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The LIF/STAT3 Pathway in ES Cell Self-renewal

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1. Introduction

Embryonic stem (ES) cells are pluripotent cells derived from the inner cell mass (ICM) of blastocysts (Evans & Kaufman, 1981; Martin, 1981). Recent progress in establishing human ES cells and human induced pluripotent stem (iPS) cells opens the possibility of utilizing these stem cells in regenerative medicine (Thomson et al., 1998; Takahashi et al., 2007; Yu et al., 2007). There are, however, several issues that remain to be resolved in relation to the application of pluripotent stem cells to cell therapy. One critical issue is how to efficiently amplify these stem cells, since their pluripotent potential means that under commonly used culture conditions they tend to spontaneously differentiate rather than undergo continued self-renewal. Cell therapy will require a large number of stem cells, and it will be necessary to understand the molecular mechanisms of ES cell self-renewal in order to establish efficient *in vitro* expansion systems.

Since the discovery that leukemia inhibitory factor (LIF) can support self-renewal of mouse ES cells (Smith et al., 1988; Williams et al., 1988), researchers have sought to understand the underlying molecular mechanisms. It is now well established that signal transducer and activator of transcription 3 (STAT3), a transcription factor downstream of LIF, plays an indispensable role in the self-renewal of mouse ES cells. Extensive studies have identified many interesting molecules downstream of LIF/STAT3 signaling, including transcription factors, epigenetic regulators, and oncogenes. In this chapter, we will introduce these downstream molecules and discuss their roles in ES cell self-renewal.

2. The LIF signaling pathway and ES cell self-renewal

LIF belongs to the interleukin-6 cytokine family. LIF binds to a heterodimeric receptor consisting of the low-affinity LIF receptor and gp130, with downstream signals being transmitted through gp130. There are a number of signaling pathways downstream of gp130, including the STAT3, phosphatidylinositol 3-kinase (PI3K) and Ras/Erk pathways. In the STAT3 pathway, signaling through gp130 leads to activation of Janus-associated tyrosine kinases (JAKs), which in turn phosphorylate STAT3. Phosphorylated STAT3 dimerizes and translocates to the nucleus, where it functions as a transcription factor. In the PI3K pathway, PI3K phosphorylates phosphoinositides on the 3-OH position of the inositol

ring to generate the second messengers phosphatidylinositol-3,4-bisphosphate (PI-3,4-P2) and phosphatidylinositol-3,4,5-trisphosphate (PIP3), leading to activation of the serine/threonine protein kinase Akt (also known as protein kinase B or PKB). Activated Akt then phosphorylates its target molecules, such as glycogen synthase kinase (GSK)-3 and pro-apoptotic BCL2-antagonist of death (BAD) protein. In the Ras/Erk pathway, Ras stimulates sequential activation of the Raf/MEK/Erk kinase cascade, leading to phosphorylation of Erk target molecules, including the transcription factor Elk-1, pro-apoptotic protein caspase-9, and p90 ribosomal S6 protein kinase (RSK).

In mouse ES cells, the STAT3 pathway plays a critical role in the maintenance of self-renewal. When the function of STAT3 is abrogated by expression of a dominant-negative mutant or gene disruption, ES cells undergo differentiation (Boeuf et al., 1997; Niwa et al., 1998; Ying et al., 2008). Artificial activation of the STAT3 pathway can maintain ES cell self-renewal even in the absence of LIF and can improve the efficiency of establishment of ES cells from ICM (Matsuda et al., 1999; Cinelli et al., 2008). These observations underscore the importance of STAT3 in self-renewal of mouse ES cells. The importance of the LIF/STAT3 pathway in self-renewal has been reported also for chicken and rat ES cells (Horiuchi et al. 2004; Buehr et al., 2008; Li et al., 2008). Similarly, the PI3K pathway positively regulates self-renewal (Paling et al., 2004; Watanabe et al., 2006). On the other hand, activation of the Ras/Erk pathway leads to ES cell differentiation into the endoderm lineage (Yoshida-Koide et al, 2004; Hamazaki et al. 2006), while suppression of Ras/Erk signaling promotes self-renewal (Burdon et al., 1999). Thus, the balance among the three pathways allows fine-tuning of the LIF-mediated maintenance of ES cell self-renewal.

In contrast to mouse ES cells, LIF and STAT3 signaling have no effect on self-renewal of monkey and human ES cells (Dahéron et al., 2004; Sumi et al., 2004). When these findings were reported, it was generally thought that the difference between mouse and primate ES cells reflected a genuine species difference. However, studies from stem cells established from mouse epiblasts (EpiSC) have offered an alternative explanation, since these cells show greater similarity to human ES cells than to mouse ES cells (Brons et al., 2007; Tesar et al., 2007). For example, both human ES cells and mouse EpiSC form flatter colonies than mouse ES cells; require the presence of fibroblast growth factor (FGF)-2 and activin A, but not LIF, for their self-renewal; and cannot contribute significantly to chimeras. These results suggest that human ES cells are, in fact, human EpiSC and that the observed difference between mouse and human ES cells actually reflects the difference between ES cells and EpiSC.

