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REVIEW

Molecular marks for epigenetic identification of developmental and cancer stem cells

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Abstract Epigenetic regulations of genes by reversible methylation of DNA (at the carbon-5 of cytosine) and numerous reversible modifications of histones play important roles in normal physiology and development, and epigenetic deregulations are associated with developmental disorders and various disease states, including cancer. Stem cells have the capacity to self-renew indefinitely. Similar to stem cells, some malignant cells have the capacity to divide indefinitely and are referred to as cancer stem cells. In recent times, direct correlation between epigenetic modifications and reprogramming of stem cell and cancer stem cell is emerging. Major discoveries were made with investigations on reprogramming gene products, also known as master regulators of totipotency and inducer of pluoripotency, namely, OCT4, NANOG, cMYC, SOX2, Klf4, and LIN28. The challenge to induce pluripotency is the insertion of four reprogramming genes (Oct4, Sox2, Klf4, and c-Myc) into the genome. There are always risks of silencing of these genes by epigenetic modifications in the host cells, particularly, when introduced through retroviral techniques. In this contribution, we will discuss some of the major discoveries on epigenetic modifications within the chromatin of various genes associated with cancer progression and cancer stem cells in comparison to normal development of stem cell. These modifications may be considered as molecular signatures for predicting disorders of development and for identifying disease states.

Keywords Cancer · Development · DNA-methylation · Histone modification · Epigenetics · Cancer Stem cell

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Introduction

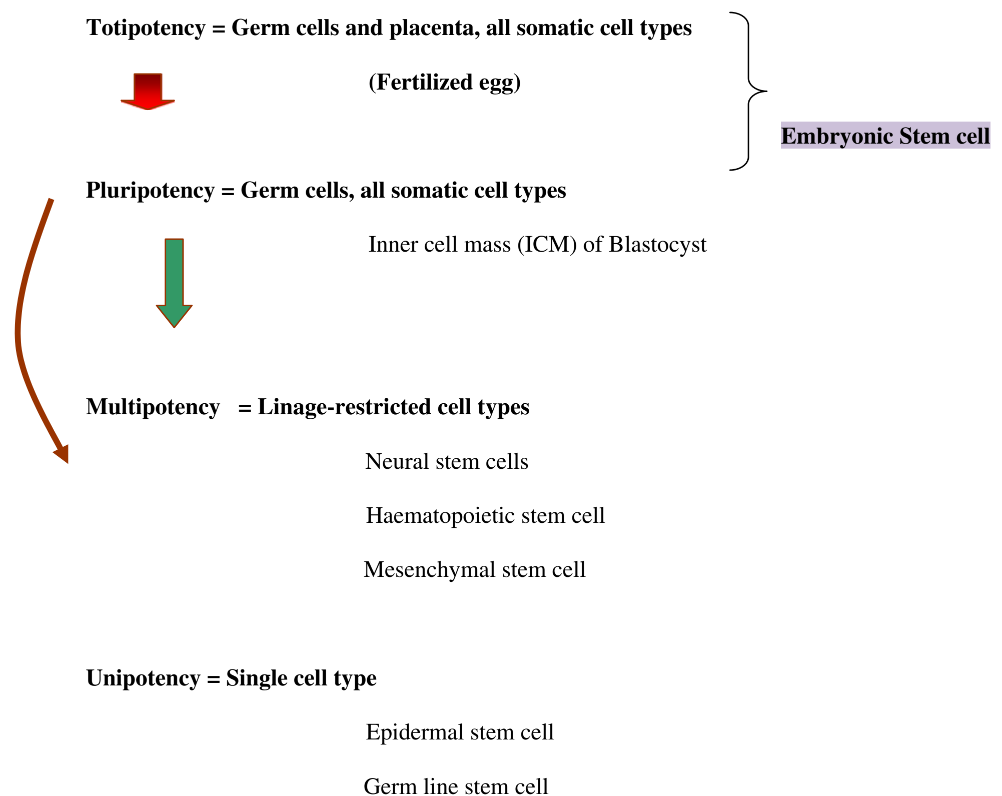
Defining the growth requirements for the maintenance and differentiation of developmental and cancer cells, and attempts to define their predictive molecular signatures have proved frustrating in the past. However, currently there has been remarkable progress in understanding molecular mechanisms of development and cancer biology. Under certain conditions of cell cycle control mechanisms, cells of many developing organs and cancer cells of those particular organs follow general mechanisms and may be compared for understanding the disease state. Some studies examining specific epigenetic features of embryonic and cancer stem cells—such as the aberrant DNA-methylation, abundance of modified histones, Polycomb group (PcG) protein binding patterns, replication timing, and chromatin accessibility have provided important insights into the unique properties of stem cells. Here, we discuss the unique epigenetic features of developmental and cancers stem cells, and explore the new questions that these findings have raised about stem cells, cancer stem cells, and their implications for practical applications.

Stem cells

Indefinite self-renewal and multipotency are two fundamental properties of Stem cells. They have the capacity to divide with at least one daughter retaining the phenotype of the mother (Pardal et al. 2003a; Gupta and Massagué 2006; Rapp et al. 2008; Michor 2008; Hart and El-Deiry 2008; Maitland and Collins 2008). Conceptually there are two major types of stem cells: namely, embryonic stem (ES) cells and adult stem cells. Embryonic development is a process of differentiation, growth, and maturation of different organs by which all the tissues and cells of an organism are derived from zygotic stem cells. This property is defined by the ways of conversion of totipotency to pluripotency to multipotency to unipotency (See Scheme 1). In adult organisms, cells of many tissues retain their stemness properties and play critical roles in tissue regeneration and repair. These adult stem cells are considered pluripotent as generally they have limited ability for differentiation and are committed to create the mature differentiated cells in the tissues where they reside. This differentiation process is part of the homeostatic system that can renew senescent differentiated cells and replace tissue loss following injury. In many tissues, it is now proven that homeostasis is maintained by a hierarchical system in which, firstly, stem cells generate transit-amplifying cells. These rapidly cycling cells maintain a degree of multipotency and can expand and differentiate into non-cycling, terminally differentiated cells. This hierarchy is prominent in most of the cells of epithelial origin, including gut, breast, lung, prostate, skin, cornea, and liver (Leedham et al. 2005; Kakarala and Wicha 2008; Otto 2002; Richardson et al. 2004a; Tsujimura et al. 2002; Alonso and Fuchs 2003; Lavker et al. 2004; Vessey and de la Hall 2001).

Embryonic stem cells are derived from embryos and the derivation of human embryonic stem cell (hESC) line has involved embryo destruction. Many people have ethical objections to their use for any purpose other than reproduction. But at present, derivation of at least five hESC lines is possible without embryo destruction. hESC may be cultured in three different ways. In first two processes blastomere cocultured with green fluorescent protein (GFP)-labeled hESC for 12–24 and 12 h, respectively, in a modified medium (blastocyst medium supplemented with laminin and fibronectin) which is approximately similar to the inner cell mass (ICM) niche. In another process, blastomeres were cultured in blastocyst medium without GFP-hESC. In these three processes, stable hESC generated 3.8%, 20% and 50%, respectively, and cultures were stable hESC to re-differentiate in vivo and vitro. Blastomere culture medium was supplemented with laminin and fibronectine. Laminin is a component of basement membranes and associated with induction of apical/basal polarity, possibly which suppressed trophectoderm differ-

Scheme 1 Levels of stem-cell state



entiation (Chung et al. 2008). Stable induced pluripotent stem cell was also generated from human fibroblasts by directly delivering four reprogramming proteins (octamer binding transcription factor, Oct4; Sox2; Klf4; and c-Myc) fused with cell membrane penetrating peptide. These four proteins induced pluripotent stem cells morphology, proliferation, and expression profiles of characteristic pluripotency markers were similar to human embryonic stem cell (Kim et al. 2009). Adult stem cells derived from skin cells or bone marrow cells upon treatment with reprogramming proteins also have acquired the properties of embryonic stem cells. But this work is not widely accepted because of higher time required for the protein-mediated process (poor efficiency) in comparison to the gene delivery-mediated process. However, scientists believe that by exploiting the potential of embryonic stem cells to develop into any cells of the body, they may be able to treat many incurable conditions, but there is lack of full understanding of the mechanisms of epigenetic silencing or activation of genes.

Epigenetics related to development and cancer biology

Epigenetics is the study of the stable inheritance of phenotype without altering the genotype manifested by changes in gene expression (Probst et al. 2009). Epigenetic changes in eukaryotic biology are best observed during cellular differentiation (Probst et al. 2009; Klose and Bird 2006; Jaenisch and Bird 2003; Feinberg et al. 2006; Jones and Baylin 2007). During embryogenesis, totipotent stem cells become the various pluripotent cell lines of the embryo which in turn become fully differentiated cells. In other words, a single fertilized egg cell—the zygote—changes into the many cell types found in vertebrates. This process is regulated by activating some genes while silencing many others by complex processes of epigenetic regulations. It involves a unique modification of DNA at the cytosine 5-carbon position (hereafter, DNA-methylation), and numerous modifications in histones for the activation or repression of certain genes (Jones and Baylin 2007). Additionally, various proteins associated with the chromatin folding and dynamics may be activated or silenced. What this means is that every cell in our body has the same programming/instruction manual, but different cell types are using different chapters. Most epigenetic changes that are involved with the chromatin modifications related to gene expression only occur within the course of one individual organism's lifetime, but some epigenetic changes are inherited from one generation to the next. Epigenetic processes include paramutation (the result of heritable changes in gene expression that occur upon interaction between alleles), gene bookmarking (a mechanism of epigenetic memory that functions to transmit through mitosis the pattern of active genes and/or genes that can be activated to daughter cells), imprinting, gene silencing, X chromosome inactivation, position effect, reprogramming, transvection (an epigenetic phenomenon that results from an interaction between an allele on one chromosome and the corresponding allele on the homologous chromosome), maternal effects, the progress of carcinogenesis, and many effects of teratogens, regulation of histone modifications and heterochromatin formation, and technical limitations affecting parthenogenesis and cloning (Probst et al. 2009; Klose and Bird 2006; Jaenisch and Bird 2003; Feinberg et al. 2006; Jones and Baylin 2007; Christman 2002; Patra et al. 2008; Vaissiere et al. 2008; Patra and Szyf 2008; Patra 2008a).

