JAK-STAT 2:4, e25155; October/November/December 2013; © 2013 Landes Bioscience

LIF-STAT signaling and trophoblast biology

Pankaj Suman, Sudha Saryu Malhotra, and Satish Kumar Gupta*

Reproductive Cell Biology Laboratory; National Institute of Immunology; Aruna Asaf Ali Marg; New Delhi, India

Keywords: leukemia inhibitory factor, embryo implantation, JAK-STAT, trophoblast, pregnancy, trophoblast invasion, syncytialization

Abbreviations: CAECAM1, carcinoembryonic antigen-related cell adhesion molecule 1; CCL2, chemokine (C-C motif) ligand 2; CTB, cytotrophoblast; EDIL3, EGF-like repeats and discoidin I-like domains 3; EGF, epidermal growth factor; EVT, extravillous trophoblast; HB-EGF, heparin binding-epidermal growth factor; HGF, hepatocyte growth factor; ICAM1, intercellular adhesion molecule 1; ID1, DNA-binding inhibitor protein; IFN, interferon; IGF, insulin-like growth factor; ITGB3, integrin β3; IVF, in vitro fertilization; JAK, Janus kinase; LIF, leukemia inhibitory factor; MAPK, mitogen activated protein kinase; MMP, matrix metalloproteinase; OMR, oncostatin M receptor; PAPPA, pregnancy associated plasma protein A; PDGFR, platelet-derived growth factor receptor; PDPN, podoplanin; PI3K, phosphatidylinositol-3-kinase; RAS, rat sarcoma; SERPINB3, squamous cell carcinoma antigen-1; Smad, mothers against decapentaplegic homolog; SOCS, suppressor of cytokine signaling; STAT, signal transducer and activator of transcription; STB, syncytiotrophoblast; TGF-β, transforming growth factor-beta; TIMP, tissue inhibitor of metalloproteinase

Leukemia inhibitory factor (LIF) is a pleiotropic growth factor that regulates several biological functions. This review focuses on the LIF-dependent STAT activation and its impact on modulation of trophoblast functions during embryo implantation. LIF is mainly produced by the maternal endometrium at the time of implantation while its receptors are present both on the endometrium and trophoblasts. It might influence blastocyst attachment through STAT3 activation and expression of integrins. After attachment of the blastocyst, trophoblasts undergo proliferation and differentiation into invasive EVTs and non-invasive STBs. Under in vitro conditions, LIF regulates all these processes through activation of STAT and MAPK-dependent signaling pathways. The observations that LIF and STAT3 knockout mice are infertile further strengthen the notion about the critical involvement of LIF-mediated signaling during embryo implantation. Hence, a better understanding of LIF-STAT signaling would help in improving fertility as use of LIF in in vitro blastocyst culture improves the implanting ability of blastocyst after IVF.

Introduction

After fertilization, the zygote undergoes several rounds of cell division to form the blastocyst during its journey from the fallopian tube to the uterine cavity. Attainment of successful implantation of blastocyst depends upon the synchronized changes in the endometrium before and after arrival of the blastocyst into the uterine cavity. The cues obtained from the receptive endometrium initially helps in the attachment of the blastocyst to the endometrial epithelium and later proliferation and differentiation of trophoblasts to form functional placenta.[1] After blastocyst hatching, the trophectodermal cells become accessible for paracrine signaling through growth factors and other soluble bioactive molecules present in the uterine fluid.[2] The blastocyst, freed from the zona pellucida, can now interact with uterine luminal epithelium where there could be possibility for the juxtracrine signaling through the growth factors like HB-EGF present on the cell surface that induce the expression of integrin α5β1 on the trophoblasts (**Fig. 1**).[3,4] This imparts competence to the blastocyst to make an attachment with the fibronectin present in the extracellular matrix (ECM), marking the beginning of the cross-signaling across the trophoblast and endometrial cells that lead to firm attachment of the blastocyst.[2] Adhesion of the blastocyst to the maternal endometrium acts as anchor and trigger for differentiation of trophoblasts into the outer syncytiotrophoblast (STB) and the inner cytotrophoblast (CTB).[2] STBs have the inherent ability to produce several lytic enzymes, which degrade the ECM and secrete factors that trigger apoptosis of the endometrial epithelial cells. This way, they enter through the endometrial epithelium and breach the barrier of basal lamina to embed the blastocyst into the stroma of the endometrium. In addition, STBs perform many different functions, including exchange of substrates, gases, and other factors between the maternal and fetal circulation and synthesis and secretion of protein and steroid hormones, growth factors, and other substances vital for regulation of maternal and fetal metabolism. With this, the process of early implantation events finishes by the end of the 2nd week of fertilization.[1] After this, CTBs undergo extensive proliferation to form a compact cell column and later differentiate into highly invasive form of extravillous trophoblasts (EVTs). These are produced either to anchor the chorionic villi into the Nitabuch layer or to profoundly infiltrate the endometrial decidua. Invasive trophoblast cells ultimately reach to the