In addition to STAT3, the transcription factors Oct3/4 and Sox2 are also indispensable for ES cell self-renewal (Niwa et al., 2000; Masui et al., 2007). Oct3/4 is a POU family transcription factor encoded by the *pou5f1* gene, and deficiency of this transcription factor during development results in loss of ICM (Nichols et al., 1998). Sox2 is an SRY-related HMG-box protein and *Sox2*-deficient mouse embryos die shortly after implantation (Avilion et al., 2003). In ES cells, both transcription factors show self-renewal-specific expression and are downregulated upon LIF removal. When the expression of Oct3/4 or Sox2 is shut off, ES cells differentiate into trophectoderm cells or trophectoderm-like cells (Niwa et al., 2000; Masui et al., 2007). Interestingly, Oct3/4 and Sox2 have been identified as reprogramming factors that can generate iPS cells from fibroblast cells (Takahashi & Yamanaka, 2006), suggesting a strong role for these factors in determining pluripotency. It has been proposed that STAT3, Oct3/4, and Sox2, together with several other important transcription factors such as Nanog, form transcriptional networks to maintain the self-renewal of ES cells (Boyer et al., 2005; Loh et al., 2006; Chen et al., 2008).

3. Target molecules of the LIF/STAT3 pathway in ES cells

When LIF is removed from the culture medium, mouse ES cells rapidly lose the capacity for self-renewal and differentiate into a variety of cell types. During this process, the expression of self-renewal genes declines and the expression of differentiation-associated genes increases. This suggests that LIF signaling in ES cells promotes expression of self-renewal genes and simultaneously suppresses induction of differentiation-associated genes. How is the STAT3 pathway involved in LIF-mediated maintenance of self-renewal? ChIP-on-chip and ChIP-seq analyses have revealed that STAT3 binds to the regulatory regions of several self-renewal genes in ES cells (Chen et al., 2008; Kidder et al., 2008). Which STAT3 target molecules are involved in the maintenance of ES cell self-renewal? Several groups including ourselves have extensively searched for downstream target genes of STAT3 that regulate ES cell self-renewal and identified a range of genes, as discussed below.

3.1 Transcription factors

GA-binding protein (GABP) belongs to the Ets transcription factor family and forms a heterotetramer consisting of two α-subunits and two β-subunits (Rosmarin et al., 2004). The α-subunit (GABPα) mediates DNA binding, while the β-subunit (GABPβ) enhances the transcriptional activity of the α-subunit. GABPα-deficient embryos die prior to implantation and fail to form a blastocyst (Ristevski et al., 2004). Knockdown of GABPα in ES cells results in the downregulation of Oct3/4 expression and the induction of differentiation-associated genes (Kinoshita et al., 2007). Although expression of GABPα alone is not sufficient for the maintenance of ES cells, overexpression of this transcription factor delays the downregulation of Oct3/4 that occurs during differentiation (Kinoshita et al., 2007). These findings suggest that GABPα is involved in the maintenance of ES cells by positively regulating expression of Oct3/4.

JunB is a member of the AP-1 transcription factor family (Shaulian & Karin, 2001). Like other Jun proteins (c-Jun and JunD), JunB forms either homo- or heterodimers with members of the Fos and ATF protein families. Although JunB-null mouse embryos die between E8.5 and E10.0, JunB-null ES cells are viable and have normal growth potential (Schorpp-Kistner et al, 1999), suggesting that JunB is not necessary for the self-renewal of ES cells.

Krüppel-like factor (Klf)-4 belongs to the KLF zinc finger protein family that shares homology with the *Drosophila* Krüppel segmentation protein (Rowland & Peeper, 2006). Klf4 is expressed in a variety of tissues and plays an important role in many physiological processes, including cell proliferation and terminal differentiation. Klf4 can either activate or repress transcription, depending on the specific target gene, and it can function as an oncogene or a tumor suppressor gene in certain cellular contexts. In ES cells, Klf4 acts as a co-factor for Oct3/4 and Sox2 (Nakatake et al., 2006). Klf4 activates the transcription of Sox2 (Niwa et al., 2009). Overexpression of Klf4 delays or prevents ES cell differentiation (Li et al., 2005; Niwa et al., 2009). Moreover, Klf4 has been shown to be a reprogramming factor (Takahashi & Yamanaka, 2006), and overexpression of Klf4 can drive the conversion of EpiSC to ES cells (Guo et al., 2009). These observations strongly suggest that Klf4 is important in ES cell self-renewal. However, knockdown of Klf4 has no effect on ES cell self-renewal (Nakatake et al., 2007; Jiang et al., 2008), possibly due to redundancy within the Klf family, since simultaneous depletion of Klf2, Klf4 and Klf5 leads to ES cell differentiation (Jiang et al., 2008).