Epigenetic regulation of genes by DNA methylation and histone modifications

Methylation at the carbon-5 position of cytosine base is the only known stable modification of DNA, which occurs primarily in CpG dinucleotides and is often altered in cancer cells (Jones and Baylin 2007). This modification consists of the covalent addition of a methyl group catalyzed by a family of enzymes called DNA methyltransferases (DNMTs), using S-adenosylmethionine as the donor of the methyl group (Klose and Bird 2006; Christman 2002; Patra et al. 2008; Vaissiere et al. 2008; Patra and Szyf 2008; Patra 2008a). In mammals, the de novo methylation generally does not occur during normal postgastrulation development but is seen frequently during the establishment of cell lines in vitro and in tumor tissues (Jones et al. 1990; Kawai et al. 1994). It was suggested that the maintenance of DNA methylation depends on DNMT1 that specifically recognizes hemi-methylated DNA and methylates the complementary strand and de novo methylation is carried out by DNMT3a and DNMT3b proteins (Klose and Bird 2006; Jaenisch and Bird 2003). DNMT3a is involved in the nucleolar inactivation of resting and growth-arrested cells. Methylation of DNA repeats in the region of centromeric satellite DNA is specially maintained through Dnmt3b (Thompson et al. 2010). Recent data suggest that the DNMT1 protein is methylated by the histone methyltransferase (e.g., SET7) and demethylated by histone demethylase (e.g., LSD1) (Esteve et al. 2009; Wang et al. 2009a), which increase or decrease respectively the DNMT1 activity in the different stage of cell cycle. DNA methylation can inactivate a gene in a number of ways; for example by attracting CpG-methylated-DNA binding proteins, by attracting histone deacetylases (HDACs) and by inducing variations in histone methylation (Jones and Baylin 2007; Patra et al. 2008; Patra and Szyf 2008; Patra 2008a). Only a small fraction of the eukaryotic genome is transcriptionally competent. The state of chromatin in these regions must be dynamic to meet the changing transcriptional requirements of a cell. This balance between

euchromatin and heterochromatin ensures that the gene-expression pattern of a given cell type is stably maintained in daughter cells as a heritable state. In terms of CpG-density, there are two types of gene promoters. One type accounts for ~50% of the genes in the mammalian genome and contains unmethylated CpG-islands. The other promoter type is CpG-poor in composition, as is the rest of the genome. Among the genes that have CpG-poor promoters, it is not known for how many of those CpG-methylation might have a modulatory role in their transcription? High-CpG-density promoters (HCPs) are associated with two classes of genes: ubiquitous "housekeeping" genes and highly regulated "key developmental" genes (Saxonov et al. 2006). In ES cells, HCPs at housekeeping genes are enriched with the transcription initiation mark H3K4me3 ("univalent") and genes are generally highly expressed, whereas HCPs at developmental genes are enriched with both H3K4me3 and the repressive mark H3K27me3 ("bivalent") and genes are generally silent (Meissner et al. 2008; Mikkelsen et al. 2007; Bernstein et al. 2006).

Genome-wide decrease of DNA methylation certainly contributes to development of cancers (Bedford and van Helden 1987). This hypomethylation has been associated with the emergence of chromosome instability both in mouse (Dodge et al. 2005), and human neoplasms (Schulz et al. 2002). However, it is the regional hypermethylation of critical genes that has attracted immense interest in understanding the pathogenesis of cancer (Patra et al. 2002). Methylated genes commonly found in cancer affect diverse cellular processes, some of which have roles in tumor physiology and signaling such as the hormonal response (androgen receptor, AR; estrogen receptor 1/2; retinoic acid receptor beta; retinoic acid receptor responder protein 1), tumor invasion/architecture (adenomatous polyposis coli, APC; caveolin 1; cluster of differentiation 44; E-cadherin 1; E-cadherin 13; laminin subunit alpha-3; laminin subunit beta-3; laminin subunit gamma-2; urokinase type plasminogen activator), cell cycle control (G1/S-specific cyclin-D2; Cyclin-dependent kinase inhibitor 2 A), repair of DNA damage (glutathione-S-transferase P; O-6 methylguanine DNA methyltransferase), and signal transduction (disabled homolog 2-interacting protein; death-associated protein kinase 1; endothelin receptor type B; Ras Association domain Family 1A; Patra and Szyf 2008; Patra 2008a; Patra et al. 2002; Li et al. 2005; Joshua et al. 2008). Some other genes are also demethylated, e.g., uPA (Pulukuri et al. 2007; Patra and Bettuzzi 2007), heparanase (Ogishima et al. 2005), and clusterin (Rauhala et al. 2008) which may have functional importance, especially for the invasive phenotype. These epigenetic signatures and changes have been reviewed extensively (Schulz and Hatina 2006; Dobosy et al. 2007; Cooper and Foster 2009).

Eukaryotic genomes undergo hundreds of modifications in their core histones tails. One of the best-studied histone modifications is acetylation, and histone acetylation events play crucial roles in all kinds of nuclear phenomena involving DNA, namely: replication, recombination, repair, condensation, and transcription. Acetylation is catalyzed by histone acetyltransferases (HATs) such as monocytic leukemia zinc finger protein (MOZ), MOZ-related factor, males-absent on the first protein, HIV-1 tat-interacting protein (TIP60), and human acetylase binding to origin of replication complex—ORC1 (Esteller 2007; Clayton et al. 2006; Kouzarides 2007), where acetyl-coenzyme A is the donor of acetyl-group. Primary site of acetylation is the side chain of lysine (K) residues of the all histones, but frequencies are moderate for H4 and highest for H3. Downstream molecular consequences of histone acetylation are (a) binding of chromodomain proteins at sites where lysines are acetylated and (b) alteration of histone–DNA binding. The later event opens faces of DNA for further interactions with macromolecules/enzymes for the events like replication, repair, or transcription. The amount of residual acetylation level depends on the precise balance between the action of HATs and histone deacetylases (HDACs). Until now, four classes of HDACs have been identified: Class I are ubiquitously expressed in human cell lines and tissues. Class II has tissue specific expression and can shuttle between the nucleus and cytosol. The class III family is numbers of NAD+dependent proteins (Liu et al. 2010; Ropero and Esteller 2007); and the class IV is homologous to the class I and II cytoplasm, and it may be responsible for the acetylation of non-histone proteins (Ropero and Esteller 2007). HDAC1 and HDAC2 have been shown to directly interact with DNMT1 (Robertson et al. 2000; Rountree et al. 2000). We and others have shown that HDACs are associated with cancer development (Liu et al. 2010; Patra et al. 2001). Recently, it is observed that inhibition of HDAC by chidamide in colon cancer cells increase acetylation level in histone H3, and arrests the cancer cell cycle at G1 phase by inhibiting the PI3K/Akt and MAPK/Ras signaling pathways and promotes the apoptosis of cancer cell (Liu et al. 2010). The addition of an acetyl group to the lysine residue neutralizes the charge, which relaxes the bound DNA from the histone complex. As a consequence, portions of DNA with largely acetylated histones result in euchromatin formation and activation of gene transcription, while histone deacetylation is associated with chromatin condensation (heterochromatinization) and gene suppression. A survey of results published over the last decades described that histone acetylation can modulate gene transcription at global (genome-wide) and gene-specific levels (Jones and Baylin 2007; Patra et al. 2008; Vaissiere et al. 2008; Patra and Szyf 2008; Clayton et al. 2006; Kouzarides 2007; Liu et al. 2010; Ropero and

Esteller 2007; Robertson et al. 2000; Rountree et al. 2000; Patra et al. 2001; Bedford and Clarke 2009; Li and Zhao 2008; Schulz and Hoffmann 2009a; Seligson et al. 2005; Brown et al. 2000). It is now clearer that promoter-specific hyperacetylation occurs in at the backyard of global acetylation, preventing deacetylation on those sites that activates basal transcription. This facilitates a rapid return to the default state of acetylation when transcription is turned on (Vaissiere et al. 2008; Patra and Szyf 2008; Clayton et al. 2006; Kouzarides 2007; Brown et al. 2000)