*Correspondence to: Satish Kumar Gupta; Email: skgupta@nii.ac.in
Submitted: 05/02/2013; Accepted: 05/23/2013
Citation: Suman P, Malhotra SS, Gupta SK. LIF-STAT signaling and trophoblast biology. JAK-STAT 2013; 2:e25155; http://dx.doi.org/10.4161/jkst.25155

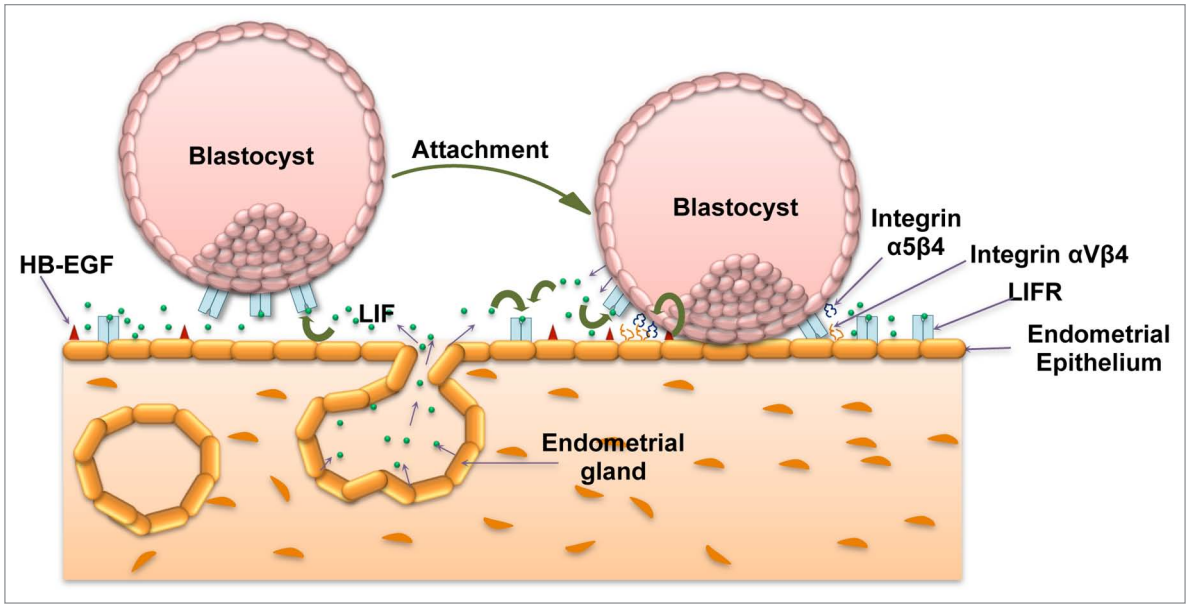

Figure 1. Significance of LIF-mediated signaling in blastocyst attachment. LIF is expressed by the receptive endometrial luminal and glandular epithelium. At the same time LIFR is expressed by the endometrial luminal epithelium as well as by the blastocyst. At the time of implantation, endometrial epithelial cells express integrin αVβ3 as well as osteopontin (not shown in figure) that forms the part of pinopodes essential for the initiation of implantation. A juxtacrine signaling through HB-EGF expressed on the endometrial epithelium leads to the expression of integrin α5β3 by trophoblast cells. These changes collectively bring out attachment of the blastocyst to the endometrium. Once the blastocyst gets attached, it also starts expressing LIF that can act in autocrine or paracrine way on trophoblast and endometrial cells, respectively.

maternal spiral arteries and replace the existing endothelial layer by forming the endovascular trophoblasts.[5]

LIF, interleukin (IL)-6, IL-11, HGF, IGF, IL-1, IL-15, IL-8, EGF, cytokines of TGF-β super family, IFN-α, IFN-γ, etc. are the major cytokines and growth factors present during the peri-implantation period and play vital role in the accomplishment of successful pregnancy through influencing the trophoblast function.[6-9] Apart from these, studies have also been performed which suggest that for attainment of successful implantation, activation of signaling pathways like JAK-STAT, MAPK, Notch, Smad, PI3K, etc. plays a crucial role.[1,5-7,9-12] In this review, we will focus on the LIF-mediated activation of the STAT signaling pathway in the regulation of blastocyst attachment followed by trophoblast proliferation, invasion, and differentiation during the course of implantation.

