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Regulation of Embryonic Stem Cell Self-Renewal

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Abstract

Embryonic Stem Cells (ESC) are type of cells capable of self-renewal and multi-directional differentiation. The self-renewal of ESCs is regulated by factors including signaling pathway proteins, transcription factors, epigenetic regulators, cytokines, and small molecular compounds. Similarly, non-coding RNAs, small and miRNAs also play an important role in the process. Functionally, the core transcription factors interact with helper transcription factors to activate the expression of genes that contribute to maintenance of pluripotency, thereby inhibiting expression of differentiation-related genes. In addition, cytokines such as leukemia suppressor factor (LIF) also activate downstream signaling pathways and promote self-renewal of ESC. Particularly, LIF binds onto its receptor (LIFR/gp130) to activate downstream Jak-Stat3 signaling pathway. In the absence of serum, BMP4 activates the downstream pathway and acts in combination with Jak-Stat3 to promote pluripotency of ESCs. In addition, activation of another signaling pathway, Wnt-FDZ, has been observed to facilitate self-renewal of ESC. Small molecule modulators of the above pathway proteins are widely used in *in vitro* culture of stem cells. The aspect of epigenetic status of ESCs is also critical in maintenance of ESC self-renewal with various epigenetic regulators involved in maintaining this process. Similarly, non-coding RNAs have been described to promote maintenance of self-renewal in ESCs. The described factors form signal networks that regulate self-renewal and differentiation of embryonic stem cells. This paper reviews the role of the above factors in the self-renewal of embryonic stem cells.

Keywords: Embryonic stem cells (ESC), Self-renewal, Transcription factors, Signaling pathways, Small molecular compounds, Epigenetics, Non-coding RNA

In vitro Culture and Stemness Maintenance of Embryonic Stem Cells

Embryonic stem cells are a group of cells derived from embryo blastocyst period, type of cell capable of self-renewal and multi-directional differentiation [1]. During development, ESCs can differentiate into cells of the ectoderm, mesoderm and endoderm [2]. The entire development process is controlled by multiple factors. For instance, ESCs maintains their pluripotency by specifically expressing essential transcription factors, while spatial and temporal expression and silencing of genes related to cell fate determines cell differentiation [3].

Since the establishment of the first mouse ESC line by Martin Evans et al. in 1981, research on ESC has been a hotspot in regenerative medicine research [4], after then the ESC derivation from earlier stages of embryos were reported [5,6]. Unlike adult stem cells, ESCs are pluripotent and capable of differentiating into all tissues and organs of adult animals including germ cells under appropriate induction conditions [7]. Meanwhile, under appropriate culture conditions, ESCs are capable of indefinitely multiplying and maintaining self-renewal. Despite the rapid development in adult stem cell research in recent years, ESCs still play an irreplaceable role in gene function analysis, developmental biology, drug design and development [8]. Research in and utilization of ESCs are therefore still one of the core issues in the field of stem cell research.

During *in vitro* culture of ESCs, specific culture conditions are adopted to generate either a "steady", or "ground" state [9]. In

the initial stages of establishment and maintenance of ESC, mouse fibroblasts were used as trophoblast cells and were cultured on the feeder after irradiation or drug treatment. Subsequent studies revealed that, during this process, the role played by trophoblast cells was production of leukemia inhibitory factor (LIF) [10-12]. However, in the presence of serum, LIF can replace the function of trophoblast and maintain ESC growth without differentiation. Later, it was found that BMP4 played a beneficial role in growth of stem cells and could therefore replace the serum requirements of cells in the presence of LIF [13,14]. These discoveries enabled researchers to grow stem cells in the presence of serum and non-trophoblast cells, or in the serum replacement condition *in vitro* [15], thereby greatly facilitating research and application. Functional studies have confirmed that LIF activates downstream JAK/STAT3 by binding onto receptor LIFR and helper receptor gp130 [16], while BMP4 maintains stem cell differentiation by activating Smad, which induces helix-loop heliform basic protein production. In addition, the core transcription factors Oct3/4 [17] and Nanog [18,19] also play key roles in maintaining the ground state of stem cells. In general, a number of transcription factors, include Oct4, Nanog, Stat3, Sox2, c-Myc, Esrrb, Klf4, Ronin, Tcl1, Tbx3 and Rest, with or without their co-interaction factors, have been found to regulate mESC stemness [20-22].

Since establishment of the first human ESC (hESC) cell line in 1998, various studies have attempted to elucidate how growth of these cells are regulated, but to date, this process remains unclear [20]. Then, the haploid hESC cell lines have been isolation and maintenance [23]. Unlike in mESCs, hESCs self-renewal is not regulated by LIF and STAT3 [24-26]. A study reported growth of hESC in a serum-free culture medium containing bFGF under the condition of trophoblast cells [27]. In addition, these cells could successfully be cultured with mouse MEF conditioned medium (MEF CM) on a cell culture plate coated with gelatin or laminin under the condition of trophoblast cells [28]. Sato et al. [24] found that activation of Wnt signaling pathway could replace the need for hESC in MEF CM at the early stage (5-7 d) and maintain their undifferentiated state. However, it is not clear whether the Wnt signaling pathway plays a role in the long-term stemness of hESC. A high expression of Oct4 [29] and Nanog [24,30,31] in hESC confirmed the role played by transcription factors in maintenance stemness in these cells, similar to human long intergenic noncoding RNAs (lincRNAs) [32-34]. However, the mechanism to maintain stemness and self-renewal of hESC needs further study.

This review will focus on the role of major transcription factors, signaling pathways, small molecular compounds, epigenetic regulation and non-coding RNA in ESC self-renewal.

Transcription Factors that Regulate Pluripotency in Stem Cells

Transcription factors act as molecular switches to activate or inhibit gene expression and control the fate of cells during development. Oct3/4, Sox2 and Nanog are core transcription factors involved in maintaining pluripotency of ESC and therefore play a key role in maintaining the ground state of ESCs [35]. Of them, Oct-3/4 is the most important factor in ESC. It is encoded by the *Pou5f1* gene and contains 2 POU domains. Conditional expression of Oct-3/4 in ESCs has been shown to promote maintenance of pluripotency as well as the ability of multipotent differentiation. On the other hand, inhibition of Oct-3/4 expression results in loss of pluripotency and the inability of multipotent differentiation. Oct4 has been found to promote maintenance of pluripotency in ESCs but cannot inhibit their differentiation [36], so as the mechanism of OCT4 modifications [37,38]. Another transcription factor that plays a key role in embryo formation is Nanog [39,40]. Expression of this factor occurs in the early stages of embryonic development with an expression in undifferentiated compared to differentiated mouse or human stem cells. In addition, its overexpression promotes unlimited expansion of ESC and maintains the Oct3/4 expression levels in ESC [41]. Sox2 has synergistic effect with Oct-3/4 and activates Oct-Sox enhancers. This activation, in turn, enhances its expression as well as that of Nanog and Oct3/4 thereby maintaining pluripotency of ESC [42].

These core transcription factors exist in mouse and human ESCs. They have also been shown to bind onto a regulatory region of common target genes and exert synergistic effects [43-45]. In hESCs, the common area occupied by these factors during gene regulation is at least 353 genes. In mESCs, Oct3/4 and Sox2 also synergistically activate transcription of downstream target genes [46-48].

Several other transcriptional and regulatory factors are also involved in maintaining self-renewal and pluripotency of stem cells. In the core transcriptional regulatory network, core Oct 3/4 modules include Oct4, Sox2, Nanog, BMP, LIF, Wnt signals, and downstream Smad1, STAT3, and Tcf3. These extracellular signaling pathways and core transcription factors form a regulatory network, jointly regulating stem cell pluripotency. The transcriptional factors including Nanog, Oct3/4, STAT3, Smad1, Sox2, Zfx, c-Myc, n-Myc, Klf4, Esrrb, Tcfcp2l1, E2f1, CTCF as well as their regulators p300 and Suz12, form various transcriptional complexes and play an important role in regulation of self-renewal of stem cells [49,50]. The core transcriptional regulatory clusters formed by Nanog-Oct3/4-Sox2 show ESC-specific transcriptional enhancer activity, which interacts with Smad1 and STAT3 downstream of BMP and LIF signaling pathways. This process plays a key role in stabilizing transcription factor complexes thereby enhancing the transcriptional activity of ESC-specific genes [51,52]. Wnt signaling pathways downstream of transcription factor Tcf3 acts together with Oct3/4 and Nanog to occupy downstream gene promoter regions and regulate expression of these

genes. This transcription regulation of composite components is responsible for maintaining pluripotency of ESC and a steady state condition [53].