Another major modification of histone tails is the methylation at several lysine (K) and arginine (R) residues. Histone methylations are linked to both transcriptional activation and repression. Methylated K residues, obtained in H3 at positions −4, −9, −27, −36, and −79, and in H4 at K 20. Lysine can be mono-, di-, and trimethylated, whereas arginine (R) can additionally be tetramethylated. The dimethylated form of R may again be symmetric or asymmetric (Bedford and Clarke 2009). Histone methylation is reversible and catalyzed by two families of enzymes called histone lysine methyltransferases and protein arginine methyltransferases, and the methyl group can be removed by histone demethylases (Kouzarides 2007; Bedford and Clarke 2009). Stunning numbers of reversible modifications along with different combinations of modification involving physical interactions of enzymes and regulatory proteins in both directions throws insights of complexity of epigenetic regulation by histone modification (Patra and Szyf 2008; Kouzarides 2007). In reality, the consequences of histone methylation depend on the modified residue, as we know that methylation of H3K4, H3K36, or H3K79 correlates with the active gene transcription; however, methylation at H3K9, H3K27, or H4K20 is usually linked to gene repression. Moreover, mono-, di-, and trimethylation at the same K residues lead to different levels of gene activation or repression and are involved in distinct cellular pathways (Patra and Szyf 2008; Li and Zhao 2008). H3K27me3 represses the gene when located at promoter region. It is specifically coupled with HOX gene repression and X chromosome inactivation (Schulz and Hoffmann 2009a). Histone modifications are altered in cancer, and since these are mitotically heritable, they can play the same roles and undergo the same selective processes as genetic alterations in the development of a cancer. It is essential to identify the usual patterns of normal tissues before determining the altered patterns of histone modification in cancer. To date, some examples from recent studies are available: for example, lower level of H4K12-Ac is an indicator of recurrence in prostate cancer (Seligson et al. 2005) and increased H3K4 dimethylation and H3K18 acetylation activation mark correlated with poor prostate cancer prognosis (Schulz and Hoffmann 2009a). Moreover, specific epigenetic gene silencing can also occur by aberrant targeting of HDACs to the gene promoter, which causes histone hypoacetylation (Seligson et al. 2005). Typical genes silenced in this manner in various human cancers include the tumor-suppressor gene p21WAF1 (cyclin-dependent kinase inhibitor 1; Patra et al. 2001). Among the members of polycomb repressive complex (PRC), the most studied is the polycomb protein enhancer of zeste homologue 2 (EZH2), an essential component of a protein complex that catalyzes methylation of histone H3 at K9 contributing to transcriptional repression of a large number of specific genes. PRC1 complex was also required for trimethylation at H3K27, which is responsible for stable maintenance of gene repression (Schulz and Hoffmann 2009a). EZH2 is overexpressed in high percentage in prostate cancer patients, with moderate increases in localized tumors, and higher expression in metastatic cases. EZH2 overexpression may lead to the stable downregulation of approximately 100 genes and increased expression of a smaller number (Varambally et al. 2002). Moreover, global patterns of histone modification are shown to be linked to the risk of prostate cancer recurrence (Cooper and Foster 2009). Specifically, the activating histone modifications H3K18 acetylation and H4 R3 dimethylation have been reported to occur in many cases of prostate cancer and to be associated with higher grades and a worse prognosis (Seligson et al. 2005). New evidences are accumulating against the convention: histone modifications that mark inactive chromatin are associated with DNA-hypermethylated promoters, whereas histone marks for active chromatin are normally associated with hypomethylated promoters. Using a ChIP-based microarray approach, it was found that in prostate cancer and PC3 cell line around 5% of promoters (16% with CpG islands and 84% without CpG islands) were enriched with H3K27me3, a modification that marks inactive chromatin. The genes containing this mark were specifically silenced in PC3 compared with normal prostate epithelial cells even though most of the promoter of the genes with CpG islands showed low levels of DNA methylation (Kondo et al. 2008). Apart from changes of chromatin state and dynamicity by methylation and acetylation of histones, emerging evidence suggest a role for phosphorylation, beyond chromatin condensation (Patra and Szyf 2008; Kouzarides 2007).

Molecular marks for identifying stem-cells

Each cell in our body has its unique epigenetic mark. These epigenetic marks establish their genotype, developmental history and it reflects into the phenotype of the cell (Table 1). During the process of fertilization, paternal genome exchange protamines and DNA demethylation and histone modifications occur (Santos et al. 2002). But some area of heterochromatin in and around centromere (Santos et al. 2002; Rougier et al. 1998), including intracisternal A particle (IAP) retrotransposons (Lane et

Table 1 Molecular signatures observed on chromatin in normal and cancer cells

Epigenetic changes	Chromatin modification observed in normal cell			Modification observed in cancer cell		
	Type of modification on respective gene	Effect of modification	Reference	Type of cancer	Type of modification on respective gene	Reference
DNA methylation	In hESCs, cancer-related genes, including tumor suppressor genes, are repressed by the establishment of “bivalent chromatin domains” consisting of activating (H3 lysine 27 methylation) and repressing (H3 lysine 4 methylation) histone marks that make them poised for activation.	Balance those gene expression or suppression.	(Schlesinger et al. 2007; Ohm et al. 2007; Widschwendter et al. 2007)	Adult cancer cell	In adult cancer cell, the promoter region of the gene immediately hypermethylated and fully stopped them from activation.	(Schlesinger et al. 2007; Ohm et al. 2007; Widschwendter et al. 2007)
	Class A-I (genes are primarily involved in early differentiation processes and more enriched in Polycomb and bivalent marks) cancer-related genes were not hypermethylated in hESC and normal tissue.	Regulation of normal developmetal process	(Calvanese et al. 2008)	Adult cancer cell.	Class-I genes were hypermethylated in adult cancer cell.	(Calvanese et al. 2008)
	Class A-II genes were sometimes methylated in normal tissue but uncommon in hESC.	Methylation may be important for linage specification	(Calvanese et al. 2008)	Cancer cell	These genes were frequently methylated in cancer cell line and it became abnormal when it was not hypermethylated in the corresponding normal tissue.	(Calvanese et al. 2008)
	Promoter region of Class B-I genes (excluding ASCL2, NPY, and SLC5A8 genes) are frequently hypermethylated in hESCs cells lines but never in normal tissues.	DNA methylation maintain pluripotency in cancer sten cell and hESC.	(Calvanese et al. 2008)	cancer cell	Class B-I gene are also hypermethylated in cancer stem-cell line and responsible for their pluripotency.	(Calvanese et al. 2008)
	Class B-II genes(associated with those linked to lineage specification) are often hypermethylated in hESCs and sometimes methylated in normal tissues	Important for linage specification	(Calvanese et al. 2008)	Cancer cell	These genes are also hypermethylated in cancer cell line. Their hypermethylation in cancer were considered aberrant in tumor types when the related gene is completely unmethylated in the	(Calvanese et al. 2008)

				normal cell.	
AIM2 and RUNX3, that were hypermethylated and repressed in CD34+ hematopoietic progenitor cells and that became unmethylated and overexpressed in myeloid and lymphoid lineages, respectively	Maintain linage specification	(Li et al. 2002; Woerner et al. 2007)	Gastric cancer, colon cancer	Hemizygous deletion and hypermethylation in the promoter region of RUNX3 gene was observed in gastric cancer and in colon cancer promoter region of AIM2 was hypermethylated.	(Li et al. 2002; Woerner et al. 2007)
Stat3 and Tcf3 binding site on the upstream (−4,880 to −3,790 bp) of Nanog gene is hypomethylated in ES		(Hattori et al. 2007; Gu et al. 2005; Pereira et al. 2006)			
Gcnf binding site on upstream (−2,050 to −1,800 bp) is hypermethylated in ES		(Hattori et al. 2007)			
Six proximal CpG site of Oct-4, Sox2 and p53 regulator binding site in nenog gene (−1 to −1,000) is completely unmethylate and another ten distal CpG site is 29% methylated.	Activate Nanog expression	(Kuroda et al. 2005; Lin et al. 2005; Hattori et al. 2007; Rodda et al. 2005)			
Oct-4 gene regulatory region displays quite a unique DNA methylation pattern regulated by specific *cis*-elements such as Sp1 or Sp3 binding sites.	Expression control of the gene	(Hattori et al. 2004)			
the SOX2 protein is expressed in normal gastric mucosae	Inhibited cell proliferation through cell-cycle (G1) arrest and apoptosis	(Otsubo et al. 2008)	Gastric cancer	Half of the case of gastric cancer (three out of six) promoter region of Sox2 gene is hypermethylated and lowering the expression of SOX2 protein.	(Otsubo et al. 2008)
The sex-determining region Y-box 7 (Sox7) normally express in embryonic stem cell and some differentiated depend on cell type	Is a transcription factor, essential for embryonic development and endoderm differentiation		Prostate adenocarcinomas and primary prostate tumors	A study shows that in 47% of the prostate adenocarcinomas and 48% of the primary prostate tumors SOX7 gene were downregulated through promoter hypermethylation.	(Guo et al. 2008)

Table 1 (continued)

