymphocytes and endometrial stroma. Expression of the hPRL gene in decidual cells results in a PRL message larger than that expressed in pituitary cells because it contains a unique 5′-untranslated exon (25, 29). Accordingly, we demonstrated with Northern blot analysis and RT-PCR, using a 5′-primer specific for exon 1a sequence, that the nature of the PRL transcript induced in St-2 cells in response to elevation of intracellular CAMP is consistent with it being transcribed from the dPRL promoter. Transient transfection of St-2 cells with a reporter gene construct carrying 3000 bp 5′-flanking DNA to the dPRL and stimulation with 8-bromo-cAMP was used to demonstrate that the factors necessary for activation of the distal dPRL promoter were present in St-2 cells. Unstimulated St-2 cells did not activate the promoter and, therefore, displayed properties similar to those of transiently transfected undifferentiated ESC (25). MPA alone was unable to induce de novo expression of either protein or message, but synergized with 8-bromo-cAMP to increase the amount of PRL detected in conditioned medium and the apparent abundance of dPRL mRNA. However, such synergy between cAMP and MPA was not observed on the dPRL promoter-reporter construct. Although activation of an estragonomic promoter/reporter construct may not entirely reflect that of the endogenous gene, our observations are in agreement with those of Tang et al. (14). These workers reported a putative role for CAMP in human PRL gene expression in primary ESC cultures and postulated a posttranscriptional role for MPA in modulating PRL secretion, potentially by inhibition of phosphodiesterase activity, stabilization of PRL mRNA, or other cAMP/progesterone-coordinated mechanisms (14).

The observation that ZK98,299, a pure PR antagonist, could abolish the synergistic effect of MPA on cAMP-induced PRL production demonstrated that MPA exerts its effect through activation of the PR. Interestingly, ZK98,299 also partially antagonized cAMP-induced PRL secretion in the absence of MPA, suggesting that activation of the protein kinase A pathway by cAMP may result in ligand-independent activation of PR in St-2 cells. Activation of human PR in the absence of cognate hormone has been reported in response to cAMP (30), although there was no evidence for altered phosphorylation of PR itself (30, 31). The mechanism through which alternate signal transduction pathways can activate PR in the absence of steroid ligand in a cell-specific context and its biological relevance to ESC differentiation remain unresolved.

IGFBP-1 is considered to be a major secretory product of decidual cells and has been widely used as a marker of
decidualization of ESC in culture (32). In St-2 cells, expression of mRNA for IGFBP-1 was low or undetectable in untreated cells and induced by 8-bromo-cAMP. This observation provides further evidence that St-2 cell differentiation is at least in part mediated through the cAMP signaling pathway and demonstrates biochemical correlates of differentiation consistent with a decidualization response.

In summary, we have successfully established an immortalized cell line from enriched ESC cultures that is fibroblastic by morphological and immunocytochemistry criteria. St-2 cells displayed the ability to differentiate, as illustrated by phenotypic transformation and the induction of PRL, IGFBP-1, and estrone sulfatase activity after stimulation with MPA plus 8-bromo-cAMP. Furthermore, we demonstrated that the cAMP signaling cascade appears to mediate activation of the dPRL gene promoter and that MPA, acting via the PR, synergistically enhances the level of dPRL mRNA and protein. The mechanism of such synergy between the CAMP and PR pathways remains to be established; however, St-2 cells provide a model in which to define such interaction in a biochemical context relevant to induction of ESC differentiation in preparation for implantation.

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References