Leukemia Inhibitory Factor

LIF is a pleiotropic cytokine of IL-6 family that is considered as one of the cytokines essential for the successful completion of human pregnancy.[13,14] It was initially identified as a cytokine having the ability to inhibit proliferation of mouse myeloid leukemic cells and induce their differentiation into macrophages.[15] However, in humans, it is also produced by several other cell types like endometrial cells, fibroblasts, hepatocytes, osteoblasts, monocytes, macrophages, T cells, etc. to regulate varying degree of functions.[16-18] It is also expressed by granulosa-lutein and ovarian stroma cells.[19] A higher concentration of LIF in follicular fluid correlates with the embryo quality suggesting an important role of LIF in the physiology of ovulation and early embryo development. In humans, LIF controls the uterine receptivity for blastocyst implantation, trophoblast behavior by promoting proliferation, invasion, and differentiation.[11,20] In the endometrium, both glandular and luminal epithelial cells express LIF with a higher expression by glandular epithelium, which peaks during the secretory/postovulatory phase of the menstrual cycle (**Fig. 1**).[20-22] In contrast to LIF, expression of LIFR is higher in the endometrial epithelial cells as compared with glandular epithelial cells. After blastocyst attachment to the endometrium, trophoblasts also start expressing LIF that might influence their physiological functions in an autocrine way.[20,23-25] Both villous and extravillous trophoblasts express LIF and its receptor throughout pregnancy.[26]

LIF and Its Influence on Pregnancy

First observation about the critical involvement of LIF in embryo implantation came through experimentation in LIF knockout mouse. LIF-deficient female mice showed inability to attach the implanting blastocyst and it was fascinating to note that infusion of LIF into the uterus allowed the blastocyst to attach and grow until the full term.[27] However, mice knocked out for LIF receptor (LIFR) had normal implantation but newborns died within 24 h of birth due to impaired placental function.[28] Disruption of gp130, the STAT3-activating subunit shared by all members of the IL-6 receptor family, leads to an identical phenotype as knocking out of LIF.[29] This suggests that for the initial phase of implantation, LIF might be influencing the trophoblast function. Clinically, it has been observed that the endometrial cells obtained from several cases of infertility have a diminished

expression of LIF and these are represented with repeated abortions or unexplained infertility.[30,31] Not only the expression of LIF but, mutations in the LIF gene expression have been associated with unexplained infertility in woman. In a case study, analysis of the mutations in the coding region and critical regulatory regions of the LIF gene has revealed that there were increasing number of heterozygous point mutations in close proximity to the start codon of exon 1 and in exon 3.[32] These mutations could also be the reason for the poor outcome of fertility following IVF than the control group of woman.[33] These regions are functionally important for the biological activity of LIF. Thus, heterozygosity for a LIF gene mutation could contribute to rising level of functionally inactive LIF in the uterus leading to implantation failure. But, mutation in the LIF gene is not the only reason for the unexplained infertility or recurrent implantation failure.[34]

LIF-STAT Signaling

LIF, like other members of IL-6 family of cytokines transduces its signal through formation of heterodimer with specific LIFR and the common co-receptor for IL-6 family (gp130).[13] Binding of the LIF to its receptor leads to activation of both STAT and RAS/MAPK signaling cascade in trophoblasts.[35,36] In this section, of all the signaling pathways getting activated in trophoblast cells, STAT-dependent downstream signaling pathways will be discussed in detail.

