LIF-Dependent Signaling: New Pieces in the Lego

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Abstract LIF, a member of the IL6 family of cytokine, displays pleiotropic effects on various cell types and organs. Its critical role in stem cell models (e.g.: murine ES, human mesenchymal cells) and its essential non redundant function during the implantation process of embryos, in eutherian mammals, put this cytokine at the core of many studies aiming to understand its mechanisms of action, which could benefit to medical applications. In addition, its conservation upon evolution raised the challenging question concerning the function of LIF in species in which there is no implantation. We present the recent knowledge about the established and potential functions of LIF in different stem cell models, (embryonic, hematopoietic, mesenchymal, muscle, neural stem cells and iPSC). We will also discuss EVO-DEVO aspects of this multifaceted cytokine.

Keywords LIF cytokine · IL6 · Pleiotropy · Stem cells · Signaling · Chromatin · miRNA · EVO-DEVO · Reprogrammation · p53

Abbreviations

AK7 · Adenylate Kinase 7
ALDH · AcetaLdehyde DeHydrogenase
API · Activator Protein 1
BFU-E · Burst Forming Unit Erythroid
BMP · Bone Morphogenetic Protein
cDNA · complementary DeoxyriboNucleic Acid
Ceacam1 · Carcinoembryonic antigen-related cell adhesion molecule 1
CFU-Blast · Colony-Forming Units Blast
CFU-E · Colony-Forming Units Erythroid
CFU-Eo · Colony-Forming Units Eosinophil
CFU-GM · Colony-Forming Units Granulocyte Macrophage
CFU-Mix · Colony-Forming Units Mix
Chd1 · Chromodomain-helicase-DNA-binding protein 1
CHIP-seq · Chromatin ImmunoPrecipitation followed by sequencing
CIS · Cytokine-Inducible SH2
CNTF · Ciliary NeuroTrophic Factor
CLC · Cardiotrophin-Like Cytokine
CT1 · CardioTrophin-1
Cobra1 · cofactor of BRCA1 (breast cancer 1)
CrxOs · Crx Opposite strand
Dapp1 · Dual adaptor for PY and PI3K
DG · Dentate Gyrus
| Abbreviation | Full Name |
|--------------|-----------|
| EED          | Polycomb protein Embryonic Ectoderm Development |
| EGF          | Epidermal Growth Factor |
| EpiSC        | epiblast stem cells |
| ERK          | Extracellular signal-Regulated Kinases |
| ES           | Embryonic Stem |
| ESRRB        | EStrogen-Related Receptor beta |
| FGFR         | Fibroblast Growth Factor Receptor |
| Gja1         | Gap junction membrane channel protein alpha 1 |
| Gjb3         | Gap junction membrane channel protein beta 3 |
| GM-CSF       | Granulocyte Macrophage Colony Stimulating Factor |
| GP           | GlycoProtein |
| GSK3b        | Glycogen Synthase Kinase 3 beta |
| hES          | human Embryonic Stem |
| HSC          | Hematopoietic Stem Cell |
| ICM          | Inner Cell Mass |
| IL6/11/27    | InterLeukin 6, 11, 27 |
| IL6ST        | InterLeukin 6 Signal Transducer |
| iPSC         | induced Pluripotent Stem Cells |
| IRAK 3       | Interleukin 1 Receptor-Associated Kinase 3 |
| JAK          | Janus Kinase |
| JNK          | Stress-Jun-activated protein Kinase |
| JunB         | Oncogene JUN-B |
| KD           | Knock Down |
| kDa          | kilo Dalton |
| KLF4/5       | Kruppel-Like Factor 4/5 |
| KO           | Knock-Out |
| KRT42        | Keratin 42 |
| LIF          | Leukemia Inhibitory Factor |
| Lifind       | LIF-induced |
| LIFR         | LIF Receptor |
| Lin          | Lineage |
| L-Myc        | Avian myelocytomatosis viral oncogene homolog 1, lung carcinoma-derived |
| Ly6          | Ly6g6e lymphocyte antigen6 complex |
| MAPK         | Mitogen Activated Protein Kinase |
| MARCH7       | Membrane Associated Ring finger (C3HC4) 7 |
| MEK          | MAP Kinase Kinase |
| mES cells    | mouse Embryonic Stem cells |
| miRNA        | micro RNA |
| MRAs         | Muscle and Microspikes ras |
| MSC          | Mesenchymal Stem Cells |
| MW           | Molecular Weight |
| MYO D        | MYOgenic Differentiation Antigen |
| NANOG        | Homeobox transcription factor Nanog |
| NFKb         | Nuclear Factor Kappa-B |
| N-Myc        | v-myc avian myelocytomatosis viral-related oncogene, neuroblastoma-derived |
| NPC          | Neural Precursor Cells |
| NSC          | Neural Stem Cells |
| OCT4         | OCTamer 3/4 |
| OSM          | Oncostatin M |
| Pem          | Placenta and embryonic expression protein |
| PI3K         | Phosphatidylinositol 3-Kinase |
| PIAS         | Protein Inhibitor of the Activated STAT |
| Pim3         | Proviral integration site 3 |
| Plei-         | Pleiotropic LIF-Induced |
| TGFbeta      | Transforming Growth Factor beta |
| TNFalpha     | Tumor Necrosis Factor alpha |
| RALDH        | RetinALdehyde Deshydrogenase |
| Rex1         | Reduced expression protein 1 (or Zfp-42, Zinc finger protein 42) |
| Sca1         | Stem Cell antigen 1 |
| SH           | Src Homology |
| SHP          | SH2-containing Phosphatase |
| Spe-Lifind   | Specific LIF-Induced |
| SOCS         | Suppressor Of Cytoking Signaling |
| SOX2         | Sex-determining region Y-box2 |
| STAT         | Signal Transducer and Activator of Transcription |
| Susd2        | Sushi domain containing 2 |
| Tcfcp211     | Transcription factor CP2-like 1 |
| TYK2         | Tyrosine Kinase 2 |
| WT           | Wild Type |
| Yap          | Yes associated protein |
| ZAP70        | Zeta-chain-Associated Protein kinase 70 |
| Zic3         | Zinc-chain protein of the cerebellum 3 |
| Zfx          | Zinc finger protein, X-linked |
| Zscan4       | Zinc finger and SCAN domain containing 4 |

