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Epigenetic mechanisms of tumorigenicity manifesting in stem cells

Po-Yuan Tung[1,2,3,4] and **Paul S. Knoepfler**[#,1,2,3,4]

[1]Department of Cell Biology and Human Anatomy, University of California Davis School of Medicine

[2]UC Davis Genome Center, University of California Davis, Davis, CA 95616, USA

[3]UC Davis Comprehensive Cancer Center

[4]Institute of Pediatric Regenerative Medicine, Shriners Hospital For Children Northern California, Sacramento, CA 95817, USA

Abstract

One of the biggest roadblocks to using stem cells as the basis for regenerative medicine therapies is the tumorigenicity of stem cells. Unfortunately, the unique abilities of stem cells to self-renew and differentiate into a variety of cell types are also mechanistically linked to their tumorigenic behaviors. Understanding the mechanisms underlying the close relationship between stem cells and cancer cells has therefore become a primary goal in the field. In addition, knowledge gained from investigating the striking parallels between mechanisms orchestrating normal embryogenesis and those that invoke tumorigenesis may well serve as the foundation for developing novel cancer treatments. Emerging discoveries have demonstrated that epigenetic regulatory machinery plays important roles in normal stem cell functions, cancer development, and cancer stem cell identity. These studies provide valuable insights into both the shared and distinct mechanisms by which pluripotency and oncogenicity are established and regulated. In this review, the cancer-related epigenetic mechanisms found in pluripotent stem cells and cancer stem cells will be discussed, focusing on both the similarities and the differences.

Cancer hallmarks in stem cells

Stem cells, by definition, are endowed with the capacities to self-renew and to maintain multi- or pluripotency. Self-renewal is the ability to proliferate while the cells consistently remain in an undifferentiated state in order to maintain stem cell homeostasis during discrete developmental windows or even throughout the lifetime of the organism for homeostasis or repair. This replicative potential of stem cells is analogous in a number of ways to that of transformed cancer cells. In fact, limitless proliferation potential, termed immortality, is one of the most fundamental hallmarks of malignant tumors (1, 2). In addition, the maintenance

[#]Corresponding author knoepfler@ucdavis.edu.

of "stemness" is achieved by restricted differentiation, apoptosis, and cellular senescence, all of which happen to be important cancer characteristics.

Notably, characterizations of pluripotent stem cells were initiated in the 1950s when teratoma (benign) and teratocarcinoma (malignant), tumors composed of tissues from all three germ layers, were described and studied in the mouse strain 129. This strain shows an incidence of spontaneous testicular teratoma of approximately 1% (3). The pluripotent embryonic carcinoma cells (ECCs) isolated from teratocarcinomas are capable of self-renewal as well as differentiation into a very wild range of cell types. Later more extensive studies and increased understanding of ECCs, including the derivation of several key pluripotency makers and the isolation of the cells, have grounded the foundations of embryonic stem cells (ESCs) research (4-6). Further studies of cultured human ESCs demonstrated that ECCs constitute the abnormal malignant counterparts of ESCs, emphasizing the close relationship between the two cell types (7, 8).

The cancer stem cell (CSCs) hypothesis postulates that immortality is a pathological offshoot of the normally exquisitely controlled proliferation machinery in normal stem cells from which mis-regulated cell expansion occurs due to oncogenic mutations (9, 10). This CSC model further proposes that there is a subpopulation of cancer cells within tumors that possesses some stem cell-related properties such as self-renewal and that give rise to tumors (11). However, whether CSCs originate from normal stem cells or from differentiated cells, which reacquire stem cell capabilities through a dedifferentiation process, is a long-standing question (12). The answer to this key open question may vary depending on tumor type and stage as well. Take the hematopoietic system for example, leukemia stem cells have been shown to arise from both self-renewing stem cells and also from transient repopulating progenitors, providing evidence that stem cells and late-stage precursors can both undergo oncogenic transformation and result in similar tumor phenotypes (13).

The existence of CSCs in tumors is still debated because many studies cannot successfully verify the similarities between normal and cancer stem cells, nor can they provide any clear and consistent distinction between the two types (14). The traits used to define CSCs do not rely on knowledge of their cellular origin within normal tissues, rather on the basis of experimental characterizations of cancer cell populations (15). Thus, the CSC model that argues for a hierarchy of cells analogous to normal stem cell development is yet to be validated (16). If CSCs arise through mutations that occur in previously normal stem cells, another valuable related question to address is the extent to which uncontrolled self-renewal molecular machinery specifically contributes to oncogenesis.