Epigenetic changes	Chromatin modification observed in normal cell			Modification observed in cancer cell		
	Type of modification on respective gene	Effect of modification	Reference	Type of cancer	Type of modification on respective gene	Reference
				Lung carcinoma	SOX7, SOX18 promoter region methylated in lung carcinoma.	(Dammann et al. 2005)
				Colorectal cancer	SOX17 promoter region hypermethylated in colorectal cancer.	(Zhang et al. 2008a)
	The sex-determining region Y-box 7 (Sox7) normally express in embryonic stem cell and some differentiated depend on cell type			Colorectal cancer	Sox7 promoter region is hypermethylated in colorectal cancer	(Zhang et al. 2009)
		sFRPs and DKK1 as secreted Wnt antagonists acting at cell membrane to prevent ligand-receptor interactions and APC degrade b-catenin or export b-catenin from the nuclear to cytoplasm		Colorectal cancer	Promoter hypermethylation occurs at sFRPs, DKK1, and APC genes, which are key genes in colorectal cancer development.	(Caldwell et al. 2004; Esteller et al. 2000; Aguilera et al. 2006)
	In normal embryonic ureteal and bladder cell, only the sixth binding site of CTCF insulator protein on the promoter region of human H19 gene is allele-specific methylation.	Controlled the normal expression of H19 gene	(Takai et al. 2001)	Bladder cancer	Aberrant hypomethylation observed at sixth CTCF binding site in parenal allele causes overexperssion of H19.	(Takai et al. 2001)
	Allele-specific normal methylation pattern at the sixth CTCF binding site at H19 promoter region in normal cell.	Controlled the normal expression of H19 gene	(Takai et al. 2001)	Wilms' tumor and colon cancer	Hypermethylation occur at sixth CTCF binding site in maternal allele.	(Takai et al. 2001)
	Allele-specific normal methylation pattern at the sixth CTCF binding site at H19 promoter region in normal cell.	Controlled the normal expression of H19 gene	(Takai et al. 2001)	Lung cancer	Hypomethylation of that site and biallelic expression of H19 gene observe	(Takai et al. 2001)
	In normal squamous cell, repetitive sequence classes including SINEs, LINEs, subtelomeric repeats, and segmental duplications are hypermethylated.		(Pfeifer and Rauch 2009)	Squamous cell tumors	In squamous cell tumors, repetitive DNA sequences are hypomethylated at CpG island.	(Pfeifer and Rauch 2009)

			(Pfeifer and Rauch 2009)		Multiple methylated CpG islands present in four *HOX* gene loci on chromosomes 2, 7, 12, and 17, so these are the hotspots region for tumor-associated methylation	(Pfeifer and Rauch 2009)
	The *p57KIP2* gene, a cyclin-dependent kinase inhibitor in the kinase-interacting protein (KIP) family is unmethylated in normal tissue	Tumor suppressor gene	(Hagiwara et al. 2010)	Lymphocytic leukemia and lung cancer	DNA methylation inactive this tumor suppressor gene.	(Hagiwara et al. 2010; Shen et al. 2003; Pateras et al. 2006)
	DNMT3L promoter region is methylated in normal differentiated cell.		(Gokul et al. 2009)	Squamous cell carcinoma of cervix and cervical cancer cell	Loss of DNA methylation at the DNMT3L promoter region observe in this cancer cell. Overexpression of DNMT3L regulates DNMT3A and DNMT3B activity which result into cell proliferation and induce independent growth	(Gokul et al. 2009)
	Hypermethylation not observed in normal placenta.		(Zhang et al. 2008b)	Choriocarcinoma. seminoma and embryonal carcinoma	Minimal promoter and exon 1 regions of Oct4 are both hypermethylated in choriocarcinoma. In gonadal germ cell tumors, specifically seminoma and embryonal carcinoma Oct4 expression become lower.	(Zhang et al. 2008b; Lau and Chang 2006)
Histone modification	The level of H3-K9 and H3-K27 methylation of the Nanog proximal and distal tissue specific differentially methylated regions (T-DMR) is low in ICM cells then TE cells and differentiated cell.	Activate nanog expression in ES cell	(Hattori et al. 2007)			
	H3-K4 trimethylation and H3-K4 dimethylation level is high at the *Nanog* proximal and distal T-DMR in ICM then TE cells	Assist chromatin relaxation in ICM	(Hattori et al. 2007)			

Table 1 (continued)

Epigenetic changes	Chromatin modification observed in normal cell			Modification observed in cancer cell		
	Type of modification on respective gene	Effect of modification	Reference	Type of cancer	Type of modification on respective gene	Reference
	H3-K9 and H3-K27 methylation of Oct4 gene T-DMR is low in both ES and TS cell compare to nanog gene	H3-K9 and H3-K27 is not more important for Oct4 gene expression	(Hattori et al. 2007)			
	H3-K4 tri- and di-methylation level is high at the Oct-4 gene T-DMR in ICM then TE cells	Assist chromatin relaxation in ICM	(Hattori et al. 2007)			
	In normal cell early growth response 1 (EGR1) gene, inhibition is not observed.	EGR1 gene expressed.	(Lubieniecka et al. 2008)	Synovial sarcoma	In these cancer cells, H3K27me3 modification and polycomb group protein recruitment occur at the promoter region of the tumor suppressor gene EGR1 by the SS18-SSX protein.	137
	In ESCs, Nanog gene H3 and H4 is hyperactylated in the proximal and distal T-DMR then differentiated cell	Nanog expression ES cell	(Hattori et al. 2007)			
	H3 and H4 is also hyperacetylated in Oct4 gene upstream region.	Nanog expression ES cell	(Hattori et al. 2007)			
	The SOX2 protein is expressed in normal gastric mucosae	Inhibited cell proliferation through cell-cycle (G1) arrest and apoptosis	(Otsubo et al. 2008)	Gastric cancer	Some SOX2 expression-negative cases did not show any DNA methylation but they re-establish SOX2 expression after treatment with a histone deacetylase inhibitor TSA in gastric cancer cell line MKN7. It may represent that histone modification also involves in SOX2 expression.	(Otsubo et al. 2008)
	H3K4 and H3K27 methylation two of the most important histone methylation marks in normal cell.	Regulate gene expression	(Chi et al. 2010)	Human myeloid and lymphoid leukemias	H3K4 methylation is increased by the MLL (mixed lineage leukemia) gene rearrangement and increase level of H3K4-specific HMT expression.	(Chi et al. 2010; Krivtsov and Armstrong 2007; Milne et al. 2002; Nakamura et al. 2002)

				Prostate, breast, colon, skin and lung cancer	EZH2, an H3K27-specific methyltransferase, overexpression in various solid tumors may increase the H3K27 methylation and disrupt the normal activity in cancer cell.	(Chi et al. 2010)
	LSD1 demethylates H3K4me2/1.	Repress the invasiveness and metastasis of breast cancer cells.	(Wang et al. 2009b)	Breast carcinoma	LSD1 downregulates.	(Wang et al. 2009b)
Ubiquitination	In normal cell, polycomb group protein Bmi1 and histone H2A monoubiquitination suppress oncogene expression.		(Barco et al. 2009)	Synovial sarcoma	SYT-SSX2 recruitment promotes the displacement and/or degradation of Bmi1 protein and histone H2A hypoubiquitination which is responsible for the reactivation of polycomb-repressed genes.	(Barco et al. 2009)
miRNA	let-7 has very important role in the maintenance of stemness of stem cell and cancer stem cell. In ESCs, accumulation of let-7 related to the reduction of the level of LIN28, LIN28B and MYC (promote induction of pluripotency).	MYC, RAS and HMGA2 oncogene expression and cell-cycle progression controlled by let -7.	(Viswanathan et al. 2008; Newman et al. 2008; Johnson et al. 2007; Takamizawa et al. 2004; Lee and Dutta 2007; Mayr et al. 2007; Shell et al. 2007; Hebert et al. 2007; Wang et al. 2007; Johnson et al. 2005; Büssing et al. 2008)	Breast cancer cell line, non-small-cell lung carcinoma	In cancer, stem-cell let-7 expression is repressed.	(Büssing et al. 2008; Yu et al. 2007b)
	miR-145 negatively regulate the pluripotency of hESCs. miR-145 directly targeted the 3′ un-translated region of NANOG (in murine ESCs), OCT4, SOX2, KLF4 and inhibit the expression of these protein.	Necessary for downregulation of pluripotency genes during differentiation.	(Xu et al. 2009)	Breast cancer, colorectal cancer, cervical cancer and lung adenocarcinoma	miR-145 downregulate	(Cho et al. 2009)