The oncoprotein c-Myc binds to the regulatory regions of numerous self-renewal genes in ES cells (Chen et al., 2008; Kidder et al., 2008; Lin et al., 2009a). Expression of a dominant-negative c-Myc induces ES cell differentiation, and artificial activation of this transcription factor maintains self-renewal even in the absence of LIF (Cartwright et al., 2005), indicating the importance of c-Myc in ES self-renewal. In addition, it has been shown that c-Myc regulates expression of several miRNAs to promote ES cell self-renewal (Lin et al., 2009b).
Pem (also known as Rhox5) is an X-linked homeobox-containing gene product. Overexpression of Pem is sufficient for maintenance of ES cells in the absence of LIF (Fan et al., 1999; Cinelli et al., 2008). On the other hand, Pem-null ES cells do not differ from wild-type ES cells in their morphology and Oct3/4 expression (Fan et al., 1999), suggesting that, although Pem can promote ES cell self-renewal, it is dispensable. Interestingly, when Pem-null ES cells are allowed to differentiate, the level of Oct3/4 does not decline as rapidly as in wild-type ES cells (Fan et al., 1999). These results suggest that Pem plays important roles in both self-renewal and differentiation.
Zinc finger protein (Zfp)-57 is a transcription factor containing a zinc finger motif and a Krüppel-associated box (KRAB) domain. Zfp57 was originally identified as an undifferentiated state-specific gene in embryonal carcinoma cells (Okazaki et al., 1994). Expression of Zfp57 in ES cells is also self-renewal-specific and is lost following LIF removal (Akagi et al., 2005). The successful establishment of Zfp57-null ES cells, however, indicates that expression of this transcription factor is dispensable for the maintenance of ES cell self-renewal (Akagi et al., 2005). Interestingly, detailed analysis of Zfp57-null ES cells has revealed that this transcription factor is involved in imprinting during development (Mackay et al., 2008; Li et al., 2008).
β-catenin is a downstream molecule in the Wnt signaling pathway and plays an important role in tumorigenesis (Reya & Clevers, 2005). In the absence of Wnt signaling, the so-called "degradation complex", which contains GSK-3, phosphorylates β-catenin, leading to its rapid degradation via the ubiquitin/proteasome pathway. On the other hand, Wnt signaling leads to the accumulation of a cytoplasmic pool of β-catenin, which translocates into the nucleus and functions as a co-activator of the T-cell factor (TCF) family proteins. Several reports have shown that activation of the Wnt/ β-catenin pathway promotes ES cell self-renewal (Kielman et al. 2002; Sato et al., 2004; Hao *et al*., 2006; Ogawa *et al*., 2006; Singla *et al*., 2006).
In addition to the Wnt signal, LIF can also enhance nuclear accumulation of β-catenin (Takao et al., 2007), suggesting an additional pathway by which LIF regulates ES cell self-renewal. The mechanism by which LIF signaling stabilizes β-catenin protein is not known. A LIF-stimulated pathway leading to inactivation of GSK-3 through the PI3K/Akt pathway has been described in ES cells (Paling et al., 2004), although the importance of this pathway is unclear since treatment with a PI3K inhibitor did not inhibit the activity of β-catenin and expression of constitutively active Akt fails to promote the nuclear localization and activity of β-catenin (Watanabe et al., 2006). Thus, it is not yet clear whether LIF regulates the stability of β-catenin through activation of the PI3K/Akt pathway in ES cells.
It is likely that multiple molecular mechanisms contribute to the promotion of ES cell self-renewal by β-catenin (Fig. 1). Activation of β-catenin results in the upregulation of STAT3 mRNA (Hao et al., 2006), and c-Myc is also a target gene of the β-catenin/TCF complex. Furthermore, β-catenin physically associates with Oct-3/4 and can upregulate Nanog in an Oct3/4-dependent manner (Takao et al., 2007). Although several studies have shown the importance of β-catenin in the self-renewal of ES cells, it is not essential since the establishment of β-catenin-null ES cells has been reported (Huelsken et al., 2000). This may

reflect functional compensation by γ-catenin, a protein highly homologous to β-catenin (Takao et al., 2007).