The JAK-STAT pathway was first defined as the signal transduction pathway downstream of cytokine receptors. Later, it was demonstrated that this pathway is responsible for the control of several biological responses, including cell growth, differentiation, longevity, and migration.[37] STATs were discovered as molecules associated with interferon-γ-mediated signaling and gene expression with DNA-binding ability.[38] STAT proteins are made up of about 750 to 848 amino acids (90–155 kDa). Out of the six STAT families of proteins, STAT1, STAT3, and STAT5 also generate their splice variants.[39] All six STAT proteins are encoded by separate genes. All STAT proteins have a typical six domain structure, namely an oligomerization domain, a coiled-coil domain, a DNA-binding domain that determines the DNA-binding specificity, a linker domain, a SH2 domain that allows receptor binding and dimerization, and a transcription activation domain that contains a conserved serine residue (except in STAT2 and STAT6). In addition, all STAT proteins possess a critical tyrosine near the SH2 domain, approximately at amino acid position 700 (at position 705 in STAT3). Phosphorylation of this tyrosine is essential for dimerization, nuclear translocation, and DNA binding of STAT proteins. Apart from tyr phosphorylation, STAT1, STAT3, and STAT5 proteins also get phosphorylated at a C-terminal serine (for example, ser727 in STAT3) that is required for maximal transcriptional activity. Truncated isoforms of STAT proteins that lack the C-terminal transcription activation domain may act as dominant-negative isoforms and regulate the STATs biological activity.

STATs are present in latent form in the cytoplasm until the time they get activated by extracellular ligands like cytokines, growth factors, and hormones. Each of them is differentially activated by specific extracellular ligands, allowing differential intracellular processing of signals transduced across the plasma membrane. They regulate distinct biological functions in normal human physiology and development but also regulate oncogenic signaling in many different tumors.[40] STAT3 and STAT5 are the STAT proteins that have been mostly implicated in the progression of cancer. In cancer cells, activation of STAT3 and STAT5 leads to increased expression of downstream target genes, which increases proliferation, survival, angiogenesis, and immune evasion.[41] STAT target genes that regulate cell survival and proliferation include the B-cell lymphoma 2 (Bcl-2) family members, survivin, cyclin D1, and myc. STAT3 activation also promotes the cellular invasion by activating the transcription of MMP1, MMP2, MMP9, and MMP10.[42,43] In certain circumstances, STATs can be activated independent of JAKs by other non-receptor tyrosine kinases.[44] This kind of activation is mostly linked with the downstream signaling activation through growth factor receptors. For example, STAT1, STAT3, and STAT5 are activated directly through EGFR while PDGFR can directly activate STAT5.[45-48]

Most of the information about the functional role of STAT proteins in the regulation of biological function comes from the studies conducted on knockout mouse for a specific STAT protein.[49] Among these, only *STAT3* knockout mouse showed remarkable loss of fertility due to embryonic lethality in early gestation. *STAT3* knockout embryos degenerate and die in the early post-implantation period on E7.5 but, can be rescued through substitution with an alternative splice form of STAT3, STAT3β, in which the C-terminal transactivation domain is replaced with a seven amino acid extension.[49-51] Furthermore, the inhibition of STAT3 activation in the mouse endometrium also prevents the embryo implantation.[52] This invites the hypothesis that LIF-STAT signaling might have a critical involvement in the process of implantation, possibly acting as a critical modulator of trophoblast invasion.[27,28,49,52]

STAT3 gets activated through phosphorylation at tyrosine residue 705 as well at serine residue 727 in response to external ligands.[53] Tyrosine(705) phosphorylation facilitates STAT3 dimerization and translocation to the nucleus, where they bind to the specific DNA response elements and enable gene transcription.[53,54] A phosphorylation event at serine residue 727 also modulates the transcriptional activity of STAT3, and is required for maximal transcriptional activity.[53,55] In J774.2 macrophages, leptin-induced ERK activation corresponded with an increase in both phosphorylation of ser727 and STAT3 DNA binding activity.[56] However, there is also a notion that STAT3(ser727) phosphorylation has no bearing on their DNA binding or transcriptional activity.[57] Phosphorylation of STAT3(ser727) has been linked with the activation of MAPK family members, whose activation are mainly dependent in the cellular context and the stimulus used.[58-62] Although, it is undetermined whether serine phosphorylation is dependent on tyrosine phosphorylation, phosphorylation at ser727 of STAT3 may be essential for STAT3 activity.[53,55,63] For example, serine phosphorylation of STAT3 is essential for post-natal survival and growth, since knock-in of STAT3SA cDNA, which replaces serine residue 727