On the other hand, the discovery of induced pluripotent stem cells (iPSCs) supports the idea that CSCs may in some cases arise from differentiated cells through a process of dedifferentiation or reprogramming. This hypothesis is based on the fact that iPSC reprogramming and tumorigenesis share striking molecular similarities at multiple stages of oncogenesis, from the initial oncogenic transformation to the development of an actual complex tumor (17, 18). Although cancer hallmarks and cancer-related changes, both genetic and epigenetic, have been found in some cases in iPSCs (19), oncogenic transformed cells and iPSCs generated from common parental fibroblasts are highly-related yet distinct

cell types based on expression profiling (20). Importantly, transient expression of reprogramming factors *in vivo* generates tumors with altered epigenetic states that cause abnormal growth of the incompletely-reprogrammed cells, supporting the idea that premature termination of induced pluripotency can result in cancer development (21).

Related epigenetic signatures of stem cells and cancer cells

Epigenetic mechanisms, including DNA methylation and histone modifications, play important roles in stem cell identity, especially that of pluripotent ESCs (22, 23). The studies of Polycomb group (PcG) proteins in ESCs and various cancers indicate the presence of epigenetic stem cell signatures in cancers (24, 25). PcG proteins are key epigenetic regulators in ESCs due to their important roles to impede the transcriptions of developmental genes through generating repressive histone marks (26). Many DNA-hypermethylated cancer-specific genes are also targets of the PcG repressor complex in ESCs (24). Furthermore, the identification of a specific DNA hypermethylation module from adult stem/progenitor cells in cancers indicates that these epigenetic signatures may contribute to the "stemness" of cancers (27).

Significant progress has been made in our understanding of the epigenetic alterations involved in the abnormal cellular processes that lead to the development of malignancy. A host of abnormal epigenetic events, termed "epi-mutations", such as DNA methylation aberrations, histone modification changes, deregulated nucleosome remodeling, and miRNA changes, are present in various forms and combinations in almost all human cancers (28). Each cancer type may harbor a specific 'epigenetic signature', which will provide useful information for diagnosis and the design of new specific treatments (29). Interestingly, epigenetic cancer-related signatures are also seen in apparently normal stem cells (30-32). Genome-wide analysis during iPSC generation showed that cancer-related epigenetic changes, such as DNA hypermethylation of normally unmethylated CpG island-containing promoters, were observed in both partially and fully reprogrammed cells (33). In addition, altered epigenetic regulation, including changes in global DNA methylation patterns, is responsible for the cancer development induced by incomplete reprogramming *in vivo* (21). Therefore, many cancer-associated epigenetic signatures are also commonly found in stem cells, just as the epigenetic stem cell signatures are in cancer cells.

The DNA methylation writers: DNMTs

In mammals, DNA methylation at the C5 position of cytosine, predominantly in the context of CpG dinucleotides, is a mechanism for long-term gene silencing and plays multiple functional roles during development (Figure. 1) (34). Specific methylation patterns are established and maintained by the DNA methyltransferases (DNMTs), which are divided into two classes according to their activities. The maintenance methyltransferases, like DNMT1, are responsible for delivering specific methylation patterns to daughter cells during cell division (35, 36), while the *de novo* methyltransferases, DNMT3A and 3B, function to establish methylation patterns during development (36). In addition, dynamic regulated changes in DNA methylation patterns play essential roles in reprogramming during iPSC generation(37). DNMT3A and 3B functions are also essential for ESC

differentiation (36). Hypermethylation of specific genomic domains by Dnmt3a and 3b in ESCs is important for teratoma formation, indicating that these proteins are responsible for the establishment and maintenance of functionally important methylation patterns in ESCs (38). However, mouse ESCs in which Dnmt1, Dnmt3a, and Dnmt3b are disrupted by gene targeting exhibit a loss of DNA methylation, but surprisingly retain self-renewal function (39). This suggests that DNA methylation is not entirely essential to ESCs and plays more specific roles. Similarly, iPSCs generated from Dnmt3a and Dnmt3b-deficient fibroblasts still exhibited reactivated pluripotency genes and possessed self-renewal, but showed restricted developmental potential that was rescued upon reintroduction of Dnmt3a and Dnmt3b, indicating that *de novo* DNA methylation by Dnmt3a and Dnmt3b is dispensable for reprogramming but is critical for proper differentiation (40).

Aberrant hypermethylation of tumor-suppressor genes and genetic inactivation of DNA methyltransferases are well-characterized events in cancers (41, 42). For example, DMNT3A mutations are found in human leukemias (42), and deletion of DNMT3A leads to accelerated lung tumor progression (43), implying a strong tumor-suppressor role for DNMT3A. On the other hand, DNMT1 mutations have not been reported in cancers, yet DNMT1 plays an essential role in promoting cancer progression via repressing the expression of tumor suppressors. Inhibition of DNMT1 function results in demethylation and re-expression of tumor suppressor genes, suggesting that DNMT1 is necessary to maintain the aberrant gene silencing in human cancer cells (44). In a mouse disease model, haploinsufficiency of Dnmt1 is sufficient to impair leukemia stem cell self-renewal and proliferation through hypomethylation and derepression of specific tumor suppressor genes, indicating that Dnmt1 activity is required for CSC identity and tumorigenesis (45). Although the mechanisms of how these DNMTs participate in cancer-associated progression events, such as cell cycle control and apoptosis, are still under investigation, it is clear that they may play important but markedly different roles in various cellular contexts.