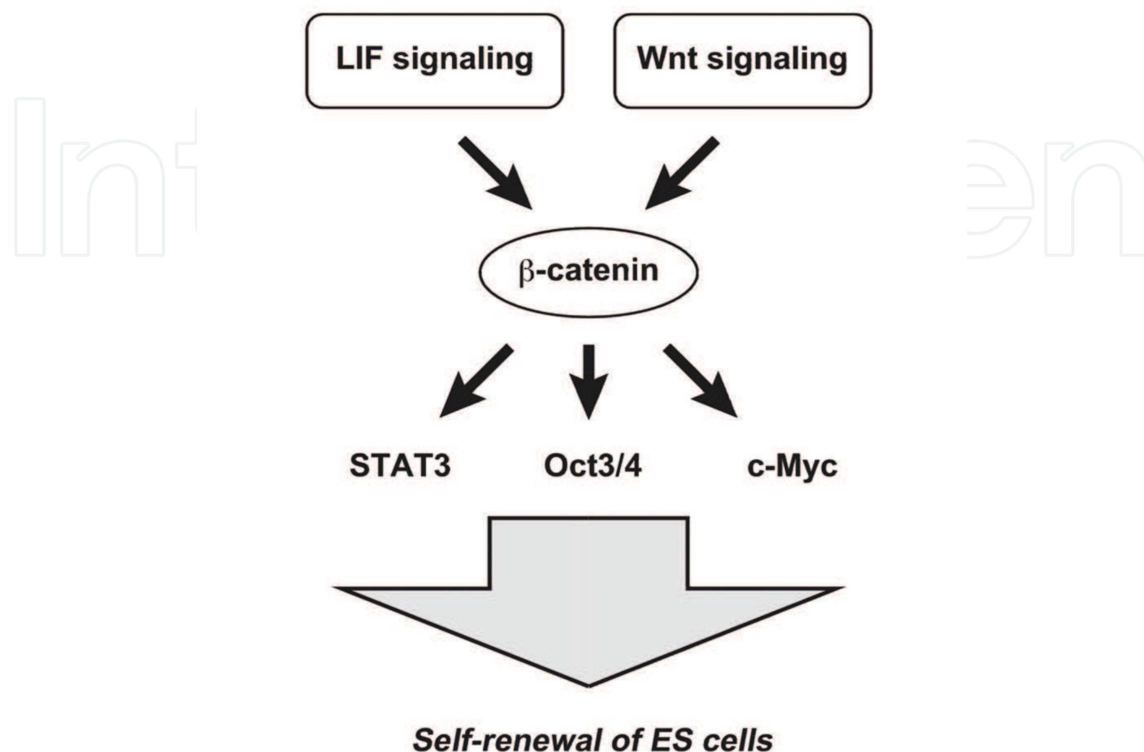

Fig. 1. LIF and Wnt signalings promote ES cell self-renewal by enhancing the stabilization of β-catenin. As a result, β-catenin translocates to the nucleus and acts as a co-activator of TCF, leading to the induction of c-Myc. Activation of β-catenin also results in the induction of STAT3 by unknown mechanism. In addition, β-catenin forms a complex with Oct3/4, which can stimulate Nanog expression

3.2 Transcriptional repressors

The orphan nuclear receptor Dax1 (DSS-AHC critical region on the X-chromosome gene 1; also known as Nr0b1 and Ahch) was originally identified as a gene responsible for the congenital disease dosage-sensitive sex reversal (DSS) and for adrenal hypoplasia congenita (AHC); in humans, gene duplication causes male-to-female sex reversal, while mutations in DAX1 result in AHC (Niakan & McCabe, 2005). Dax1 also plays an important role in the establishment and maintenance of steroid-producing tissues such as testis and adrenal cortex. Dax1 is expressed in self-renewing ES cells under the regulation of STAT3 and Oct3/4 (Clipsham et al., 2004; Sun et al., 2008), and its importance in ES cell self-renewal has been suggested by both knockdown and knockout experiments (Niakan et al., 2006; Wang et al., 2006; Khalfallah et al., 2009). Interestingly, Dax1 binds Oct3/4, leading to suppression of the DNA binding activity and transcriptional activity of Oct3/4 (Sun et al., 2009). The observation that Dax1-overexpressing ES cells have a similar phenotype to Oct3/4-knockdown ES cells further supports the role of Dax1 as a negative regulator of Oct3/4 in ES cells. Since overexpression of Oct3/4 activity results in ES cell differentiation (Niwa et al., 2000), Dax1 may have a major role in neutralizing the excess activity of Oct3/4. The

existence of a regulatory loop between Dax1 and Oct3/4 raises the possibility that Dax1 is not just a negative regulator but a fine-tuner of Oct3/4 activity (Fig. 2).

