Stem Cell Rev and Rep (2012) 8:1–15
DOI 10.1007/s12015-011-9261-7

LIF-Dependent Signaling: New Pieces in the Lego

Marie-Emmanuelle Mathieu · Claire Saucourt · Virginie Mournetas · Xavier Gauthereau · Nadine Thézé · Vincent Praloran · Pierre Thiébaud · Hélène Bœuf

Published online: 3 May 2011

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

M.-E. Mathieu · C. Saucourt · V. Mournetas · X. Gauthereau · N. Thézé · V. Praloran · P. Thiébaud · H. Bœuf (✉)
Univ. de Bordeaux, CIRID, UMR5164,
F-33000 Bordeaux, France
e-mail: helene.boeuf@u-bordeaux2.fr

M.-E. Mathieu · C. Saucourt · V. Mournetas · X. Gauthereau · N. Thézé · V. Praloran · P. Thiébaud · H. Bœuf
CNRS, CIRID, UMR5164,
F-33000 Bordeaux, France

Present Address:
M.-E. Mathieu · X. Gauthereau · N. Thézé · V. Praloran · P. Thiébaud · H. Bœuf
University Bordeaux Segalen; CNRS-UMR5164-CIRID,
Bat. 1B, BP 14, 146 rue Léo Saignat,
33076 Bordeaux, France

Present Address:
C. Saucourt
IRTH/Cell Prothera,
Mulhouse, France

Present Address:
V. Mournetas
Institut of Integrative Biology, University of Liverpool,
Liverpool, United Kingdom

Abbreviations

AK7	Adenylate Kinase 7
ALDH	AcetaLdehyde DeHydrogenase
AP1	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-Inductible 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

EED	Polycomb protein Embryonic Ectoderm Development
EGF	Epidermal Growth Factor
EpiSC	epiblast stemcells
ERK	Extracellular signal-Regulated Kinases
ES	Embryonic Stem
ESRRB	EStrogen-Related Receptor beta
FGF	Fibroblast Growth Factor
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
Pleio-Lifind	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
Tcfcp2l1	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 finger 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	Corti1 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.		[191]
	Normal and tumoral mammary derived cell lines.	LIF inhibits (in non-tumorigenic cells) or induces (in tumorigenic cells) cell survival.	[192]
	ARMS and ERMS rhabdomyosarcoma cell lines.	LIF is involved in rhabdomyosarcoma-derived metastasis.	[193]
	In vivo and in vitro studies.	LIF is involved in melanoma tumor growth.	[194]
	In vivo and in vitro studies: BalbC/mice; SEKI cells; 8 human melanoma-derived cell lines.	LIF has a potential role in melanoma-induced bone metastasis.	[195]
	In vivo and in vitro studies: human glioma cell lines: U373MG and A172 and human primary cell cultures.	LIF and TGFbeta induce self renewal of glioma-initiating cells, promoting oncogenesis in vivo.	[196]

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 haematopoietic 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]

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/PI3K/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

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

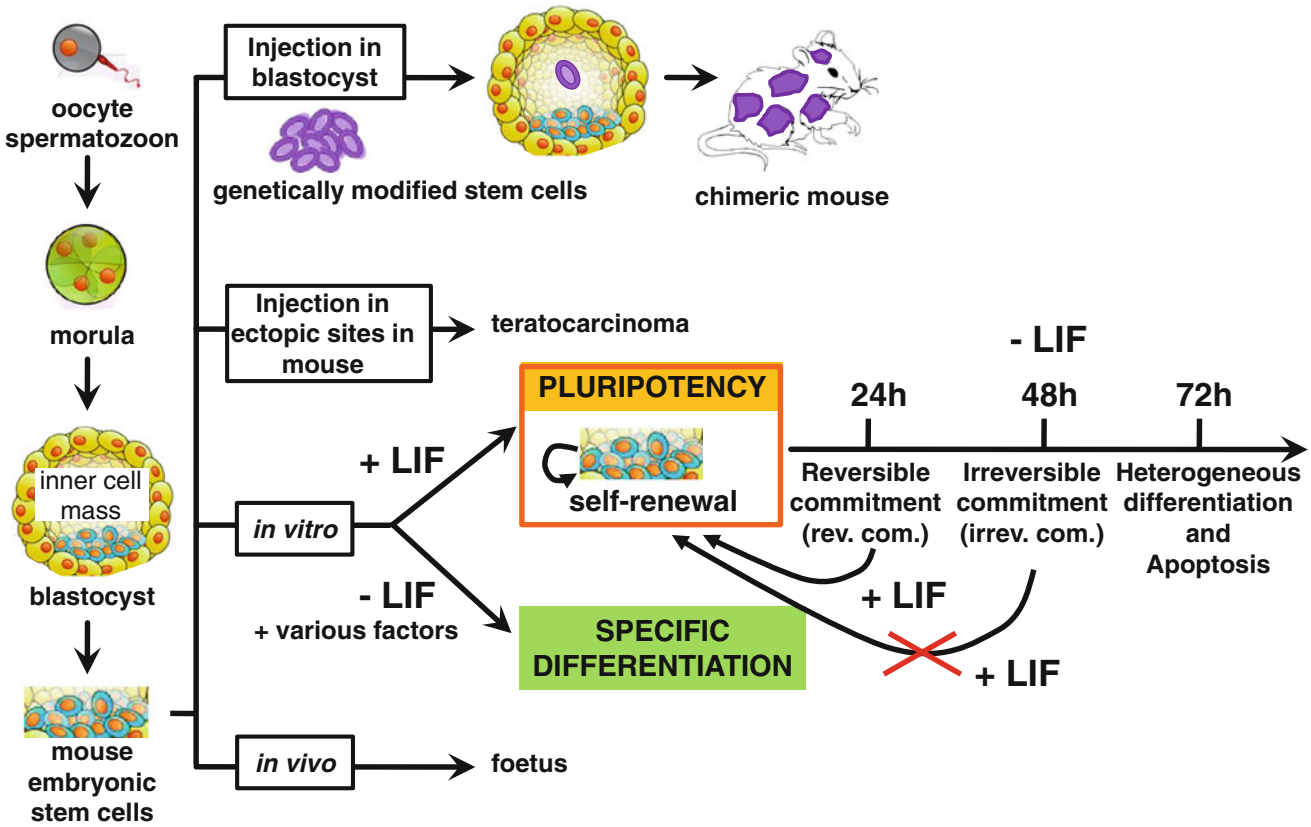

Fig. 1 The mES system: the «three in one» cell model to study LIF mechanisms in pluripotent, committed and differentiated cells. Part of this figure has been taken from the Web site: http://en.wikipedia.org/wiki/Stem_cell, available under the «Creative Commons Attribution-ShareAlike 2.5 Generic License ». 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 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], *Pdcd2* [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, Tcfcp2l1, 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/NANOG 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

(induced by LIF after different periods of LIF starvation, 24 h, 48 h or 10 days after LIF withdrawal, this latter condition corresponding to differentiated cells which re-express LIF and its receptors [4, 7, 105, 111]). Since withdrawal of LIF for 24 h leads to reversible commitment [112–114], we postulate that some of the *Spe-Lifind* genes could be involved in the reversible process.

Klf4 (a well known member of the "Yamanaka" cocktail, involved in reprogrammation of somatic cells to iPSC) and *Klf5* are both *Spe-Lifind* genes shown to be critical actors of maintenance of pluripotency in mES [115–120]. Both genes are also direct STAT3 targets and block endoderm (Klf4) or mesoderm (Klf5) differentiation [48]. Klf4 and 5 are also 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 "ressetors" 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 reprogrammation 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 reprogrammation 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 reprogrammation.