The DNA methylation erasers: TETs in stem cells and cancer

Another prominent cytosine modification is the conversion of 5-methylcytosine (5-mC) to 5-hydroxymethylcytosine (5hmC) catalyzed by the 2-oxoglutarate (2OG)- and Fe(II)-dependent enzymes encoding by the *TET* family genes (Ten Eleven Translocations; Figure. 1) (46). The oxidation mediated by TETs to generate 5hmC subsequently leads to conversion to unmodified cytosine, which gives the net result of demethylation events in the genome (47). Interestingly, increased 5hmC was recently found in ESCs cultured in 2i medium, media supplemented with two small-molecule kinase inhibitors (48), compared to ESCs grown in media with serum supplemented with leukemia inhibitory factor (LIF) (49-51). Adding 2i to ESCs originally grown in serum-containing medium induces a shift in methylation profile. Culturing of ESCs in 2i medium results in increased 5hmC levels and a coincident rapid decline of *Dnmt3a, 3b,* and *3l* gene expression (51), as well as enhanced Tet1/2 activity, generating the epigenetic ground state of pluripotency (50, 51). Even though Tet1 and Tet2 together are responsible for the bulk of 5hmC production in ESCs cultured in the presence of LIF, no major changes were observed in ESC morphology and the expression of key pluripotency markers upon siRNA-mediated depletion of either Tet1 alone or both Tet1 and Tet2 (52). Instead, Tet1 and Tet2 depletions resulted in altered cell lineage

specification in ESCs. Just as Tet1-depletion skews ESC differentiation toward the endoderm-mesoderm lineage, Tet2 deletion in ESCs causes lineage bias toward neuroectoderm in teratoma, suggesting that Tet1 and Tet2 are important for cell lineage specification during ESC differentiation (52). Interestingly, although homozygous mutation of *Tet3* led to neonatal lethality, highlighting its essential roles during embryogenesis (53), Tet3 is not associated with the pluripotent state of ESCs and iPSCs (52).

Recently, many studies have focused on the involvement of TET proteins during reprogramming (54). iPSCs generated from overexpression of *Oct4, Sox2, Klf4,* and *c-Myc* (OSKM) in mouse embryonic fibroblasts (MEFs) have elevated *Tet2* expression and increased global 5hmC levels, while *Tet1* and *Tet3* levels remain unaltered (55). Consistent with the idea that Tet2 can mediate hydroxylation during cellular reprogramming, in Tet2-knockdown MEFs, which possessed significantly reduced 5hmC levels at the *Nanog* locus compared with the control MEFs, OSKM introduction failed to generate iPSC colonies. In the same OSKM reprogramming system, *Oct4* gene demethylation and transcriptional reactivation are promoted by TET1 through 5hmC conversion, which might be the reason why *Tet1* can be used to replace *Oct4* during the early stage of reprogramming (56). Notably, both TET1 and TET2 proteins also interact with NANOG, and overexpression of either TET1 or TET2 together with NANOG increased the efficiency of iPSC colony formation (57). Mechanistically, TET1 and NANOG act together to increase 5hmC levels and co-occupy genomic loci of genes associated with the maintenance of pluripotency and also lineage commitment in ESCs, including *Oct4* and *Esrrb* (57). Taken together, global and specific hypomethylation events mediated by TET1 and TET2 activities play important roles to establish the native epigenetic state required for the maintenance of pluripotency in ESCs and for cellular reprogramming. While a few specific targets of TET1 and TET2 have been verified, the extent and the characteristics of their overlapping targets remain to be elucidated.

Global DNA hypomethylation has long been recognized as an epigenetic hallmark in many tumors, both benign and malignant (58). Hypomethylation and activation of specific oncogenic-associated genes also occurs. For example, hypomethylation and transcriptional activation of the *R-Ras* proto-oncogene occurs in gastric cancer (59, 60). Based on the oxidation function of TETs to convert 5mC to 5hmC, which leads to demethylation of cytosine, they could contribute to the global DNA hypomethylation state and in that way could promote carcinogenesis. However, substantially reduced expression of all three *TET* genes, which is associated with a decrease of 5hmC, is often observed in human cancers compared with the matched surrounding normal tissue, suggesting that decreased 5hmC can be used as a biomarker during cancer development (61). Additionally, genome-wide mapping of 5mC and 5hmC in melanoma revealed that loss of 5hmC is an epigenetic hallmark of melanoma that correlates with tumor progression and prognosis (62).