Other pluripotent regulators, including Dax1, Nac1, Zfp281, Nr5a2 and Klf4 [54-56], form protein complexes with Oct3/4 core regulatory modules, bind to the distal region of the transcription initiation site and regulate expression of Oct3/4. An interaction between Oct3/4 and these transcription regulators plays a key role in the complex. Other transcription factors c Myc, n Myc, E2f1, Zfx, Rex1 and Ronin bind to sites near the site of transcription initiation and regulate metabolism of downstream target proteins [52,57].

Studies have reported that excessive expression of genes involved in stemness in mESC is not a prerequisite for maintenance of pluripotency of the stem cells. In fact, continuous expression of Oct3/4 and Sox2 was found to disrupt homeostasis of stem cells and lead to stem cell differentiation [17,19]. Similarly, a high expression of stemness-related genes disrupts the balance of the stem cells themselves as well as that of most of the key factors (Oct3/4, Sox2, Nanog, Esrrb, Sall4, Dax1, Klf2, Klf4, Klf5, Stat3, Tet and Tcf3) [58-64].

An Important Signaling Pathway that Regulates the Fate of Embryonic Stem Cells

Signal transduction among mammalian cells can be achieved by direct contact with the adjacent cells. During this process, specific proteins secreted by the cells and a combination of chemical substances interact with cell surface receptors, thereby activating transcription factors through downstream signaling pathways for regulation of downstream gene expression. Multiple signaling pathways, are involved in regulating the fate of stem cells, including proliferation, differentiation and maintenance of stemness. These processes can be regulated by adding small molecule modulators to activate or inhibit the signaling pathways. The important signaling pathways that regulate the fate of stem cells are described below.

Bidirectional regulation of downstream LIF-LIFR /gp130 signaling pathway

LIF-mediated activation of the Jak-Stat3 pathway promotes mESC stemness maintenance: Initial establishment and maintenance of initial ESC by MEF cells involved the use of trophoblast. Later studies revealed that the role played by feeder cells in maintenance of pluripotency of stem cells is largely through production of leukemia inhibitory factor (LIF), a member of the IL-6 cytokine family [65]. The IL-6 cytokine family comprises IL-6, IL-11, LIF, Ciliary neutrophilic factor (CNTF), Cardiotrophin-1(CT-1), Oncostatin M (OSM) and other cytokines with similar biological effects. Among them, OSM, CNTF, CT-1 and LIF are known to control non-differentiation in ESCs [66,67]. ESCs do not express a receptor for IL-6, therefore IL-6 does not exhibit the afore mentioned effects. On the other hand, IL-6 and its soluble receptor act on ESCs together to maintain the non-differentiation of ES cells. Different IL-6 families share a common helper receptor, gp130, a transmembrane protein with no kinase activity belonging to the cytokine receptor superfamily. Its signal transmission relies on the intracellular non-receptor tyrosine protein kinase JAK [68]. Phosphorylation of gp130 at specific Tyr residues sites activates downstream signaling molecules such as transcription factors, SH2, STAT3 to initiate expression of target genes [69], Studies have shown that gp130 binds to STAT3, mainly through phosphorylation of Tyr in four YXXQ sequences. Deletions or mutations in these sites hinders the gp130 binding and activates STAT3 [70], self-renewal of the resultant ESCs cannot be maintained. LIF-STAT3 signal maintains mESC self-renew instead of hESC [16,24]. Similarly, decreased expression of STAT3 was found to promotes ESCs differentiation [71,72], indicating that activation of STAT3 is required for maintenance of self-renewal in mESC.

Activation of PI3K signaling pathway is conducive to mES cell self-renewal

Studies have demonstrated that LIF not only activates the Jak-Stat3 pathway through gp130, for maintenance of self-renewal in mouse ESCs, but also acts on phosphoinosidide 3 kinase (PI3K) through gp130. This plays an important role in maintenance of stemness of ESCs in humans and mice [73]. PI3K catalyzes phosphorylation of PI (4,5) P2 to form PI (3,4,5) P3, while Akt is phosphorylated by PDK1 downstream of PI (3,4,5) P3 to enable it act on downstream targets for regulation of various life activities. In mESC, LIF can activate the PI3K pathway and increase phosphorylation levels of intracellular Akt, thus promoting self-renewal of ES cells and inhibiting cell differentiation. In the event of gene mutations, inactivation of catalytic subunit PI3K occurs and the PI3K pathway is blocked by its specific inhibitor LY294002. The rate of phosphorylation of Akt is reduced leading to an increase in intracellular ERK and in turn causing ESCs differentiation [74]. Effect of the PI3K signaling pathway on regulation of proliferation was reported by Sun., et al. following knockout of PTEN in ESCs. PTEN, is a phosphatase that can dephosphorylate PI (3,4,5) P3 thus inhibiting the PI3K signaling pathway [75,76]. Knocking out of PTEN could not dephosphorylate PI (3,4,5) P3. As a result, levels of intracellular PI (3,4,5) P3 as well as phosphorylation of downstream signaling molecule Akt remained high leading to accelerated cell proliferation and reduced cell apoptosis, maintain pluripotency of ESC [75,77].

Activation of SHP2-Ras-ERK pathway leads to differentiation of ES cells: In addition to activating the JAK-STAT3 pathway and PI3K pathway through gp130, LIF can also activate another major downstream signaling pathway, SHP2-Ras-ERK, through gp130. The protein phosphatase SHP2 downstream of gp130 is a signal molecule containing the SH2 domain, which mediates the binding between gp130 and Ras-

ERK, thereby activating ERK, inhibiting self-renewal of ES cells and accelerating their differentiation. By blocking the binding of SHP2 to gp130 through gene mutation, Ras-ERK pathway can be inhibited, and the activation time of STAT3 induced by LIF stimulation is prolonged [78]. If the junction protein between SHP2 and downstream Ras is absent, ES cells will express inhibited H-Ras and block the activation of ERK in Ras-ERK signaling pathway [79], which will promote the self-renewal of ESC. Activation of the SHP2-Ras-ERK pathway is necessary for normal differentiation of ES cells, and blocking the SHP2-Ras-ERK pathway will lead to abnormal differentiation of ES cells [80,81]. Mice with SHP2 mutation cannot differentiate into hematopoietic cells and fibroblasts [82].

In summary, LIF-mediated signal network is bidirectional in maintaining mESC pluripotency. When LIF acts on ES cells, it can activate the JAK-STAT3 signaling pathway through gp130 to promote cell self-renewal, activate the PI3K signaling pathway to promote cell proliferation and growth, and at the same time may activate the SHP2-Ras-ERK pathway to lead to ES cell differentiation. ES cell can only maintain self-renewal if it maintains a precise balance among the three pathways.

BMP combined with LIF promotes self-renewal of ES cells in serum free condition

In the presence of serum, LIF can replace the function of trophoblast and maintain the growth process of mESC without differentiation. In the absence of serum, LIF alone cannot completely prevent mESC differentiation, and a small number of cells will differentiate into nerve cells [13]. The above results indicate that there are other cytokines in serum, which can inhibit the differentiation of ESC into nerve cells and facilitate the self-renewal of ESC, and these cytokines play a role by combining with LIF. It was found that BMP in serum could inhibit the differentiation of ES cells into nerve cells. BMP can inhibit the differentiation of ES cells into nerve cells, BMP 4/2 induces the expression of helix-loop heliform (HLH) basic protein Id by activating the Smad pathway, which inhibits the differentiation of ES cells into nerve cells, suggesting that the combined action of LIF and BMP may completely block the differentiation of ES cells and maintain a high degree of self-renewal of ES cells [83]. As mentioned earlier, phosphorylation of ERK leads to ES cell differentiation. Small molecule PD98059 is an inhibitor of ERK upstream kinase MEK, and the addition of PD98059 is conducive to the maintenance of ESC self-renewal [78]. BMP4 can also inhibit ESC differentiation and promote stemness maintenance by inhibiting the phosphorylation of ERK in ESC [84]. The schematic representation of LIF-mediated signaling pathways regulate mES pluripotency **(Figure 1)** [72,85].