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 aproximatively 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.

Acknowledgements This work was supported by CNRS, University Bordeaux Segalen and fundings under the sixth Research framework program of the European Union (FungenES, LSHG-CT-2003- 503494), the Association de la Recherche contre le Cancer, the Ligue Nationale contre le cancer (comité Charentes Aquitaine), the Association Française contre les Myopathies, IFR66/SFR Trans Biomed Bordeaux and the Etablissement Français du Sang, Aquitaine.

We thank JF Moreau and P Veschambre for critical reading of the manuscript.

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

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

References

1. Taupin, J. L., Pitard, V., Dechanet, J., Miossec, V., Gualde, N., & Moreau, J. F. (1998). Leukemia inhibitory factor: part of a large ingathering family. *International Reviews of Immunology, 16*(3–4), 397–426.
2. Metcalf, D. (2003). The unsolved enigmas of leukemia inhibitory factor. *Stem Cells, 21*(1), 5–14.

3. Moreau, J. F., Donaldson, D. D., Bennett, F., Witek-Giannotti, J., Clark, S. C., & Wong, G. G. (1988). Leukaemia inhibitory factor is identical to the myeloid growth factor human interleukin for DA cells. *Nature, 336*(6200), 690–692.
4. Smith, A. G., Nichols, J., Robertson, M., & Rathjen, P. D. (1992). Differentiation inhibiting activity (DIA/LIF) and mouse development. *Developmental Biology, 151*(2), 339–351.
5. Gough, N. M., Gearing, D. P., King, J. A., Willson, T. A., Hilton, D. J., Nicola, N. A., et al. (1988). Molecular cloning and expression of the human homologue of the murine gene encoding myeloid leukemia-inhibitory factor. *Proceedings of the National Academy of Sciences of the United States of America, 85*(8), 2623–2627.
6. Shellard, J., Perreau, J., & Brûlet, P. (1996). Role of leukemia inhibitory factor during mammalian development. *European Cytokine Network, 7*(4), 699–712.
7. Trouillas, M., Saucourt, C., Guillotin, B., Gauthereau, X., Taupin, J., Moreau, J., et al. (2009). The LIF cytokine: towards adulthood. *European Cytokine Network, 20*(2), 51–62.
8. Heinrich, P. C., Behrmann, I., Haan, S., Hermanns, H. M., Müller-Newen, G., & Schaper, F. (2003). Principles of interleukin (IL)-6-type cytokine signalling and its regulation. *The Biochemical Journal, 374*(Pt 1), 1–20.
9. Murakami, M., Kamimura, D., & Hirano, T. (2004). New IL-6 (gp130) family cytokine members, CLC/NNT1/BSF3 and IL-27. *Growth Factors, 22*(2), 75–77.
10. Hermanns, H. M., Müller-Newen, G., Heinrich, P. C., & Haan, S. (2005). Bow to your partner for signaling. *Nature Structural & Molecular Biology, 12*(6), 476–478.
11. Rousseau, F., Chevalier, S., Guillet, C., Ravon, E., Diveu, C., Froger, J., et al. (2008). Ciliary neurotrophic factor, cardiotrophin-like cytokine, and neuropoietin share a conserved binding site on the ciliary neurotrophic factor receptor alpha chain. *The Journal of Biological Chemistry, 283*(44), 30341–30350.
12. Rousseau, F., Basset, L., Froger, J., Dinguirard, N., Chevalier, S., Gascan, H. (2010). IL-27 structural analysis demonstrates similarities with ciliary neurotrophic factor (CNTF) and leads to the identification of antagonistic variants. *Proceedings of the National Academy of Sciences of the United States of America*.
13. Gearing, D. P. (1993). The leukemia inhibitory factor and its receptor. *Advances in Immunology, 53*, 31–58.
14. Blanchard, F., Duplomb, L., Wang, Y., Robledo, O., Kinzie, E., Pitard, V., et al. (2000). Stimulation of leukemia inhibitory factor receptor degradation by extracellular signal-regulated kinase. *The Journal of Biological Chemistry, 275*(37), 28793–28801.
15. Gao, W., Thompson, L., Zhou, Q., Putheti, P., Fahmy, T. M., Strom, T. B., et al. (2009). Treg versus Th17 lymphocyte lineages are cross-regulated by LIF versus IL-6. *Cell Cycle, 8*(9), 1444–1450.
16. Blanchard, F., Duplomb, L., Raher, S., Vusio, P., Hoflack, B., Jacques, Y., et al. (1999). Mannose 6-Phosphate/Insulin-like growth factor II receptor mediates internalization and degradation of leukemia inhibitory factor but not signal transduction. *The Journal of Biological Chemistry, 274*(35), 24685–24693.