TET family members have been identified as a major pathologically target of isocitrate dehydrogenases 1 and 2 (IDH1 and IDH2) mutations, which are frequently found in multiple human cancers (63). In a distinct subset of glioblastomas known as methylator phenotype, hypermethylation at the glioma-CpG island loci is associated with *IDH1* mutations (64). In addition, *IDH1* mutation is sufficient to cause the hypermethylation phenotype in

glioblastomas (65). Impaired hematopoietic differentiation and disrupted TET2 functions are induced by the leukemic IDH1 and IDH2 mutations (66). Importantly, the at least partially linear IDH-TET pathway is demonstrated by the finding that mutant IDH1 and IDH2 can lead to inhibited TET activity (67). Even though the function of IDH varies in different cancer types, the important epigenetic roles played by the IDH-TET pathway in stem cell differentiation and tumor suppression demonstrates interplay of genetic elements during tumorigenesis.

When looking into each individual member of the TET family, interestingly TET2 has been characterized as a powerful tumor-suppressor, whereas TET1 has been recognized as both a tumor-suppressor and a potential oncogene in different cancers. Mutant TET2 with concomitant impaired hydroxylation of 5mC was identified in myeloid cancers (68). In an animal model of conditional TET2 loss, increased hematopoietic stem cell self-renewal and myeloid transformation were observed (69). TET2 is a downstream target of the oncogenic microRNA miR-22 that promotes hematopoietic stem cell self-renewal and malignant transformation (70). Over-expression of TET2 in human melanoma cells can re-establish 5hmC levels and suppress tumor growth (62). Similarly, enhanced TET1 activity in breast cancer suppresses cancer growth and metastasis, while also being a prognostic signature for patient survival (71). Mechanically, as both a direct downstream target and a fusion protein partner of the mixed lineage leukemia (MLL) protein, TET1 plays a powerful oncogenic role in MLL-related leukemia (72).Overall, the roles of TET1 and TET2 are highly context specific, possibly due to different subsets of downstream targets. Structurally, both TET1 and TET2 have a CD domain that contains Cys-rich and DSBH regions and exhibits 2-oxoglutarate (2-OG)- and iron (II)-dependent dioxygenase activity. The N-termini of TET1, but not TET2, contains a CXXC domain that mediates direct DNA-binding ability (73, 74). Although TET1 and TET2 share the same catalytic functions, their pathological roles in leukemia are completely opposite. Similarly, while TET1 acts as a tumor-suppressor in solid tumors, such as breast cancer, it also displays an oncogenic role that is essential in leukemogenesis.

Histone modification and chromatin regulators

In addition to DNA methylation, another major epigenetic mechanism at work in some common ways in both stem cells and cancer cells is post-translational modification (PTMs) of histones. These modifications include but are not limited to methylation, acetylation, phosphorylation, ubiquitination, and sumoylation (75, 76). Together with DNA methylation discussed above, specific epigenetic landmarks manifesting as histone PTMs are established to maintain unique gene expression profiles during reprogramming and oncogenesis (77). In Eukaryotes, histone PTMs mediated by different types of chromatin regulators are critical for normal chromatin structure, gene expression, cell behavior, and developmental events (78). Mutations of histone-modifying genes are frequently observed in human cancers (79-81). Accordingly, mutations of histones, including the non-canonical histone H3.3, and perturbation of histone modifications have been strongly implicated in tumorigenesis (82). Importantly, chromatin regulators contribute to malignancy in many different ways. Besides being *bona fide* oncogenes or tumor suppressors, they also are involved in regulation of many cancer-related processes, such as genome stability, senescence, metastasis, and

epithelial-mesenchymal transition (EMT)(83). Notably, the *Myc* proto-oncogene, which is also an important player in iPSC generation, regulates global chromatin structure contributing to oncogenesis via histone PTMs by targeting epigenetic machinery (84, 85), indicating histone regulators might play the central roles that link cancer cells and stem cells. Here we will focus on the chromatin regulators that have been shown to function in both CSCs and pluripotent stem cells (Figure. 2).

BMI1, a component of PRC1

Polycomb group (*PcG*) genes, which encode components of multimeric transcriptional repressor complexes that epigenetically modify chromatin, are crucial in directing cell fate during developmental processes and also play a central role in stem cell maintenance and lineage specification (86). In addition, *PcG* genes are also candidate determination genes for the activities of both normal and cancer stem cells because of their regulation of cell cycle, senescence, apoptosis, and differentiation (87). Two intensively studied components, BMI1 (B-cell-specific Moloney murine leukemia virus insertion site 1) and EZH2 (enhancer of zeste homolog 2), will be discussed for their importance in CSCs and cancer development.