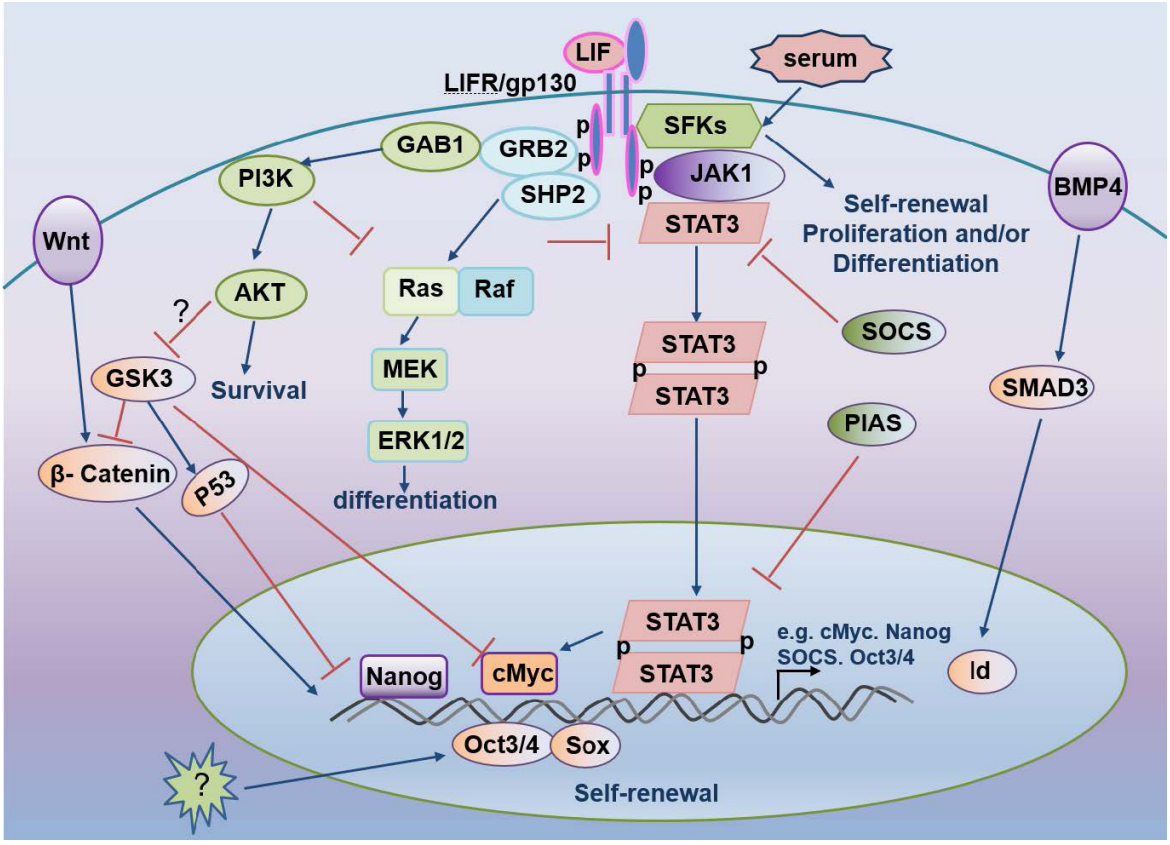

Figure 1: Lif mediated signaling pathways regulate mES pluripotency.

Activation of the Wnt signaling pathway promotes self-renewal of ES cells

Activation of LIF-STAT3 signaling pathway by adding LIF can maintain mESC self-renewal, but LIF has no effect on hESC. Activation of the Wnt signaling pathway promoted self-renewal of ESC cells in both humans and mice. The receptor of Wnt is frizzled (Frz), which is a seven-fold transmembrane receptor, the extracellular N-terminal of Frz has a cysteine rich domain (CRD), which can combine with Wnt. Frz act on the cytoplasm of dishevel protein (Dsh or Dvl), Dsh degrades β-catenin complex (including the APC, Axin, GSK-3β, CK1), so as to cut off the β-catenin degradation pathway, lead to β-catenin accumulate in the cytoplasm, enter into the nuclei and activate related gene transcription involved in stemness regulating [86,87]. The specific inhibitor of GSK3β on ESC, BIO, induces activation of the β-catenin-dependent Wnt pathway and up-regulates the expression of multi-competent transcription factors Oct-3/4, Rex -1 and Nanog, thereby promoting self-renewal of human and mouse embryonic stem cells. Activation of the Wnt signaling pathway is critical not only in maintaining the pluripotency of ESC [27, 88], at the same time, it plays an important role in maintaining the self-renew of adult stem cells (such as skin stem cells and hematopoietic stem cells) [89, 90]. Activation of Wnt signaling pathway promotes ES cell self-renewal **(Figure 2)** [91].

Small Molecular Compounds that Promote Stemness Maintenance

The addition of some cytokines such as LIF and BMP4 during ESC culture *in vitro* is beneficial to the growth and stemness maintenance of ESC. Based on the in-depth study of signal pathways related to stemness maintenance and differentiation, scientists found that small molecule modulators added with specific signal pathway proteins are beneficial to stemness maintenance of ESC. Small molecular compounds that have been reported to maintain the self-renewal of stem cells including the inhibitor CHIR99021 of GSK3β, the inhibitor PD0325901 of MEK, and the inhibitor SU5402 of FGFR signaling pathway, etc. These small molecules mainly maintain ESC pluripotency *in vitro* culture by inhibiting differentiation. Among them, CHIR99021 can improve the growth ability of ESC and promote the self-renewal of stem cells by inhibiting phosphorylation of downstream ERK and reducing differentiation. PD0325901 maintains the self-renewal and pluripotency of stem cells by inhibiting the MEK-ERK signaling pathway and reducing the phosphorylation level of PKB. SU5402 is an FGFR inhibitor. In the FGF signaling pathway, FGF4 can activate both phosphokinase PKB and extracellular signal Ras-MRK-ERK to lead to ESC differentiation, and the addition of compound SU5402 inhibits the above process. AG1478 is an inhibitor of EGFR, which maintains ESC

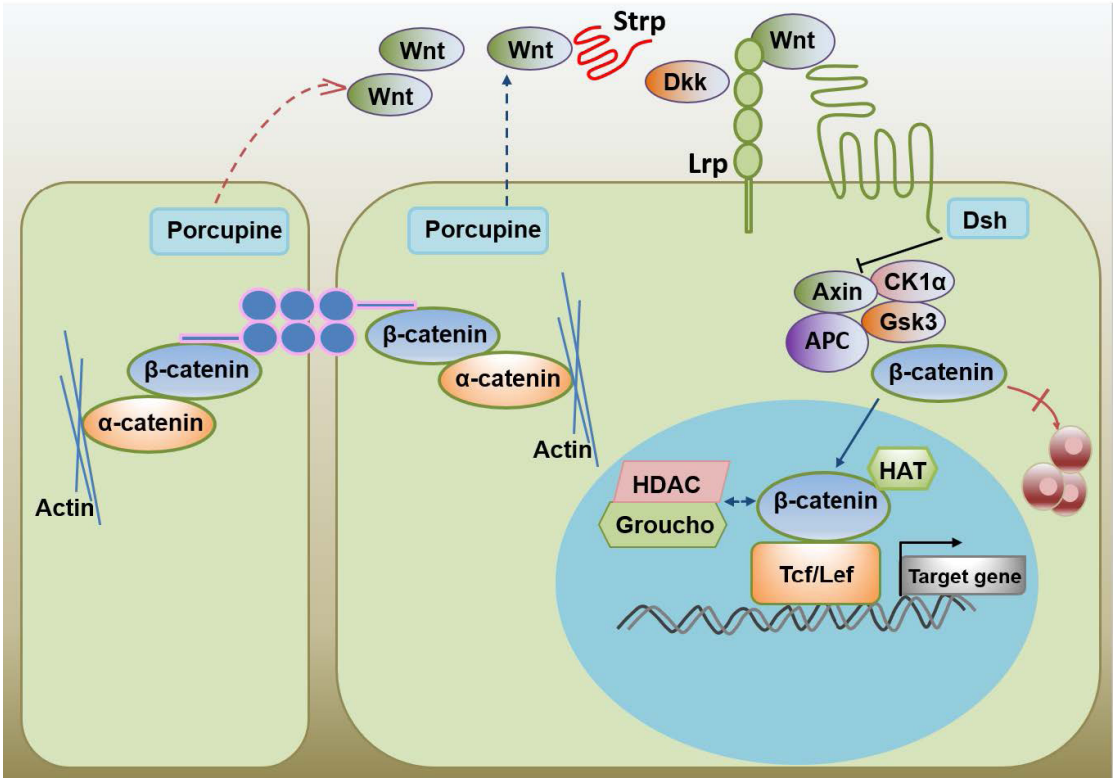

Figure 2: Activation of Wnt signaling pathway promotes ES cell self-renewal.