17. Larsen, J. V., Hansen, M., Møller, B., Madsen, P., Scheller, J., Nielsen, M., et al. (2010). Sortilin facilitates signaling of ciliary neurotrophic factor and related helical type 1 cytokines targeting the gp130/leukemia inhibitory factor receptor beta heterodimer. *Molecular and Cellular Biology, 30*(17), 4175–4187.
18. Bhatt, H., Brunet, L. J., & Stewart, C. L. (1991). Uterine expression of leukemia inhibitory factor coincides with the onset of blastocyst implantation. *Proceedings of the National Academy of Sciences of the United States of America, 88*(24), 11408–11412.
19. Stewart, C. L., Kaspar, P., Brunet, L. J., Bhatt, H., Gadi, I., Köntgen, F., et al. (1992). Blastocyst implantation depends on maternal expression of leukaemia inhibitory factor. *Nature, 359*(6390), 76–79.
20. Escary, J. L., Perreau, J., Duménil, D., Ezine, S., & Brûlet, P. (1993). Leukaemia inhibitory factor is necessary for maintenance of haematopoietic stem cells and thymocyte stimulation. *Nature, 363*(6427), 361–364.
21. Kritikou, E. A., Sharkey, A., Abell, K., Came, P. J., Anderson, E., Clarkson, R. W., et al. (2003). A dual, non-redundant, role for LIF as a regulator of development and STAT3-mediated cell death in mammary gland. *Development, 130*, 3459–3468.
22. Bauer, S., & Patterson, P. H. (2006). Leukemia inhibitory factor promotes neural stem cell self-renewal in the adult brain. *The Journal of Neuroscience, 26*(46), 12089–12099.
23. Oshima, K., Teo, D. T. W., Senn, P., Starlinger, V., & Heller, S. (2007). LIF promotes neurogenesis and maintains neural precursors in cell populations derived from spiral ganglion stem cells. *BMC Developmental Biology, 7*, 112.
24. Simamura, E., Shimada, H., Higashi, N., Uchishiba, M., Otani, H., & Hatta, T. (2010). Maternal leukemia inhibitory factor (LIF) promotes fetal neurogenesis via a LIF-ACTH-LIF signaling relay pathway. *Endocrinology, 151*(4), 1853–1862.
25. Hunt, L. C., Tudor, E. M., & White, J. D. (2010). Leukemia inhibitory factor-dependent increase in myoblast cell number is associated with phosphotidylinositol 3-kinase-mediated inhibition of apoptosis and not mitosis. *Experimental Cell Research, 316*(6), 1002–1009.
26. Spangenburg, E. E., & Booth, F. W. (2002). Multiple signaling pathways mediate LIF-induced skeletal muscle satellite cell proliferation. *American Journal of Physiology. Cell Physiology, 283*(1), C204–C211.
27. Broholm, C., & Pedersen, B. K. (2010). Leukaemia inhibitory factor—an exercise-induced myokine. *Exercise Immunology Review, 16*, 77–85.
28. Bozec, A., Bakiri, L., Hoebertz, A., Eferl, R., Schilling, A. F., Komnenovic, V., et al. (2008). Osteoclast size is controlled by Fra-2 through LIF/LIF-receptor signalling and hypoxia. *Nature, 454*(7201), 221–225.
29. Sendtner, M., Götz, R., Holtmann, B., Escary, J. L., Masu, Y., Carroll, P., et al. (1996). Cryptic physiological trophic support of motoneurons by LIF revealed by double gene targeting of CNTF and LIF. *Current Biology, 6*(6), 686–694.
30. Holtmann, B., Wiese, S., Samsam, M., Grohmann, K., Pennica, D., Martini, R., et al. (2005). Triple knock-out of CNTF, LIF, and CT-1 defines cooperative and distinct roles of these neurotrophic factors for motoneuron maintenance and function. *The Journal of Neuroscience, 25*(7), 1778–1787.
31. Aghajanova, L. (2010). Update on the role of leukemia inhibitory factor in assisted reproduction. *Current Opinion in Obstetrics & Gynecology, 22*(3), 213–219.
32. Slaets, H., Hendriks, J. J. A., Stinissen, P., Kilpatrick, T. J., & Hellings, N. (2010). Therapeutic potential of LIF in multiple sclerosis. *Trends in Molecular Medicine, 16*(11), 493–500.
33. Hu, W., Feng, Z., Teresky, A. K., & Levine, A. J. (2007). p53 regulates maternal reproduction through LIF. *Nature, 450*(7170), 721–724.
34. Hu, W., Feng, Z., Atwal, G. S., & Levine, A. J. (2008). p53: a new player in reproduction. *Cell Cycle, 7*(7), 848–852.
35. Stewart, C. L. (2007). Reproduction: the unusual suspect. *Nature, 450*(7170), 619.
36. Kawamura, T., Suzuki, J., Wang, Y. V., Menendez, S., Morera, L. B., Raya, A., et al. (2009). Linking the p53 tumour suppressor pathway to somatic cell reprogramming. *Nature, 460*(7259), 1140–1144.
37. Hong, H., Takahashi, K., Ichisaka, T., Aoi, T., Kanagawa, O., Nakagawa, M., et al. (2009). Suppression of induced pluripotent

