Chapter 2

Embryonic Stem Cells and Oncogenes

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Additional information is available at the end of the chapter

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1. Introduction

Stem cells possess two main attributes, namely, a self-renewal capacity and multipotency. Self-renewal refers to the ability of a cell to replicate itself without differentiating or losing multipotency. Multipotency is the ability to differentiate into more than one cell lineage. Recent studies have revealed that there are several types of stem cells. For example, neural stem cells can differentiate into neurons, astrocytes, and oligodendrocytes, while hematopoietic stem cells can differentiate into all types of blood cells. Embryonic stem (ES) cells were originally established from the inner cell mass (ICM) of mouse blastocysts [1, 2]. As the ICM gives rise to the embryo, ES cells can differentiate into most cell types in the body and are therefore pluripotent. The successful establishment of human pluripotent cells, namely, human ES cells and human induced pluripotent (iPS) cells, opens up the possibility of using these cells in regenerative medicine [3-5]. However, several issues remain to be resolved. One such issue is unanticipated tumor formation by transplanted pluripotent cells, which seems to be associated with the self-renewal capacity of these cells.

The self-renewal of mouse ES cells [6, 7] can be maintained by the presence of leukemia inhibitory factor (LIF) in the culture medium. LIF binds to LIF receptor (LIFR) and induces formation of gp130/LIFR heterodimers, leading to activation of the downstream transcription factor signal transducer and activator of transcription (STAT)-3. The LIF/STAT3 pathway plays a critical role in ES cell self-renewal by upregulating several self-renewal genes [8]. The expression of a set of self-renewal genes is also regulated by networks of important transcription factors, including Oct3/4, Sox2, and Nanog [9].

Cancer is one of the most feared diseases throughout the world. Approximately 10 million people die from cancer every year and the number of cancer patients is increasing. This disease is characterized by loss of cellular growth control, which is induced by genome alterations, such as DNA sequence changes, copy number aberrations, chromosomal rearrangements, and

modifications in DNA methylation. Recent studies have provided evidence that tumor tissue contains a small subset of stem-like cells called cancer stem cells. Cancer stem cells have stem cell-like attributes, namely, self-renewal and differentiation, which enables them to produce tumors by self-renewing and giving rise to differentiated progeny. The concept of cancer stem cells first arose from studies of leukemia stem cells [10] and subsequently of solid tumors [11, 12]. Given that cancer stem cells play a prominent role in cancer cell growth, it may be reasonable to expect similarities between ES cells and cancer cells.

Indeed, ES cells are similar to cancer cells in several respects. When injected into immunodeficient mice, ES cells and cancer cells can produce benign and malignant tumors, respectively. Both cell types have a rapid cell cycle, which results in fast proliferation. Telomerase activity is very high in both cell types, which allows them to proliferate indefinitely. Both cell types contain a "side population of cells" with a high drug efflux capacity, which gives rise to their drug-resistance phenotype. In addition, several signal transduction pathways seem to be used in both ES cell self-renewal and cancer cell growth. For example, the STAT3 pathway, which plays a central role in ES cell self-renewal, is activated in several types of cancer cells. On the other hand, the Wnt/β-catenin pathway, whose activation is associated with tumorigenesis in many tissues, is involved in ES cell self-renewal. Moreover, poorly differentiated tumors preferentially overexpress genes that are normally enriched in ES cells [13].

The similarities between ES cells and cancer cells raise the possibility that certain molecules that are involved in ES cell self-renewal play important roles in cancer cell growth, while certain oncogenes play critical roles in ES cell self-renewal (Fig. 1). In this chapter, I will provide examples of such molecules and describe their roles in ES cell self-renewal and tumorigenesis.