self-renewal by stimulating ESC proliferation and inhibiting MAPK signaling pathway activation [92]. Small molecule SC1 inhibits differentiation by double inhibiting RasGAP and ERK1 to maintain self-renewal of ESC [93]. Currently, 2i (a combination of two small molecule inhibitors CHIR99021 3 μM and PD0325901 3 μM) has been used as an important medium additive in stem cell culture. However, the combination of these two compounds has a large concentration and an inhibitory effect on cell proliferation, which brings some inconvenience to the research. In our study group, VEGFR signaling pathway inhibitor Sunitinib was found to inhibit VEGFR to maintain the embryonic stem cells self-renewal and undifferentiated state of long-term culture *in vitro* [94]. *In vitro* culture of stem cells, with the extension of culture time and the increased volume of stem cell clones, the expression of HIF1α increased, then the VEGF expression was up-regulated, and cell differentiation was induced. Sunitinib inhibits the effect of VEGF by inhibiting the VEGFR signaling pathway, while feedback regulation reduces the expression of VEGF, which is beneficial to self-renew maintenance. The discovery of these small molecular compounds is conducive to further elucidate the mechanism of ESC stemness maintenance, and can be used as small molecular tools and even drug leads. We have also reviewed the small molecular compounds in stem cell maintaining and reprogramming [95]. Therefore, the discovery of more small molecules that promote the stemness maintenance of ES cells not only has important theoretical significance, but also has important application value.

Epigenetic Characteristics and Important Epigenetic Modification Factors of ES Cells

The self-renewal and differentiation of ESC are dynamically regulated by epigenetics. Epigenetic regulatory modifications include DNA methylation modifications, histone modifications, and regulatory modifications mediated by non-coding RNA [96]. These epigenetic regulatory modifications play an important role in the regulation of self-renewal and differentiation of stem cells [97-99]. Generally, DNA demethylation occurs in the promoter region of important transcription factors in ESC cells, lead to the expression of transcription factors increases, while the reverse is true in differentiated cells. For example, in differentiated ESC, the expression of core transcription factors Oct4 and Nanog decreased after methylation of Dnmt3a and Dnmt3b [81]. Meanwhile, histone modification of ESC is also different from that of differentiated cells. In ESC, 87% of histones do not undergo trimethylation of H3K4 and H3K27, while differentiated cells show high trimethylation of H3K4 and H3K27 [100,101] and both transcriptionally activation of H3K4me3 and repression of H3K27me3 [102]. And the TRIM28 and interacting KRAB-ZNFs control self-renewal of hESC by modulating H3K9me3 and DNA methylation. Some epigenetic regulators regulate these processes. For example, histone regulators Jmjd1a and Jmjd2c encode histone H3K9 demineralization enzymes, which prevent the accumulation of methylation in the promoter region of ESC pluripotent genes, thus maintaining the high expression level of ES stemness genes and promoting the self-renewal of ES cells [103]. When genes Jmjd1a and Jmjd2c are knocked out or deleted, the expression of pluripotent genes Tcl1 and Nanog will be down-regulated, and ES cells tend to differentiate. Jarid2 and Mtf2, components of PRC2, mediate histone H3K27 methylation, down-regulate Oct4 expression, and accelerate ES cell differentiation [104,105]. Meanwhile, Oct4 expression can increase the expression level of H3K9 demethylase and the expression of components of PRC2 complex. The Oct4 and Sox2 downstream targeted bivalent chromatin component, Utf1, promote the ESCs pluripotency and proliferation by limiting Histone 3 lysine-27 trimethylation and PRC2 loading [106]. Nowadays, regulation and maintenance of tumorigenicity and self-renewal in tissue stem cells as cancer stem cells are regulated by some epigenetic proteins [107-110], or non-coding RNA mediated regulated modifications [111,112]. Our previous work uncovers that the m6 A methylation-mediated HIF-ALKBH5-SOX2 axis induces an endometrial cancer stem-like cell expansion in hypoxia [113]. Thus, the epigenetic modification factors are essential for stem cells self-renewal for ESC cells and other tissue stem cells.

New Role of Non-Coding RNA in Stemness Maintenance

Non-coding RNAs (ncRNAs) are various RNA molecules that do not translate into proteins. A growing body of research is showing that RNA can do much more than just be a messenger, or a component of RNA in the ribosome. A variety of non-coding RNA are involved in gene expression, protein translation and post-translation modification, and play an important role in life activities [114-118]. Currently, many non-coding RNA studied in stemness maintenance include long non-coding RNA (lncRNA) and small RNA (microRNA, miRNA). lncRNA are a class of RNA that are located between protein-coding genes with a length of no less than 200 bases. miRNAs are small non-coding RNAs about 22nt long, with high interspecific conservation and spatio-temporal specificity, and they play an important regulatory role in the self-renewal and differentiation of stem cells [119-121]. miRNAs that promote mESC stemness maintenance include *mir302* and *mir290* clusters. They may shorten the G1 phase of ES cells by inhibiting the expression of key cell cycle regulators *Cdkn1a*, *Rbl1* and *Lats2* [122]. The Core transcription factors Oct4, Sox2, and Nanog regulate the expression of *mir302* and *mir290* clusters, and these miRNAs are inhibited by miRNA let-7. Transcription factor Lin28 inhibits the expression of *let-7*, and down-regulation of Lin28 can increase the level of *let-7* in ES cells, thereby inhibiting the activity of Myc, reducing the expression of downstream genes of core transcription factor, and accelerating the differentiation of ES cells [123].

It also reports that the Oct4 and Nanog regulated conserved long ncRNAs AK028326 and AK141205 modulate mESC pluripotency [124]. And the other conserved long ncRNA TUNA, also control pluripotency and neural differentiation of ESCs [125]. Moreover, the small nucleolar RNA host gene 3 (Snhg3) [126], is essential for mESC self-renewal and pluripotency. The stably expressed long intergenic ncRNA Cyrano, maintain ESC self-renew by restraining the action of mir-7 [127]. A divergent lncRNA of lncKdm2b, controls self-renewal of ESCs by activating expression of transcription factor Zbtb3 and ATPase activity [128]. The long ncRNAs growth arrest specific 5 (Gas5) regulates mESC pluripotency by Dicer-miR291a-cMyc axis [129] and can also maintain human embryonic stem cell self-renewal by NODAL signal [130,131]. In hESC, *mir302* acts on *LEFTY1* and *LEFTY2*, regulatory factors of Nodal signaling pathway, regulating the pluripotency of hESC [132]. Other studies have shown that Oct4 can activate more than 200 nucleic acid length of non-coding RNA (*lincRNA*) and maintain the pluripotency state of ESC [133]. Deletion of *lincRNA-ROR* in hESC can lead to the increase of cell growth defects and apoptosis. The above results show that lncRNA and miRNA play an important role in self-renew maintenance of ES cells. At present, research on the role of long non-coding RNA and miRNA in self-renew maintenance of ESC has gradually become an important frontier direction of stem cell research.

The maintenance of pluripotency of ES cells is a multi-level regulatory process, which forms a complex regulatory network of epigenetic factors, non-coding RNA and various signaling pathways, and finally maintains a steady state (ground state) characterized by specific stemness related gene expression. Among them, core transcription factors Oct4, Sox2, Nanog and the common role of transcription factors, LIF and BMP4 regulation of signaling pathways, apparent modification and small molecule compounds play an important role in embryonic stem cell self-renewal. Stem cells can be cultured *in vitro* for a long time and maintain self-renewal, which lays a foundation for the research of stem cell biology and provides a guarantee for the clinical application of stem cells. At present, scientists are continuing to make further research on the mechanism of self-renewal of stem cells and make new progress. These research results are bound to further promote the development of regenerative medicine and make new contributions to human health.