stem cell generation by the p53-p21 pathway. *Nature, 460*(7259), 1132–1135.
38. Liu, Y., Hoya-Arias, R., & Nimer, S. D. (2009). The role of p53 in limiting somatic cell reprogramming. *Cell Research, 19*(11), 1227–1228.
39. Marión, R. M., Strati, K., Li, H., Murga, M., Blanco, R., Ortega, S., et al. (2009). A p53-mediated DNA damage response limits reprogramming to ensure iPS cell genomic integrity. *Nature, 460* (7259), 1149–1153.
40. Menendez, S., Camus, S., & Belmonte, J. C. I. (2010). p53: guardian of reprogramming. *Cell Cycle, 9*(19), 3887–3891.
41. Li, H., Collado, M., Villasante, A., Strati, K., Ortega, S., Cañamero, M., et al. (2009). The Ink4/Arf locus is a barrier for iPS cell reprogramming. *Nature, 460*(7259), 1136–1139.
42. Hilton, D. J. (1992). LIF: lots of interesting functions. *Trends in Biochemical Sciences, 17*(2), 72–76.
43. Lim, W. A., & Pawson, T. (2010). Phosphotyrosine signaling: evolving a new cellular communication system. *Cell, 142*(5), 661–667.
44. Ingley, E. (2008). Src family kinases: regulation of their activities, levels and identification of new pathways. *Biochimica et Biophysica Acta, 1784*(1), 56–65.
45. Yagil, Z., Nechushtan, H., Kay, G., Yang, C. M., Kemeny, D. M., & Razin, E. (2010). The enigma of the role of protein inhibitor of activated STAT3 (PIAS3) in the immune response. *Trends in Immunology, 31*(5), 199–204.
46. Smith, A. G. (2001). Embryo-derived stem cells: of mice and men. *Annual Review of Cell and Developmental Biology, 17*, 435–462.
47. Niwa, H. (2007). How is pluripotency determined and maintained? *Development, 134*(4), 635–646.
48. Bourillot, P., Aksoy, I., Schreiber, V., Wianny, F., Schulz, H., Hummel, O., et al. (2009). Novel STAT3 target genes exert distinct roles in the inhibition of mesoderm and endoderm differentiation in cooperation with Nanog. *Stem Cells, 27*(8), 1760–1771.
49. Ura, H., Usuda, M., Kinoshita, K., Sun, C., Mori, K., Akagi, T., et al. (2008). STAT3 and Oct-3/4 control histone modification through induction of Eed in embryonic stem cells. *The Journal of Biological Chemistry, 283*(15), 9713–9723.
50. Boeuf, H., Hauss, C., Graeve, F. D., Baran, N., & Kedinger, C. (1997). Leukemia inhibitory factor-dependent transcriptional activation in embryonic stem cells. *The Journal of Cell Biology, 138*(6), 1207–1217.
51. Matsuda, T., Nakamura, T., Nakao, K., Arai, T., Katsuki, M., Heike, T., et al. (1999). STAT3 activation is sufficient to maintain an undifferentiated state of mouse embryonic stem cells. *The EMBO Journal, 18*(15), 4261–4269.
52. Minami, M., Inoue, M., Wei, S., Takeda, K., Matsumoto, M., Kishimoto, T., et al. (1996). STAT3 activation is a critical step in gp130-mediated terminal differentiation and growth arrest of a myeloid cell line. *Proceedings of the National Academy of Sciences of the United States of America, 93*(9), 3963–3966.
53. Yoshida, T., Iwamoto, T., Adachi, K., Yokota, T., Miyake, Y., & Hamaguchi, M. (2005). Functional analysis of the effect of forced activation of STAT3 on M1 mouse leukemia cells. *International Journal of Molecular Medicine, 15*(2), 269–275.
54. Yamanaka, Y., Nakajima, K., Fukada, T., Hibi, M., & Hirano, T. (1996). Differentiation and growth arrest signals are generated through the cytoplasmic region of gp130 that is essential for Stat3 activation. *The EMBO Journal, 15*(7), 1557–1565.
55. Silver, J. S., & Hunter, C. A. (2010). gp130 at the nexus of inflammation, autoimmunity, and cancer. *Journal of Leukocyte Biology, 88*(6), 1145–1156.
56. Fischer, P., Lehmann, U., Sobota, R. M., Schmitz, J., Niemand, C., Linnemann, S., et al. (2004). The role of the inhibitors of interleukin-6 signal transduction SHP2 and SOCS3 for desensitization of interleukin-6 signalling. *The Biochemical Journal, 378*(Pt 2), 449–460.
57. Lin, C., Jackson, A. L., Guo, J., Linsley, P. S., & Eisenman, R. N. (2009). Myc-regulated microRNAs attenuate embryonic stem cell differentiation. *The EMBO Journal, 28*(20), 3157–3170.
58. Tarantino, C., Paolella, G., Cozzuto, L., Minopoli, G., Pastore, L., Parisi, S., et al. (2010). miRNA 34a, 100, and 137 modulate differentiation of mouse embryonic stem cells. *The FASEB Journal, 24*(9), 3255–3263.
59. Oskowitz, A. Z., Lu, J., Penfornis, P., Ylostalo, J., McBride, J., Flemington, E. K., et al. (2008). Human multipotent stromal cells from bone marrow and microRNA: regulation of differentiation and leukemia inhibitory factor expression. *Proceedings of the National Academy of Sciences of the United States of America, 105*(47), 18372–18377.
60. Hao, J., Li, T., Qi, X., Zhao, D., & Zhao, G. (2006). WNT/beta-catenin pathway up-regulates Stat3 and converges on LIF to prevent differentiation of mouse embryonic stem cells. *Developmental Biology, 290*(1), 81–91.
61. Ogawa, K., Nishinakamura, R., Iwamatsu, Y., Shimosato, D., & Niwa, H. (2006). Synergistic action of Wnt and LIF in maintaining pluripotency of mouse ES cells. *Biochemical and Biophysical Research Communications, 343*(1), 159–166.
62. Boiani, M., & Schöler, H. R. (2005). Regulatory networks in embryo-derived pluripotent stem cells. *Nature Reviews. Molecular Cell Biology, 6*(11), 872–884.
63. Pera, M. F., & Tam, P. P. L. (2010). Extrinsic regulation of pluripotent stem cells. *Nature, 465*(7299), 713–720.
64. Kochegarov, A. (2009). Small molecules for stem cells. *Expert Opinion on Therapeutic Patents, 19*(3), 275–281.
65. Niwa, H. (2010). Mouse ES cell culture system as a model of development. *Development, Growth & Differentiation, 52*(3), 275–283.
66. Silva, J., & Smith, A. (2008). Capturing pluripotency. *Cell, 132* (4), 532–536.
67. Ying, Q., Wray, J., Nichols, J., Batlle-Morera, L., Doble, B., Woodgett, J., et al. (2008). The ground state of embryonic stem cell self-renewal. *Nature, 453*(7194), 519–523.
68. Chambers, I., & Tomlinson, S. R. (2009). The transcriptional foundation of pluripotency. *Development, 136*(14), 2311–2322.
69. Yang, W., Wei, W., Shi, C., Zhu, J., Ying, W., Shen, Y., et al. (2009). Pluripotin combined with leukemia inhibitory factor greatly promotes the derivation of embryonic stem cell lines from refractory strains. *Stem Cells, 27*(2), 383–389.
70. Chen, L., Yang, M., Dawes, J., & Khillan, J. S. (2007). Suppression of ES cell differentiation by retinol (vitamin A) via the over-expression of Nanog. *Differentiation, 75*(8), 682–693.
71. Chen, L., & Khillan, J. S. (2010). A novel signaling by vitamin A/retinol promotes self renewal of mouse embryonic stem cells by activating PI3K/Akt signaling pathway via insulin-like growth factor-1 receptor. *Stem Cells, 28*(1), 57–63.
72. Chazaud, C., Yamanaka, Y., Pawson, T., & Rossant, J. (2006). Early lineage segregation between epiblast and primitive endoderm in mouse blastocysts through the Grb2-MAPK pathway. *Developmental Cell, 10*(5), 615–624.
73. Carter, M. G., Stagg, C. A., Falco, G., Yoshikawa, T., Bassey, U. C., Aiba, K., et al. (2008). An in situ hybridization-based screen for heterogeneously expressed genes in mouse ES cells. *Gene Expression Patterns, 8*, 181–198.
74. Toyooka, Y., Shimosato, D., Murakami, K., Takahashi, K., & Niwa, H. (2008). Identification and characterization of subpopulations in undifferentiated ES cell culture. *Development, 135*, 909–918.
75. Kim, M. O., Lee, Y. J., & Han, H. J. (2010). Involvement of Cx43 phosphorylation in 5′-N-ethylcarboxamide-induced migra-