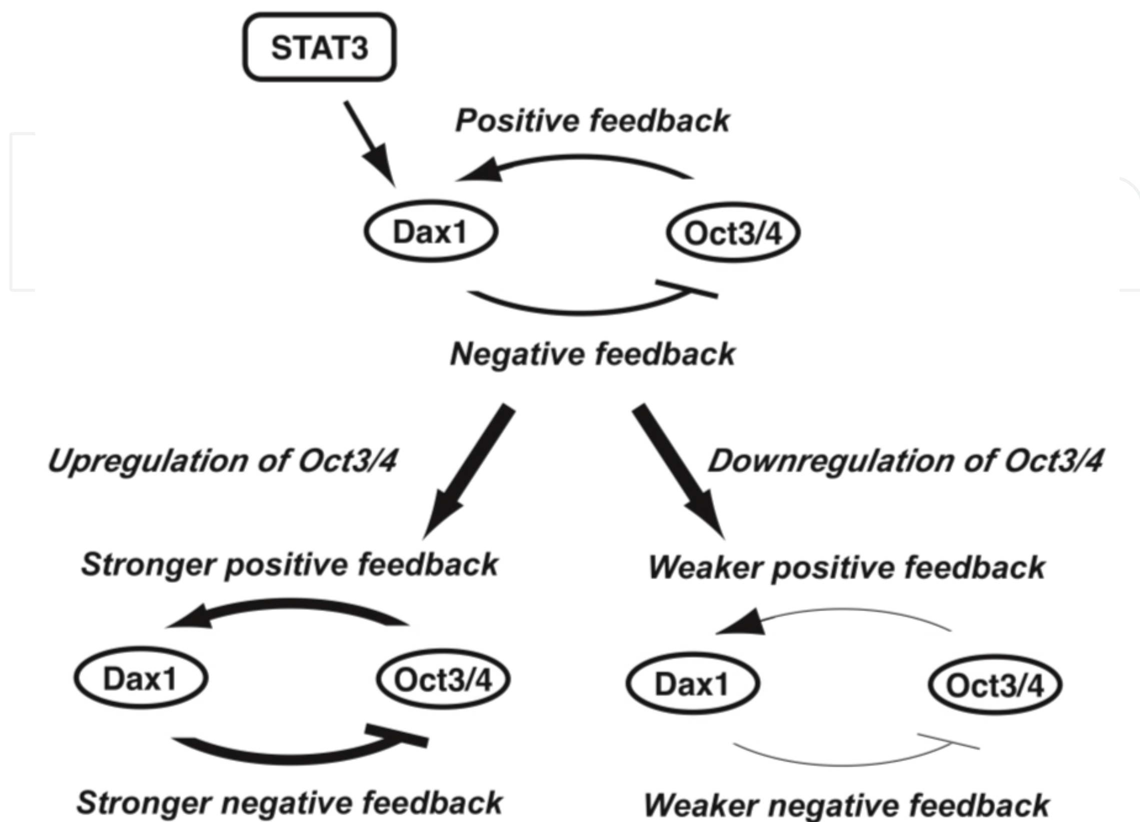

Fig. 2. Dax1 may function as a fine-tuner of Oct3/4 activity in ES cells. Dax1 negatively regulates the transcriptional activity of Oct3/4, whereas Oct3/4 and STAT3 positively regulate the expression of Dax1. When an excess amount of Oct3/4 exists in ES cells, it triggers the induction of Dax1 protein, which in turn suppresses the activity of Oct3/4. On the other hand, when Oct3/4 is downregulated, the expression level of its negative regulator, Dax1, also decreases, which allows Oct3/4 to maintain its activity at the normal level. Thus, the suppression of Oct3/4 by Dax1 depends on the activity of Oct3/4, and this dependency enables Dax1 to act as a "fine-tuner" that maintains the Oct3/4 activity at an appropriate level in ES cells.

Sall4 is a zinc-finger transcription factor that was originally cloned based on sequence homology to *Drosophila spalt* (*sal*), which is a homeotic gene essential for the development of posterior-head and anterior-tail segments (de Celis & Barrio, 2009). In humans, mutations in SALL4 cause an autosomal dominant disorder known as Okihiro syndrome or Duane-radial ray syndrome. Sall4 functions as a strong transcriptional repressor by associating with the Mi-2/Nucleosome remodeling and deacetylase (NuRD) complex (Lu et al., 2009; Yuri et al., 2009). Sall4-null ICM proliferates poorly, and Sall4-deficient mice die shortly after implantation (Sakaki-Yumoto et al., 2006). In agreement with these findings, Sall4-null ES cells exhibit retarded proliferation and express the trophectodermal marker Cdx2. ChIP-on-chip analysis reveals that Sall4 binds to a broad variety of genes that may be important for stem cell functions (Yang et al., 2008). Furthermore, Sall4 forms a complex (or complexes) with Nanog and Oct3/4 (Wang et al., 2006; Wu et al., 2006; Liang et al., 2008). On the other hand, Sall4-null

ES cells can be maintained for long periods, express Oct3/4 at normal level, and generate chimeric mice (Sakaki-Yumoto et al., 2006; Yuri et al., 2009). These observations suggest that Sall4 is essential for stabilization, but not for pluripotency, of ES cells.
Amino-terminal enhancer of split (Aes1, also known as Grg5) belongs to the Gro/TLE family and is a direct target of STAT3 in ES cells (Sekkaï et al., 2005). Gro/TLE family proteins function as transcriptional repressors and negatively regulate several signaling pathways such as Wnt and Notch (Gasperowicz & Otto, 2005). Aes1 lacks the C-terminal region common to most Gro/TLE family proteins and under certain circumstances can block Gro/TLE-mediated transcriptional repression in a dominant-negative fashion. Aes1-null mice show no severe phenotype except postnatal growth retardation (Mallo et al., 1995), suggesting that Aes1 is dispensable for ES cell self-renewal.

3.3 Epigenetic modulators

Embryonic ectoderm development (Eed) was originally identified as a gene that regulates early ectoderm development. Eed belongs to the polycomb family and forms the PRC2 complex together with polycomb proteins Suz12 and Ezh2 (Simon & Kingston, 2009). The PRC2 complex can suppress gene expression by catalyzing the methylation of Lys-27 on histone H3. Eed deficiency in ES cells results in the loss of PRC2 activity and the induction of a variety of differentiation-associated genes (Azura et al., 2006; Boyer et al., 2006; Ura et al., 2008), suggesting that the role of Eed in ES cells is to suppress the expression of differentiation-associated genes via the PRC2 complex. Since expression of Eed is also regulated by Oct3/4 (Ura et al., 2008), it is likely that STAT3 and Oct3/4 both regulate PRC2 activity by controlling Eed expression (Fig. 3). Despite the expression of differentiation-associated genes, Eed-null ES cells can be cultured for a long time and maintain the expression level of Oct3/4, suggesting that Eed is required for the stabilization of ES cell self-renewal.