See also Supplementary Table-II for Class A-I, Class A-II, Class B-I, and Class B-II genes

al. 2003) and permanently methylated imprinted genes remain methylated (Olek and Walter 1997) for maintaining the normal chromosome stability. The real mechanism and cause of demethylation is unknown. It may be a part of the process by which gametic genome return to embryonic totipotency. The maternal genome remains epigenetically unaffected. Before DNA demethylation, highly acetylated histones are generally incorporated into the paternal pronucleus (Santos et al. 2002; Adenot et al. 1997). Then immediately, histones are deacetylated by HDACs and monomethylated (Lepikhov and Walter 2004; Erhardt et al. 2003) with specific histone methyltransferase, including SET 7/9 for H3K4me (Olek and Walter 1997), G9a and ERG-associated protein with SET domain (ESET) for H3K9me (Beaujean et al. 2004; Dodge et al. 2004), and Ezh2 free unknown HMTase for H3K27me. These modifications may be of help to protect the specific regions from DNA demethylation. Recent data suggest that in maternal pronucleus protection against DNA methylation is related to H3K9me2 (Erhardt et al. 2003). Before blastocyte stage when one cell develops to eight-cell stage, passive demethylation takes place due to failure of maintenance methylation that usually follows the DNA replication. Oocytes containing DNMT1o leave the nucleus for first three cleavage divisions (Carlson et al. 1992; Bestor 2000), but at eight-cell stage it is only present in the nucleus (Mertineit et al. 1998). After that in 16-celled embryo, morula and blastocyte DNMT1o again appeared in cytoplasm. Cellular maintenance DNMT1 is expressed after implantation (Howell et al. 2001). Remodeling of histone during passive DNA demethylation is not clear. Firstly, different epigenetic lineages appear in blastocyst as embryonic and extraembryonic lineage, which are pluripotent. Oct4 and Sox2 express in preimplantation stage which maintains the pluripotenty of ES and Cdx2 express in trophectoderm (TE). There are so many global differences in DNA methylation and acetylation between two lineages. H3K27me1, me2, and me3 more frequently found in ICM than TE (Lepikhov and Walter 2004). DNA methyltransferases DNMT1, DNMT3a and DNMT3b, Ezh2, ESET, and G9a (euchromatic H3K9 and perhaps H3K27 HMTase; Dodge et al. 2004; Morgan et al. 2005) are important for embryonic growth, not for TE lineage (Morgan et al. 2005). After implantation, methylation levels increase in the blastocyst inner cell mass, which is the progenitor of the embryo proper. Parent-of-origin-specific-imprinting marks must be removed in primordial germ cells (PGCs) and thereafter established according to the sex of the individual. Evidence indicates that this demethylation might also be active [removal of the methyl group from methylated DNA with the help of DNA repair based mechanisms or by a direct DNA demethylase] (Patra et al. 2008; Patra and Szyf 2008). After fertilization at 7.5 days, PGCs arise from epiblast in the posterior primitive and enter the genital ridge between 8.5 and 11.5 days (Morgan et al. 2005). Until day 13.5, they contentiously proliferate and then enter either meiotic prophase I (females) or mitotic arrest until birth (males, see Fig. 1). In between 11.5 and 13.5 days, most methyl marks are erased in imprinted genes and single copy genes (Gehring et al. 2009). PGCs at this stage display an overall increase in nuclear size, loss, or down-regulation of linker histone H1, H3K9me3, H3K27me3, H4/H2AR3me2s, H2A.Z, and H3R26me2, and the disappearance or redistribution of factors that are associated with facultative or constitutive heterochromatin (Sasaki and Matsui 2008; Hemberger et al. 2009). During this process, presence of DNMT1 indicates that it is an active rather than passive demethylation process, and implicates for the presence of a DNA demethylase (Patra et al. 2008; Morgan et al. 2005). Transposable elements, like IAP and long interspersed nuclear element (LINE) resist demethylation to a variable extent (Rakyan et al. 2003). Methylation patterns are reestablished at later stages during gametogenesis by de novo methyltransferases.

Pluripotent cells are characterized by distinctive cellular markers and functions that relate to their uncommitted state. Evidence from various sources has indicated that chromatin might generally be less compact and more “transcription-permissive” in undifferentiated ES cells compared with differentiated cells. Differentiation of mouse and human ES cell increases histone H4 deacetylation in pericentromeric region and becomes heterochromatin in nature (Keohane et al. 1996). Heterochromatinization pattern in ES cell and differentiated cell is different. In case of lymphocyte cell, many inactive genes are present near centromeric heterochromatin (Brown et al. 1999). But in ES cell, this type of gene inactivation was not observed (Smale 2003). Bi-valent chromatin structure, in which active and repressive marker are closely arranged, is a special epigenetic signature in stem cell. This bi-valent chromatin structure decided which of the highly conserved genes in ES cell, including transcription factors of the Sox, Fox (forkhead box protein), Pax (paired box gene), Irx, and Pou families become switch on or off in later stages of embryonic development. A gene with H3K27me3 is expressed when jointly associated with di- or tri-methylated H3K4 in ES cell, but in differentiated cell, including T-cell and neural progenitor cell genes with only H3K27me3 was expressed when remained in promoter region of many non-transcribed developmental genes (Spivakov and Fisher 2007). In ES cell, a multiprotein complex repressor protein, PcG plays a crucial role in maintenance of pluripotency. At least four PcG have been identified. Among them polycomb repressor complex (PRC) 1 and 2 are important for ES cells function maintenance. PRC2 mainly consists of embryonic ectoderm development (EED), suppressor of zeste 12 and the HMTase enhancer

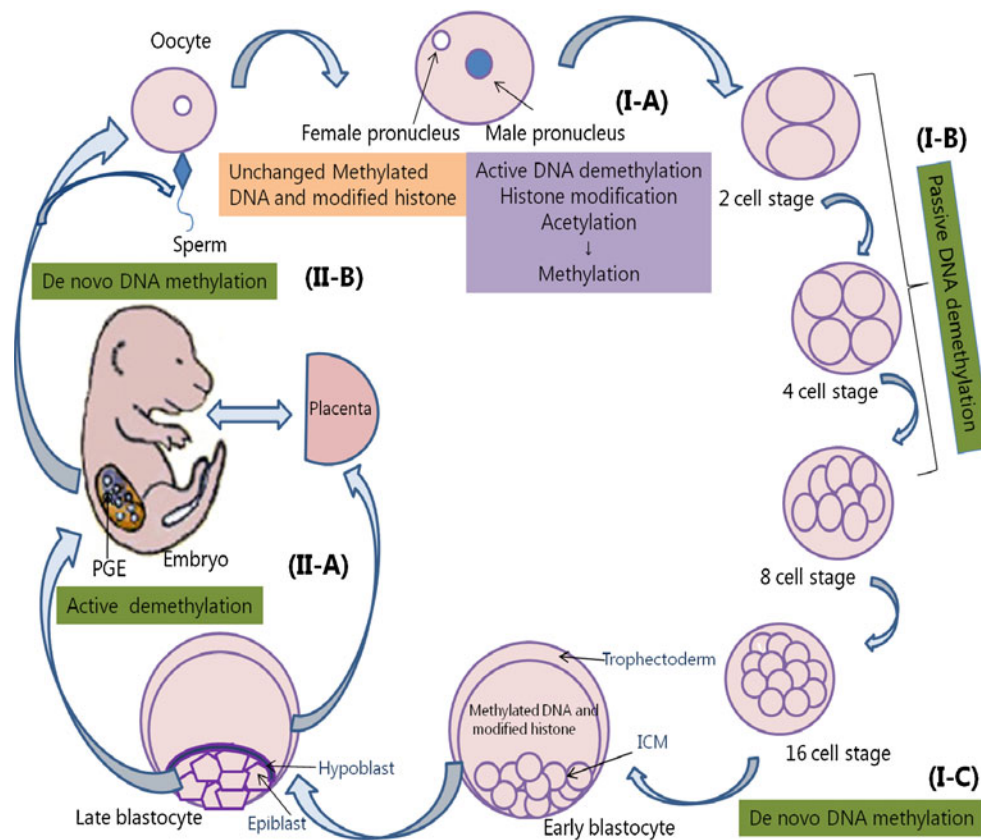


Fig. 1 Embryo development and epigenetic reprogramming cycle. Epigenetic modifications take place in two phases during the embryo development. Fertilization signals the reprogramming during preimplantation development. *I-A* In preimplantation development just after fertilization, DNA demethylation takes place in *male pronucleus* but *female pronucleus* remains unchanged. *I-B* Thereafter, both genomes are passively demethylated, except at imprinted genes and some transposons, for several rounds of cell division. This demethylation occurs due to disruption of maintenance methylation mechanism. *I-C* The genome is de novo methylated around the blastocyst stage, which responsible for differentiation of the first two lineages of the blastocyst stage, the inner cell mass (*ICM*) and the *trophectoderm*. All embryonic lineages differentiate from the ICM. *II-A* PGCs arise from somatic tissue and develop into mature gametes during gametogenesis stage. Their genome undergoes DNA demethylation in the embryo between day 11.5 and 12.5, including all imprinted genes. *II-B* Following demethylation, the genomes of the gametes are de novo methylated and acquire imprints; this process continues up to 18.5 in males and in maturing oocytes before ovulation in females