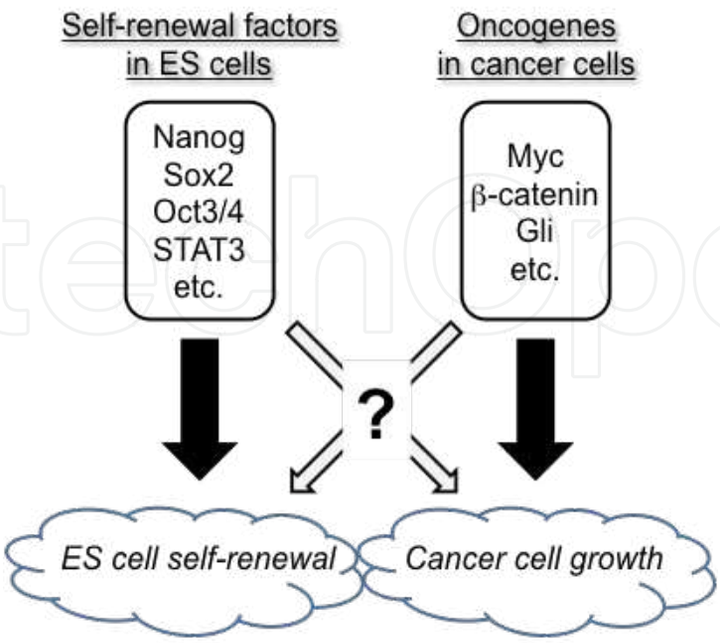

Figure 1. Similarity between ES cells and cancer cells.

2. ES cell self-renewal genes that are involved in cancer cell growth

2.1. Oct3/4

Oct3/4 was originally isolated by three groups [14-16]. Since Oct1 and Oct2 had already been identified, two groups named this protein Oct3, while the other group named it Oct4. Therefore, I will use the term "Oct3/4" to describe this protein. Oct3/4 is a transcription factor belonging to the POU family and is encoded by the *pou5f1* gene. The Oct3/4 protein contains three domains, namely, the N-terminal, POU, and C-terminal domains. The N- and C-terminal domains are transactivation domains with redundant functions, while the POU domain is a bipartite DNA-binding domain consisting of the POU-specific domain and the POU homeodomain. In mouse development, deficiency of this transcription factor results in loss of the ICM [17]. In agreement with this, conditional knockout of this gene in ES cells results in their differentiation into trophectoderm cells [18], indicating that Oct3/4 is a central player in the self-renewal of ES cells. Furthermore, the recent finding that Oct3/4 is one of the four factors required for the production of iPS cells indicates the importance of Oct3/4 for the acquisition of pluripotency [19].

Not only suppression, but also overexpression of Oct3/4 induces ES cell differentiation [18], suggesting that the activity of Oct3/4 needs to be sustained at the correct level to maintain ES cell self-renewal. Oct3/4 expression in ES cells is positively and negatively regulated by multiple factors. The upstream region of the *pou5f1* gene contains proximal and distal enhancers, which regulate stem cell-specific expression of Oct3/4 [20]. An orphan nuclear receptor Lrh1 (also known as Nr5a2) binds to the proximal enhancer to maintain Oct3/4 expression [21], whereas Oct3/4 and Sox2 associate with the distal enhancer to stimulate Oct3/4 expression [22]. Negative regulators of Oct3/4 expression include Gcnf, Coup-tfs, and Cdx2, whose expression are induced upon ES cell differentiation. In addition to regulation of Oct3/4 expression, the transcriptional activity of Oct3/4 protein is regulated by Oct3/4-binding proteins. For example, it is well-established that Sox2 is a co-factor of Oct3/4. β-catenin binds to Oct3/4 and functions as a co-activator that enhances the transcriptional activity of Oct3/4 [23]. The orphan nuclear receptor Dax1 also binds to Oct3/4, but acts as a negative regulator by interfering with the binding of Oct3/4 to DNA [24].

In adult human tissues, expression of Oct3/4 is restricted to germline cells and is very low in other tissues. By contrast, Oct3/4 is expressed in several types of human cancers, including prostate, breast, oral, bladder, and seminoma [25], suggesting the importance of this transcription factor in cancer development. Moreover, Oct3/4 is highly expressed in cancer stem-like cells in breast cancer, lung cancer, and bone sarcoma [26-28]. In lung cancer, Oct3/4 maintains the properties of cancer stem-like cells [28]. In agreement with these findings, high expression of Oct3/4 significantly correlates with poor overall survival of nasopharyngeal carcinoma patients [29].