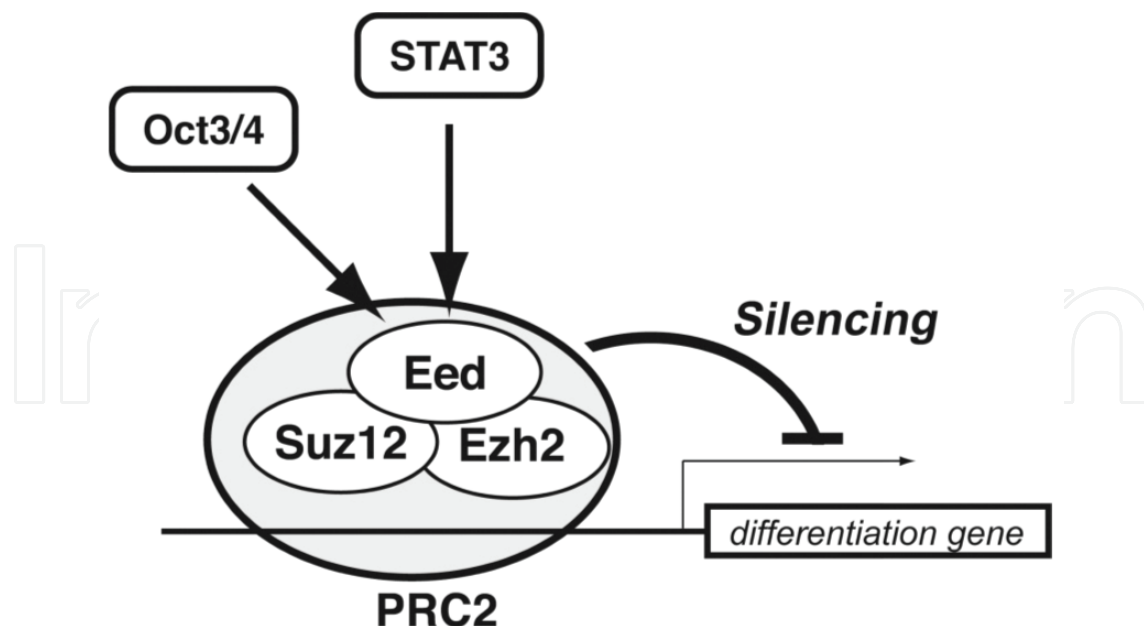

Fig. 3. Eed is involved in the silencing of differentiation-associated genes. PRC2 mainly consists of three subunits, Eed, Ezh2 and Suz12. STAT3 and Oct3/4 regulate Eed expression to maintain the activity level of PRC2, and thereby suppress the induction of differentiation-associated genes

In addition to Eed, which regulates methylation of histone H3, STAT3 controls the expression of jumonji domain containing 1A (Jmjd1a), a demethylase of Lys-9 of histone H3 (Ko et al., 2006). Lys-9 methylation also contributes to the suppression of gene expression, indicating that the demethylase activity of Jmjd1a may be involved in the activation of gene expression in ES cells. Indeed, Jmjd1a is known to regulate expression of self-renewal genes in ES cells, including Tcl1, Zfp57 and Tcfcp2l1 (Loh et al, 2007). Knockdown of Jmjd1a results in ES cell differentiation, which can be partially reversed by overexpression of Tcl1. Since Tcl1 has been shown to regulate self-renewal of ES cells (Ivanova et al. 2006; Matoba et al. 2006), these results suggest that Jmjd1a plays an important role in the maintenance of ES cells by upregulating Tcl1 expression.

3.4 Kinases

In addition to transcription-related genes, the LIF/STAT3 pathway regulates expression of other classes of genes, for example, kinases. The *pim* genes encode serine/threonine kinases, Pim-1, Pim-2 and Pim-3, which regulate cell growth and apoptosis (Bachmann & Möröy, 2005). The expression of both Pim-1 and Pim-3 is regulated by STAT3 in ES cells (Aksoy et al., 2007). Overexpression of Pim-1 and Pim-3 promotes self-renewal and knockdown of these genes increases the rate of spontaneous differentiation and apoptosis (Aksoy et al., 2007), suggesting that these kinases have important roles in ES cell self-renewal.

The Akt-related kinase serum/glucocorticoid-regulated kinase (SGK) can phosphorylate and inactivate GSK-3, a kinase that is known to regulate β-catenin levels in ES cells (Tessier & Woodgett, 2006). SGK is downstream of both the STAT3 and PI3K pathways (Kobayashi & Cohen, 1999; Park et al., 1999; Bourillot et al., 2009). As described above (Section 3.1), the molecular mechanism behind the LIF-mediated activation of β-catenin is unknown. The ability of SGK to inactivate GSK-3 raises the intriguing possibility that the LIF/STAT3 pathway can stabilize β-catenin protein through SGK-mediated inactivation of GSK-3.

3.5 Other target molecules

Stem cell-derived differentiation regulator (Sddr, also known as Moep19, Ooep and Floped) contains the domain similar to KH domain, which binds to RNA. Sddr is highly expressed in self-renewing ES cells, and is downregulated when ES cells undergo differentiation (Miura et al., 2010). Despite the self-renewal-specific expression, studies with Sddr-null ES cells indicate that this protein is dispensable for self-renewal (Miura et al., 2010). However, since Sddr deficiency promotes ES cell differentiation, Sddr may play a role in switching from self-renewal and differentiation.

Embryonic stem cell-specific gene 1 (Esg1, also known as H34, Ecat2 and Dppa5) is another KH domain-containing protein that is specifically expressed in self-renewing ES cells (Tanaka et al., 2002). Similar to the case of Sddr, Esg1 is likely to be dispensable for self-renewal, since Esg1-null ES cells can be established and show no abnormality (Amano et al., 2006).