EZH2, and catalyzes the methylation of H3K27. PRC1 can bind with the methylated H3K27. Polycomb groups are correlated with transcriptional repression of Nanog, Oct4, and Sox2 which are the key controllers of Human ES cell pluripotency. RING1A and RING1B which are main proteins of PRC1 have ubiquitin ligase activity in case of mono-ubiquitination of H2AK119 transcriptional regulator, including Msx1 (muscle segment homeobox gene 1), HoxA7, Gata4 are repressed by EED and RING1B which are the key components of PRC2 and PRC1, respectively (Spivakov and Fisher 2007). Other main important protein groups are trithorax (trxG) group proteins, jumanji protein, and SetDB1 proteins. Function of these proteins depends on each other during ES cell development and differention. TrxG proteins mainly proceed as transcriptional activator protein. trxG has several groups including TAC1 (Trithorax Acetylation Complex), SW1/SNF (SWItch/sucrose nonfermentable), NURF, Ash1 (the absent, small or homeotic discs1 gene), and MLL1–3 (mixed lineage leukemia1–3; Ringrose and Paro 2004; Schuettengruber et al. 2007). TrxG and PcG maintain transcriptional expression and repression antagonistically by posttranslational modification of histone. Two multiprotein complexes of trxG proteins have HMTase activity. TAC1 group containing trithorax (Trx) protein posseses both HATase and H3K4 HMTase activity (Petruk et al. 2008). Another protein group, Ash1 methylates H3K4, H3K9, H3K20, and H3K36. Human Trx homolog MLL1–3 group of trxG protein is responsible for H3K4 trimethylation at human HOXA9 locus (Schuettengruber et al. 2007). The PcG- and trxG-conserved group of proteins epigenetically regulate several hundred important developmental genes throughout the development, including abdA, AbdB in 2–6-h embryo, Ubx in 2–6-h embryo, larval brain, and larval third leg disc, tumor-infiltrating lymphocyte gene in embryo and larval brain, slam (signaling lymphocytic activation molecule) in *Drosophila* embryo; HoxD cluster in human

adult fibroblasts and Hox cluster in embryonic placenta and adult tissue, etc. (Hekimoglu and Ringrose 2009). In *Drosophila* or fly, they bind to the PcG and trxG response elements; but in human, very little is known about these regions (Hekimoglu and Ringrose 2009; Papp and Müller 2006). In ES cell, PcG protein complex and TrxG protein maintain the "bivalent chromatin structure". EZH2 catalyzes H3K27Me3 and the ASH1 and Trx/MLL proteins catalyze H3K4Me3 in ES cell which are determined the target gene will become silent or active in differentiated cell (Hekimoglu and Ringrose 2009). Misbalance in PcG/TrxG maintenance may cause cancer or several rare genetic diseases. Mutation in TrxG protein may cause leukemogenesis. After mutation, a TrxG protein lin-59 that maintained the transcription of Hox gene lin-39 is aberrantly activated. A LIN-39/CHE-20 (TALE-class Hox cofactor) complex binds to the promoter of pro-apoptotic BH3-only gene egl-1 and inhibits the transcription and allows survival of ventral cord (VC) neuron. Downregulation of LIN-39 disrupted the regulatory mechanism by allowing egl-1/BH3 transcription and may be the cause of immature VC neuron death. LIN-39 overexpressions due to mutation suppress the normal apoptosis and promote leukemia (Potts et al. 2009). With PcG and TrxG protein, jumanji group proteins are required for ES cells differentiation and normal development. Large family of jumanji-domain-containing proteins functions as histone lysine demethylases, although functions of remaining few members are unknown (Glass and Rosenfeld 2008)—jumanjiC domain-containing histone demethylase (JHDMs) such as JHDM 2A, 2C, 2B, and 2D are Fe(II) and α-ketoglutarate-dependent protein that oxygenated methylated histone lysine residue (Hamada et al. 2009). The UTX (ubiquitously transcribed tetratricopeptide repeat X), UTY (ubiquitously transcribed tetratricopeptide repeat Y) and JMJD3 protein also are jumanjiC domain-contaning protein, capable to demethylating H3K27Me3 at promoter region and activate the gene expression. They are highly expressed in ES cell and maintained demethylation of the Hox gene promoter region and help Hox gene expression. They have a significant role in X-chromosome inactivation and maintenance (Karl et al. 2007; Sen et al. 2008). Jumanji and ARID domain containing protein JARID2 forms a stable complex with PRC2 that is responsible for recruit PcG protein to heterologous promoter in ES cell. PCR2-JARID2 complex is required for ES cell differentation (Pasini et al. 2010). JHDMs has role in cancer development. It was shown that JMJD2C is correlated with the abnormal growth of the oesophagal squamous cancer and JMJD2A, 2B, and 2C are involved in prostate cancer (Hamada et al. 2009). Apart from these proteins, a H3K9 methyltransfarase named as SetDB1 has a very important role in maintaining ES cell development and cell differentiation. Thirty-eight percent of repressed gene in ES cell chromosome is co-occupied by SetDB1 and PcG subunit Suz12 and repress the expression of genes involve in cell differentiation. SetDB1 act as a transcriptional repressor through H3K9Me3 (Bilodeau et al. 2009). Recent study suggests that at leatst two pathways control the ES cell pluripotency and self renewal activity. One is control by Nanog, Oct4, and Sox2 group which are related with up or down-regulation of 474 genes. Another group has estrogen-related receptor-β (Esrrb), T-box 3 (Tbx3), T-cell lymphoma breakpoint 1 (Tcl1) and developmental pluripotency-associated 4 (Dppa4) genes which couple with up or down regulation of 272 genes (Spivakov and Fisher 2007, see Fig. 2)

Molecular marks for identifying cancer stem cells

Classical analyses of carcinogenesis implicated that every cell within a tumor can develop a new primary tumor, and this reasoning formed the basis for most tumor therapies to the present day (Pardal et al. 2003b). Current findings in the field of tumor biology have suggested a stem-cell model of carcinogenesis, which assumes that only a subset of tumor cells are carcinogenic and are defined on their capability to initiate tumor growth in serial transplantation models (Clarke and Fuller 2006). These self-renewing cancer stem cells (CSCs) or tumor-initiating cells (TICs) must not to be confused with normal adult stem cells, which are pluoripotent organ-specific cells that have the biological properties of self-renewal and with each division they divided both progenitor cells and at least one offspring that maintains the stem-cell phenotype. There are a few similarities but the main mechanism of SC and CSC formation are different. There are three different models behind cancer propagation including the CSC model, the clonal evolution model, and the interconversion model. But it is not precisely restricted that a cancer cell followed only one model. It may follow more than one pathway which finally depends on genetic or epigenetic changes in the cancer cell (Shackleton 2010a). Mainly two theories behind the formation of CSC are related to epigenetic changes. One theory is that CSCs arise from already differentiated cell after some alteration and epigenetic changes (Reya et al. 2001); another is transformation of immature tissue stem cell or progenitor cell in tumor cell (Pardal et al. 2003a; Miyoshi et al. 2009). Normal adult stem cells and cancer cells occur from same origin/tissue and sometime express same markers. Human mammary stem cells and some breast cancers, both lack CD24 expression (Al-Hajj et al. 2003; Lim et al. 2009). Human acute myelogenous leukemia stem cells and normal human hematopoietic stem cells both are supplemented with the CD34+CD38− bone marrow markers and also lack CD24 expression (Lapidot et al. 1994; Shackleton 2010b). Although normal and cancer stem cells arise from same tissue and sometimes express same markers, it is not mandatory that they always express same

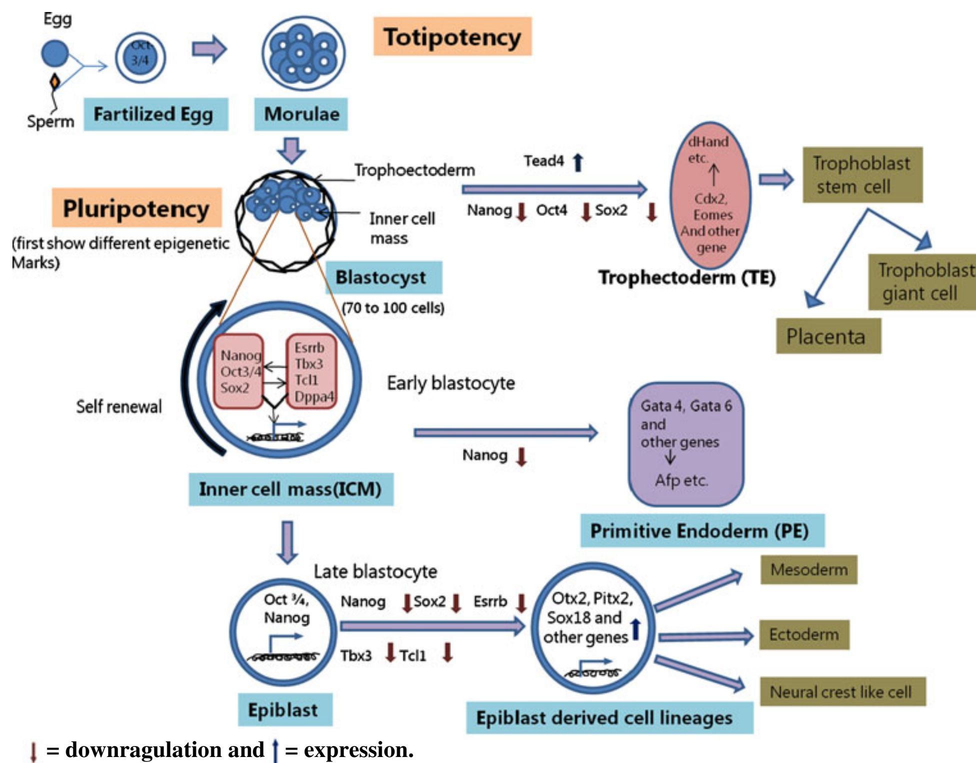


Fig. 2 During early embryogenesis, master transcriptional regulatory genes and signaling pathways play essential roles in cell line differentiation. Esrrb, Tbx3 and Tcl1, as well as Nanog, Oct4 and Sox2, are required for self-renewal property of ES cells. Oct4 is required to prevent trophectodermal differentiation; Nanog and Sox2 appear to be global regulators that repress multiple differentiation programs, whereas Esrrb, Tbx3 and Tcl1 are essential to block the differentiation into epiblast-derived lineages. These regulators couple with transcriptional network and control the expression ofdifferent genes through distinct molecular pathways. Downregulation of Nanog, SOX2, ESRRB, Tbx3 or TCL1 leads to the immediate induction of Otx2 (orthodenticle homolog 2), Pitx2 (paired-like homeodomain transcription factor-2), Sox18 (SRY (Sex determining region Y)-box 18), and probably additional genes, which help in the differentiation of cell lineages in epiblast. Tead4 expresses when Oct4, Nanog and Sox2 are repressed. Tead4 expression is responsible for Cdx2 gene expression that is nesessary for placenta development. Nanog directly repress GATA6, which results in repression of GATA4, thereby inhibiting primitive endoderm differentiation