BMI1 itself has no known enzyme activity but is a key regulatory component of the polycomb repressive complex 1 (PRC1) that leads to histone H2A ubiquitination and gene silencing (88). PRC1 has been shown to regulate cell proliferation and self-renewal of CSCs, contributing to cancer recurrence and chemoresistance in several human cancers (89). One of the first indications that PcG proteins play a role in cancer was BMI's identification as a c-Myc-collaborating oncogene (90). Numerous studies have demonstrated that BMI1, overexpressed in a variety of cancers, correlates with poor prognosis and is a predictor of early relapse (91, 92). For example, in glioblastoma multiforme (GBM), one of the most common and lethal types of brain tumors, BMI1 sustains the clonogenic potential of CD133-positive tumor initiating cells and promotes tumor formation through prevention of cell apoptosis and differentiation (93). Besides repressing the INK4a/ARF locus which regulates cell proliferation and senescence (94), BMI1 also suppresses alternate tumor suppressor pathways that can overcome INK4a/ARF/P53 inactivation and PI3K/AKT hyperactivaion. Thus, BMI1 regulates multiple cancer-related pathways during gliomagenesis (93). Moreover, BMI1 plays essential roles in the induction of EMT (95), a developmental program that can promote cancer cell metastasis and CSC self-renewal capability (96). Hence, BMI1 has strong potential to be developed as a cancer therapeutic target because of its key role in CSCs (97). Importantly, a recent study focusing on an oncogenic microRNA miR-22 in breast cancer indicated that *BMI1* is upregulated via hypermethylation and epigenetic silencing of miR-200 promoter mediated by miR-22-inhibited TET family proteins (98). These observations provide fundamental mechanistic insights into how different epigenetic layers and players, starting from microRNAs, to DNA demethylation, and then histone modifications, as well as their regulatory enzymes, coordinate as an integrated epigenomic system to orchestrate stem cell identity during tumor progression (99).

BMI1 plays a central role in the self-renewal of somatic stem cells in a variety of tissues and organs (87, 89). However, Bmi1-knockout ESCs have been established and grow normally

(100). While Bmi1 expression is not normally detectable in ESCs and ectopic expression of Bmi1 has no effect on ESC self-renewal, Bmi1 can effectively enhance development of hematopoietic cells upon ESC differentiation (101). Inhibition of Bmi1 via shRNAs reduces the efficiency of reprogramming induced by OSKM, indicating that Bmi1 acts as a facilitator of reprogramming (102). Overexpression of Bmi1 can cause transdifferentiation of mouse fibroblasts into neural stem cell-like cells, and in combination with Oct4, can replace Sox2, Klf4 and c-Myc during iPSCs generation (103). Overall, in ESCs, BMI1 is more important in regulating the transition of cell identities, both differentiation and reprogramming, than in the establishment and maintenance of pluripotent status.

EZH2, a component of PRC2

EZH2 is the catalytic subunit of the polycomb repressive complex 2 (PRC2) and is involved in repressing gene expression through methylation of histone H3 on lysine 27 (H3K27). Emerging evidence suggests that increased activity of EZH2 might induce dedifferentiation of normal cells back to a stem cell–like state through epigenetically repressing cell-fate regulatory genes and tumor suppressor genes, resulting in tumorigenesis (104-107). Overexpression of EZH2 has been implicated in aggressive tumor progression and correlates with poor prognosis in several cancers (108, 109). Elevated expression of EZH2 in breast tumor initiating cells (BTICs) leads to reduced expression of RAD51 and increased genomic damage, which promotes BTICs expansion and tumor growth via accumulation of recurrent RAF1 amplification (110). Attenuating expression of EZH2 via pharmacological inhibitors disrupts CSC function and tumor growth in pancreatic cancer, prostate cancer, and glioblastoma (111-114). Importantly, the expression of the oncogene c-Myc, which is essential for CSCs in glioblastoma (115), is significantly suppressed upon EZH2 depletion, suggesting that EZH2 can promote cancer progression through positively regulating c-Myc (114). Notably, PRC2 and EZH2 specifically are also emerging as important targets of mutated histone variant H3.3 in pediatric glioblastoma and diffuse intrinsic pontine glioma (82).

Notably, EZH2 also interacts with and recruits DNA methyltransferases at certain target genes mediating establishment of repressive chromatin domains (116). In *Dnmt1* haploinsufficient mice with impaired leukemia stem cell survival and self-renewal, Ezh2-controlled target genes were also derepressed, suggesting that PRC2 might cooperate with DNA methyltransferases to regulate genes involved in leukemia stem cell function and tumor progression (45). Taken together these findings indicate that EZH2 can be recognized as a CSC factor that is involved in not only the induction of uncontrolled cell proliferation but also the maintenance of stemness characteristics, both critical hallmarks of cancer cells and stem cells.

Additionally, H3K27me3 and associated PRC2 repressors play essential roles in the regulation of development and lineage commitment both in ESCs and adult stem cells (86). Specific developmental regulatory genes are silenced via PRC2-mediated H3K27me3 in their regulatory regions in mouse and human ESCs (117, 118). Such silent genes are derepressed in EZH2-deficient ESCs, causing severe defects in ESC differentiation, emphasizing its critical role in maintaining an ESC gene expression repertoire and in

executing proper developmental program during differentiation (119). Unexpectedly, EZH2 is not required for either *de novo* establishment or maintenance of ESCs (119). However, cell fusion-based reprogramming studies using Ezh2-depleted ESCs demonstrated that Ezh2 is required to direct successful conversion of differentiated somatic cells toward pluripotency (120). Inhibition of Ezh2 by shRNAs significantly reduced the efficiency of human iPSC generation induced by OSKM, highlighting the importance of somatic gene silencing during reprogramming (102). Recent studies have shown that Ezh2 is a direct downstream target of c-Myc and the high Ezh2 expression level required for normal ESC function is c-Myc dependent (121). Mechanistically, Ezh2 functions at least in part through repressing a major barrier of iPSC generation, the CDK inhibitor Ink4a/Arf (122). Therefore, Ezh2 serves as a key epigenetic player that in part functions to connect cell cycle machinery to the transition of cell fate through repressing the genes that involves in cell cycle regulation, reprogramming, and differentiation. .