Suppressor of cytokine signaling (Socs)-3 is an SH2 domain-containing protein that plays an important role in placental development and immunological processes (Kubo et al., 2003). Socs3 inhibits JAK tyrosine kinase activity through a kinase inhibitory region in its N-terminal domain. As a result, Socs3 functions as a feedback regulator of LIF/STAT3 signaling and its overexpression blocks the self-renewal of ES cells (Ying et al, 2003). It was therefore surprising that Socs3-null ES cells also exhibit impaired self-renewal and increased differentiation into primitive endoderm (Forrai et al., 2006). Detailed analysis revealed that Socs3 deficiency enhances Ras/Erk signaling in addition to its effect on STAT3, and that the

self-renewal activity of Socs3-null ES cells can be recovered by inhibiting the Ras/Erk pathway. It is likely therefore that Socs3 maintains the balance between the STAT3 and the Ras/Erk pathways in ES cells.
STAT3 also regulates the expression of the plasma membrane-associated molecule CD9 (Oka et al., 2002). ES cell colony formation and cell viability are reduced by the addition of anti-CD9 antibody to the culture medium (Oka et al., 2002). However, CD9-null ES cells exhibit normal morphology and growth properties, and normal expression of self-renewal markers (Akutsu et al., 2009), indicating that expression of CD9 is not essential for ES cell self-renewal.

4. The role of the LIF/STAT3 pathway in ES cell self-renewal

Identification of STAT3 targets helps us to understand the molecular mechanisms by which the LIF/STAT3 pathway regulates ES cell self-renewal. The known STAT3 targets can be divided into several groups based on their functions and it is likely that the LIF/STAT3 pathway plays multiple roles in the maintenance of self-renewal (Fig. 4).
One major role of the LIF/STAT3 pathway is to form transcriptional networks with other key transcription factors such as Oct3/4, Sox2, Nanog, c-Myc, Klf4, Esrrb and Sall4. Some of these transcription factors are themselves regulated by the LIF/STAT3 pathway, including c-Myc, Klf4, and Sall4. Although Oct3/4 is not a downstream molecule of the LIF/STAT3 pathway, its activity and expression are regulated in various ways by STAT3 targets. For example, the expression and transcriptional activity of Oct3/4 are positively regulated by GABPα and β-catenin, respectively, while Oct3/4 activity is negatively regulated by Dax1. In turn, STAT3-regulated transcription factors bind to the regulatory regions of other important factors and induce their expression. In this way, the LIF/STAT3 pathway participates in the formation of transcriptional networks.
The LIF/STAT3 pathway also regulates chromatin structure. The chromatin in self-renewing ES cells exhibits increased accessibility due to fewer and more loosely bound histones and architectural proteins (Meshorer and Misteli, 2006). When ES cells undergo differentiation, their chromatin structure changes dynamically in response to global histone modifications, and this mechanism regulates gene activation and repression during development (Kouzarides, 2007). For example, methylation of Lys-4 of histone H3 is associated with transcriptional activation, whereas methylation of Lys-9 or Lys-27 of histone H3 is linked to transcriptional silencing. The LIF/STAT3 pathway regulates expression of molecules such as Eed and Jmjd1a that are involved in histone modifications, and thereby controls chromatin structure in ES cells.
In addition to transcription factors, the LIF/STAT3 pathway also communicates with other signaling pathways. For example, there may be cross-talk between the LIF/STAT3 and Wnt signaling pathways due to their shared effects on β-catenin (Fig. 1). In fact, LIF and Wnt exhibit a synergistic effect on the maintenance of ES cell self-renewal (Ogawa et al., 2006). The LIF/STAT3 and Wnt pathways may interact with each other through Aes1, which is believed to exert dominant-negative activity on Gro/TLE family proteins that, in turn, can negatively regulate the Wnt pathway. The LIF/STAT3 pathway may also communicate with PI3K through its ability to regulate the expression of Tcl1 through Jmjd1a. Tcl1 can bind to Akt and enhance its kinase activity (Laine et al., 2000; Pekarsky et al., 2000), suggesting a plausible link between the PI3K/Akt and LIF/STAT3 pathways. The identification of SGK as a downstream molecule of STAT3 raises the possibility that this

kinase may act as an additional connector molecule between STAT3, PI3K and Wnt, since the activity of SGK is regulated by the PI3K pathway, and SGK can control the Wnt pathway through GSK-3.