markers. As for example, in induced mouse leukemia which was induced by MLL-ENL (MLL-eleven nineteen leukemia), MLL-AF9, and MOZ-TIF2 (monocytic leukemia zinc finger-transcriptional mediators/intermediary factor 2) fusion gene product had more similar phenotype to differentiated hematopoietic cells than hematopoietic stem cells, and tumorigenic mammary cancer cells arising in mouse contain markedly lower levels of CD29 expression than normal mouse mammary stem cells (Shackleton 2010b). Several studies show that many kind of developmental genes including Nanog, Ssea4 (Stage-Specific Embryonic-Antigen-4), Tra-1–60 (Tumor Rejection Antigen 1–60), and Tra-1–80 expression are misbalanced in esophageal, stomach, colorectal, liver, pancreatic, and cholangiocellular cancer cells. These cancer cells are not able to differentiate in different kind of cell line but when induced pluoripotent stem (iPS) cells were prepared from this type of cancer cell by inducing those genes that are able to differentiate in ectoderm, mesoderm, and endoderm. These iPS cells are capable to maintain almost all epigenetic status including methylation of DNA strands and the H3K4 residue in promoter regions of pluripotency-associated genes such as NANOG (Miyoshi et al. 2009). More examples of such epigenetic differences that are markedly observed in various developmental and cancer cells are provided in Table 1 and references 108–157 (cited in the Table).

Evidences are accumulating in favor of putative marker candidates for cancers, including prostate TICs having CD44, while the normal prostate stem cell lies within the basal compartment (Tang et al. 2007). Various subpopulations have been delineated as putative prostate TICs on the basis of various cell-surface markers (Signoretti and Loda 2006). For instance, since most prostate tumors resemble luminal cells (CK8+/18+AR+CD44−p63−), it had been proposed that prostate TICs are due to the dedifferentiation and transformation of luminal cells (Nagle et al. 1987; Liu et al. 1999). Keratin profile and intrinsic androgen independence, respectively, are other modes for analyzing prostate cancer including the transit-amplifying cell and basal cell. Characterization of prostate TICs have been most promising with a basal cell subpopulation of

CD44+α2β1CD133+ cells, which possess remarkable in vitro proliferative potential and can reconstitute prostatic-like acini in immune-compromised male nude mice with concomitant expression of differentiation markers, such as AR, PAP, and K18. Prostate TICs typically represent 0.01% to 0.5% of the total cell population and they appear to express many of the same markers as PSCs. Ultimately, the characterization of TICs and the nature of their involvement in prostatic carcinogenesis remain promising areas of investigation (Joshua et al. 2008; van Leenders and Schalken 2001; Verhagen et al. 1992; De Marzo et al. 1998; Bonkhoff and Remberger 1996; Richardson et al. 2004b). It has been demonstrated that prostate TICs are, like PSCs, negative for androgen receptor expression and predominantly express the basal cell cytokeratins (Richardson et al. 2004b; Collins et al. 2005) and express higher mRNA levels of several ESCs genes, including OCT3/4, BMI1, β-catenin, and SMOOTHENED (Lawson et al. 2007; Patrawala et al. 2006). Deleted in liver cancer 1 (DLC1), a gene that encodes a Rho GTPase activating protein, is a tumor suppressor gene in liver and other carcinomas. Methylation of DLC1 gene increases in the prostate of older man and expression of the DLC1 gene decreased. This kind of gene repression may involve in early stage of prostate cancer formation. From various studies, it is revealed that hypermethylation is associated with early stage cancer development and hypomethylation help in progression of prostate cancer (Jaenisch and Bird 2003; Feinberg et al. 2006; Jones and Baylin 2007; Patra et al. 2008; Schulz and Hoffmann 2009b).

Signaling cascades from membrane to nucleus

CSC's behaviors are constantly affected by external signals from their niche, including neighboring stromal, immune, and non-stem tumor cells. Extracellular and paracrine effects are mediated commonly from cell-surface ligand-receptor systems. Accumulating evidence has demonstrated that cancer cells and CSC functions hinge on major receptor-mediated pathways. For example, receptor tyrosine kinases (RTK) family mediates the effects of multiple oncogenic growth factor pathways, among which the EGFR (epidermal growth factor receptor) is one of the best characterized in cancers, including prostate cancer. Malignant cancer cells frequently have increased EGFR signaling as a result of either amplified EGFR copy number or reciprocal crosstalk with TGF-β. The signal initiated by RTKs is transduced and amplified through downstream molecule cascades, such as Ras-MAPK, Ras-Raf-MEK-ERK-Elk, and the pro-survival AKT/phosphoinositide 3-hydroxykinase pathway [for an outstanding review, see Patra 2008a, 2008b; for abbreviations, like EGFR etc. see Supplementary Table 1].

Embryonic signaling, programming, and re-derived pluripotency

Role for OCT4

The POU homeodomain containing, class 5, transcription factor 1; POU5F1 (OMIM 164177), alternatively known as octamer-binding transcription factor 3; OCT3/OTF3 and octamer-binding transcription factor 4; OCT4/OTF4 have been shown to be important regulators of tissue-specific gene expression in early mammalian development and in lymphoid and pituitary differentiation. Takeda et al. (1992) amplified POU-related sequences in human pancreatic islet mRNA by PCR and degenerating oligonucleotide primers specific for the homeodomain (Takeda et al. 1992). The sequences of both of the PCR products were identical to human OCT1 (OMIM 164175), and two others were homologous to mouse Oct3. They showed that OCT3 gene spans about 7 kb and consists of five exons, and two forms of OCT3 mRNA are expressed in adult tissues as a result of alternative splicing—OCT3A and OCT3B. OCT3A and OCT3B are composed of 360 and 265 amino acids, respectively, of which the 225 amino acids at the COOH-termini are identical. The sequence of human OCT3A showed 87% amino acid identity with mouse Oct3. Reverse transcriptase PCR showed low level of expression of both OCT3A and OCT3B mRNA in all adult human tissues examined (Takeda et al. 1992). The gene is specifically expressed in embryonic stem (ES) cells but can also be detected in adult stem cells such as bone marrow-derived mesenchymal stem cells. Expression of Oct4 is downregulated during stem-cell differentiation. Oct4 plays a critical role in maintaining pluripotency and self-renewal of ES cells (Niwa et al. 2000), but its utility as a marker of pluripotency has been challenged recently by studies suggesting that it is expressed in a variety of differentiated cells, including peripheral blood mononuclear cells. Requirements for Oct3/4 in the maintenance of developmental potency in murine embryonic stem (ES) cells were tested by conditional expression and repression (Niwa et al. 2000). Although transcriptional determination has usually been considered as a binary on–off control system, Niwa et al. (2000) found that the precise level of Oct3/4 governs three distinct fates of ES cells (Niwa et al. 2000). A less-than-twofold increase in expression causes differentiation into primitive endoderm and mesoderm. In contrast, repression of Oct3/4 induces loss of pluripotency and dedifferentiation to trophectoderm. Thus, a critical amount of Oct3/4 is required to sustain stem cell self-renewal, and up- or downregulation induces divergent developmental programs. Those findings established a role for Oct3/4 as a master regulator of pluripotency that controls lineage commitment and illustrated the sophistication

of critical transcriptional regulators. However, detection of Oct4 expression by RT-PCR could be prone to artifacts generated by pseudogene transcripts. We therefore suggest to analyze the sequences of human Oct4 and its pseudogenes and designed PCR primers that can avoid false-positive detection of Oct4 expression. Oct3 plays many other roles, including cardiac development in the early mouse embryo (Zeineddine et al. 2006).