Active chromatin states mediated by the MLL complex

Whereas maintenance of repression of specific transcription programs requires the PcG proteins, the trithorax group (TrxG) proteins are critical to ensure continued expression of specific genes. Trimethylation of H3K4 (H3K4me3) by the TrxG proteins, often described as the antagonists of PcG proteins, contributes to an open chromatin conformation that facilitates active gene transcription (123). TrxG proteins are involved in ESC self-renewal, cell fate choice, cellular proliferation, and tumorigenesis (124). The mixed-lineage leukemia (MLL) protein possesses histone H3K4-specific methyltransferase activity and is the catalytic component of TrxG complex (125). Interestingly, MLL fusion oncoproteins generated by chromosomal translocation efficiently transform hematopoietic cells into leukemia stem cells (126). Although MLL fusion proteins typically lack the catalytic SET (Suvar3-9, Enhancer-of-zeste, Trithorax) domain, they can function via the recruitment of cofactors (127). Functional collaboration between MLL fusion and Bmi1 of PRC1 is also important for the development of both normal hematopoietic and leukemia stem cells (128). Significantly, mutant, leukemogenic *MLL* gene alleles can initiate acute myeloid leukemia not only from multipotent stem cells, but also from short-lived myeloid progenitors, suggesting that oncogenic *MLL* is sufficient for acquiring self-renewal functions (13). Furthermore, wild-type MLL1 has also been implicated in CSC activity. Knockdown of MLL1 suppresses tumor growth in nude mice and down-regulates various factors related to tumor growth and angiogenesis, including HIF1α (hypoxia- inducible factor-α), through decreasing H3K4me3 level at promoter regions (129). In glioma stem cells, MLL1 expression is elevated together with the expression of HIF1α and HIF2α under hypoxic conditions that promote reprogramming towards a CSC phenotype (130). Moreover, decreased MLL1 level not only suppressed HIF2α levels, but also reduced CSC self-renewal and growth, suggesting that MLL1 is required for the maintenance and the tumorigenic capacity of CSCs (131).

MLL1 also plays roles in ESC maintenance and iPSC generation. MLL1 associates with a subcomplex containing WD repeat protein-5 (WDR5), retinoblastoma-binding protein-5 (RbBP5), absent-small-homeotic-2-like protein (ASH2L), and dumpy-30 (DPY-30), which together form the MLL1 core complex that is required for the methylation of H3K4 (132).

The levels of Wdr55, being the core member of the complex, positively correlate with the undifferentiated state and regulate ESC self-renewal. Wdr5 directly interacts with Oct4 protein and has overlapping gene regulatory functions with Oct4 demonstrated by genome-wide protein localization and transcriptome analyses (133). Significantly decreased reprogramming efficiency was observed in Wdr5-knockdown fibroblasts during OSKM induction, indicating that H3K4 methylation is essential for efficient iPSC generation (133, 134). Moreover, Dpy-30 can also directly regulate H3K4me3 globally throughout the genome in mouse ESCs. Depletion of Dpy-30 or RbBP5 does not affect ESC self-renewal, but significantly impairs ESC differentiation upon LIF withdrawal by causing defects in gene induction and in H3K4 methylation at several key developmental loci (135). Hence, MLL1 complex-mediated H3K4 methylation events are crucial for iPSC reprogramming and ESC fate transition. Taken together, MLL1 is required for both normal development and tumorigenesis, and is a good example of a link between epigenetic cell memory and cell identity transition.

LSD1, a histone demethylase

Histone demethylases, such as lysine-specific demethylase 1 (LSD1), also regulate embryonic development and tumorigenesis (136). LSD1, which suppresses gene expression by converting dimethylated H3K4 to mono- and unmethylated H3K4, can also relieve repressive marks by demethylation of H3K9 (137, 138). LSD1 expression is correlated with poorly differentiated neuroblastoma and poor prognosis in both prostate and breast cancer (139-141). In a mouse model of human MLL-AF9 leukemia, LSD1 functions as a key effector to block differentiation and apoptosis in leukemia stem cells (142). Notably, knockdown of LSD1 by specific siRNA and inhibition of LSD1 activity by small molecules both enhances global H3K4 methylation and leads to reduced proliferation of cancer cells that express pluripotent cell markers OCT4 and SOX2, including teratocarcinoma, seminoma, and embryonic carcinoma (143). Importantly, the growth of non-pluripotent cancer or normal somatic cells was not affected by reduced LSD1 activity, suggesting that LSD1 and H3K4 methylation are essential for cancer cells with pluripotent stem cell properties (143).

