Expression of leukemia inhibitory factor and its receptors is increased during differentiation of human embryonic stem cells

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Objective: To investigate gene expression profiles during the early spontaneous differentiation of human embryonic stem cells (hESCs), with particular emphasis on leukemia inhibitory factor (LIF)–induced pathways and the ultrastructural surface morphology of the undifferentiated and spontaneously differentiated hESCs.
Design: Prospective experimental study.
Setting: University laboratory.
Patient(s): Four hESC cell lines.
Intervention(s): The effect of LIF on receptor expression level was studied in cultures.
Main Outcome Measure(s): Gene expression in the hESC line HS237 was analyzed using microarrays. Real-time reverse-transcription polymerase chain reaction was used to validate the microarray results in four hESC lines (HS181, HS235, HS237, HS293). Immunohistochemistry was used to assay LIF, LIF receptor, and gp130 protein expression. Cell surface morphology was studied using scanning electron microscopy.
Result(s): The expression of LIF, LIF receptor, and gp130 messenger RNA and protein was increased in spontaneously differentiated HS237 cells compared with undifferentiated cells, with high expression of an inhibitor of LIF-mediated signaling, suppressor of cytokine signaling–1, in undifferentiated hESCs. Genes, those expressed specifically and those shared in undifferentiated hESCs, differentiated cells, and in fibroblasts, were identified. Supplementation with LIF did not affect the LIF receptor expression.
Conclusion(s): The expression of LIF and its receptors is low in undifferentiated hESCs but increases during differentiation. Added LIF does not prevent spontaneous differentiation. Suppressor of cytokine signaling–1 may prevent LIF signaling in hESCs. (Fertil Steril® 2006;86(Suppl 3):1193–209. ©2006 by American Society for Reproductive Medicine.)

Key Words: Human embryonic stem cells, leukemia inhibitory factor, LIF receptor, gp130, SOCS-1, gene expression

Human embryonic stem cells (hESCs) are unique cells that are derived from the inner cell mass of the blastocyst (1).

These pluripotent cells maintain their ability to self-renew, and they give rise to differentiated progeny representing all three embryonic germ layers (2, 3). A high-quality hESC line expresses specific cell-surface markers (stage-specific embryonic antigen-4, tumor-related antigen (TRA)-1-60, and TRA-1-81) and transcription factors (Oct-4 and Nanog), is karyotypically normal, and has high telomerase and alkaline phosphatase enzyme activities (2). In vitro, cultured hESCs can, under optimal conditions, divide and give rise to new undifferentiated cells indefinitely (self-renewal), and cells recover after freezing and thawing. Differentiation of hESCs in vitro into several cell types, such as cardiac, neural, hematopoietic, pancreatic, and hepatic lineages, has been described elsewhere (4–8). Derivatives of hESCs thus could potentially be used for cell transplantation therapies in various severe degenerative diseases (9, 10).

Human ESCs have been cocultured with feeder cell layers (2). These cells have a tendency to differentiate in cultures. When they are left without a feeder layer or medium that has been conditioned in the presence of feeder cells, they will begin spontaneous differentiation. The use of feeder cells supports the undifferentiated growth of hESCs by an as-yet unidentified mechanism. The expression of several genes is decreased or increased during the differentiation process of hESCs (11–15), but the mechanisms behind spontaneous differentiation largely are unknown.

Received August 22, 2005; revised and accepted December 22, 2005.
Supported by the Academy of Finland, Helsinki (H.S. and R.L.), the Finnish Cultural Foundation, Helsinki, and NorFA, Oslo (H.S.), and by the Swedish Research Council, Stockholm/Juvenile Diabetes Research Foundation (JDRF), New York (O.H.).
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It is not clear which cytokines and signaling pathways are involved and what the molecular mechanisms are for the maintenance of hESC self-renewal versus induction of differentiation. Signal transducer and activator of transcription (STAT)–3 and extracellular signal-regulated kinase (ERK) critically influence mouse ESC differentiation and proliferation (16). However, there is no evidence indicating the importance of these proteins in hESCs. Mouse ESCs stay undifferentiated without a fibroblast feeder layer if leukemia inhibitory factor (LIF) is added to the culture medium (17). Mummery et al. (18) showed that in the absence of LIF in culture medium, mouse ESCs lose their pluripotency and start differentiating into a monolayer. Currently, the role of LIF supplementation of culture medium when culturing hESCs is not clear. Thomson et al. (2) reported that in both the presence and absence of LIF, hESCs differentiated when cultured in the absence of mouse fibroblast feeder layers.