2.2. Sox2

Sox2 is a transcription factor that belongs to the SRY-related HMG-box protein (Sox) family. The Sox2 protein contains one HMG box, as well as a transactivation domain in its C-terminus. Expression of Sox2 in ES cells is mainly regulated by Oct3/4 and Sox2 itself. In addition, microRNAs (miRNAs) are involved in the control of Sox2 expression. For example, miR-9 binds to the 3'-untranslated region of *Sox2* mRNA and represses Sox2 expression [30]. In addition to regulation at the transcriptional level, the activity of Sox2 protein is regulated by post-translational modifications. Mouse Sox2 protein can be sumoylated at Lys-247, which impairs its binding to the *Fgf4* enhancer [31]. Additionally, acetylation of Lys-75 by p300/CBP promotes the nuclear export of Sox2 [32]. Akt directly interacts with Sox2 to phosphorylate Thr-118, leading to stabilization of the Sox2 protein [33].

Sox2-deficient mouse embryos die shortly after implantation [34], indicating that Sox2 is required for early development. In ES cells, Sox2 usually cooperates with Oct3/4 to regulate the expression of several self-renewal factors, including Nanog, and disruption of the *sox2* gene results in the differentiation of ES cells into trophectoderm-like cells [35]. In addition, Sox2 regulates expression of histone acetyltransferases, including Tip60 and Elp3, and forms a positive feedback loop with the polycomb group protein Eed to control the levels of histone acetylation and methylation [36]. Similar to Oct3/4, Sox2 is one of the four factors required for iPS cell production [19].

Besides its pivotal role in maintaining ES cell self-renewal, Sox2 is closely associated with many types of cancer [37]. The *Sox2* gene is located at chromosome 3q26, a region that is frequently amplified in carcinomas. Amplification of the *Sox2* gene has been observed in human squamous cell carcinomas of the lung and esophagus [38]. Sox2 is involved in the tumorigenesis of several types of tumors, such as lung, breast, skin, prostate, ovarian, and sinonasal. For example, Sox2 is expressed in early-stage breast tumors, and high Sox2 expression is associated with large tumor size [39, 40]. Sox2 expression in breast cancer enhances cancer stem cell-like properties [40]. Sox2 is also reportedly involved in regulation of cancer stem-like cells in ovarian carcinoma [41].

2.3. Nanog

Nanog was originally identified as a gene whose overexpression can bypass the LIF requirement of mouse ES cells for self-renewal [42, 43]. Nanog protein can be ubiquitinated at its PEST domain, resulting in its degradation through the proteasome pathway in ES cells [44]. Phosphorylation of Nanog prevents this ubiquitination, thereby increasing the stability of Nanog [45].

Although *Nanog*-deficient ES cells expand at a slower rate than wild-type cells, they can self-renew and retain expression of Oct3/4 and Sox2 [46]. These observations suggest that Nanog is involved in the growth of ES cells, but is dispensable for ES cell self-renewal. Human Nanog is a reprogramming factor that can produce human iPS cells [5]. In murine cells, mouse Nanog accelerates reprogramming and promotes the transition of pre-iPS cells into mature iPS cells [47].

Normally, Nanog is expressed at early embryonic stages and in germline stem cells, but not in adult tissues. However, Nanog is expressed at high levels in several types of cancers, including breast, cervical, oral and kidney [48]. Nanog is also highly expressed in germ cell tumors [49], which are characterized by the gain of the short arm of chromosome 12, at which the *Nanog* gene is located. In addition, several reports have suggested that Nanog is involved in the epithelial-mesenchymal transition and metastasis. For example, Nanog stimulates cell migration and invasion in ovarian cancer through downregulation of *E-cadherin* and *Foxj1* [50]. Nanog expression is higher in hepatocellular carcinoma cell lines that are highly metastatic than in those that are lowly metastatic, and the Nodal/Smad3 pathway plays an important role in the Nanog-stimulated epithelial-mesenchymal transition [51]. More importantly, Nanog overexpression is associated with poor prognosis in some types of cancer, such as colorectal, ovarian, and breast [48]

Nanog has 11 highly homologous pseudogenes in human cells. Of these, *NanogP8* encodes a full-length protein with only one amino acid difference from Nanog, and is involved in tumorigenesis [52, 53]. It was recently shown that NanogP8 can function as a reprogramming factor, with similar activity to Nanog [54].