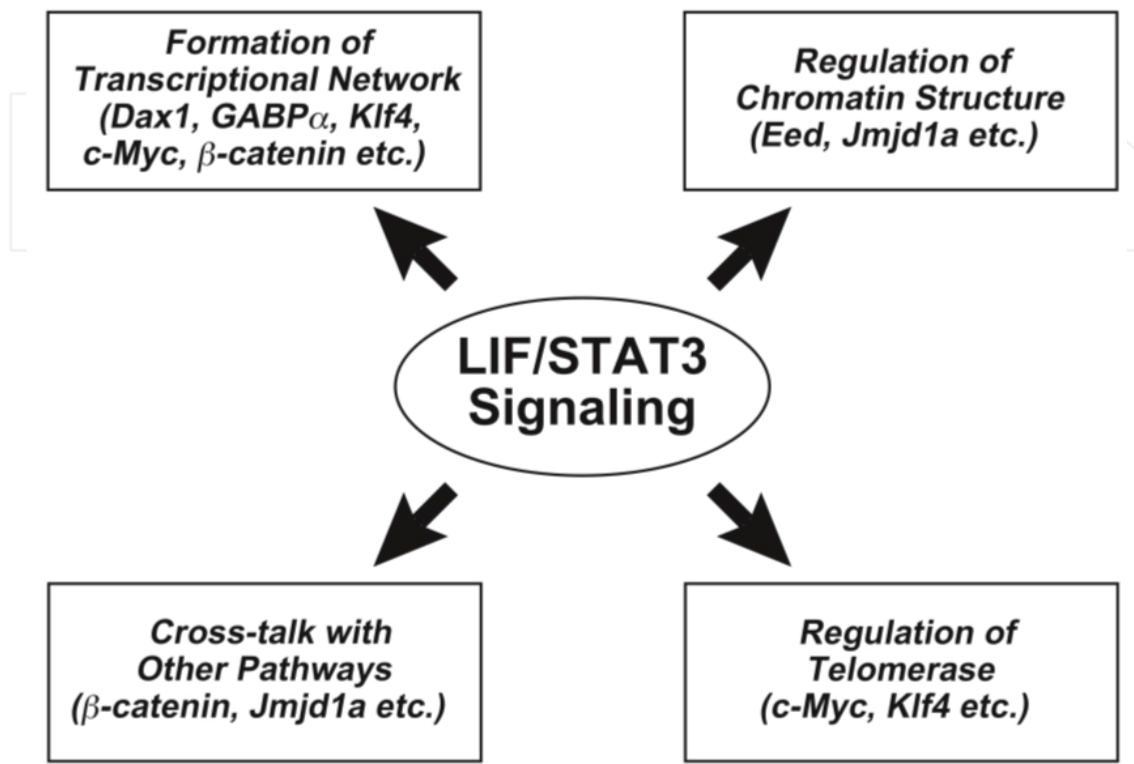

Fig. 4. Role of the LIF/STAT3 pathway in the self-renewal of mouse ES cells. The LIF/STAT3 pathway regulates the expression of a variety of genes, and thereby plays multiple roles in the maintenance of self-renewal in mouse ES cells

Unlike most somatic cells, ES cells are immortal and capable of indefinite self-renewal. This unique ability is supported by high levels of telomerase activity in self-renewing ES cells. The expression of telomerase reverse transcriptase (Tert) is essential for this telomerase activity (Liu et al., 2000), although the mechanisms that regulate Tert expression in ES cells are not known. Among the STAT3 targets described above, c-Myc has been shown to regulate Tert expression in primary human fibroblasts (Greenberg et al., 1999) and Klf4 can stimulate Tert expression in human cancer cells and ES cells (Wong et al., 2010). GABPα and c-Myc have also been identified as candidate regulators of Tert in mouse ES cells, based on RNAi screening (Coussens et al., 2010). Therefore, the LIF/STAT3 pathway may regulate expression of Tert through its target genes such as c-Myc.

5. Conclusion

This chapter summarizes current knowledge on the role of the LIF/STAT3 pathway in the self-renewal of mouse ES cells. The LIF/STAT3 pathway stimulates the expression of several classes of genes, which play important roles in self-renewal through a range of activities such as the formation of transcriptional networks, suppressing expression of differentiation-associated genes, and communicating with other signaling pathways. In addition, we would also add that STAT3 can function as a transcriptional repressor. STAT3 associates with co-

activators, such as steroid receptor co-activator 1 (SRC1/NcoA) and p300/cAMP response element binding protein (CBP), but it can also associate with co-repressors, including KRAB-associated protein-1 (Kap1/Tif1β), to inhibit transcription (Tsuruma et al., 2008). STAT3 binds to the regulatory regions of many differentiation-associated genes (Chen et al., 2008; Kidder et al., 2008) and it is therefore likely that STAT3 suppresses expression of differentiation-associated genes in ES cells by both direct and indirect effects on gene expression.

Our understanding of the LIF/STAT3 pathway is not restricted to ES cell self-renewal; for example, STAT3 is activated in a variety of tumors and has been described as an oncogene (Yu & Jove, 2004). It is likely that ES cells and cancer cells may use several STAT3 targets in common for their growth, and this hypothesis is supported by the fact that the list of STAT3 target genes contains well-established tumor-related genes such as c-Myc, β-catenin, and Pim. Since the discovery of cancer stem cells, it has been demonstrated that tumor growth and stem cell self-renewal share several signaling pathways including the Wnt pathway (Reya & Clevers, 2005). Therefore, it would be interesting to perform a detailed comparison of the key molecular mechanisms in STAT3-mediated self-renewal of ES cells and STAT3-mediated tumorigenesis.

Due to the finding that human ES cells do not respond to LIF, the importance of the LIF/STAT3 pathway has been neglected. However, we now know that so-called human ES cells are not true ES cells but EpiSC. Furthermore, it has been shown recently that human "ES" cells, which are LIF-dependent and show greater similarity to ES cells than to EpiSC, can be generated, although we still need some genetic manipulations (Hanna et al., 2010). Understanding the role of the LIF/STAT3 pathway in mouse ES cells may give us valuable clues to support the development of an efficient expansion system for human ES and iPS cells in future.

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7. References

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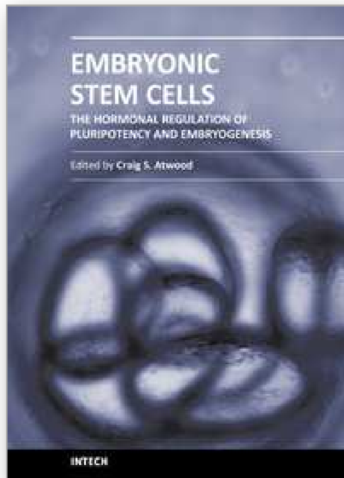

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