Leukemia inhibitory factor is a multifunctional cytokine that acts on a wide range of cell types. It is a member of the interleukin-6 (IL-6) family and first was described as a factor that induces the differentiation of mouse myeloid leukemic M1 cells into macrophages (19). Later, it was rediscovered as an inhibitor of the differentiation of mouse ESCs (20). The pleiotropic effects of LIF in many physiological systems include proliferation, differentiation, and cell survival (21, 22). Leukemia inhibitory factor acts on cells by binding to the heterodimeric LIF receptor, which consists of two transmembrane proteins, the LIF receptor itself (LIFR) and gp130. The latter binds several cytokines that share a signal-transducing subunit in their receptor–transducer mechanism (23).

The LIFR activates several signaling pathways in diverse cell types, including the Janus kinase (JAK)–STAT3, the mitogen-activated protein kinase pathway, and the phosphoinositol 3-kinase pathway, whereas gp130 participates in the activation of STAT1, STAT3, and STAT5b (24).

Characterization of the LIF signaling pathway in hESCs appears to be essential for understanding self-renewal and pluripotency. The results of large-scale gene expression studies in hESCs to date are contradictory as regards the expression and activity of LIF signaling pathways (11, 25–28). The results of our previous studies have suggested that the expression of LIFR and gp130 may be increased during hESC differentiation (29).

The aim of the current study was to investigate the gene expression profiles of undifferentiated hESC and their spontaneously differentiating counterparts, focusing on analysis of LIF-induced pathways. On the basis of our previous results, we specifically were interested in protein expression of LIF and its receptor in undifferentiated hESCs, as well as in their early differentiated counterparts. We also wanted to investigate the ultrastructural surface morphology of the cell populations and the effect of LIF supplementation to hESC cultures.

MATERIALS AND METHODS

Human ESC Source

Human ESC lines HS181, HS235, HS237, and HS293 that were derived at our laboratory in the Karolinska University Hospital Huddinge were used for analyses. Derivation and characterization of line HS181 has been described elsewhere (30), and lines HS235, HS237, and HS293 have been characterized accordingly. Lines HS181, HS235, and HS237 have an XX karyotype, and line HS293, an XY karyotype. They express markers typical of hESCs, such as alkaline phosphatase, stage-specific embryonic antigen–4, TRA-1-60, TRA-1-81, and Oct-4, but are stage-specific embryonic antigen–1 negative. Pluripotency has been demonstrated by formation of teratomas when injected into severe combined immune deficiency mice and by in vitro differentiation of embryoid bodies expressing markers from three embryonic germ cell layers (30–32).

The Ethics Committee of the Karolinska Institute approved the studies with hESCs, and informed consent was obtained from all couples who donated embryos for hESC research.

Cell Culture

Human foreskin fibroblasts (CRL-2429; American Type Culture Collection, Manassas, VA), used as feeder cells, were mitotically inactivated by irradiation (35 Gy) and were cultured in Iscove's medium supplemented (10%) with fetal calf serum (Stem Cell Technologies, Vancouver, British Columbia, Canada). After formation of a confluent monolayer, feeder cells were cultured in hESC serum replacement (SR) medium. This hESC SR medium consisted of knockout Dulbecco's modified essential medium, 20% knockout SR, 2 mM L-glutamine, 1% penicillin–streptomycin, 1% nonessential amino acids, 0.5 mM β-mercaptoethanol, 1% insulin-transferrin-selenium (Sigma-Aldrich, St. Louis, MO), and 8 ng/mL of basic fibroblast growth factor (R&D Systems, Oxford, United Kingdom).

To study the effect of LIF concentration on growth, differentiation, and expression of LIF receptors (LIFR and gp130), the HS293 line was cultured for 3 weeks without supplementary LIF and was cultured in the presence of supplementary LIF at 5 ng/mL (Chemicon International, Temecula, CA) or at 10 ng/mL in SR medium. The light-microscopic morphology and growth and differentiation were observed. All of the culture chemicals were from GIBCO-Invitrogen Corporation (Grand Island, NY) unless stated otherwise.

Light Microscopy

In this study, we used undifferentiated and spontaneously differentiated (early differentiation) colonies from hESC lines HS181, HS235, HS237, and HS293. Undifferentiated (Fig. 1A) and spontaneously differentiated colonies (Fig. 1B) used for microarray studies were isolated by microdissection