2.4. STAT3

STAT3 is a downstream transcription factor of gp130, and is therefore activated by several cytokines, including interleukin (IL)-6, IL-11, and LIF. This transcription factor was initially identified as APRF (acute phase response factor), an inducible DNA-binding protein that binds to the IL-6 response element within the promoters of genes that encode hepatic acute phase proteins [55]. Human and mouse STAT3 proteins both have 770 amino acid residues and are highly homologous to each other (only three amino acid differences). In addition to the DNA-binding domain, STAT3 protein contains a SH2 domain, which facilitates dimer formation upon phosphorylation by upstream kinases including JAK2. STAT3 protein is usually in an inactive form and is localized in the cytoplasm. Upon cytokine stimulation, STAT3 is tyrosine-phosphorylated by activated JAKs. Thereafter, the phosphorylated STAT3 protein forms a homodimer or a heterodimer and translocates into the nucleus, where it stimulates the transcription of its target genes. In mouse development, STAT3 activity is detected during early post-implantation development [56] and *Stat3*-deficient mice die prior to gastrulation [57], suggesting that STAT3 plays an important role in early embryogenesis.

STAT3 plays an indispensable role in the self-renewal of mouse ES cells, and deficiency of STAT3 leads to the differentiation of these cells. By contrast, human ES cells do not require STAT3 activation for their self-renewal. This discrepancy in the requirement for STAT3 is most likely due to differences between mouse and human ES cells, as mouse ES cells are derived from the ICM, whereas human ES cells are derived from the epiblast. Extensive studies have identified many self-renewal factors that are downstream of STAT3 signaling, such as transcription factors, epigenetic regulators, and oncogenes [8].

Although the STAT3 protein is normally only activated in response to signals that control cell growth, overactivation of STAT3 protein has been observed in several types of cancer, including breast, prostate, and pancreas, as well as leukemia and lymphoma [58]. Indeed,

STAT3 is constitutively activated in nearly 70% of tumors. Because of the high frequency of its overactivation, STAT3 is considered to be a valuable target for anti-cancer therapy.

2.5. Krüppel-like factor (Klf) 4

Klfs are a family of transcription factors that play important roles in many fundamental biological processes. They were named "Krüppel-like" owing to their strong homology with the *Drosophila* gene product Krüppel, which is involved in segmentation of the developing embryo. Klf family proteins contain three C2H2-type zinc fingers that bind DNA. *Klf4* was independently cloned by two groups and named "gut-enriched KLF" and "epithelial zinc finger" owing to its high expression in the intestine and skin epithelium, respectively [59, 60]. However, it was later discovered that this transcription factor is expressed in several other tissues, such as lung, testis, and thymus. In addition to a C-terminal DNA-binding domain consisting of zinc fingers, Klf4 protein contains an activation domain in its N-terminus and a repressive domain in its central region. Probably owing to this structure, Klf4 is a bi-functional transcription factor that can either activate or repress transcription of its target genes. Similar to Oct3/4 and Sox2, Klf4 is one of the four factors that induce reprogramming of murine cells [19].

Klf4 is highly expressed in self-renewing ES cells, but not in differentiated ES cells. Klf4 regulates self-renewal-specific expression of Lefty1, in cooperation with Oct3/4 and Sox2 [61]. Klf4 is also involved in Oct3/4 expression [62]. Overexpression of Klf4 results in the inhibition of ES cell differentiation, possibly through upregulation of *Nanog* [63, 64]. In addition, there is a marked overlap between genes that are regulated by Nanog and those that are regulated by Klf4. These observations suggest the importance of Klf4 in ES cell self-renewal. However, *Klf4*-null mice have no detectable defects during embryogenesis [65]. Furthermore, a recent study reported that the function of Klf4 in ES cell self-renewal is partially redundant because combined knockdown of *Klf4*, *Klf2*, and *Klf5*, but not any one gene individually, results in spontaneous ES cell differentiation [62], suggesting that Klf4 is dispensable for ES cell self-renewal.

In cancer cells, Klf4 acts as a tumor suppressor or an oncogene, possibly owing to its bi-functionality. Whether Klf4 acts as a tumor suppressor or an oncogene likely depends on the tumor type. For example, Klf4 functions as a tumor suppressor in the intestinal and gastric epithelium, and expression of *Klf4* is downregulated in human colorectal and gastric carcinomas [66, 67]. On the other hand, overexpression of Klf4 in the skin results in squamous epithelial dysplasia, eventually leading to squamous cell carcinoma [68, 69]. A high expression level of Klf4 significantly correlates with a poor prognosis in hepatocellular carcinoma [70].