tion and proliferation of mouse embryonic stem cells. *Journal of Cellular Physiology, 224*(1), 187–194.
76. Todorova, M. G., Soria, B., & Quesada, I. (2008). Gap junctional intercellular communication is required to maintain embryonic stem cells in a non-differentiated and proliferative state. *Journal of Cellular Physiology, 214*(2), 354–362.
77. Wörsdörfer, P., Maxeiner, S., Markopoulos, C., Kirfel, G., Wulf, V., Auth, T., et al. (2008). Connexin expression and functional analysis of gap junctional communication in mouse embryonic stem cells. *Stem Cells, 26*(2), 431–439.
78. Wong, R. C. B., Pera, M. F., & Pébay, A. (2008). Role of gap junctions in embryonic and somatic stem cells. *Stem Cell Reviews, 4*(4), 283–292.
79. Saito, R., Yamasaki, T., Nagai, Y., Wu, J., Kajiho, H., Yokoi, T., et al. (2009). CrxOS maintains the self-renewal capacity of murine embryonic stem cells. *Biochemical and Biophysical Research Communications, 390*(4), 1129–1135.
80. Lian, I., Kim, J., Okazawa, H., Zhao, J., Zhao, B., Yu, J., et al. (2010). The role of YAP transcription coactivator in regulating stem cell self-renewal and differentiation. *Genes & Development, 24*(11), 1106–1118.
81. Fan, Y., Melhem, M. F., & Chaillet, J. R. (1999). Forced expression of the homeobox-containing gene Pem blocks differentiation of embryonic stem cells. *Developmental Biology, 210*(2), 481–496.
82. Lim, L. S., Loh, Y., Zhang, W., Li, Y., Chen, X., Wang, Y., et al. (2007). Zic3 is required for maintenance of pluripotency in embryonic stem cells. *Molecular Biology of the Cell, 18*(4), 1348–1358.
83. Lim, L. S., Hong, F. H., Kunarso, G., & Stanton, L. W. (2010). The pluripotency regulator Zic3 is a direct activator of the Nanog promoter in ESCs. *Stem Cells, 28*(11), 1961–1969.
84. Galan-Caridad, J. M., Harel, S., Arenzana, T. L., Hou, Z. E., Doetsch, F. K., Mirny, L. A., et al. (2007). Zfx controls the self-renewal of embryonic and hematopoietic stem cells. *Cell, 129*(2), 345–357.
85. Mu, W., Munroe, R. J., Barker, A. K., & Schimenti, J. C. (2010). PDCD2 is essential for inner cell mass development and embryonic stem cell maintenance. *Developmental Biology, 347*(2), 279–288.
86. Amleh, A., Nair, S. J., Sun, J., Sutherland, A., Hasty, P., & Li, R. (2009). Mouse cofactor of BRCA1 (Cobra1) is required for early embryogenesis. *PLoS ONE, 4*(4), e5034.
87. Hu, G., Kim, J., Xu, Q., Leng, Y., Orkin, S. H., & Elledge, S. J. (2009). A genome-wide RNAi screen identifies a new transcriptional module required for self-renewal. *Genes & Development, 23*(7), 837–848.
88. Ding, L., Paszkowski-Rogacz, M., Nitzsche, A., Slabicki, M. M., Heninger, A., de Vries, I., et al. (2009). A genome-scale RNAi screen for Oct4 modulators defines a role of the Paf1 complex for embryonic stem cell identity. *Cell Stem Cell, 4*(5), 403–415.
89. Ivanova, N., Dobrin, R., Lu, R., Kotenko, I., Levorse, J., DeCoste, C., et al. (2006). Dissecting self-renewal in stem cells with RNA interference. *Nature, 442*(7102), 533–538.
90. Loh, Y., Zhang, W., Chen, X., George, J., & Ng, H. (2007). Jmjd1a and Jmjd2c histone H3 Lys 9 demethylases regulate self-renewal in embryonic stem cells. *Genes & Development, 21*(20), 2545–2557.
91. Gaspar-Maia, A., Alajem, A., Polesso, F., Sridharan, R., Mason, M. J., Heidersbach, A., et al. (2009). Chd1 regulates open chromatin and pluripotency of embryonic stem cells. *Nature, 460*(7257), 863–868.
92. Dejosez, M., Krumenacker, J. S., Zitur, L. J., Passeri, M., Chu, L., Songyang, Z., et al. (2008). Ronin is essential for embryogenesis and the pluripotency of mouse embryonic stem cells. *Cell, 133*(7), 1162–1174.
93. Dejosez, M., Levine, S. S., Frampton, G. M., Whyte, W. A., Stratton, S. A., Barton, M. C., et al. (2010). Ronin/Hcf-1 binds to a hyperconserved enhancer element and regulates genes involved in the growth of embryonic stem cells. *Genes & Development, 24*(14), 1479–1484.
94. Smith, K. N., Singh, A. M., & Dalton, S. (2010). Myc represses primitive endoderm differentiation in pluripotent stem cells. *Cell Stem Cell, 7*(3), 343–354.
95. Tay, Y., Zhang, J., Thomson, A. M., Lim, B., & Rigoutsos, I. (2008). MicroRNAs to Nanog, Oct4 and Sox2 coding regions modulate embryonic stem cell differentiation. *Nature, 455*, 1124–1128.
96. Bernstein, E., Kim, S. Y., Carmell, M. A., Murchison, E. P., Alcorn, H., Li, M. Z., et al. (2003). Dicer is essential for mouse development. *Nature Genetics, 35*(3), 215–217.
97. Kanellopoulou, C., Muljo, S. A., Kung, A. L., Ganesan, S., Drapkin, R., Jenuwein, T., et al. (2005). Dicer-deficient mouse embryonic stem cells are defective in differentiation and centromeric silencing. *Genes & Development, 19*(4), 489–501.
98. Murchison, E. P., Partridge, J. F., Tam, O. H., Cheloufi, S., & Hannon, G. J. (2005). Characterization of Dicer-deficient murine embryonic stem cells. *Proceedings of the National Academy of Sciences of the United States of America, 102*(34), 12135–12140.
99. Babiarz, J. E., Ruby, J. G., Wang, Y., Bartel, D. P., & Blelloch, R. (2008). Mouse ES cells express endogenous shRNAs, siRNAs, and other Microprocessor-independent, Dicer-dependent small RNAs. *Genes & Development, 22*(20), 2773–2785.
100. Wang, Y., Medvid, R., Melton, C., Jaenisch, R., & Blelloch, R. (2007). DGCR8 is essential for microRNA biogenesis and silencing of embryonic stem cell self-renewal. *Nature Genetics, 39*, 380–385.
101. Wang, Y., Baskerville, S., Shenoy, A., Babiarz, J. E., Baehner, L., & Blelloch, R. (2008). Embryonic stem cell-specific microRNAs regulate the G1-S transition and promote rapid proliferation. *Nature Genetics, 40*, 1478–1483.
102. Niwa, H., Miyazaki, J., & Smith, A. G. (2000). Quantitative expression of Oct-3/4 defines differentiation, dedifferentiation or self-renewal of ES cells. *Nature Genetics, 24*(4), 372–376.
103. Rizzino, A. (2008). Transcription factors that behave as master regulators during mammalian embryogenesis function as molecular rheostats. *The Biochemical Journal, 411*(2), e5–e7.
104. Kopp, J. L., Ormsbee, B. D., Desler, M., & Rizzino, A. (2008). Small increases in the level of Sox2 trigger the differentiation of mouse embryonic stem cells. *Stem Cells, 26*(4), 903–911.
105. Trouillas, M., Saucourt, C., Guillotin, B., Gauthereau, X., Ding, L., Buchholz, F., et al. (2009). Three LIF-dependent signatures and gene clusters with atypical expression profiles, identified by transcriptome studies in mouse ES cells and early derivatives. *BMC Genomics, 10*, 73.
106. Palmqvist, L., Glover, C. H., Hsu, L., Lu, M., Bossen, B., Piret, J. M., et al. (2005). Correlation of murine embryonic stem cell gene expression profiles with functional measures of pluripotency. *Stem Cells, 23*(5), 663–680.
107. Schulz, H., Kolde, R., Adler, P., Aksoy, I., Anastassiadis, K., Bader, M., et al. (2009). The FunGenES database: a genomics resource for mouse embryonic stem cell differentiation. *PLoS ONE, 4*(9), e6804.
108. Loh, Y., Wu, Q., Chew, J., Vega, V. B., Zhang, W., Chen, X., et al. (2006). The Oct4 and Nanog transcription network regulates pluripotency in mouse embryonic stem cells. *Nature Genetics, 38*(4), 431–440.
109. Cinelli, P., Casanova, E. A., Uhlig, S., Lochmatter, P., Matsuda, T., Yokota, T., et al. (2008). Expression profiling in transgenic FVB/N embryonic stem cells overexpressing STAT3. *BMC Developmental Biology, 8*, 57.
110. Storm, M. P., Kumpfmueller, B., Thompson, B., Kolde, R., Vilo, J., Hummel, O., et al. (2009). Characterization of the phosphoi-