**Generalities on LIF**

LIF cytokine is a glycosylated protein (MW of 37–62 kDa depending on its degree of glycosylation) secreted by extraembryonic part of the embryo at the egg cylinder stage as well as by many cell types in adult organs (e.g.: endometrial cells, fibroblasts, hepatocytes, osteoblasts, monocytes, macrophages, T cells) [1, 2]. Three laboratories simultaneously discovered and cloned the LIF cytokine through its pleiotropic biological activities on i) the proliferation of adult human T cells (HILDA; [3]), ii) the maintenance of ES cells pluripotency (DIF [4]) and iii) the inhibition of leukemic cell differentiation (LIF; [5]). LIF was thus characterized as a pleiotropic cytokine with pro or anti-differentiation, pro or anti-survival effects depending upon cell maturity and cell types [6, 7].

LIF belongs to the “helical type 1” Interleukin 6 family, which includes IL11, IL27, CNTF, CT1, CLC, and OSM [8–12]. These cytokines interact with homo- or heteromeric
receptors, all including the common gp130 subunit (IL6 signaling transducer, (IL6ST)) [13]. LIF receptor is composed of two subunits, gp130 and gp190 (LIF receptor beta). The gp130 common subunit is believed to explain the functional redundancies with several members of the IL-6 family [1]. The gp190 subunit is under ERK MAPK and axotrophin/March7 E3 ligase-dependent degradation pathways, respectively in liver cell models and in T cells [14, 15]. Glycosylated LIF can also hold mannose phosphate residues able to bind the Mannose 6 phosphate receptor to the core gp130/GP190 complex and allowing recycling of LIF ligand [16]. Recently, Sortilin, an intracellular sorting receptor, member of vacuolar protein sorting-10 (Vps10) domain-containing proteins, has been shown to facilitate the signaling of all helical type 1 cytokines which engage the gp130/LIFR beta complex [17].

Studies of LIF Knock-Out (KO) mice revealed that LIF is essential for the implantation process of blastocysts, for the maintenance of hematopoietic stem cell pools and for the not so well understood mechanisms leading to cachectic animals ([18–20] and reviewed in [7]). In addition, in LIF rescued KO mice model, it was shown that LIF is essential for mammary gland involution after lactation [21]. More recent studies performed with LIF KO mice challenged for injury responses, demonstrated the importance of LIF at various stages of neurogenesis and for tissue regeneration after brain or spinal cord injury [22–24]. Also, LIF is