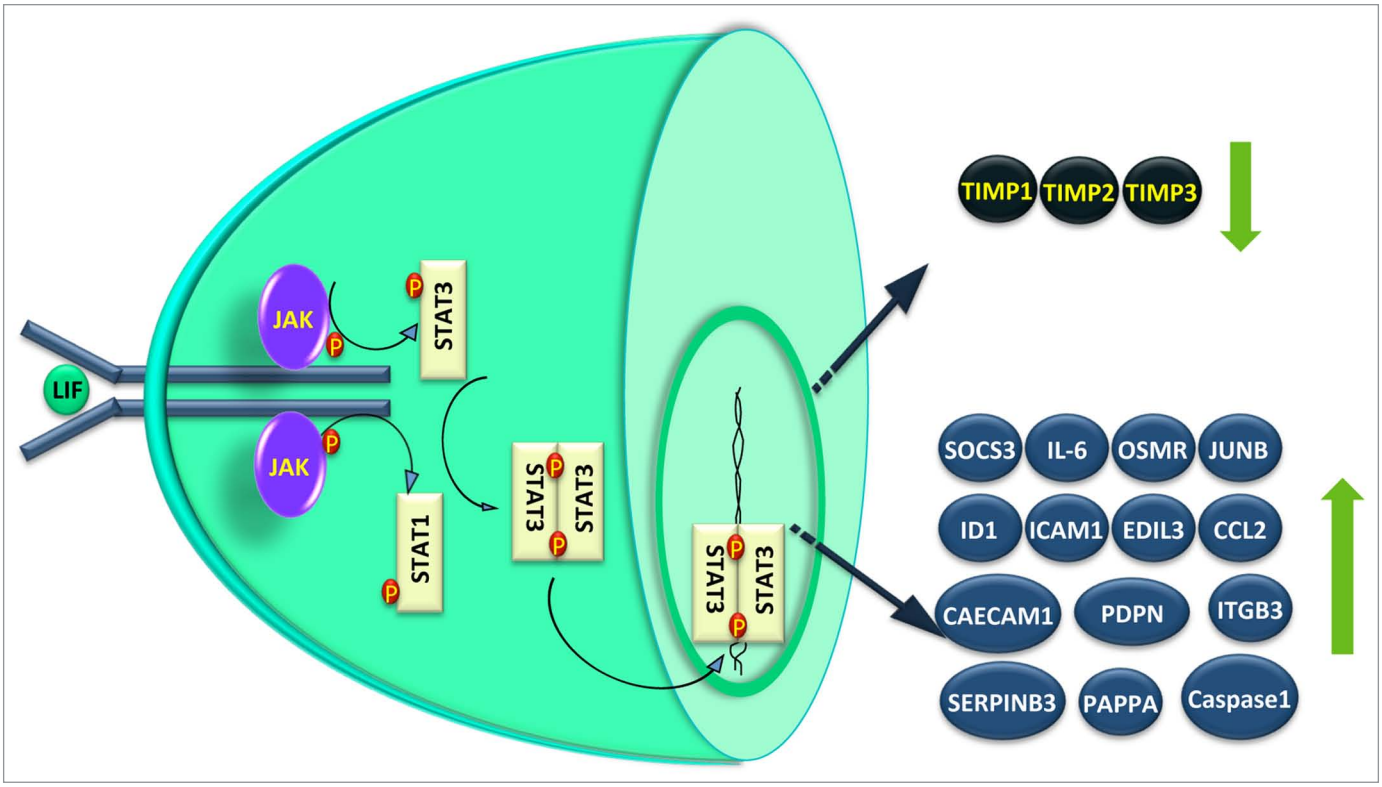

Figure 2. STAT-dependent signaling and gene expression in LIF treated trophoblastic cells. LIF upon binding to the gp130-LIF receptor complex present on the plasma membrane of trophoblastic cells activate JAKs that ultimately phosphorylate the STAT3 and or STAT1 in the cytoplasm. These activated STATs form the homo- or hetero-dimers and move inside the nucleus to influence the expression of various genes that could regulate different functions like cytokine and signaling (IL-6, OSMR, SOCS3, and JUNB), adhesion (CECAM1, PDPN, and ITGB3), invasion (PAPPA, Caspase1, SERPINB3, TIMP1, TIMP2, and TIMP3), angiogenesis (ID1, ICAM1, EDIL3, and CCL2), etc. The genes whose expression is downregulated following LIF treatment are shown with a down arrow, while those showing upregulation are depicted with an up arrow.