During development, LSD1 is essential for cell-lineage determination, and loss of LSD1 leads to embryonic lethality (144). LSD1-deficient ESCs could be established, but had growth and differentiation defects, suggesting that LSD1 is not required for the establishment of ESCs but regulates their differentiation (145). Based on the recruitment of distinct LSD1-containing coactivator or corepressor complexes to the targets, LSD1 can both activate or repress gene regulation (144). As a component of NuRD (nucleosome remodeling and histone deacetylase) complex in ESCs, LSD1 is involved in decommissioning enhancers of genes involved in the regulation of the pluripotency program during differentiation, leading to a shutdown of the ESC gene expression program (146). Interestingly, LSD1 also demethylates and stabilizes DNMT1 in ESCs, emphasizing its role in the maintenance of global methylation and in coordination of histone methylation and DNA methylation (145). Currently, no loss-of-function study has been reported yet on investigating the role of LSD1 in iPSC generation. However, RCOR2 (RE1-silencing transcription factor corepressor 2), which forms a complex with LSD1 in ESCs, can regulate

LSD1-mediated histone methylation and substitutes for SOX2 in reprogramming of both mouse and human fibroblast into iPSCs, indicating that LSD1 also likely plays a role in promoting iPSC generation (147).

Conclusions

Epigenetic regulatory mechanisms, such as DNA methylation and histone modifications, play important roles in both stem cells and cancer cells. As the field of reprogramming continues to decipher the genetic and epigenetic codes for shaping cellular identity, research is progressing rapidly to dissect tumor heterogeneity determined by different genetic programs and epigenetic states that influence cell plasticity and fate. Characterization of the role of epigenetic changes in CSC development, iPSC generation, and ESC differentiation has demonstrated how cell fate changes might be orchestrated. Reprogramming from somatic cells to iPSCs serves as an invaluable cellular system for understanding the epigenetic regulations during cell fate transition, while ESCs provide a cellular model for studying the epigenetic mechanisms of cell-fate control. In addition, iPSC generation can recapitulate the dedifferentiation mechanisms that might drive CSC formation during tumorigenesis. Abnormal control of cellular differentiation is involved in carcinogenesis (148). Therefore, understanding how ESC differentiation is controlled and also how iPSC reprogramming is achieved, in both cases at the epigenetic level, will provide valuable insights into the mechanisms of tumorigenesis. In the CSC model, stem-like cancer cells generated through oncogenic transformation from somatic stem cells or through oncogenic reprogramming of cancer cells contribute to tumorigenesis (12). Thus, elucidating the epigenetic circuitry that defines the specific identities of cells could answer not only how stemness and oncogenicity are related, but also provide insight into how to design better stem cell-based regenerative medicine and cancer therapeutic strategies. There is also growing interest in the concept of reprogramming cancer cells away from a neoplastic state, a process that should be facilitated by a greater understanding of epigenetic mechanisms at work in both stem and cancer cells.

The CSC model has major clinical implications and the feasibility of developing therapeutic agents that target CSCs has been demonstrated (149, 150). Further, epigenetic regulators can be the therapeutic targets of self-renewal. For example, the elimination of CSCs but not normal stem cells can be achieved by selectively targeting BMI1 (151, 152). Recently, a small molecule, PTC-209, has also been shown to block colon cancer initiating cells self-renewal by inhibiting BMI1 expression and also effectively block tumor growth *in vivo* (153). In addition to the development of small molecule inhibitors that block the methyltransferase active site of EZH2 (154, 155), an alternative strategy of dismantling the PRC2 complex through disruption of protein interactions has also been developed (156). Many potential epigenetic cancer therapies, including inhibitors of DNA methyltransferases and histone deacetylase, have been developed and are currently being studied for safety and efficacy (157).

In addition to DNA methylation and histone modifications, ATP-dependent chromatin remodeling complexes, which utilize the energy from ATP hydrolysis to mobilize nucleosomes and alter the structure of chromatin, can also modulate transcription factor

access to chromosomal DNA and alter the chromatin function (158). In mammalian cells, BAF (Brg/Brahma-associated factors) and PBAF are two closely related SWI/SNF modeling complexes that are related to ESC differentiation and oncogenesis (159, 160). BAF complexes are required not only for the self-renewal and pluripotency of mouse ESCs (161) but also for the maintenance of hematopoietic stem and for progenitor cells (162). However, the roles of BAF complexes in CSCs remain unclear. It is reasonable to speculate that BAF complexes might be involved in the dedifferentiation process during CSC establishment, based on the fact that they have been reported to facilitate reprogramming during iPSC generation (163).