nositide 3-kinase-dependent transcriptome in murine embryonic stem cells: identification of novel regulators of pluripotency. *Stem Cells, 27*(4), 764–775.
111. Rathjen, P. D., Nichols, J., Toth, S., Edwards, D. R., Heath, J. K., & Smith, A. G. (1990). Developmentally programmed induction of differentiation inhibiting activity and the control of stem cell populations. *Genes & Development, 4*(12B), 2308–2318.
112. Burdon, T., Stracey, C., Chambers, I., Nichols, J., & Smith, A. (1999). Suppression of SHP-2 and ERK signalling promotes self-renewal of mouse embryonic stem cells. *Developmental Biology, 210*(1), 30–43.
113. Duval, D., Trouillas, M., Thibault, C., Dembelé, D., Diemunsch, F., Reinhardt, B., et al. (2006). Apoptosis and differentiation commitment: novel insights revealed by gene profiling studies in mouse embryonic stem cells. *Cell Death and Differentiation, 13*(4), 564–575.
114. Boeuf, H., Merienne, K., Jacquot, S., Duval, D., Zeniou, M., Hauss, C., et al. (2001). The ribosomal S6 kinases, cAMP-responsive element-binding, and STAT3 proteins are regulated by different leukemia inhibitory factor signaling pathways in mouse embryonic stem cells. *The Journal of Biological Chemistry, 276*(49), 46204–46211.
115. Li, Y., McClintick, J., Zhong, L., Edenberg, H. J., Yoder, M. C., & Chan, R. J. (2005). Murine embryonic stem cell differentiation is promoted by SOCS-3 and inhibited by the zinc finger transcription factor Klf4. *Blood, 105*(2), 635–637.
116. Jiang, J., Chan, Y., Loh, Y., Cai, J., Tong, G., Lim, C., et al. (2008). A core Klf circuitry regulates self-renewal of embryonic stem cells. *Nature Cell Biology, 10*(3), 353–360.
117. Niwa, H., Ogawa, K., Shimosato, D., & Adachi, K. (2009). A parallel circuit of LIF signalling pathways maintains pluripotency of mouse ES cells. *Nature, 460*(7251), 118–122.
118. Parisi, S., Passaro, F., Aloia, L., Manabe, I., Nagai, R., Pastore, L., et al. (2008). Klf5 is involved in self-renewal of mouse embryonic stem cells. *Journal of Cell Science, 121*(Pt 16), 2629–2634.
119. Takahashi, K., & Yamanaka, S. (2006). Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. *Cell, 126*(4), 663–676.
120. Zhang, P., Andrianakos, R., Yang, Y., Liu, C., & Lu, W. (2010). Kruppel-like factor 4 (Klf4) prevents embryonic stem (ES) cell differentiation by regulating Nanog gene expression. *The Journal of Biological Chemistry, 285*(12), 9180–9189.
121. Hall, J., Guo, G., Wray, J., Eyres, I., Nichols, J., Grotewold, L., et al. (2009). Oct4 and LIF/Stat3 additively induce Krüppel factors to sustain embryonic stem cell self-renewal. *Cell Stem Cell, 5*(6), 597–609.
122. Moreau, J. F., Bonneville, M., Godard, A., Gascan, H., Gruart, V., Moore, M. A., et al. (1987). Characterization of a factor produced by human T cell clones exhibiting eosinophil-activating and burst-promoting activities. *Journal of Immunology, 138*(11), 3844–3849.
123. Dührsen, U., & Metcalf, D. (1988). A model system for leukemic transformation of immortalized hemopoietic cells in irradiated recipient mice. *Leukemia, 2*(6), 329–333.
124. Leary, A. G., Wong, G. G., Clark, S. C., Smith, A. G., & Ogawa, M. (1990). Leukemia inhibitory factor differentiation-inhibiting activity/human interleukin for DA cells augments proliferation of human hematopoietic stem cells. *Blood, 75*(10), 1960–1964.
125. Verfaillie, C., & McGlave, P. (1991). Leukemia inhibitory factor/human interleukin for DA cells: a growth factor that stimulates the in vitro development of multipotential human hematopoietic progenitors. *Blood, 77*(2), 263–270.
126. Metcalf, D., & Gearing, D. P. (1989). Fatal syndrome in mice engrafted with cells producing high levels of the leukemia inhibitory factor. *Proceedings of the National Academy of Sciences of the United States of America, 86*(15), 5948–5952.
127. Metcalf, D., Nicola, N. A., & Gearing, D. P. (1990). Effects of injected leukemia inhibitory factor on hematopoietic and other tissues in mice. *Blood, 76*(1), 50–56.
128. Ware, C. B., Horowitz, M. C., Renshaw, B. R., Hunt, J. S., Liggitt, D., Koblar, S. A., et al. (1995). Targeted disruption of the low-affinity leukemia inhibitory factor receptor gene causes placental, skeletal, neural and metabolic defects and results in perinatal death. *Development, 121*(5), 1283–1299.
129. Dagoneau, N., Scheffer, D., Huber, C., Al-Gazali, L. I., Di Rocco, M., Godard, A., et al. (2004). Null leukemia inhibitory factor receptor (LIFR) mutations in Stuve-Wiedemann/Schwartz-Jampel type 2 syndrome. *American Journal of Human Genetics, 74*(2), 298–305.
130. García-Gómez, I., Elvira, G., Zapata, A. G., Lamana, M. L., Ramírez, M., Castro, J. G., et al. (2010). Mesenchymal stem cells: biological properties and clinical applications. *Expert Opinion on Biological Therapy, 10*(10), 1453–1468.
131. Lorgeot, V., Rougier, F., Fixe, P., Cornu, E., Praloran, V., & Denizot, Y. (1997). Spontaneous and inducible production of leukaemia inhibitory factor by human bone marrow stromal cells. *Cytokine, 9*(10), 754–758.
132. Metcalfe, S. M. (2005). Axotrophin and leukaemia inhibitory factor (LIF) in transplantation tolerance. *Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences, 360*, 1687–1694.
133. Nasef, A., Mazurier, C., Bouchet, S., Francois, S., Chapel, A., Thierry, D., et al. (2008). Leukemia inhibitory factor: role in human mesenchymal stem cells mediated immunosuppression. *Cellular Immunology, 253*, 16–22.
134. Szilvassy, S. J., Weller, K. P., Chen, B., Juttner, C. A., Tsukamoto, A., & Hoffman, R. (1996). Partially differentiated ex vivo expanded cells accelerate hematologic recovery in myeloablated mice transplanted with highly enriched long-term repopulating stem cells. *Blood, 88*(9), 3642–3653.
135. Schraml, E., Fehrer, C., Brunauer, R., Lepperdinger, G., Chesnokova, V., & Schauenstein, K. (2008). lin-Sca-1+ cells and age-dependent changes of their proliferation potential are reliant on mesenchymal stromal cells and are leukemia inhibitory factor dependent. *Gerontology, 54*(5), 312–323.
136. Dhawan, J., & Rando, T. A. (2005). Stem cells in postnatal myogenesis: molecular mechanisms of satellite cell quiescence, activation and replenishment. *Trends in Cell Biology, 15*(12), 666–673.
137. Montarras, D., Morgan, J., Collins, C., Relaix, F., Zaffran, S., Cumano, A., et al. (2005). Direct isolation of satellite cells for skeletal muscle regeneration. *Science, 309*(5743), 2064–2067.
138. Buckingham, M., & Montarras, D. (2008). Skeletal muscle stem cells. *Current Opinion in Genetics & Development, 18*(4), 330–336.
139. Austin, L., & Burgess, A. W. (1991). Stimulation of myoblast proliferation in culture by leukaemia inhibitory factor and other cytokines. *Journal of the Neurological Sciences, 101*(2), 193–197.
140. Barnard, W., Bower, J., Brown, M. A., Murphy, M., & Austin, L. (1994). Leukemia inhibitory factor (LIF) infusion stimulates skeletal muscle regeneration after injury: injured muscle expresses lif mRNA. *Journal of the Neurological Sciences, 123*, 108–113.
141. Diao, Y., Wang, X., & Wu, Z. (2009). SOCS1, SOCS3, and PIAS1 promote myogenic differentiation by inhibiting the leukemia inhibitory factor-induced JAK1/STAT1/STAT3 pathway. *Molecular and Cellular Biology, 29*(18), 5084–5093.
142. Yang, Y., Xu, Y., Li, W., Wang, G., Song, Y., Yang, G., et al. (2009). STAT3 induces muscle stem cell differentiation by interaction with myoD. *Cytokine, 46*(1), 137–141.
143. Spangenburg, E. E., & Booth, F. W. (2006). Leukemia inhibitory factor restores the hypertrophic response to increased loading in the LIF(−/−) mouse. *Cytokine, 34*(3–4), 125–130.