2.6. Zinc-finger protein (Zfp)-57

Zfp57 is a transcription factor that was originally identified as an undifferentiated cell-specific gene in F9 embryonal carcinoma cells [71]. Mouse Zfp57 protein contains one Kruppel-associated box (KRAB) domain and five zinc fingers, while human Zfp57 protein has one KRAB domain and seven zinc fingers. In adult mouse, Zfp57 is highly expressed in testis and

brain [71, 72]. Loss of the zygotic function of Zfp57 leads to partial lethality, while eliminating both the maternal and zygotic functions of Zfp57 results in complete embryonic lethality [73]. Through its KRAB domain, Zfp57 interacts with KRAB-associated protein 1 (Kap1), a scaffold protein for heterochromatin-inducing factors, and thus participates in genome imprinting by recruiting Kap1 to multiple imprinting control regions [73, 74]. Mutations in the *Zfp57* gene cause transient neonatal diabetes mellitus type 1 [75]. Zfp57 is a downstream molecule of STAT3 and Oct3/4 in ES cells, and is therefore specifically expressed in self-renewing ES cells [76]. Zfp57 deficiency has no effect on the self-renewal or growth in ES cells, suggesting that this transcription factor is dispensable for ES cell self-renewal.

Based on our prediction that a molecule expressed in self-renewing ES cells may play an important role in cancer cell growth, we recently screened several ES cell-specific transcription factors for their tumor-promoting activity, and found that Zfp57 can promote anchorage-independent growth of human fibrosarcoma HT1080 cells [77]. Zfp57 overexpression enhances, while its knockdown suppresses, HT1080 tumor formation in nude mice. Zfp57 regulates the expression of insulin-like growth factor 2, which plays a critical role in Zfp57-induced anchorage-independent growth and tumor formation. Furthermore, overexpression of Zfp57 causes anchorage-independent growth of the mouse immortal fibroblast cell line NIH3T3, and immunohistochemical analysis revealed the overexpression of Zfp57 in several cancers, including pancreatic, gastric, breast, colon, and esophageal. These results suggest that *Zfp57* is an oncogene in some types of cancer. Moreover, we also found that Zfp57 is involved in anchorage-independent growth of ES cells and that *Zfp57*-null ES cells form smaller teratomas than the parental ES cells in immunodeficient mice, suggesting the importance of Zfp57 in teratoma formation by ES cells.

3. Oncogenes that are involved in ES cell self-renewal

3.1. β-catenin

The importance of the Wnt pathway in tumorigenesis was recognized by identification of adenomatous polyposis coli (APC) mutations in familial adenomatous polyposis [78, 79]. In Wnt signaling, the Apc protein functions as a negative regulator and is involved in degradation of β-catenin, the mammalian homologue of *Drosophila* Armadillo. β-catenin contains multiple armadillo repeats in its central region and a transcriptional activator domain in its C-terminal region. Human and mouse β-catenin proteins both have 781 amino acid residues and are almost identical to each other (only one amino acid difference). β-catenin acts as a transcriptional co-activator and an adaptor protein for intracellular adhesion. In epithelial tissues, β-catenin interacts with cadherins and α-catenin, and regulates epithelial cell growth and intracellular adhesion. By contrast, in Wnt signaling, β-catenin is a major transcriptional modulator and plays a crucial role in embryogenesis. In the absence of Wnt signaling, Apc forms a complex with β-catenin and Axin. This leads to phosphorylation of β-catenin by glycogen synthase kinase (GSK)-3β, which triggers degradation of β-catenin. When Wnt binds to its receptor Frizzled, Disheveled is hyper-phosphorylated, which results in release of

GSK3β from the β-catenin degradation complex and prevents phosphorylation of β-catenin by GSK3β. Unphosphorylated β-catenin translocates into the nucleus, where it forms a complex with Tcf/Lef and functions as a transcriptional co-activator.

In human cancers, such as colon cancer, the β-catenin/Tcf/Lef complex positively regulates the expression of a variety of cancer-associated genes, including *cyclin D*, *Tert*, and *c-Myc*, to promote tumorigenesis. Apc mutation leads to stabilization and accumulation of β-catenin in nuclei. However, in some cancers, mutation of β-catenin itself renders this protein unable to be phosphorylated, resulting in its stabilization.