The data reviewed here suggest that it is relatively common for pluripotency-related factors to also be implicated in cancer through epigenetic mechanisms. As our understandings of the epigenetic landscapes in stem and cancer cells continues to increase and with rapid improvements in epiggenomics technology, the promise of emerging epigenetic therapies that target cancer stem cells continues to grow. Additionally, understanding the interplay between the different epigenetic pathways is a fundamental step toward learning the cause of cancers from perturbations of stem cells. We predict that many as yet unidentified additional levels of epigenetic regulatory machinery are shared between pluripotent stem cells and cancer. Future studies on these overlapping areas of cell fate regulation will be proven fruitful in enhancing our knowledge of stem cells and cancer cells, which can also further serve as the foundations for safer stem cell-based therapies.

Acknowledgements

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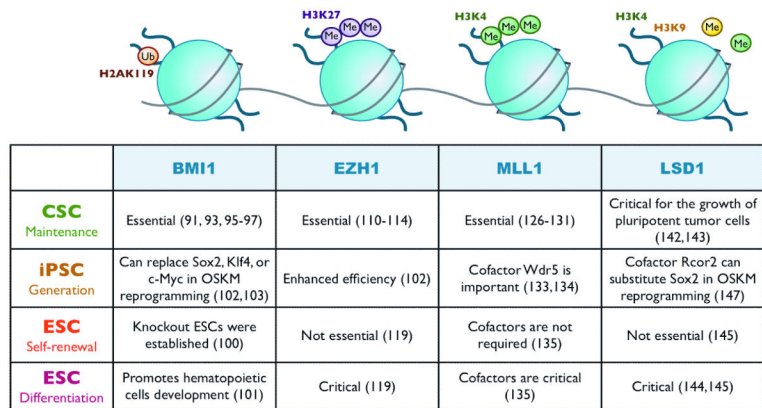
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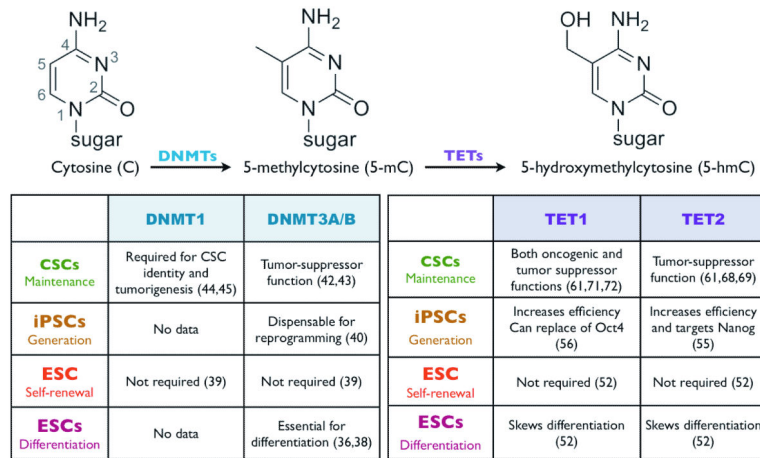
Figure. 1.

The roles of DNMT and TET proteins in CSCs, iPSCs, and ESCs. The upper panel depicts the DNMT-mediated methylation of cytosine at the 5 position (5-mC) and the TET-mediated oxidation of 5-mC to generate 5-hmC. The table below summarizes the functions of DNMTs and TETs in CSC establishment, iPSC generation, ESC self-renewal, and ESC differentiation.

	BMI1	EZH1	MLL1	LSD1
CSC Maintenance	Essential (91, 93, 95-97)	Essential (110-114)	Essential (126-131)	Critical for the growth of pluripotent tumor cells (142,143)
iPSC Generation	Can replace Sox2, Klf4, or c-Myc in OSKM reprogramming (102,103)	Enhanced efficiency (102)	Cofactor Wdr5 is important (133,134)	Cofactor Rcor2 can substitute Sox2 in OSKM reprogramming (147)
ESC Self-renewal	Knockout ESCs were established (100)	Not essential (119)	Cofactors are not required (135)	Not essential (145)
ESC Differentiation	Promotes hematopoietic cells development (101)	Critical (119)	Cofactors are critical (135)	Critical (144,145)

Figure. 2.

Histone modifying proteins that regulate CSCs, iPSCs, and ESCs. The upper panel depicts the histone modifications mediated by different histone modification complexes. As a key regulatory component of PRC1, BMI1 is involved in establishing a repressive epigenetic mark, histone H2A K119 ubiquitylation (H2AK119ub). EZH2 is the catalytic component of PRC2 that places a repressive modification of H3K27me3. On the other hand, H3K4me3, an active modification, is deposited by the MLL1 histone methyltransferase complex. LSD1 is a histone demethylase that removes the methyl group from both H3K4 and H3K9. The table is a summary of the regulatory roles played by these histone modification proteins in CSC establishment, iPSC generation, ESC self-renewal, and ESC differentiation.