Abbreviations

ESCs: Embryonic Stem Cells; LIF: Leukemia Suppressor Factor; GSK-3β: Glycogen Synthesis Kinase 3β; PI3K: Phosphatidyl Inositol-3-Kinase; HLH: Helix-Loop Heliform; CRD: Cysteine Rich Domain; lncRNA: Long non-coding RNA; lincRNA: Length of non-coding RNA

Ethical Approval

No ethical issues exist in the submission of this manuscript.

Consent to Participate

We consent to participate and publish this manuscript. No objection exists in the authors contributions and submission of this manuscript.

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Conflict of Interest

All authors have read and approved this manuscript. Neither the submitted paper nor any similar paper, in whole or in part, has been or will be published in any other primary scientific journal. No conflict of interest exists in the submission of this manuscript.

References

1. Martin GR. Isolation of a pluripotent cell line from early mouse embryos cultured in medium conditioned by teratocarcinoma stem cells. Proceedings of the National Academy of Sciences. 1981 Dec 1;78(12):7634-8.

2. Goumans MJ, Ward-van Oostwaard D, Wianny F, Savatier P, Zwijsen A, Mummery C. Mouse embryonic stem cells with aberrant transforming growth factor β signalling exhibit impaired differentiation in vitro and in vivo. Differentiation. 1998 Jul;63(3):101-13.

3. Ivanova NB, Dimos JT, Schaniel C, Hackney JA, Moore KA, Lemischka IR. A stem cell molecular signature. Science. 2002 Oct 18;298(5593):601-4.

4. Evans MJ, Kaufman MH. Establishment in culture of pluripotential cells from mouse embryos. Nature. 1981 Jul;292(5819):154-6.

5. Tesar PJ. Derivation of germ-line-competent embryonic stem cell lines from preblastocyst mouse embryos. Proceedings of the National Academy of Sciences. 2005 Jun 7;102(23):8239-44.

6. Lee KH, Chuang CK, Guo SF, Tu CF. Simple and efficient derivation of mouse embryonic stem cell lines using differentiation inhibitors or proliferation stimulators. Stem Cells and Development. 2012 Feb 10;21(3):373-83.

7. Gottlieb DI, Huettner JE. An in vitro pathway from embryonic stem cells to neurons and glia. Cells Tissues Organs. 1999;165(3-4):165-72.

8. Rathjen PD, Lake J, Whyatt LM, Bettess MD, Rathjen J. Properties and uses of embryonic stem cells: prospects for application to human biology and gene therapy. Reproduction, Fertility and Development. 1998;10(1):31-48.

9. Ying QL, Wray J, Nichols J, Batlle-Morera L, Doble B, Woodgett J, et al. The ground state of embryonic stem cell self-renewal. Nature. 2008

May;453(7194):519-23.

10. Smith AG, Heath JK, Donaldson DD, Wong GG, Moreau J, Stahl M, et al. Inhibition of pluripotential embryonic stem cell differentiation by purified polypeptides. Nature. 1988 Dec;336(6200):688-90.

11. Williams RL, Hilton DJ, Pease S, Willson TA, Stewart CL, Gearing DP, et al. Myeloid leukaemia inhibitory factor maintains the developmental potential of embryonic stem cells. Nature. 1988 Dec;336(6200):684-7.

12. Stewart CL, Kaspar P, Brunet LJ, Bhatt H, Gadi I, Köntgen F, et al. Blastocyst implantation depends on maternal expression of leukaemia inhibitory factor. Nature. 1992 Sep;359(6390):76-9.

13. Ying QL, Nichols J, Chambers I, Smith A. BMP induction of Id proteins suppresses differentiation and sustains embryonic stem cell self-renewal in collaboration with STAT3. Cell. 2003 Oct 31;115(3):281-92.

14. Ying QL, Stavridis M, Griffiths D, Li M, Smith A. Conversion of embryonic stem cells into neuroectodermal precursors in adherent monoculture. Nature Biotechnology. 2003 Feb;21(2):183-6.

15. Chaudhry MA, Vitalis TZ, Bowen BD, Piret JM. Basal medium composition and serum or serum replacement concentration influences on the maintenance of murine embryonic stem cells. Cytotechnology. 2008 Nov;58(3):173-9.

16. Matsuda T, Nakamura T, Nakao K, Arai T, Katsuki M, Heike T, et al. STAT3 activation is sufficient to maintain an undifferentiated state of mouse embryonic stem cells. The EMBO Journal. 1999 Aug 2;18(15):4261-9.

17. Niwa H, Miyazaki JI, Smith AG. Quantitative expression of Oct-3/4 defines differentiation, dedifferentiation or self-renewal of ES cells. Nature Genetics. 2000 Apr;24(4):372-6.

18. Chambers I, Colby D, Robertson M, Nichols J, Lee S, Tweedie S, et al. Functional expression cloning of Nanog, a pluripotency sustaining factor in embryonic stem cells. Cell. 2003 May 30;113(5):643-55.

19. Mitsui K, Tokuzawa Y, Itoh H, Segawa K, Murakami M, Takahashi K, et al. The homeoprotein Nanog is required for maintenance of pluripotency in mouse epiblast and ES cells. Cell. 2003 May 30;113(5):631-42.

20. Thomson JA, Itskovitz-Eldor J, Shapiro SS, Waknitz MA, Swiergiel JJ, Marshall VS, et al. Embryonic stem cell lines derived from human blastocysts. Science. 1998 Nov 6;282(5391):1145-7.

21. Reubinoff BE, Pera MF, Fong CY, Trounson A, Bongso A. Embryonic stem cell lines from human blastocysts: somatic differentiation in vitro. Nature Biotechnology. 2000 Apr;18(4):399-404.

22. King HW, Klose RJ. The pioneer factor OCT4 requires the chromatin remodeller BRG1 to support gene regulatory element function in mouse embryonic stem cells. Elife. 2017 Mar 13;6:e22631.

23. Sagi I, Chia G, Golan-Lev T, Peretz M, Weissbein U, Sui L, et al. Derivation and differentiation of haploid human embryonic stem cells. Nature. 2016 Apr;532(7597):107-11.

24. Dahéron L, Opitz SL, Zaehres H, Lensch WM, Andrews PW, Itskovitz-Eldor J, et al. LIF/STAT3 signaling fails to maintain self-renewal of human embryonic stem cells. Stem Cells. 2004 Sep;22(5):770-8.

25. Amit M, Carpenter MK, Inokuma MS, Chiu CP, Harris CP, Waknitz MA, et al. Clonally derived human embryonic stem cell lines maintain pluripotency and proliferative potential for prolonged periods of culture. Developmental Biology. 2000 Nov 15;227(2):271-8.

26. Xu C, Inokuma MS, Denham J, Golds K, Kundu P, Gold JD, et al. Feeder-free growth of undifferentiated human embryonic stem cells. Nature Biotechnology. 2001 Oct;19(10):971-4.

27. Sato N, Meijer L, Skaltsounis L, Greengard P, Brivanlou AH. Maintenance of pluripotency in human and mouse embryonic stem cells through activation of Wnt signaling by a pharmacological GSK-3-specific inhibitor. Nature Medicine. 2004 Jan;10(1):55-63.

28. Ginis I, Luo Y, Miura T, Thies S, Brandenberger R, Gerecht-Nir S, et al. Differences between human and mouse embryonic stem cells. Developmental Biology. 2004 May 15;269(2):360-80.

29. Richards M, Tan SP, Tan JH, Chan WK, Bongso A. The transcriptome profile of human embryonic stem cells as defined by SAGE. Stem Cells. 2004 Jan;22(1):51-64.

30. Keller G. Embryonic stem cell differentiation: emergence of a new era in biology and medicine. Genes & Development. 2005 May 15;19(10):1129-55.

31. Doetschman TC, Eistetter H, Katz M, Schmidt W, Kemler R. The in vitro development of blastocyst-derived embryonic stem cell lines: formation of visceral yolk sac, blood islands and myocardium. J Embryol Exp Morphol, 1985. 87: p. 27-45.

32. Durruthy-Durruthy J, Sebastiano V, Wossidlo M, Cepeda D, Cui J, Grow EJ, et al. The primate-specific noncoding RNA HPAT5 regulates pluripotency during human preimplantation development and nuclear reprogramming. Nature Genetics. 2016 Jan;48(1):44-52.