**Table 1** Pleiotropic effects of LIF: in vitro models

| Model                  | Effects of LIF                                                                 | Ref       |
|------------------------|-------------------------------------------------------------------------------|-----------|
| Reproduction/fertility | Healthy women volunteers and 8-week-old female mice.                         | LIF induces oocytes and graafian follicle expansion. | [176]     |
|                        | In vitro studies from germ and testis cell culture.                          | LIF increases male germ cell maturation during spermatogenesis. | [177]     |
|                        | Primary human endometrial epithelial cells and human endometrial epithelial cell line. | LIF and IL11 synergize to regulate cell adhesion of endometrial epithelial cells with blastocyst during implantation process. | [178]     |
| Hormone regulation     | NCI-H295R adrenocortical cells                                               | LIF stimulates adrenal steroidogenesis.                     | [179]     |
| Hematopoiesis          | Bone marrow from 2 month old C3H/HeJ mice.                                  | LIF and multi-CSF synergize to stimulate murine megakaryocyte production in vitro. | [180]     |
|                        | Human mesenchymal stem cells, Treg cells.                                   | LIF is involved in transplantation tolerance.               | [15, 181] |
| Muscle                 | C2C12 myoblast cell line                                                    | LIF induces the proliferation of myoblasts.                | [182]     |
|                        | Model of JNK1−/− mice                                                        | There is a link between TNFalpha/LIF/JNK1 in myoblast proliferation/differentiation. | [183]     |
| Vessels                | IEM, murine endothelial cell line.                                           | LIF, in synergy with FGF, regulates signals controlling capillary outgrowth. | [184]     |
| Neurons                | Neural crest cultures from CBA mouse embryos at stage E9.                   | LIF stimulates the generation of sensory neurons in culture of mouse neural crest. | [185]     |
|                        | Human midbrain precursor cells.                                              | LIF favours neurogenic differentiation of long-term propagated human midbrain precursor cells. | [186]     |
| Eyes                   | Human retinal cells.                                                         | LIF allows CD133+ adult retinal cells to remain undifferentiated. | [187]     |
| Ears                   | Cort1 cells.                                                                | LIF is involved in auditory cell proliferation.             | [188]     |
| Kidneys                | Culture of rat metanephric mesenchyme.                                     | LIF converts kidney mesenchyme to epithelia which then form nephrons. | [189]     |
| Liver                  | Human hepatoma cell lines (HepG2).                                          | LIF, with oncostatin M, induces hepcidin production in hepatoma cell lines. Hepcidin is considered to be the main factor responsible for the development of anemia in inflammatory conditions. | [190]     |
| LIF in cancer          | Cell lines derived from thyroid tumor specimen in human patients.           | LIF inhibits (in non-tumorigenic cells) or induces (in tumorigenic cells) cell survival. | [191]     |
|                        | Normal and tumoral mammary derived cell lines.                              | LIF is involved in rhabdomysarcoma-derived metastasis.     | [192]     |
|                        | ARMS and ERMS rhabdomyosarcoma cell lines.                                  | LIF is involved in melanoma tumor growth.                  | [193]     |
|                        | In vivo and in vitro studies.                                                | LIF has a potential role in melanoma-induced bone metastasis. | [194]     |
|                        | In vivo and in vitro studies; BalbC/mice; SEK1 cells; 8 human melanoma-derived cell lines. | LIF and TGFbeta induce self renewal of glioma-initiating cells, promoting oncogenesis in vivo. | [195]     |
|                        | In vivo and in vitro studies; human glioma cell lines: U373MG and A172 and human cell cultures. | LIF inhibits (in non-tumorigenic cells) or induces (in tumorigenic cells) cell survival. | [196]     |
potentially involved in particular contexts of muscle stimulation and regeneration [25–27] and analysis of LIF KO newborn mice revealed a 40% decrease in bone volume [28]. Double and triple KO model mice with other members of the IL6 family, as CT1 and CNTF, revealed also the importance of LIF and CNTF for motor neuron functions [29, 30]. The therapeutic potential of LIF in neurodegenerative and autoimmune diseases and in reproduction failure treatments has recently been reviewed stressing the importance to dissect LIF outcomes in the different cell contexts [31, 32]. Exemples of LIF functions in vitro and in muso are presented in Tables 1 and 2.

Recent studies have also demonstrated that LIF expression is under the control of the p53 pathway for the implantation process [33–35]. This recent finding of a crosstalk between p53 (the so called “guardian of the genome” also recently involved in somatic cell reprogrammation) and LIF opens new perspectives for LIF studies in relation with the resetting of the pluripotent program, from committed or mature differentiated cells [36–41].

### LIF Signaling and Pleiotropy: As a Lego

We propose to view LIF signaling as a Lego built with different combinations of similar pieces, leading to various outcomes, which range from cell proliferation and survival to differentiation and apoptosis, depending on maturity and cell types [1, 2, 7, 42]. This Lego includes the “ptyr signaling toolkit” described recently by Lim and Pawson [43]. Indeed, major pieces, always present in the core of the Lego, are kinases (as JAKs, SRC members, ZAP70 cytosolic tyrosine kinases and MAPK family members), activated transcription factors (STAT, AP1 (e.g.:JunB/cfos), NFKb, MYC family members) and feedback loop components like SHP1 and SHP2 phosphatases, PIAS (Protein Inhibitor of the Activated STAT) family of proteins and SOCS (Suppressor of cytoking Signaling). In both mouse and human genomes, there are four JAKs (JAK1, 2, 3 TYK2), nine SRC [44], two ERK/MAPK (ERK1 and ERK2), eight STATs, two SHPs (i.e., SHP-1 and SHP-2), eight SOCS (SOCS1 to SOCS7 and CIS) and four PIAS (PIAS1, -3, -x, and -y) proteins [45]. Many combinations of these proteins will result in pleiotropic effects of this cytokine. For example, the LIF/JAK1/ERK1/2/P13K/STAT3/JunB-cfos/KLF4/5/SOCS3 combination leads to maintenance of the pluripotency in the murine ES cell model, with ERK signaling pushing towards differentiation while STAT3 and its targets allow cells to remain undifferentiated by repressing endoderm and mesoderm differentiation programs [46–48]. Epigenetic marks, not yet studied in details, represent an additional level of sophistication.