Accumulated evidence suggests that Wnt/β-catenin signaling contributes to the maintenance of ES cell self-renewal. For example, *Apc*-null ES cells show severe differentiation defects [80]. Undifferentiated ES cells can be maintained in a self-renewing state by using conditioned medium from Wnt3a-expressing cells [81]. Furthermore, enforced expression of an activated form of β-catenin maintains the self-renewal of ES cells, even in the absence of LIF [23, 82]. Expression of γ-catenin, which has a similar structure to β-catenin, partially sustains the self-renewal of ES cells in the absence of LIF [23]. β-catenin binds to Oct3/4 to enhance its transcriptional activity in ES cells, leading to upregulation of *Nanog*, a target gene of Oct3/4 [23]. The Wnt/β-catenin pathway upregulates expression of *STAT3*, and this signaling converges with that of LIF [83]. Similarly, Wnt and LIF work in synergy to maintain the pluripotency of mouse ES cells [84]. On the other hand, the self-renewal of ES cells can be maintained without β-catenin [85]. Taken together, these results suggest that β-catenin promotes, but is dispensable, for ES cell self-renewal. Moreover, it was recently shown that ES cells lacking Wnt signaling resemble epiblast stem cells in terms of their morphology and gene expression [86, 87]. This suggests that Wnt/β-catenin signaling prevents the transition of ES cells from a naïve to a primed pluripotent state. In addition, β-catenin regulates Tert expression in ES cells [88], as is the case in cancer cells.

3.2. Gli

The zinc-finger transcription factor Gli is a central player in the Hedgehog (Hh)-mediated signaling pathway, which plays a critical role during embryogenesis. Gli belongs to the Klf family and has three isoforms in mammals, namely, Gli1, Gli2, and Gli3. Gli1 and Gli2 usually act as transcription activators, while Gli3 is a transcription suppressor. All Gli proteins have a DNA-binding domain consisting of C2-H2 class zinc fingers. In addition, Gli1 and Gli2 contain a C-terminal transactivation domain, while Gli2 and Gli3 have an N-terminal repression domain. Hh family proteins, namely, Sonic Hh, Indian Hh, and Desert Hh, function as ligands of the transmembrane receptor Ptch1. In the absence of a Hh ligand, Ptch1 inhibits the activity of the G-protein coupled receptor-like protein Smo, resulting in formation of a complex of Gli2 and Gli3 with the inhibitory protein Sufu. This results in cleavage of Gli2 and Gli3 into their repressor forms, which translocate into the nucleus. By contrast, binding of Hh ligands to Ptch1 results in the release and activation of Smo, leading to activation and nuclear translocation of Gli2, which results in transactivation of target genes, including *Gli1*.

Gli1 was originally identified as an amplified gene in a human glioma cell line [89]. It was recently shown that Hh/Gli signaling regulates the self-renewal of glioma stem cells, as well

as their expression of stemness genes, including *Oct3/4* and *Sox2* [90]. Gli1 forms a positive feedback loop with Nanog, and the Nanog/Gli1 signaling axis is indispensable for regulation of glioma stem cells [91]. In Ewing sarcoma, expression of Gli1 is regulated in an Hh-independent manner: EWS-FLI, an oncogenic transcription factor that is produced by chromosomal translocation, directly upregulates *Gli1* expression to promote tumor growth [92, 93].

Gli1 and Gli2 are both highly expressed in undifferentiated ES cells, while Gli3 expression level is low [94]. Gli1 and Gli2 are downstream molecules of Oct3/4 and Nanog, and their expression is downregulated upon differentiation. When Gli1 and Gli2 are suppressed by a dominant-negative mutant of Gli2, expression of the self-renewal marker Sox2 decreases, whereas that of the differentiation markers Gata4 and Cdx2 increases, suggesting the importance of Gli activity for ES cell self-renewal. However, expression of this dominant-negative Gli2 mutant does not affect expression of the self-renewal markers Oct3/4 and Nanog. These findings suggest that Gli activity is involved in repressing ES cell differentiation, but is dispensable for ES cell self-renewal. In addition, Gli is involved in ES cell growth [94, 95].