with an alanine, into STAT3 knockout mice fails to compensate the phenotype.[64] In addition, STAT3β, a truncated form of STAT3 without the serine residue 727-containing C-terminus, works as a negative regulator of STAT3-mediated activity in breast cancer cells.[39,65,66] Phosphorylation of STAT3 at ser727 is associated with the malignant phenotype of several cancers including breast cancer.[67] In human first trimester placentae, pSTAT3(ser727) protein is detectable in both cytoplasm and nuclei of CTB, STB, and vCTB while this expression profile disappears in the term placenta.[68] Placental trophoblastic cancers also show higher nuclear pSTAT3(ser727) localization than their normal trophoblast counterpart. In trophoblastic cells, activation of STAT3 by serine phosphorylation is mainly mediated via mammalian target of rapamycin (mTOR).[62] STAT3 expression or activation profile is constitutively higher in several malignancies, including those pertaining to the reproductive system.[37,69,70] Choriocarcinoma cells also have higher STAT3 DNA binding activity which correlated with their malignant phenotype.[68] In trophoblasts, upon LIF treatment, cytoplasmic STATs get phosphorylated by activated JAKs (tyrosine kinase) through phosphorylation at STAT3(tyr705) or STAT1(tyr727) (**Fig. 2**). Extent of phosphorylation of STAT1 has always been lower as compared with STAT3 upon LIF stimulation. In JEG-3 choriocarcinoma cells, STAT3 also gets phosphorylated at ser727 by activated ERK1/2 as inhibition of ERK1/2 activation following LIF treatment abrogated the LIF-mediated STAT3(ser727) phosphorylation (unpublished observation). Activated STATs form homo-/hetero-dimers through binding of the phosphotyrosine of one STAT molecule to the SH2 domain of its partner (**Fig. 2**). Upon dimerization, the STATs are translocated to the nucleus, where they act as transcription factors. One of the transcribed proteins is the SOCS3 that can negatively modulate the duration of the cytokine signaling response by binding to phosphotyrosine residues on JAKs (**Fig. 2**).[71-74] LIF suppresses its own effects by means of negative feedback regulation of the JAK-STAT pathway through SOCS3.[73,75] In trophoblast cells, STAT3-dependent expression of SOCS3 is essential for the negative regulation of trophoblast giant cell differentiation.[76] We have observed a significant increase in the expression of SOCS3 in JEG-3 choriocarcinoma as well as in HTR-8/SVneo trophoblastic cells treated with LIF (unpublished observations).

Role of LIF in Blastocyst Attachment and Implantation

In humans, appearance of pinopodes (ectoplasmic protrusions from the endometrial epithelial cells) is considered as morphologic marker for the uterine receptivity.[77] It is present for a very

brief period of time and coincides with the window of implantation. Role of LIF in the initial phase of blastocyst attachment and implantation becomes more speculative with the fact that the peak of LIF and LIFR expression by uterine epithelium coincides with the appearance of pinopodes.[22] In addition to LIFR, pinopodes also have higher expression of molecules like osteopontin and integrin αVβ3 that help in embryo implantation (**Fig. 1**).[77-79] Establishment of a possible regulatory role of LIFR mediated signaling in the expression of osteopontin and integrin αVβ3 would help in understanding the LIF-STAT signaling in embryo implantation. Another way to confirm the significance of LIF in embryo implantation is to ablate the LIF-mediated signaling that can be achieved by neutralization of LIF present in the uterine lumen by using antibodies. Infusion of LIF antibodies in the uterine horn of pregnant mice led to reduced number of embryos implanted on day 8 of pregnancy.[80] Under in vitro conditions, treatment of blastocyst with LIF enhances the blastocyst outgrowth which also gets compromised upon addition of LIF antibodies. In rhesus monkeys, infusion of anti-LIF monoclonal antibodies also reduced the implantation rate and the pregnancy in experimental group as compared with controls.[81]