#### Table 2 Pleiotropic effects of LIF revealed by LIF−/− KO mice model analysis

| Effects of LIF                                      | Ref   |
|----------------------------------------------------|-------|
| Implantation                                       | LIF is required for implantation of blastocysts. [19, 20] |
| Hematopoiesis                                      | LIF is involved in the maintenance of HSC pools. [20] |
|                                                    | LIF is secreted by mesenchymal stromal cells to stimulate survival and proliferation of hematopoietic stem cells. [135] |
|                                                    | LIF regulates the differentiation potential of MSC. [59] |
| Muscles                                            | LIF contributes to regenerate muscle. [197] |
|                                                    | LIF is critical for the development of skeletal muscle hypertrophy in the functional overload model. [143] |
| Vessels                                            | LIF modulates oxygen-dependant VEGF expression and is essential for ensuring proper capillary density. [198] |
| Bones                                              | LIF regulates osteoclast size. [199] |
| Neurons                                            | LIF is a key regulator of neural injury. [200] |
|                                                    | LIF is involved in glia phenotypes. [201] |
|                                                    | LIF prevents oligodendrocytes destruction and improve remyelination of neurons in mice suffering from multiple sclerosis. [202] |
|                                                    | LIF is required for normal development of hippocampal astrocytes, a process regulated by spontaneous neural impulse activity through the release of ATP. [203] |
|                                                    | LIF is required for correct myelination for a short time window, during postnatal mouse optic nerve development. [152] |
|                                                    | LIF may activate an endogenous rescue pathway that protects viable photoreceptor cells, leading to an increased photoreceptor survival in stressed retina. [204] |
|                                                    | LIF signalling pathway is required for the initiation of the astrogliosis-like reaction of retinal Müller cells after optic nerve injury. [205] |
|                                                    | LIF is necessary for injury-induced neurogenesis. [206] |
| Inflammation                                       | LIF is a major anti-inflammatory molecule produced in the CFA model (injection of complete Freund’s adjuvant which induces cutaneous inflammation). It is a key regulator of the cytokine cascade. [207] |
| Hormonal functions                                 | LIF regulates the production of pituitary ACTH and inhibits the production of prolactin and growth hormone. [208] |
required to understand the mechanisms of LIF pleiotropy, in cell models in which LIF mediates opposite effects. Eed protein, a LIF-dependent STAT3 target of the repressive Polycomb complex, has been shown to silence differentiation-associated genes in self-renewing mES cells [49]. We have proposed that the level of expression and/or of activation of chromatin regulators could end up to opened or closed chromatin configuration, leading to accessibility (or not) of STAT3-dependent promoters, helping to explain opposite outcomes of LIF/STAT3 pathway [7]. It might be informative to perform parallel Chromatin Immunoprecipitation (ChIP)-seq analyses with anti-phosphoSTAT3 antibody in mES and in the M1 cell line (a leukemic myeloid cell line in which LIF triggers differentiation) to characterize LIF/STAT3—dependent promoters in two cell models in which LIF drives opposite effects [50-54]. The importance of feedback loop control of LIF signaling, almost always including SOCS3, has recently been illustrated in mice engineered to express mutated forms of gp130 lacking the SOCS3-binding site. In those mice, which develop a variety of hematopoietic and immunological defects, STAT signaling is sustained highlighting the critical role of SOCS3 in limiting gp130 signaling [55]. In addition, differential kinetic of inactivation and desensitization of LIF-dependent pathways, which could be mediated by different inhibitory signaling components, as shown for the IL6 cytokine [56], could account for its various cell-dependent effects. The characterization of combinatorial LIF-dependent activated/repressed components (including proteins and also miRNAs, as demonstrated in mES and human mesenchymal cells [57-59]) and the set up of tools allowing to understand the mechanisms of action of these proteins and/or miR complexes on cell physiology, is a future challenge in cytokine and stem cell fields.