3.3. Akt

The serine/threonine protein kinase Akt was independently identified by three different groups. Two groups identified this kinase as being homologous to protein kinase C and protein kinase A, giving rise to the names "protein kinase B" and "RAC-PK" (related to the A and C kinases) [96, 97]. The other group identified this kinase as the cellular counterpart of the oncogene *v-akt* of the acutely transforming retrovirus AKT8 that is found in a rodent T-cell lymphoma [98]. Here, I will describe this protein as "Akt". Akt has three isoforms, namely, Akt1, Akt2, and Akt3. Each Akt family member has an N-terminal pleckstrin homology domain, a short α-helical linker, and a C-terminal kinase domain. Akt is directly downstream of phosphatidylinositol-3-OH kinase, and is a key player in the regulation of cell growth and survival.

As expected from its identification as a counterpart of a viral oncogene, Akt plays an important role in human malignancy [99, 100]. Several studies have identified amplification of the *Akt* gene in human cancers. Amplification of *Akt1* was detected in a human gastric cancer [101]. Amplification and overexpression of Akt2 were detected in ovarian and pancreatic cancers. Artificial activation of Akt1 or Akt2 can transform NIH3T3 cells [102, 103], and Akt2 anti-sense RNA inhibits the tumorigenic phenotype of pancreatic carcinoma cell lines [104]. Furthermore, Akt1 kinase activity is often increased in prostate and breast cancers and is associated with a poor prognosis [102].

A constitutively activated Akt mutant can maintain the undifferentiated phenotype of mouse ES cells, even in the absence of LIF, although the mechanism underlying Akt-mediated maintenance of ES cell self-renewal is unclear [105]. Bechard and Dalton demonstrated that Akt phosphorylates, and thereby inactivates, GSK3β in ES cells, suggesting that Akt maintains ES cell self-renewal by inactivating GSK3β and thus stimulating activation of β-catenin [106]. By contrast, Watanabe *et al.* did not observe the accumulation of β-catenin in nuclei or activation of the transcriptional activity of β-catenin in mouse ES cells [105]. Another possible

mechanism is that Akt induces expression of Tbx3, which in turn stimulates expression of Nanog to maintain ES cell self-renewal [107].

3.4. c-Myc

c-Myc is a cellular counterpart of the *v-myc* gene, which was isolated from the avian retrovirus MC29 [108], and belongs to a family of helix-loop-helix/leucine zipper transcription factors. c-Myc forms a complex with Max, which results in the increased stability of c-Myc protein [109]. Phosphorylation of Thr-62 by Erk also stabilizes c-Myc, while phosphorylation of Thr-58 by GSK3β reduces the stability of c-Myc [110]. c-Myc regulates expression of its target genes through binding to E-box sequences and recruiting histone acetyltransferases. Under normal conditions, when cells are stimulated by an internal or external growth-promoting signal, the level of c-Myc rapidly and transiently increases to induce cell proliferation, and the level of c-Myc subsequently returns to a low level in quiescent cells.

It is well-established that many, if not most, human tumors have elevated levels of c-Myc owing to gene amplification and translocation [111, 112]. Gene amplification of *c-Myc* has been reported in several cancers, including breast, ovarian, and colon. A common human translocation involving *c-Myc* is t(8;14), which is critical for the development of Burkitt's lymphoma. Although c-Myc was one of the four factors originally identified as being required for iPS cell production [19], generation of iPS cells without this transcription factor has been reported [113], suggesting that c-Myc is dispensable for cell reprogramming.

During the self-renewal of ES cells, levels of c-Myc are elevated [114]. By contrast, upon LIF withdrawal, the level of *c-Myc* mRNA decreases and c-Myc protein is phosphorylated on Thr-58 by GSK3β, which triggers degradation of c-Myc. Expression of a stable c-Myc mutant, in which the Thr-58 residue is mutated to alanine, allows ES cells to self-renew in the absence of LIF. By contrast, expression of a dominant-negative form of c-Myc inhibits the self-renewal of ES cells and induces their differentiation. These findings suggest that c-Myc is critically involved in maintaining the self-renewal of ES cells. Moreover, analysis of *Max*-null ES cells revealed that the function of c-Myc/Max in ES cell self-renewal seems to be largely independent of the Oct3/4, Sox2, and Nanog regulatory networks [115].