144. Gearing, D. P., Ziegler, S. F., Comeau, M. R., Friend, D., Thoma, B., Cosman, D., et al. (1994). Proliferative responses and binding properties of hematopoietic cells transfected with low-affinity receptors for leukemia inhibitory factor, oncostatin M, and ciliary neurotrophic factor. *Proceedings of the National Academy of Sciences of the United States of America, 91*(3), 1119–1123.
145. Bazan, E., Alonso, F. J., Redondo, C., Lopez-Toledano, M. A., Alfaro, J. M., Reimers, D., et al. (2004). In vitro and in vivo characterization of neural stem cells. *Histology and Histopathology, 19*, 1261–1275.
146. Abrous, D. N., Koehl, M., & Le Moal, M. (2005). Adult neurogenesis: from precursors to network and physiology. *Physiological Reviews, 85*(2), 523–569.
147. Suh, H., Deng, W., & Gage, F. H. (2009). Signaling in adult neurogenesis. *Annual Review of Cell and Developmental Biology, 25*, 253–275.
148. Andersen, R. K., Zimmer, J., Wahlberg, L. U., & Meyer, M. (2008). Effect of leukemia inhibitory factor on long-term propagation of precursor cells derived from rat forebrain subventricular zone and ventral mesencephalon. *Experimental Neurology, 211*(1), 301–310.
149. Müller, S., Chakrapani, B. P. S., Schwegler, H., Hofmann, H., & Kirsch, M. (2009). Neurogenesis in the dentate gyrus depends on ciliary neurotrophic factor and signal transducer and activator of transcription 3 signaling. *Stem Cells, 27*(2), 431–441.
150. Sun, Y., Pollard, S., Conti, L., Toselli, M., Biella, G., Parkin, G., et al. (2008). Long-term tripotent differentiation capacity of human neural stem (NS) cells in adherent culture. *Molecular and Cellular Neurosciences, 38*(2), 245–258.
151. Covey, M. V., & Levison, S. W. (2007). Leukemia inhibitory factor participates in the expansion of neural stem/progenitors after perinatal hypoxia/ischemia. *Neuroscience, 148*(2), 501–509.
152. Ishibashi, T., Lee, P. R., Baba, H., & Fields, R. D. (2009). Leukemia inhibitory factor regulates the timing of oligodendrocyte development and myelination in the postnatal optic nerve. *Journal of Neuroscience Research, 87*(15), 3343–3355.
153. Yamanaka, S. (2010). Patient-specific pluripotent stem cells become even more accessible. *Cell Stem Cell, 7*(1), 1–2.
154. Takahashi, K., Tanabe, K., Ohnuki, M., Narita, M., Ichisaka, T., Tomoda, K., et al. (2007). Induction of pluripotent stem cells from adult human fibroblasts by defined factors. *Cell, 131*(5), 861–872.
155. Elefanty, A. G., Blelloch, R., Passegué, E., Wernig, M., & Mummery, C. L. (2010). On the streets of San Francisco: highlights from the ISSCR Annual Meeting 2010. *Cell Stem Cell, 7*(4), 443–450.
156. Yoshida, Y., Takahashi, K., Okita, K., Ichisaka, T., & Yamanaka, S. (2009). Hypoxia enhances the generation of induced pluripotent stem cells. *Cell Stem Cell, 5*(3), 237–241.
157. Zhao, T., & Xu, Y. (2010). p53 and stem cells: new developments and new concerns. *Trends in Cell Biology, 20*(3), 170–175.
158. Cotterman, R., & Knoepfler, P. S. (2009). N-Myc regulates expression of pluripotency genes in neuroblastoma including lif, klf2, klf4, and lin28b. *PLoS ONE, 4*(6), e5799.
159. Auernhammer, C. J., & Melmed, S. (2000). Leukemia-inhibitory factor-neuroimmune modulator of endocrine function. *Endocrine Reviews, 21*(3), 313–345.
160. Brivanlou, A. H., & Darnell, J. E. (2002). Signal transduction and the control of gene expression. *Science, 295*(5556), 813–818.
161. Pires-daSilva, A., & Sommer, R. J. (2003). The evolution of signalling pathways in animal development. *Nature Reviews. Genetics, 4*(1), 39–49.
162. Huising, M. O., Kruiswijk, C. P., & Flik, G. (2006). Phylogeny and evolution of class-I helical cytokines. *The Journal of Endocrinology, 189*(1), 1–25.
163. Hombría, J. C. G., & Brown, S. (2002). The fertile field of Drosophila Jak/STAT signalling. *Current Biology, 12*(16), R569–R575.
164. Horiuchi, H., Furusawa, S., & Matsuda, H. (2006). Maintenance of chicken embryonic stem cells in vitro. *Methods in Molecular Biology, 329*, 17–34.
165. Fujiki, K., Nakao, M., & Dixon, B. (2003). Molecular cloning and characterisation of a carp (Cyprinus carpio) cytokine-like cDNA that shares sequence similarity with IL-6 subfamily cytokines CNTF, OSM and LIF. *Developmental and Comparative Immunology, 27*(2), 127–136.
166. Abe, T., Mikekado, T., Haga, S., Kisara, Y., Watanabe, K., Kurokawa, T., et al. (2007). Identification, cDNA cloning, and mRNA localization of a zebrafish ortholog of leukemia inhibitory factor. *Comparative Biochemistry and Physiology. Part B: Biochemistry & Molecular Biology, 147*(1), 38–44.
167. Hanington, P. C., & Belosevic, M. (2007). Interleukin-6 family cytokine M17 induces differentiation and nitric oxide response of goldfish (Carassius auratus L.) macrophages. *Developmental and Comparative Immunology, 31*(8), 817–829.
168. Duong, C. V., Geissen, M., & Rohrer, H. (2002). The developmental expression of vasoactive intestinal peptide (VIP) in cholinergic sympathetic neurons depends on cytokines signaling through LIFRbeta-containing receptors. *Development, 129*(6), 1387–1396.
169. Hanington, P. C., Patten, S. A., Reaume, L. M., Waskiewicz, A. J., Belosevic, M., & Ali, D. W. (2008). Analysis of leukemia inhibitory factor and leukemia inhibitory factor receptor in embryonic and adult zebrafish (Danio rerio). *Developmental Biology, 314*(2), 250–260.
170. Nishinakamura, R., Matsumoto, Y., Matsuda, T., Ariizumi, T., Heike, T., Asashima, M., et al. (1999). Activation of Stat3 by cytokine receptor gp130 ventralizes Xenopus embryos independent of BMP-4. *Developmental Biology, 216*(2), 481–490.
171. Nichane, M., Ren, X., & Bellefroid, E. J. (2010). Self-regulation of Stat3 activity coordinates cell-cycle progression and neural crest specification. *The EMBO Journal, 29*(1), 55–67.
172. Brons, I. G., Smithers, L. E., Trotter, M. W., Rugg-Gunn, P., Sun, B., Lopes, S. M. C. D. S., et al. (2007). Derivation of pluripotent epiblast stem cells from mammalian embryos. *Nature, 448*, 191–195.
173. Tesar, P. J., Chenoweth, J. G., Brook, F. A., Davies, T. J., Evans, E. P., Mack, D. L., et al. (2007). New cell lines from mouse epiblast share defining features with human embryonic stem cells. *Nature, 448*, 196–199.
174. Bao, S., Tang, F., Li, X., Hayashi, K., Gillich, A., Lao, K., et al. (2009). Epigenetic reversion of post-implantation epiblast to pluripotent embryonic stem cells. *Nature, 461*, 1292–1295.
175. Guo, G., Yang, J., Nichols, J., Hall, J. S., Eyres, I., Mansfield, W., et al. (2009). Klf4 reverts developmentally programmed restriction of ground state pluripotency. *Development, 136*, 1063–1069.
176. De Matos, D. G., Miller, K., Scott, R., Tran, C. A., Kagan, D., Nataraja, S. G., et al. (2008). Leukemia inhibitory factor induces cumulus expansion in immature human and mouse oocytes and improves mouse two-cell rate and delivery rates when it is present during mouse in vitro oocyte maturation. *Fertility and Sterility, 90*, 2367–2375.
177. Kanatsu-Shinohara, M., Inoue, K., Ogonuki, N., Miki, H., Yoshida, S., Toyokuni, S., et al. (2007). Leukemia inhibitory factor enhances formation of germ cell colonies in neonatal mouse testis culture. *Biology of Reproduction, 76*, 55–62.
178. Marwood, M., Visser, K., Salamonsen, L. A., & Dimitriadis, E. (2009). Interleukin-11 and leukemia inhibitory factor regulate the adhesion of endometrial epithelial cells: implications in fertility regulation. *Endocrinology, 150*, 2915–2923.
179. Mikhaylova, I. V., Jääskeläinen, T., Jääskeläinen, J., Palvimo, J. J., & Voutilainen, R. (2008). Leukemia inhibitory factor as a