**LIF in Stem Cells**

LIF in mES Cells: Highlights on New LIF Targets and of Connections with the Trio OCT4/NANOG/SOX2

Murine ES cell model, strictly depending on LIF for self renewal and maintenance of pluripotency, is a powerful model to study its effects on cells grown at different stages of maturity (from pluripotent to early differentiated cells) and to unravel the mechanisms of pluripotency and pleiotropy (Fig. 1). LIF, in synergy with BMP4 or Wnts protein members (Wnt3a and Wnt5, [60, 61]) induces PI3K and ERK signals which are contradictory signals leading

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**Fig. 1** The mES system: the «three in one» cell model to study LIF mechanisms in pluripotent, committed and differentiated cells. Part of this figure has been taken from the Web site: http://en.wikipedia.org/wiki/Stem_cell, available under the «Creative Commons Attribution-ShareAlike 2.5 Generic License ». This model is based on references: [105, 112–114]
respectively to maintenance of pluripotency (through NANOG) or to differentiation [46, 47, 62–66]. ES cells are poised, able to respond shortly and efficiently to differentiation signals. However, a complex pluripotent program locks cells in self renewal and undifferentiated state, at least in cell culture. Years of studies of the mES cell model led to the characterization of master pieces of pluripotent program. This includes, along with the LIF signaling pathway, which represses expression of endoderm and mesoderm markers, the OCT4/SOX2 targets genes, which block differentiation towards trophectoderm lineage. Knowledge of these complementary and intricate pathways led also to the set up of defined medium complemented with specific chemicals which mimic the effects of essential components of stemness, as the 3i medium (a basic serum free medium supplemented with three chemical inhibitors, repressing MEKs, GSK3b and FGFR pathways) or the “pluripotin/LIF” medium [64, 67–69]. Recently retinol, the alcohol form of Vitamin A, which is not metabolized in retinoic acid (RA) in ES cells, because of the absence of RALDH and ALDH enzymes, has been shown as a new powerful inducer of NANOG allowing the absence of RALDH and ALDH enzymes, has been shown as a new powerful inducer of NANOG allowing maintenance of ES cell pluripotency in the absence of LIF [70, 71]. In addition, it has been demonstrated that mES cells express some markers in a salt/pepper way, as shown in Inner Cell Mass (ICM) of early blastocysts [72]. Indeed, genes as Rex1, Nanog, Zscan4 and many others have heterogeneous expression in morphologically homogeneous colonies of mES cells, probably allowing cells to respond quickly to differentiation signals [73, 74]. The importance of LIF-dependent components involved in the heterogeneity of mES cells is presently unknown.

**Genes Involved in Cell Pluripotency: Functional Involvement, One by One**

There are at least a hundred of genes individually involved in the maintenance of mES cell pluripotency. Indeed, Knock Down (KD) or Knock Out (KO) of candidate genes, proves to be a powerful way to demonstrate their role in ES cell pluripotency. Proteins in each cell compartment, from the membrane to the nucleus have been shown to be critical for maintenance of pluripotency as shown for Gap junction proteins [75–78], CrxOS [79], Yap [80], Pem [81], Zic3 [82, 83], Zfx [84], Pdc2d2 [85], Cobra1 [86] and many other genes revealed by high throughput RNAi strategies [87–89]. Also chromatin regulators (Jumonji members, [90] and Chd1, [91], which modulate respectively the level of histone methylation and the degree of chromatin compaction, and Ronin [92, 93], all able to regulate many genes together) and miRNAs (controlling cell cycle regulators via c-MYC [57, 94] or Master gene expression [95]) are also key players involved in the maintenance of mES cell pluripotency. The importance of miRNAs was established in previous studies showing that embryos or ES cells lacking proper miRNA synthesis (Dicer and Dgcr8 KO models) are not anymore pluripotent, stressing the importance of miRNA in ES cell plasticity [96–101]. It is worth stressing that some of these genes behave as “rheostat” with various level of their expression leading to different (sometimes opposite) cell phenotypes, as first shown for Oct4 and then for Nanog and Sox2 [102–104], illustrating the poised state of mES cells. However, in seeking for the “Holy Grail” of the pluripotency markers, we might attempt to dissociate genes whose repression will lead to slow destruction of cells (which could go through a pseudo-differentiation stage before death, when touching Gap junctions, adhesion or cytoskeleton cell components, for example), to genes having specific effects on the pluripotent machinery, a not easy task.

**Novel LIF Signatures**

By extensive microarray studies performed in mES cells grown with or without LIF for 24 or 48 h and reinduced with LIF for 30 min, we have defined three types of LIF signatures: Pluri, Spe-Lifind and Pleio-Lifind [105]. Genes from both Pluri and Lifind clusters are essential for the proper self renewal and maintenance of pluripotency in mES cells.