33. Lu X, Sachs F, Ramsay L, Jacques PÉ, Göke J, Bourque G, et al. The retrovirus HERVH is a long noncoding RNA required for human embryonic stem cell identity. Nature Structural & Molecular Biology. 2014 Apr;21(4):423-5.

34. Göke J, Lu X, Chan YS, Ng HH, Ly LH, Sachs F, et al. Dynamic transcription of distinct classes of endogenous retroviral elements marks specific populations of early human embryonic cells. Cell Stem Cell. 2015 Feb 5;16(2):135-41.

35. Chen X, Vega VB, Ng HH. Transcriptional regulatory networks in embryonic stem cells Cold Spring Harb Symp Quant Biol 2008. 73: 203-209.

36. Niwa H, Miyazaki JI, Smith AG. Quantitative expression of Oct-3/4 defines differentiation, dedifferentiation or self-renewal of ES cells. Nature Genetics. 2000 Apr;24(4):372-6.

37. Fang L, Zhang J, Zhang H, Yang X, Jin X, Zhang L, et al. H3K4 methyltransferase Set1a is a key Oct4 coactivator essential for generation of Oct4 positive inner cell mass. Stem Cells. 2016 Mar;34(3):565-80.

38. Abulaiti X, Zhang H, Wang A, Li N, Li Y, Wang C, et al. Phosphorylation of threonine343 is crucial for OCT4 interaction with SOX2 in the maintenance of mouse embryonic stem cell pluripotency. Stem Cell Reports. 2017 Nov 14;9(5):1630-41.

39. Chambers I, Silva J, Colby D, Nichols J, Nijmeijer B, Robertson M, et al. Nanog safeguards pluripotency and mediates germline development. Nature. 2007 Dec;450(7173):1230-4.

40. Heurtier V, Owens N, Gonzalez I, Mueller F, Proux C, Mornico D, et al. The molecular logic of Nanog-induced self-renewal in mouse embryonic stem cells. Nature Communications. 2019 Mar 7;10(1):1-5.

41. Chambers I, Colby D, Robertson M, Nichols J, Lee S, Tweedie S, et al. Functional expression cloning of Nanog, a pluripotency sustaining factor in embryonic stem cells. Cell. 2003 May 30;113(5):643-55.

42. Masui S, Nakatake Y, Toyooka Y, Shimosato D, Yagi R, Takahashi K, et al. Pluripotency governed by Sox2 via regulation of Oct3/4 expression in mouse embryonic stem cells. Nature Cell Biology. 2007 Jun;9(6):625-35.

43. Boyer LA, Lee TI, Cole MF, Johnstone SE, Levine SS, Zucker JP, et al. Core transcriptional regulatory circuitry in human embryonic stem cells. Cell. 2005 Sep 23;122(6):947-56.

44. Lee SH, Chen TY, Dhar SS, Gu B, Chen K, Kim YZ, et al. A feedback loop comprising PRMT7 and miR-24-2 interplays with Oct4, Nanog, Klf4 and c-Myc to regulate stemness. Nucleic Acids Research. 2016 Dec 15;44(22):10603-18.

45. Solari C, Petrone MV, Echegaray CV, Cosentino MS, Waisman A, Francia M, et al. Superoxide dismutase 1 expression is modulated by the core pluripotency transcription factors Oct4, Sox2 and Nanog in embryonic stem cells. Mechanisms of Development. 2018 Dec 1;154:116-21.

46. Reményi A, Schöler HR, Wilmanns M. Combinatorial control of gene expression. Nature Structural & Molecular Biology. 2004 Sep;11(9):812-5.

47. Wang Z, Oron E, Nelson B, Razis S, Ivanova N. Distinct lineage specification roles for NANOG, OCT4, and SOX2 in human embryonic stem cells. Cell Stem Cell. 2012 Apr 6;10(4):440-54.

48. Kallas A, Pook M, Trei A, Maimets T. SOX2 is regulated differently from NANOG and OCT4 in human embryonic stem cells during early differentiation initiated with sodium butyrate. Stem cells International. 2014 Jan 1;2014.

49. Soucie EL, Weng Z, Geirsdóttir L, Molawi K, Maurizio J, Fenouil R, et al. Lineage-specific enhancers activate self-renewal genes in macrophages and embryonic stem cells. Science. 2016 Feb 12;351(6274):aad5510.

50. Liao Y, Cheng J, Kong X, Li S, Li X, Zhang M, et al. HDAC3 inhibition ameliorates ischemia/reperfusion-induced brain injury by regulating the microglial cGAS-STING pathway. Theranostics. 2020;10(21):9644.

51. Chen X, Xu H, Yuan P, Fang F, Huss M, Vega VB, et al. Integration of external signaling pathways with the core transcriptional network in embryonic stem cells. Cell. 2008 Jun 13;133(6):1106-17.

52. Kim J, Chu J, Shen X, Wang J, Orkin SH. An extended transcriptional network for pluripotency of embryonic stem cells. Cell. 2008 Mar 21;132(6):1049-61.

53. Cole MF, Johnstone SE, Newman JJ, Kagey MH, Young RA. Tcf3 is an integral component of the core regulatory circuitry of embryonic stem cells. Genes & Development. 2008 Mar 15;22(6):746-55.

54. Feng B, Jiang J, Kraus P, Ng JH, Heng JC, Chan YS, et al. Reprogramming of fibroblasts into induced pluripotent stem cells with orphan nuclear receptor Esrrb. Nature Cell Biology. 2009 Feb;11(2):197-203.

55. Heng JC, Feng B, Han J, Jiang J, Kraus P, Ng JH, et al. The nuclear receptor Nr5a2 can replace Oct4 in the reprogramming of murine somatic cells to pluripotent cells. Cell Stem Cell. 2010 Feb 5;6(2):167-74.

56. Dejosez M, Levine SS, Frampton GM, Whyte WA, Stratton SA, Barton MC, et al. Ronin/Hcf-1 binds to a hyperconserved enhancer element and regulates genes involved in the growth of embryonic stem cells. Genes & Development. 2010 Jul 15;24(14):1479-84.

57. Young RA. Control of the embryonic stem cell state. Cell. 2011 Mar 18;144(6):940-54.

58. Adachi K, Kopp W, Wu G, Heising S, Greber B, Stehling M, et al. Esrrb unlocks silenced enhancers for reprogramming to naive pluripotency. Cell Stem Cell. 2018 Aug 2;23(2):266-75.

59. Zhu F, Zhu Q, Ye D, Zhang Q, Yang Y, Guo X, et al. Sin3a–Tet1 interaction activates gene transcription and is required for embryonic stem cell pluripotency. Nucleic Acids Research. 2018 Jul 6;46(12):6026-40.

60. Zhang W, Chronis C, Chen X, Zhang H, Spalinskas R, Pardo M, et al. The BAF and PRC2 complex subunits Dpf2 and Eed antagonistically converge on Tbx3 to control ESC differentiation. Cell Stem Cell. 2019 Jan 3;24(1):138-52.

61. Wang B, Wu L, Li D, Liu Y, Guo J, Li C, et al. Induction of pluripotent stem cells from mouse embryonic fibroblasts by Jdp2-Jhdm1b-Mkk6-Glis1-Nanog-Essrb-Sall4. Cell Reports. 2019 Jun 18;27(12):3473-85.

62. Nandan MO, Yang VW. The role of Krüppel-like factors in the reprogramming of somatic cells to induced pluripotent stem cells. Histology and Histopathology. 2009 Oct;24(10):1343.

63. Zhang J, Liu G, Ruan Y, Wang J, Zhao K, Wan Y, et al. Dax1 and Nanog act in parallel to stabilize mouse embryonic stem cells and induced pluripotency. Nature Communications. 2014 Oct 6;5(1):1-6.

64. Olariu V, Lövkvist C, Sneppen K. Nanog, Oct4 and Tet1 interplay in establishing pluripotency. Scientific Reports. 2016 May 5;6(1):1-1.

65. Evans MJ, Kaufman MH. Establishment in culture of pluripotential cells from mouse embryos. Nature. 1981 Jul;292(5819):154-156.