In mouse, LIF-dependent activation of downstream signaling in endometrial cells is essential for the blastocyst to establish attachment with the endometrium. Co-immunoprecipitation experiments for LIFR and gp130 at the time of blastocyst attachment showed formation of heterodimers that is required for the LIF-mediated downstream signaling.[82] In mouse luminal epithelial cells, LIF increases the activation of STAT3 through binding to LIFR and gp130 heterodimer.[13] The activation of STAT3 takes place by phosphorylation at tyr705 residue. Phosphorylated STAT3 undergo nuclear localization and binds specifically to the STAT3 consensus recognition sequence. It was interesting to note that LIF treatment to the purified luminal epithelial cells only activated STAT3 and did not increase the phosphorylation of ERK1/2 (which was activated to a higher extent even before LIF treatment). The authors reasoned that a higher level of activation of ERK1/2 even before treatment of LIF could be due to the presence of EGF that mainly act by activation of ERK1/2 and less through activation of STAT3. This suggests that activation of STAT3 and not the ERK1/2 is critical for the embryo to implant. In the luminal epithelium, STAT3 activation showed a peculiar temporal activation pattern as in spite of the presence of LIF after day 4 (day on which implantation occurs in mouse) there was no activation of STAT3. This suggests that for uterine receptivity and blastocyst attachment not only the presence of LIF but activation of STAT3 is also important.[13] However, the molecular basis behind the refractoriness of LIFR-mediated signaling in the luminal epithelial cells beyond the "window of implantation" are still speculative.

The human blastocyst expresses the LIFR at the time of implantation, the time when endometrial concentration of LIF reaches to the peak and this way trophoblasts might respond to the incoming stimuli from the endometrium (**Fig. 1**).[24] In addition to this, LIF-mediated autocrine or paracrine signaling in the endometrial cells might aid in preparing the endometrial cells to attach with the incoming blastocyst (**Fig. 1**).[20] Considering its significance in the regulation of early implantation, its usefulness has been shown in the IVF application. In IVF, it can be used as an in vitro supplement to the culture medium to enhance the quality of embryo at the stage of transfer into the uterus (United States Patent 5962321; Inventors: Gough, Nicholas Martin; Willson, Tracey Ann, Seamark, Robert Frederick [Beulah Park, AU], http://www.freepatentsonline.com/5962321.html).

Trophoblast Proliferation and Invasion: Regulation by LIF-STAT Signaling

After implantation, the trophoblast cells proliferate and breach the epithelial barrier to invade through the decidua thereby establishes close physical contact with the various cellular components of the maternal endometrium. A controlled proliferation, invasion, and self-renewal of trophoblast cells during this phase of development are important for successful establishment of pregnancy. There is extensive cross-talk between the trophoblast and the decidual cells to direct the process of proliferation and invasion. Several endometrium-derived molecules including LIF alter the proliferative and invasive potential of the trophoblasts in vitro.[83,84] LIF increases the proliferation and invasion of JEG-3 choriocarcinoma as well as trophoblastic HTR-8/SVneo cells under in vitro conditions.[7,35,36] Proliferation of different cell types is regulated by the activation of both STAT and ERK1/2 dependent signaling pathways. However, in trophoblasts, activation of ERK1/2 and not the STAT3 has been shown to regulate their proliferation upon treatment with LIF.[35] Silencing of STAT3 expression in trophoblastic cells did not alter the LIF-mediated increase in proliferation.

LIF increases the invasiveness of trophoblastic cells through activation of STAT3 as silencing of STAT3 expression in JEG-3 choriocarcinoma cells resulted in a dramatic reduction in their invasive potential regardless of LIF supplementation.[85] Recently, we have reported that in trophoblastic HTR-8/SVneo cells, LIF activates not only STAT3 but also STAT1 to a significant extent to increase their invasiveness across the Matrigel matrix.[36] This led to a dose dependent increase in their invasiveness through increase in the expression of several invasion-associated molecules. LIF increased the expression of novel regulatory molecules like pappalysin 1 or PAPPA, podoplanin, SERPINB3, ICAM1, ID1, and integrin β3 (which are also expressed by human placenta) and decreased the expression of TIMP1, TIMP2, and TIMP3 (**Fig. 2**). Silencing of pappalysin 1 expression by siRNA led to abrogation of LIF-mediated invasion of HTR-8/SVneo cells. Earlier, in JEG-3 choriocarcinoma cells, LIF-mediated increase in the invasiveness was shown to be associated with increase in the expression of caspase 1 and decrease in the expression of TIMP1 (**Fig. 2**).[7]