*Pluri* genes, whose expression is restricted to undifferentiated mES cells, at least up to 10 days of differentiation triggered by LIF withdrawal, includes Esrrb, Gjb3, Krt42, Ak7, Ly6, Susd2, Irak3, Tcfcp211, Pim3, Ceacam1 and Mras. This extends the list of known stemness genes [89, 106, 107]. By analyzing the expression profiles of the Pluri genes in Microarray data obtained with the KD of Oct4 or Nanog [108] we concluded that while the expression of Esrrb and Ly6 is repressed in OCT4 or NANOG KD cell lines, expression of Susd2, Ak7, Krt42 and Irak3 is only under OCT4 control. In addition, expression of gap junction protein encoding genes is induced in OCT4 (Gjb3/Connexin 31 and Gjb5/connexin 31.1) or NANOG (Gja1/connexin43) Knock Down cell lines. Individual KD of Irak3, Susd2 and Ly6 leads to weak increase of differentiation markers suggesting their involvement in mES cell pluripotency. Efficient approaches allowing to disrupt the expression of many genes at once should be necessary for further characterization of gene clusters in mES cells [105].

In cells grown without LIF for 24 or 48 h and reinduced with LIF for 30 min, we have identified the Lifind (Lif induced) genes. Some of them are direct targets of STAT3 and a subset of these genes is also regulated by the PI3K/ NANO pathway [48, 109, 110]. Only part of the genes induced after 24 h of LIF withdrawal is also induced after a longer period of starvation. We have made a distinction between Spe-Lifind genes (only induced in restricted time windows of LIF withdrawal, 24 h) and Pleio-Lifind genes.
Klf4 synergizes with OCT4, definitively regulating the expression of NANOG by direct binding to its between KLF4 and NANOG has been suggested since KLF4 under the control of NANOG expression and a regulatory loop between KLF4 and NANOG has been suggested since KLF4 regulates the expression of NANOG by direct binding to its promoter [120]. Klf4 synergizes with OCT4, definitively connecting master genes (OCT4/SOX2/NANOG) with LIF/STAT3 pathway [48, 117, 120, 121]. Other Spe-Lifind genes, could be essential “resets” of pluripotent program during the reversible phase of commitment [7, 114].

LIF in Adult Stem Cells

Hematopoietic Stem Cells (HSC)

Several groups have described similar, different or even contradictory results about the in vitro effects of LIF on cultured normal adult hematopoietic stem cells and progenitors. In articles devoted to its functional effects on normal hematopoietic progenitors it was shown that LIF has a growth stimulating activity on human CFU-Eo and BFU-E [122] while it is ineffective on mouse CFU-GM [123]. LIF also induces the proliferation of very primitive multipotential progenitors (so called CFU-Blast) suggesting that this cytokine could stimulate the cell cycle entry of these mostly quiescent primitive progenitors [124]. Interestingly, LIF is active on CFU-Mix only in serum containing medium and its effect is mostly synergistic or additive to other multi-lineage cytokines (IL-3, IL-6 and GM-CSF) [125]. These data suggest that LIF mostly stimulates the in vitro proliferation of primitive hematopoietic progenitors. In vivo, LIF is active on hematopoietic progenitors and play a role in the regulation of the stem cell pool [20]. Permanently elevated levels of LIF in adult mice induced a polyvisceral and rapidly lethal pathology [126]. The most important hematological modifications are a reduction of bone marrow hematopoiesis related to myelosclerosis that contrasts with a splenomegaly due to an abnormal spleen hematopoiesis, increased levels of progenitors in the spleen and thrombocytosis [126, 127]. However the most informative results concerning the physiological role of LIF in the regulation of hematopoiesis were obtained from KO mouse models. LIF deficient mice are viable. However, when compared to wild type animals the pool of BFU-E and CFU-GM progenitors were reduced in bone marrow and even more in the spleen of LIF−/− mice [20]. More surprising is the fact that the null LIFR beta (gp190) spontaneous mutations in humans and induced by gene targeting in KO mice had no hematological abnormality despite the fact that other LIF target tissues were disturbed [128, 129]. Taken altogether, these in vitro and in vivo results suggest that LIF is not a mandatory cytokine for the physiological regulation of adult hematopoiesis. This is in agreement with its potential role on some basic functions such as effect on chromatin status, for example, for which a degree of redundancy is expected.