66. Yoshida K, Taga T, Saito M, Suematsu S, Kumanogoh A, Tanaka T, et al. Targeted disruption of gp130, a common signal transducer for the interleukin 6 family of cytokines, leads to myocardial and hematological disorders. Proceedings of the National Academy of

Sciences. 1996 Jan 9;93(1):407-11.

67. Scheller J, Garbers C, Rose-John S. Interleukin-6: from basic biology to selective blockade of pro-inflammatory activities. InSeminars in immunology 2014 Feb 1 (Vol. 26, No. 1, pp. 2-12).

68. Griffiths DS, Li J, Dawson MA, Trotter MW, Cheng YH, Smith AM, et al. LIF-independent JAK signalling to chromatin in embryonic stem cells uncovered from an adult stem cell disease. Nature Cell Biology. 2011 Jan;13(1):13-21.

69. Heinrich PC, Behrmann I, Müller-Newen G, Schaper F, Graeve L. Interleukin-6-type cytokine signalling through the gp130/Jak/STAT pathway. Biochemical Journal. 1998 Sep 1;334(2):297-314.

70. Niwa H, Burdon T, Chambers I, Smith A. Self-renewal of pluripotent embryonic stem cells is mediated via activation of STAT3. Genes & Development. 1998 Jul 1;12(13):2048-60.

71. Ernst M, Novak U, Nicholson SE, Layton JE, Dunn AR. The carboxyl-terminal domains of gp130-related cytokine receptors are necessary for suppressing embryonic stem cell differentiation: involvement of STAT3. Journal of Biological Chemistry. 1999 Apr 2;274(14):9729-37.

72. Annerén C. Tyrosine kinase signalling in embryonic stem cells. Clinical Science. 2008 Jul 1;115(2):43-55.

73. Kim SJ, Cheon SH, Yoo SJ, Kwon J, Park JH, Kim CG, et al. RETRACTED: Contribution of the PI3K/Akt/PKB signal pathway to maintenance of self-renewal in human embryonic stem cells. FEBS Lett . 2005 Jan 17;579(2):534-40.

74. Paling NR, Wheadon H, Bone HK, Welham MJ. Regulation of embryonic stem cell self-renewal by phosphoinositide 3-kinase-dependent signaling. Journal of Biological Chemistry. 2004 Nov 12;279(46):48063-70.

75. Sun H, Lesche R, Li DM, Liliental J, Zhang H, Gao J, et al. PTEN modulates cell cycle progression and cell survival by regulating phosphatidylinositol 3, 4, 5,-trisphosphate and Akt/protein kinase B signaling pathway. Proceedings of the National Academy of Sciences. 1999 May 25;96(11):6199-204.

76. Chang F, Lee JT, Navolanic PM, Steelman LS, Shelton JG, Blalock WL, et al. Involvement of PI3K/Akt pathway in cell cycle progression, apoptosis, and neoplastic transformation: a target for cancer chemotherapy. Leukemia. 2003 Mar;17(3):590-603.

77. Watanabe S, Umehara H, Murayama K, Okabe M, Kimura T, Nakano T. Activation of Akt signaling is sufficient to maintain pluripotency in mouse and primate embryonic stem cells. Oncogene. 2006 May;25(19):2697-707.

78. Burdon T, Stracey C, Chambers I, Nichols J, Smith A. Suppression of SHP-2 and ERK signalling promotes self-renewal of mouse embryonic stem cells. Developmental Biology. 1999 Jun 1;210(1):30-43.

79. Cheng AM, Saxton TM, Sakai R, Kulkarni S, Mbamalu G, Vogel W, et al. Mammalian Grb2 regulates multiple steps in embryonic development and malignant transformation. Cell. 1998 Dec 11;95(6):793-803.

80. Boroviak T, Loos R, Bertone P, Smith A, Nichols J. The ability of inner-cell-mass cells to self-renew as embryonic stem cells is acquired following epiblast specification. Nature Cell Biology. 2014 Jun;16(6):513-25.

81. Wu B, Li Y, Li B, Zhang B, Wang Y, Li L, et al. DNMTs play an important role in maintaining the pluripotency of leukemia inhibitory factor-dependent embryonic stem cells. Stem Cell Reports. 2021 Mar 9;16(3):582-96.

82. Qu CK, Feng GS. Shp-2 has a positive regulatory role in ES cell differentiation and proliferation. Oncogene. 1998 Jul;17(4):433-9.

83. Chambers I. The molecular basis of pluripotency in mouse embryonic stem cells. Cloning and Stem Cells. 2004 Dec 1;6(4):386-91.

84. Qi X, Li TG, Hao J, Hu J, Wang J, Simmons H, et al. BMP4 supports self-renewal of embryonic stem cells by inhibiting mitogen-activated protein kinase pathways. Proceedings of the National Academy of Sciences. 2004 Apr 20;101(16):6027-32.

85. Guo W, Hao B, Wang Q, Lu Y, Yue J. Requirement of B-Raf, C-Raf, and A-Raf for the growth and survival of mouse embryonic stem cells. Experimental Cell Research. 2013 Nov 1;319(18):2801-11.

86. MacDonald BT, Tamai K, He X. Wnt/β-catenin signaling: components, mechanisms, and diseases. Developmental Cell. 2009 Jul 21;17(1):9-26.

87. Sato N, Meijer L, Skaltsounis L, Greengard P, Brivanlou AH. Maintenance of pluripotency in human and mouse embryonic stem cells through activation of Wnt signaling by a pharmacological GSK-3-specific inhibitor. Nature Medicine. 2004 Jan;10(1):55-63.

88. Singla DK, Schneider DJ, LeWinter MM, Sobel BE. wnt3a but not wnt11 supports self-renewal of embryonic stem cells. Biochemical and Biophysical Research Communications. 2006 Jun 30;345(2):789-95.

89. Malhotra S, Kincade PW. Wnt-related molecules and signaling pathway equilibrium in hematopoiesis. Cell Stem Cell. 2009 Jan 9;4(1):27-36.

90. Haegebarth A, Clevers H. Wnt signaling, lgr5, and stem cells in the intestine and skin. The American Journal of Pathology. 2009 Mar 1;174(3):715-21.

91. Wray J, Hartmann C. WNTing embryonic stem cells. Trends in Cell Biology. 2012 Mar 1;22(3):159-68.

92. Heo JS, Lee YJ, Han HJ. EGF stimulates proliferation of mouse embryonic stem cells: involvement of Ca2+ influx and p44/42 MAPKs. American Journal of Physiology-Cell Physiology. 2006 Jan;290(1):C123-33.

93. Chen S, Do JT, Zhang Q, Yao S, Yan F, Peters EC, et al. Self-renewal of embryonic stem cells by a small molecule. Proceedings of the National Academy of Sciences. 2006 Nov 14;103(46):17266-71.

94. Chen G, Xu X, Zhang L, Fu Y, Wang M, Gu H, et al. Blocking autocrine VEGF signaling by sunitinib, an anti-cancer drug, promotes

embryonic stem cell self-renewal and somatic cell reprogramming. Cell Research. 2014 Sep;24(9):1121-36.

95. Chen G, Guo YE, Li C, Li S, Wan X. Small molecules that promote self-renewal of stem cells and somatic cell reprogramming. Stem Cell Reviews and Reports. 2020 Jun;16(3):511-23.

96. Papatsenko D, Waghray A, Lemischka IR. Feedback control of pluripotency in embryonic stem cells: signaling, transcription and epigenetics. Stem Cell Research. 2018 May 1;29:180-188.

97. Wu HA, Sun YE. Epigenetic regulation of stem cell differentiation. Pediatric Research. 2006 Apr;59(4):21-5.

98. Zhou VW, Goren A, Bernstein BE. Charting histone modifications and the functional organization of mammalian genomes. Nature Reviews Genetics. 2011 Jan;12(1):7-18.

99. Boyer LA, Plath K, Zeitlinger J, Brambrink T, Medeiros LA, Lee TI, et al. Polycomb complexes repress developmental regulators in murine embryonic stem cells. Nature. 2006 May;441(7091):349-53.

100. Fouse SD, Shen Y, Pellegrini M, Cole S, Meissner A, Van Neste L, et al. Promoter CpG methylation contributes to ES cell gene regulation in parallel with Oct4/Nanog, PcG complex, and histone H3 K4/K27 trimethylation. Cell Stem Cell. 2008 Feb 7;2(2):160-9.