regulator of steroidogenesis in human NCI-H295R adrenocortical cells. *The Journal of Endocrinology, 199*, 435–444.
180. Metcalf, D., Hilton, D., & Nicola, N. A. (1991). Leukemia inhibitory factor can potentiate murine megakaryocyte production in vitro. *Blood, 77*, 2150–2153.
181. Nasef, A., Mazurier, C., Bouchet, S., François, S., Chapel, A., Thierry, D., et al. (2008). Leukemia inhibitory factor: Role in human mesenchymal stem cells mediated immunosuppression. *Cellular Immunology, 253*, 16–22.
182. Sun, L., Ma, K., Wang, H., Xiao, F., Gao, Y., Zhang, W., et al. (2007). JAK1-STAT1-STAT3, a key pathway promoting proliferation and preventing premature differentiation of myoblasts. *The Journal of Cell Biology, 179*, 129–138.
183. Alter, J., Rozentzweig, D., & Bengal, E. (2008). Inhibition of myoblast differentiation by tumor necrosis factor alpha is mediated by c-Jun N-terminal kinase 1 and leukemia inhibitory factor. *The Journal of Biological Chemistry, 283*, 23224–23234.
184. Paradis, H., & Gendron, R. L. (2000). LIF transduces contradictory signals on capillary outgrowth through induction of stat3 and (P41/43)MAP kinase. *Journal of Cell Science, 113*(Pt 23), 4331–4339.
185. Murphy, M., Reid, K., Hilton, D. J., & Bartlett, P. F. (1991). Generation of sensory neurons is stimulated by leukemia inhibitory factor. *Proceedings of the National Academy of Sciences of the United States of America, 88*, 3498–3501.
186. Andersen, R. K., Widmer, H. R., Zimmer, J., Wahlberg, L. U., & Meyer, M. (2009). Leukemia inhibitory factor favours neurogenic differentiation of long-term propagated human midbrain precursor cells. *Neuroscience Letters, 464*, 203–208.
187. Carter, D. A., Dick, A. D., & Mayer, E. J. (2009). CD133+ adult human retinal cells remain undifferentiated in Leukaemia Inhibitory Factor (LIF). *BMC Ophthalmology, 9*, 1.
188. Chen, H., Ma, H., Sytwu, H., Wang, H., Chen, C. V., Liu, S., et al. (2010). Neural stem cells secrete factors that promote auditory cell proliferation via a leukemia inhibitory factor signaling pathway. *Journal of Neuroscience Research, 88*(15), 3308–3318.
189. Barasch, J., Yang, J., Ware, C. B., Taga, T., Yoshida, K., Erdjument-Bromage, H., et al. (1999). Mesenchymal to epithelial conversion in rat metanephros is induced by LIF. *Cell, 99*, 377–386.
190. Kanda, J., Uchiyama, T., Tomosugi, N., Higuchi, M., Uchiyama, T., & Kawabata, H. (2009). Oncostatin M and leukemia inhibitory factor increase hepcidin expression in hepatoma cell lines. *International Journal of Hematology, 90*, 545–552.
191. Lumaki, F., Basso, S. M., Orlando, R. (2010). Cytokines, thyroid diseases and thyroid cancer. *Cytokine, 50*(3), 299–233.
192. Quaglino, A., Schere-Levy, C., Romorini, L., Meiss, R. P., & Kordon, E. C. (2007). Mouse mammary tumors display Stat3 activation dependent on leukemia inhibitory factor signaling. *Breast Cancer Research, 9*, R69.
193. Wysoczynski, M., Miekus, K., Jankowski, K., Wanzeck, J., Bertolone, S., Janowska-Wieczorek, A., et al. (2007). Leukemia inhibitory factor: a newly identified metastatic factor in rhabdomyosarcomas. *Cancer Research, 67*, 2131–2140.
194. McKenzie, R. C., & Szepietowski, J. (2004). Cutaneous leukemia inhibitory factor and its potential role in the development of skin tumors. *Dermatologic Surgery, 30*, 279–290.
195. Maruta, S., Takiguchi, S., Ueyama, M., Kataoka, Y., Oda, Y., Tsuneyoshi, M., et al. (2009). A role for leukemia inhibitory factor in melanoma-induced bone metastasis. *Clinical & Experimental Metastasis, 26*, 133–141.
196. Peñuelas, S., Anido, J., Prieto-Sánchez, R. M., Folch, G., Barba, I., Cuartas, I., et al. (2009). TGF-[beta] Increases Glioma-Initiating Cell Self-Renewal through the Induction of LIF in Human Glioblastoma. *Cancer Cell, 15*, 315–327.
197. Kurek, J. B., Bower, J. J., Romanella, M., Koentgen, F., Murphy, M., & Austin, L. (1997). The role of leukemia inhibitory factor in skeletal muscle regeneration. Muscle Nerve. *Muscle & Nerve, 20*, 815–822.
198. Kubota, Y., Hirashima, M., Kishi, K., Stewart, C. L., & Suda, T. (2008). Leukemia inhibitory factor regulates microvessel density by modulating oxygen-dependent VEGF expression in mice. *Journal of Clinical Investigation, 118*, 2393–2403.
199. Bozec, A., Bakiri, L., Hoebertz, A., Eferl, R., Schilling, A. F., Komnenovic, V., et al. (2008). Osteoclast size is controlled by Fra-2 through LIF/LIF-receptor signalling and hypoxia. Nature. *Nature, 454*, 221–225.
200. Sugiura, S., Lahav, R., Han, J., Kou, S. Y., Banner, L. R., de Pablo, F., et al. (2000). Leukaemia inhibitory factor is required for normal inflammatory responses to injury in the peripheral and central nervous systems in vivo and is chemotactic for macrophages in vitro. *The European Journal of Neuroscience, 12*, 457–466.
201. Bugga, L., Gadient, R. A., Kwan, K., Stewart, C. L., & Patterson, P. H. (1998). Analysis of neuronal and glial phenotypes in brains of mice deficient in leukemia inhibitory factor. *Journal of Neurobiology, 36*, 509–524.
202. Marriott, M. P., Emery, B., Cate, H. S., Binder, M. D., Kemper, D., Wu, Q., et al. (2008). Leukemia inhibitory factor signaling modulates both central nervous system demyelination and myelin repair. *Glia, 56*, 686–698.
203. Cohen, J. E., & Fields, R. D. (2008). Activity-dependent neuron-glial signaling by ATP and leukemiainhibitory factor promotes hippocampal glial cell development. *Neuron Glia Biology, 4*, 43–55.
204. Bürgi, S., Samardzija, M., & Grimm, C. (2009). Endogenous leukemia inhibitory factor protects photoreceptor cells against light-induced degeneration. *Molecular Vision, 15*, 1631–1637.
205. Kirsch, M., Trautmann, N., Ernst, M., & Hofmann, H. (2010). Involvement of gp130-associated cytokine signaling in Müller cell activation following optic nerve lesion. *Glia, 58*, 768–779.
206. Bauer, S., Rasika, S., Han, J., Mauduit, C., Raccurt, M., Morel, G., et al. (2003). Leukemia inhibitory factor is a key signal for injury-induced neurogenesis in the adult mouse olfactory epithelium. *The Journal of Neuroscience, 23*, 1792–1803.
207. Banner, L. R., Patterson, P. H., Allchorne, A., Poole, S., & Woolf, C. J. (1998). Leukemia inhibitory factor is an anti-inflammatory and analgesic cytokine. *The Journal of Neuroscience, 18*, 5456–5462.
208. Chesnokova, V., Kariagina, A., & Melmed, S. (2002). Opposing effects of pituitary leukemia inhibitory factor and SOCS-3 on the ACTH axis response to inflammation. *American Journal of Physiology. Endocrinology and Metabolism, 282*, E1110–E1118.