4. Future prospective

As I have described here, several common transcription factors are involved in the regulation of ES cell self-renewal and cancer cell growth (Fig. 2). This raises several intriguing possibilities. Considering that these common transcription factors are stem cell-specific and are involved in tumor growth, it is possible that they are specifically expressed in cancer stem cells and play important roles in the growth of these cells. Therefore, it is possible that these factors are good markers of cancer stem cells. If so, it might be possible to utilize these factors to isolate cancer stem cells, which will help to advance cancer stem cell research. Furthermore, identification of small compounds that specifically inhibit the functions of these factors may lead to the development of new anti-cancer drugs that can selectively kill cancer stem cells.

Since ES cells and iPS cells have similar gene expression profiles, it is likely that these common transcription factors are also expressed in iPS cells. Considering the transforming potential of these factors, it is possible that their high expression in iPS cells increases the risk of tumor formation during cell therapy using iPS-derived cells. Indeed, we have already found that *Zfp57*-null ES cells are significantly less able to form tumors than wild-type ES cells.

In this way, understanding the roles of putative oncogenes in ES cells will not only help to elucidate the molecular basis underlying the similarity between ES cells and cancers cells, but will also help to develop a novel method that can be used in cancer therapy and regenerative medicine.

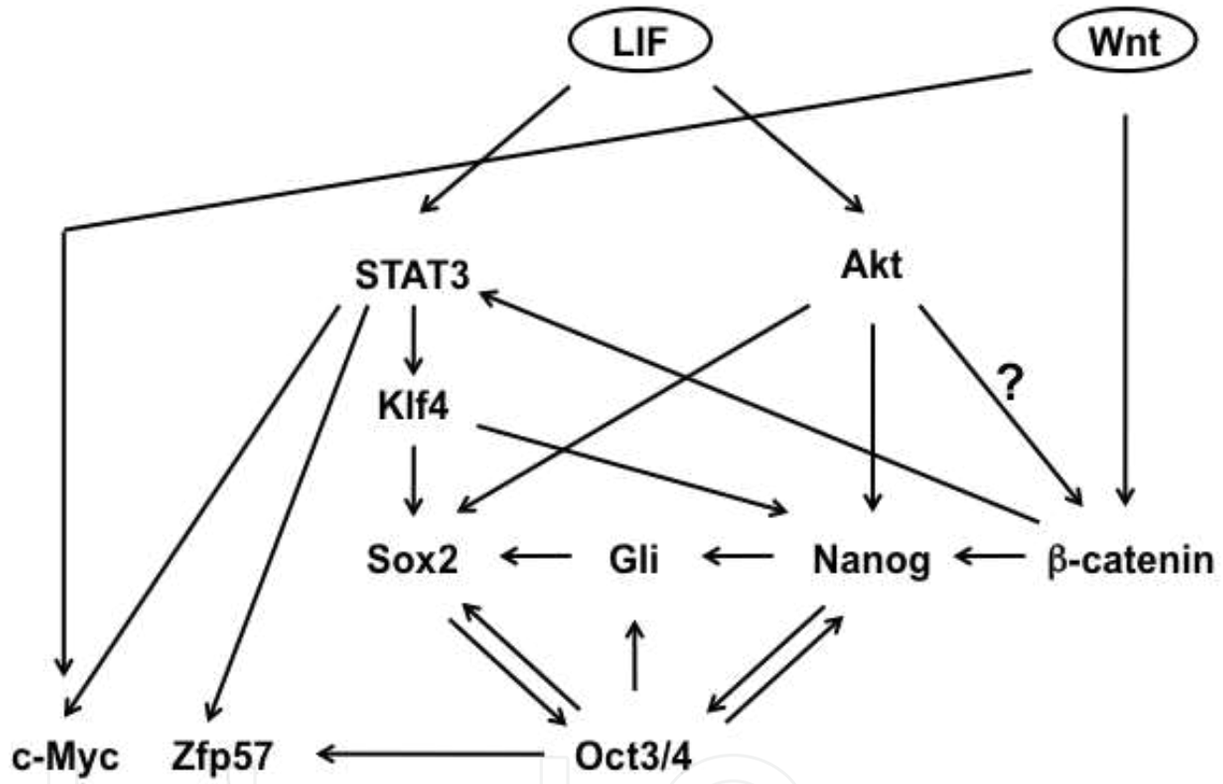

Figure 2. Transcription factor network that regulates ES cell self-renewal. Considering the similarities between ES cells and cancer cells, at least a part of this network may be used for growth regulation in cancer cells.

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