Mesenchymal Stem Cells (MSC)

Among their numerous biological functions, MSC display immune-modulatory properties. They suppress T cell proliferation induced by various stimuli in vitro and they show similar effect in vivo. Indeed several clinical trials evidenced that injection of MSC to Bone Marrow (BM) allografted patients reduced their graft versus host disease by improving the donor T cells immune tolerance [130]. Interestingly, LIF is produced by human BMMSC [131] and its overexpression and depletion in mouse models led to immune disturbances [132]. Recently, it was suggested that the MSC-induced immune tolerance is due to their production of LIF [133]. However, the cellular and molecular mechanisms (that also involve other identified MSC molecules) leading to this transplantation tolerance remain functionally ignored. Another established ex vivo effect of MSC is to improve the maintenance of HSC in liquid cocultures. Indeed, a positive effect of LIF on the in vitro maintenance of murine HSC was mediated by BM stromal cells in which it upregulates the expression of various hematopoietic cytokines [134]. By using an elegant model of young and old, wild type and LIF−/− mice for cross cocultures of MSC and Lin− Sca1+ hematopoietic progenitors and stem cells, it has been shown that LIF−/− mice had only slight disturbances of hematopoiesis when explored in culture [135] since LIF−/− and WT MSC were not significantly different in their capacity to maintain Lin− Sca1+ hematopoietic progenitors of WT and LIF−/− mice in liquid cocultures. Complementary experiments (including transplantation) should be performed if one wants to conclude about the real direct/indirect effects of LIF on HSC in vivo. However, a novel direct autocrine effect of LIF to maintain the multipotent program of MSC has recently been described. Indeed, down regulation of MSC LIF expression by specific miRNA favors their differentiation towards the osteoblast and adipogenic lineages [59]. In this context, it is worth stressing that in vivo silencing of the gp190 subunit of the LIF receptor leads to major disturbances of the bone and other mesenchy-
mal tissues suggesting a major non redundant role of LIF in the maintenance and commitment of MSC [128, 129].

Muscle Satellite Cells

Muscle satellite cells are the major cell type responsible for post-natal skeletal muscle growth and regeneration. These quiescent cells are located under the basal lamina of muscle fibers, and become activated upon injury. They proliferate and differentiate into new muscle fibers and during regeneration, the satellite cell pool is also reconstituted [136–138]. Since the first articles showing a role of LIF in stimulation of myoblast proliferation in culture [139, 140], it has been shown that LIF engages JAK1, STAT1, and STAT3 to promote cell proliferation and to repress myogenic differentiation, in primary myoblasts and in the C2C12 cell models [26, 141]. However, an opposite role for STAT3 in myoblast differentiation was shown by knock-down of endogenous STAT3 which dramatically blocked myogenic differentiation. It was then suggested that, as in the murine ES cell model, LIF/STAT3 might play distinct roles at different stages of muscle differentiation [142]. Further studies are needed to understand how STAT3 coordinates with myoD to control myogenic differentiation and how they cross-talk with other signaling pathways. It has also been demonstrated that LIF expression in skeletal muscle is critical for the development of skeletal muscle hypertrophy in the functional overload model [143]. LIF has also been suggested as being a novel myokine, secreted by muscle cells during exercise in link with activation of calcium-dependent pathway and changes in energy status [27]. However, in this study, the LIF secretion is around 3 pg/ml, which could be considered to be close to background level, based on known physical constant describing cytokine/receptor interactions [144].

Neural Stem Cells

Neural stem cells (NSCs) in the adult brain continuously provide new neurons to the hippocampal dentate gyrus (DG) and the olfactory bulb (OB). The progression from neural precursor cells (NPCs) to mature neurons is tightly controlled by coordination of cell-intrinsic programs and external signals within the neurogenic niche. Understanding signaling in adult neurogenesis is a key challenge to understand the physiological roles of neurogenesis, but also to provide knowledge required to use NSCs as potential therapy for treatment of brain diseases [145–147].

Cytokines that signal through the LIFRbeta/gp130 receptor complex, including LIF and CNTF have been suggested to promote the self-renewal of embryonic and adult mouse or rat NPCs [22, 148]. However, with CNTF or LIF KO models, it was shown that while CNTF-induced STAT3 signaling is essential for the formation and/or maintenance of the neurogenic subgranular zone in the adult dentate gyrus, LIF was not required [149]. In addition, in a model of human neural stem cells, which can be expanded under EGF and FGF2, no effect of LIF was observed [150]. In contrast, in a rat model, LIF, and not CNTF, was crucial for the expansion of NPCs in the Sub Ventricular Zone (SVZ) after perinatal brain injury [151]. LIF was also shown to control neural differentiation and maintenance of stem cell-derived murine spiral ganglion neuron precursors, a finding which could be relevant in cell replacement studies with animal models featuring spiral ganglion neuron degeneration [23]. Another study shows that during normal development of mouse optic nerve, there is a defined developmental time window when LIF is required for correct myelination [152].

Understanding of LIF signaling in adult stem cells will still depend on detailed analysis performed in LIF KO model mice, challenged with particular stimuli aiming at increasing or decreasing the natural pool of stem cells in their relevant niche.

LIF in Reprogrammation: Antagonism Between LIF and p53 Pathways. What for?