Syncytialization of Trophoblasts: Relevance of LIF-STAT Signaling

Syncytialization of trophoblastic cells is one of the essential attributes that holds the key for successful implantation of the embryo. Syncytial fusion enables the transfer of CTB-derived

nuclei and other organelles, proteins, and RNA as well as cytoplasm and membranes into the STB. Permanent acquisition of fresh cellular components, however, requires continuous disposal of aged cytosolic content to maintain the homeostasis of the STB. Thus, apoptotic material is packed into the syncytial knots at the apical plasma membrane of the STB, where these corpuscular structures are released as sealed membrane vesicles into the maternal circulation.[86] Restricted fusion, in contrast, may result in depletion of fresh cellular components within the STB, leading to exhaustion of the syncytial layer. Hence, trophoblast turnover has to be regulated within a tight range, avoiding excessive as well as restricted cytotrophoblast-syncytiotrophoblast fusion. Deregulated CTB to STB fusion may lead to preeclampsia, intrauterine growth retardation (IUGR) and implantation failure.[87,88]

LIF has been shown to regulate differentiation of trophoblast like BeWo (choriocarcinoma) cells through activation of JAK-STAT and MAPK3/1 signaling pathways. It shows a synergistic effect on forskolin-induced BeWo cell fusion.[89] It activates other signaling pathways, such as MAPK and phosphatidylinositol-3-kinase (PIK3)/AKT.[90] MAPK3/1 modulates both fusion and hCG secretion in primary STBs and BeWo cells.[91,92] Based on the fact that LIF enhances MAPK3/1 pathway activation, it is likely that the effect of LIF on cell fusion relies on a cooperative cross-talk between LIF-induced activation of MAPK3/1 and forskolin-induced activation of PKA signaling pathways. A similar converging mechanism could be proposed for the JAK-STAT pathway, where it can be speculated that homodimers and/or heterodimers of STAT1 and STAT3 directly or indirectly act as co-activators of fusion-related gene promoters, as the fusogenic capacity of BeWo cells is greatly affected by inhibiting the activation of both MAPK3/1 and JAK-STAT signaling pathways.[89]

SOCS is also an important regulator of the embryo implantation as genetic deletion of SOCS3 resulted in embryonic lethality due to placental insufficiency at around embryonic day (E)13 in mice.[93-95] In the SOCS3-null placenta, chorio-allantoic fusion occurred normally, but the labyrinthine and spongiotrophoblast layers of the mouse placenta were poorly formed, while trophoblast giant cells were increased in number and in size. To emphasize the fact that embryonic lethality associated with absence of SOCS3 is due to compromised placental differentiation, use of wild-type extraembryonic tissues, either in complementation via aggregation with tetraploid embryos (which contribute to extraembryonic tissues but not to embryo proper) or generation of chimeras composed of SOCS3-null embryos and wild-type trophoblast stem cells rescued the lethal phenotype.[95,96] Genetic crosses between mice heterozygous for deletion of SOCS3 and LIFRα (null mutants for each is lethal) revealed that the phenotype is due to dysregulation of signaling downstream of the LIFR and that the ligand responsible for this, LIF, is produced by embryonic tissues and acts in a paracrine fashion.[28,95] In human placenta SOCS1, SOCS2, and SOCS3 mRNA and protein are detectable in pre-term and term villous placenta and immunohistochemical analysis localized all three SOCS proteins to all decidual cells.[97,98] Interestingly, decreased SOCS3 expression has also been observed in the villous tissue of placentae obtained from women with preeclampsia.[99] These studies suggest that SOCS3 expression might have implication for the trophoblast invasion and their deficiency might lead to shallow invasion of the deciduas.[100] Presence of an excess of proliferative immature trophoblast in preeclampsia is indicative of lack of invasive differentiation of trophoblast cells and SOCS3 could be one of the regulator for this kind of differentiation.

Conclusion

Present understanding suggests that LIF is one of the factors behind most of the physiological changes in trophoblasts during the course of embryo implantation. Its influence ranges from embryo adhesion to the regulation of trophoblast proliferation, invasion and syncytialization. Presence of LIF in the in vitro culture medium improves the quality of the implanting blastocyst. LIF through activation of JAK-STAT signaling pathway bring out the above mentioned physiological changes. To better understand the STAT mediated invasion and differentiation of trophoblasts, substantial research efforts should be directed toward understanding the regulation of STAT responsive gene expression and their physiological relevance during these processes.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Acknowledgments

The authors are thankful to the Indian Council of Medical Research (ICMR), New Delhi, India and National Institute of Immunology, New Delhi for financial support. SS Malhotra is recipient of the Junior Research Fellowship of the Council of Scientific and Industrial Research (CSIR), Government of India.

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