101. Oleksiewicz U, Gładych M, Raman AT, Heyn H, Mereu E, Chlebanowska P, et al. TRIM28 and interacting KRAB-ZNFs control self-renewal of human pluripotent stem cells through epigenetic repression of pro-differentiation genes. Stem Cell Reports. 2017 Dec 12;9(6):2065-80.

102. Bernstein BE, Mikkelsen TS, Xie X, Kamal M, Huebert DJ, Cuff J, et al. A bivalent chromatin structure marks key developmental genes in embryonic stem cells. Cell. 2006 Apr 21;125(2):315-26.

103. Loh YH, Zhang W, Chen X, George J, Ng HH. Jmjd1a and Jmjd2c histone H3 Lys 9 demethylases regulate self-renewal in embryonic stem cells. Genes & Development. 2007 Oct 15;21(20):2545-57.

104. Landeira D, Sauer S, Poot R, Dvorkina M, Mazzarella L, Jørgensen HF, et al. Jarid2 is a PRC2 component in embryonic stem cells required for multi-lineage differentiation and recruitment of PRC1 and RNA Polymerase II to developmental regulators. Nature Cell Biology. 2010 Jun;12(6):618-24.

105. Li G, Margueron R, Ku M, Chambon P, Bernstein BE, Reinberg D. Jarid2 and PRC2, partners in regulating gene expression. Genes & Development. 2010 Feb 15;24(4):368-80.

106. Jia J, Zheng X, Hu G, Cui K, Zhang J, Zhang A, et al. Regulation of pluripotency and self-renewal of ESCs through epigenetic-threshold modulation and mRNA pruning. Cell. 2012 Oct 26;151(3):576-89.

107. Xavier PL, Cordeiro YG, Alexandre PA, Pires PR, Saranholi BH, Silva ER, et al. An epigenetic screening determines BET proteins as targets to suppress self-renewal and tumorigenicity in canine mammary cancer cells. Scientific Reports. 2019 Nov 22;9(1):1-5.

108. De Mello RA, Faleiro I, Apolonio JD, Tabori U, Price AJ, Roberto VP, et al. Hot topics in epigenetic regulation of cancer self-renewal for pancreatic tumors: future trends. Future Oncology. 2019 Mar;15(7):683-5.

109. Civenni G, Bosotti R, Timpanaro A, Vazquez R, Merulla J, Pandit S, et al. Epigenetic control of mitochondrial fission enables self-renewal of stem-like tumor cells in human prostate cancer. Cell Metabolism. 2019 Aug 6;30(2):303-18.

110. Peng Z, Zhou W, Zhang C, Liu H, Zhang Y. Curcumol controls choriocarcinoma stem-like cells self-renewal via repression of DNA methyltransferase (DNMT)-and histone deacetylase (HDAC)-mediated epigenetic regulation. Medical Science Monitor: International Medical Journal of Experimental and Clinical Research. 2018;24:461.

111. Chen X, Xie R, Gu P, Huang M, Han J, Dong W, et al. Long noncoding RNA LBCS inhibits self-renewal and chemoresistance of bladder cancer stem cells through epigenetic silencing of SOX2. Clinical Cancer Research. 2019 Feb 15;25(4):1389-403.

112. Gao S, Zhou B, Li H, Huang X, Wu Y, Xing C, et al. Long noncoding RNA HOTAIR promotes the self-renewal of leukemia stem cells through epigenetic silencing of p15. Experimental Hematology. 2018 Nov 1;67:32-40.

113. Chen G, Liu B, Yin S, Li S, Guo Y, Wang M. Hypoxia induces an endometrial cancer stem-like cell phenotype via HIF-dependent demethylation of SOX2 mRNA. Oncogenesis 2020 Sep 11;9(9):81.

114. Jeffares DC, Poole AM, Penny D. Relics from the RNA world. Journal of Molecular Evolution. 1998 Jan;46(1):18-36.

115. Hüttenhofer A, Schattner P, Polacek N. Non-coding RNAs: hope or hype?. TRENDS in Genetics. 2005 May 1;21(5):289-97.

116. Dinger ME, Amaral PP, Mercer TR, Pang KC, Bruce SJ, Gardiner BB, et al. Long noncoding RNAs in mouse embryonic stem cell pluripotency and differentiation. Genome Research. 2008 Sep 1;18(9):1433-45.

117. Guttman M, Amit I, Garber M, French C, Lin MF, Feldser D, et al. Chromatin signature reveals over a thousand highly conserved large non-coding RNAs in mammals. Nature. 2009 Mar;458(7235):223-7.

118. Guttman M, Donaghey J, Carey BW, Garber M, Grenier JK, Munson G, et al. lincRNAs act in the circuitry controlling pluripotency and differentiation. Nature. 2011 Sep;477(7364):295-300.

119. Song L, Tuan RS. MicroRNAs and cell differentiation in mammalian development. Birth Defects Research Part C: Embryo Today: Reviews. 2006 Jun;78(2):140-9.

120. Bai YS, Li L, Wei HX, Zhu C, Zhang SQ. The effect of microRNAs on the regulatory network of pluripotency in embryonic stem cells. Yi Chuan= Hereditas. 2013 Oct 1;35(10):1153-66.

121. Jia W, Chen W, Kang J. The functions of microRNAs and long non-coding RNAs in embryonic and induced pluripotent stem cells. Genomics, Proteomics & Bioinformatics. 2013 Oct 1;11(5):275-83.

122. Marson A, Levine SS, Cole MF, Frampton GM, Brambrink

T, Johnstone S, et al. Connecting microRNA genes to the core transcriptional regulatory circuitry of embryonic stem cells. Cell. 2008 Aug 8;134(3):521-33.

123. Melton C, Judson RL, Blelloch R. Opposing microRNA families regulate self-renewal in mouse embryonic stem cells. Nature. 2010 Feb;463(7281):621-6.

124. Mohamed JS, Gaughwin PM, Lim B, Robson P, Lipovich L. Conserved long noncoding RNAs transcriptionally regulated by Oct4 and Nanog modulate pluripotency in mouse embryonic stem cells. Rna. 2010 Feb 1;16(2):324-37.

125. Lin N, Chang KY, Li Z, Gates K, Rana ZA, Dang J, et al. An evolutionarily conserved long noncoding RNA TUNA controls pluripotency and neural lineage commitment. Molecular Cell. 2014 Mar 20;53(6):1005-19.

126. Lu W, Yu J, Shi F, Zhang J, Huang R, Yin S, et al. The long non-coding RNA Snhg3 is essential for mouse embryonic stem cell self-renewal and pluripotency. Stem Cell Res Ther. 2019 May 31;10(1):157.

127. Smith KN, Starmer J, Miller SC, Sethupathy P, Magnuson T. Long noncoding RNA moderates microRNA activity to maintain self-renewal in embryonic stem cells. Stem Cell Reports. 2017 Jul 11;9(1):108-21.

128. Ye B, Liu B, Yang L, Zhu X, Zhang D, Wu W, et al. LncKdm2b controls self-renewal of embryonic stem cells via activating expression of transcription factor Zbtb3. The EMBO Journal. 2018 Apr 13;37(8):e97174.

129. Tu J, Tian G, Cheung HH, Wei W, Lee TL. Gas5 is an essential lncRNA regulator for self-renewal and pluripotency of mouse embryonic stem cells and induced pluripotent stem cells. Stem Cell Research & Therapy. 2018 Dec;9(1):1-3.

130. Xu C, Zhang Y, Wang Q, Xu Z, Jiang J, Gao Y, et al. Long non-coding RNA GAS5 controls human embryonic stem cell self-renewal by maintaining NODAL signalling. Nature Communications. 2016 Nov 4;7(1):1-8.

131. Fiorenzano A, Pascale E, Patriarca EJ, Minchiotti G, Fico A. LncRNAs and PRC2: Coupled Partners in Embryonic Stem Cells. Epigenomes. 2019 Sep;3(3):14.

132. Rosa A, Spagnoli FM, Brivanlou AH. The miR-430/427/302 family controls mesendodermal fate specification via species-specific target selection. Developmental cell. 2009 Apr 21;16(4):517-27.

133. Loewer S, Cabili MN, Guttman M, Loh YH, Thomas K, Park IH, et al. Large intergenic non-coding RNA-RoR modulates reprogramming of human induced pluripotent stem cells. Nature Genetics. 2010 Dec;42(12):1113-7.