Somatic cell reprogramming which leads to the derivation of iPSC is mediated by specific set of genes (Oct4, Sox2, cMyc and Klf4 or Oct4, Sox2, Lin28 and Nanog), including LIF targets [119, 153, 154]. L-Myc has been shown recently to replace c-MYC in the reprogramming cocktail with the potential advantage to reduce the tumorigenicity of the derived iPSC [155]. In addition, growing cells at low O2 concentration (3%–5% O2), [156], and blocking the p53 pathway [36, 37, 40, 41, 157], are also critical parameters which increase the efficiency of somatic cell reprogramming.

The expression of the LIF cytokine is under the direct control of p53 (at least in mouse endometrium cells [35]), and of N-MYC (in the neuroblastoma cell model, [158]). Indeed, LIF promoter contains specific binding sites for these regulatory transcription factors. Whether N-MYC and p53 act together or not have not been established so far. LIF-induced genes and repression of p53 pathway seems to be paradoxical effectors for efficient cell reprogramming, unless both signals are not required simultaneously, a hypothesis which will deserve future attention.

LIF in EVO-DEVO

A large amount of data has been accumulated on the pleiotropic functions of LIF in mammals, but so far we have little insight on what could be its functions in non mammalian...
vertebrate with respect to early development and differentiation [159]. A limited number of signaling pathways is operating during metazoan development and most, if not all of them are conserved over a large evolutionary scale [160, 161]. Among them is the JAK/STAT pathway which conveys LIF signals. LIF belongs to class I-helical cytokines family which is considered to constitute a monophyletic group having evolved from a single ancestral gene through successive duplication events mainly in the vertebrate lineage [162]. Indeed, the core signaling elements of the JAK/STAT pathway has been found in invertebrate such as Drosophila with a unique cytokine-like peptide ligand capable of activating the pathway [163]. The knowledge of the conservation of IL6 family members, as LIF, between divergent vertebrate species and the functions of LIF during development can be very informative for understanding its pleiotropic functions. LIF ortholog has been identified through cDNA cloning and in silico analysis in several non mammalian vertebrates. Chicken cDNA LIF has been cloned and shown to be able to maintain blastodermal cells into an undifferentiated state [164]. CDNA encoding LIF ortholog has also been cloned in teleost species as zebrafish, carp and goldfish [165–167]. The finding of LIF-like cytokine in fishes indicates that the cytokine already existed before the fish-tetrapod divergence that occurred approximately 450 million years ago. LIF ortholog is also present in amphibian and we have cloned, through in silico analysis, a Xenopus LIF cDNA (our unpublished data). Although the amino acid conservation of LIF sequences between mammalian and non mammalian vertebrate species is rather low (20%–40%), those sequences share a conserved tridimensional fold. LIF receptor (LIFR) has been identified in chick and zebrafish and functional analysis performed in both species. In chicken, LIFR is involved in the control of vasoactive intestinal peptide expression in sympathetic neurons [168]. Functional experiments using morpholino based mediated knock down in zebrafish have shown that, unlike LIF, whose knock down has no obvious effects on development, LIFR knock down impairs proper neural development [169]. Although there is no functional data about LIF and LIFR functions in Xenopus development yet, it has been found that STAT3 activation, through gp130 signaling, ventralizes embryo independently of BMP4 [170]. Whether this effect can be related to LIF signaling through its receptor awaits experiments. Since LIF is essential to murine ES cells pluripotency, it is striking to note that STAT3 is able to maintain Xenopus pluripotent neural crest cells in an undifferentiated state downstream of FGF signals [171].

According to the current data and the evolutionary conservation of the components of the LIF signaling pathway, we speculate that LIF can generate distinct responses at different times during development/differentiation in different species. Most of the genes, that we have defined as LIF signatures in the mES model, is conserved in non mammalian vertebrate genomes and their study in the amphibian Xenopus model should allow for a rapid and efficient screening of the functions of the genes that make up the LIF signatures. This should also allow a better understanding of the functions of the LIF pathway during development.

Conclusions

The challenge, when studying LIF, is to catch the proper time window of its effects. Indeed, while LIF is essential for maintenance of pluripotency of murine ES cells (mES), it is not required for human ES cells (hES). It has been elegantly demonstrated that this was due to the fact that murine and human ES cells are respectively derived from early and late epiblast, stressing the differential effects of LIF that might depend on the embryonic stage. In addition, LIF/STAT3 signaling reverts murine EpiSC cells (Epiblast-derived cells, which are similar to human ES cells) to mES cells probably by LIF-dependent expression of KLF4, as suggested by recent reports [172–175].

Functions of LIF studied in KO model mice, along with functional tests performed at different time windows in the mES cells model, with new LIF targets, should increase our knowledge on this still fascinating cytokine in the near future.

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We apologise for LIF-dedicated articles not cited in this review.

Conflicts of interest The authors declare no potential conflict of interest.

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