Mammalian forkhead box protein Foxd3 and Oct4 bound identical regulatory DNA sequences in the osteopontin (SPP1; OMIM 166490) promoter (Guo et al. 2002). It was observed that Oct4 interacted directly with Foxd3, and osteopontin promoter was activated by both of the proteins, either independently or in combination. However, the activation of both Foxa1 (OMIM 602294) and Foxa2 (OMOM 600288) promoters by Foxd3 was inhibited by co-expression of Oct4 (Guo et al. 2002). More significantly, Oct4 neither bound to the forkhead box in the FoxA1 or FoxA2 promoters that FoxD3 bound to, nor did it activate transcription from these promoters. Significantly, Oct4 blocked the transcriptional activation of the FoxA1 and FoxA2 promoters by FoxD3. This repression was specific for FoxD3. Oct4 did not repress the activation of the FoxA1 or FoxA2 promoters by FoxA1 or FoxA2 proteins. Immunoprecipitation studies found that Oct-4 could physically interact with the DNA binding domain of FoxD3, which implies that when Oct-4 is not binding to DNA it can function as a corepressor to inhibit the lineage-specific promoters used here. It is possible that the dimerization and conformational changes that Oct4 undergoes when it binds DNA prevent it from acting as a corepressor of FoxD3. When Oct-4 is not binding DNA, then it can bind to the FoxD3 DNA-binding domain and repress FoxD3 transcriptional activation. Two possible mechanisms for the inhibition of FoxD3 activation of FoxA1 or FoxA2 exist. First, Oct4 could inhibit FoxD3 activation of the FoxA1 or FoxA2 promoters by blocking binding of FoxD3 to the Forkhead Box sequence in those promoters. Second, Oct-4 could function as a true corepressor by decreasing FoxD3 interaction with the transcriptosome apparatus. In either case, this inhibition prevents inappropriate activation of endodermal promoters in a totipotent ES cell. When Oct4 is downregulated after gastrulation and the initial formation of the primitive endoderm, then FoxA1 and FoxA2 can be activated appropriately by the FoxD3, which is still bound. Once activated, the proteins these promoters generate will maintain expression throughout organogenesis even as FoxD3 is downregulated (Guo et al. 2002). Oct4 is one of the partners in the protein network in which Nanog (OMIM 607937) operates in mouse ES stem cells (Wang et al. 2006). The network is highly enriched with nuclear factors that are individually critical for maintenance of the ES cell state and co-regulated on differentiation. The network is linked to multiple co-repressor pathways and is composed of numerous proteins whose encoding genes are putative direct transcriptional targets of its members and this network seems to operate as a cellular module dedicated to pluripotency (Wang et al. 2006).

Mice with targeted disruption of the Oct4, or Oct3 gene were generated by homologous recombination in ES cells (Nichols et al. 1998). Oct4-deficient embryos developed to the blastocyst stage with inner cell mass cells that were restricted to differentiation along the extra-embryonic trophoblast lineage, hence, were not pluripotent. Trophoblast proliferation was not maintained in Oct4 −/− embryos in absence of a true inner cell mass. However, expansion of trophoblast precursors was restored by an Oct4 target gene product, fibroblast growth factor-4 (FGF4, OMIM 164980). Therefore, Oct4 also determines paracrine growth factor signaling from stem cells to the trophectoderm, and the activity of Oct4 is essential for the identity of the pluripotential founder cell population in the mammalian embryo. In a very recent demonstration, Tay et al. (2008) showed the existence of many naturally occurring miRNA targets in the amino acid coding sequences of the mouse Nanog, Oct4, and Sox2 genes and concluded that the abundance of coding sequence-located miRNA targets, some of which can be species-specific (Tay et al. 2008).

Retrovirus-mediated transfection of four transcription factors, Oct3/4, Sox2 (OMIM 184429), c-Myc (OMIM 190080), and Klf4 (OMIM 602253) into mouse fibroblasts generated induced pluripotent stem (iPS), from mouse fibroblasts (Takahashi and Yamanaka 2006). Subsequently, selection for Fbx15 (OMIM 609093) expression showed that these iPS cells are similar to embryonic stem (ES) cells in morphology, proliferation and teratoma formation. However, iPS cells are different with regard to DNA methylation patterns and gene expression, and fail to produce adult chimeras. Interestingly, selection for Nanog expression results in germline-competent iPS cells with increased ES cell-like gene expression and DNA methylation patterns compared with Fbx15 iPS cells (Okita et al. 2007). The transgenes were strongly silenced in Nanog iPS cells. The generation of mouse iPS cells by repeated transfection of two expression plasmids, one containing the cDNAs of Oct3/4, Sox2, and Klf4 and the other containing the c-Myc cDNA, into mouse embryonic fibroblasts resulted in iPS cells without evidence of plasmid integration. These iPS cells produced teratomas when transplanted into mice and contributed to adult chimeras. The production of these virus-free iPS cells, albeit from embryonic fibroblasts, addresses a critical safety concern for potential use of iPS cells in regenerative medicine (Morgan et al. 2005). The adult mouse neural stem cells express higher endogenous level of Sox2 and c-Myc than

embryonic stem cells and that exogenous Oct4 together with either Klf4 or c-Myc is sufficient to generate iPS cells from neural stem cells (Kim et al. 2008).

Independently, by another group, it was demonstrated that the transcription factors Oct4, Sox2, c-Myc, and Klf4 can induce epigenetic reprogramming of a somatic genome to an embryonic pluripotent state (Wernig et al. 2007). Fibroblasts that had reactivated, in contrast to selection for Fbx15 activation (Takahashi and Yamanaka 2006), the endogenous Oct4 (Oct4-neo) or Nanog (Nanog-neo) loci grew independently of feeder cells, expressed normal Oct4, Nanog, and Sox2 RNA and protein levels, were epigenetically identical to ES cells by a number of criteria. Four factors, OCT4, SOX2, NANOG, and LIN28 (OMIM 611043), were found sufficient to reprogram human somatic cells to pluripotent stem cells that exhibited the essential characteristics of embryonic stem cells (Yu et al. 2007a). These induced pluripotent human stem cells have normal karyotypes, express telomerase activity, express cell-surface markers and genes that characterize human ES cells, and maintain the developmental potential to differentiate into advanced derivatives of all three primary germ layers (Yu et al. 2007a). Using Oct4, Sox2, Klf4, and Myc iPS cells derived from fetal, neonatal, and adult human primary cells, including dermal fibroblasts isolated from a skin biopsy of a healthy research subject (Park et al. 2008). Human iPS cells resemble embryonic stem cells in morphology and gene expression profile and in the capacity to form teratomas in immune-deficient mice. It was suggested that defined molecular components can reprogram human cells to pluripotency. A method was established to generate patient-specific cell lines in culture (Park et al. 2008). Stadtfeld et al. (2008) generated mouse iPS cells from fibroblasts and liver cells by using nonintegrating adenoviruses transiently expressing Oct4, Sox2, Klf4, and c-Myc (Stadtfeld et al. 2008). These adenoviral iPS cells showed DNA demethylation characteristic of reprogrammed cells, expressed endogenous pluripotency genes, formed teratomas, and contributed to multiple tissues, including the germ cell line, in chimeric mice.

Role for NANOG

Homeobox transcription factor NANOG (OMIM 607937). Embryonic stem (ES) cells derived from the inner cell mass (ICM) of blastocysts grow infinitely while maintaining pluripotency. Lif (OMIM, 159540) can maintain self-renewal of mouse ES cells through activation of Stat3 (OMIM 102582), but is dispensable for maintenance of ICM and human ES cells. In search of a critical factor(s) that underlies pluripotency in both ICM and ES cells, Mitsui et al. (2003) performed an in silico differential display and identified several genes specifically expressed in mouse ES cells and preimplantation embryos (Mitsui et al. 2003). One of them, encoding a homeoprotein, the authors designated Nanog (from “Tir Na Nog,” the mythologic Celtic land of the ever young) was capable of maintaining ES cell self-renewal independently of Lif/Stat3. The mouse Nanog cDNA contains an open reading frame encoding a 305-amino acid polypeptide and has a long 3-prime untranslated region containing a B2 repetitive element. The predicted Nanog protein contains a homeobox domain that is most similar to those of the Nk2 gene family (see 606727). The human Nanog protein (FLJ12581) shares 52% overall amino acid identity with the mouse protein and 85% identity in the homeodomain. Both human and mouse Nanog contain trp-rich repeats, in which trp-x-x-x is repeated eight and ten times, respectively. Human Nanog contains an Alu repetitive element in the 3-prime untranslated region. EST database searching identified clones corresponding to human Nanog in libraries from NT2 human teratocarcinoma cells, germ cell, and testis tumors, marrow, and other tumors. No EST clones were detected in libraries from normal somatic tissues. The NANOG gene contains four exons and spans 7 kb (Hart et al. 2004). The human NANOG protein contains 305 amino acids (Chamber et al. 2003; Chambers et al. 2007; Clark et al. 2004). There are three splice variants of mouse Nanog (Hart et al. 2004). The longest variant encodes a 305-amino acid protein, and both shorter variants encode a 279-amino acid protein. RT-PCR detected Nanog expression in undifferentiated mouse ES cells and embryonal carcinoma cells. In preimplantation embryo, expression was detected in morula and blastocysts, but little is known about how Nanog expression is regulated. Nanog gene is transcribed under the control of a regulatory region that lies within 332 bp upstream of the transcriptional start site. Fox D3 (Forkhead Box Protein-D3) (Pan et al. 2006), Oct3, and Sox2 are bind to the Nanog promoter region (Kuroda et al. 2005) and positively regulate the transcription of Nanog and TCF3 (Transcription factor 3, a transcription factor that functions downstream of the Wnt pathway), and p53 (Lin et al. 2005) negatively regulate the Nanog transcription after binding to the promoter region. Leukemia inhibitory factor and bone morphogenetic protein (Matsuda et al. 1999) signaling and their downstream effectors signal transducer and activator of transcription-3 (Matsuda et al. 1999; Suzuki et al. 2006) and T (Brachyury, a novel family of *putative* transcription factor) may also be involved in Nanog regulation (Suzuki et al. 2006). Expression was present after implantation, but it was downregulated after embryonic day 8.5. Low levels of Nanog were detected in many adult mouse tissues. In situ

hybridization showed Nanog confined to the inner cell mass in mouse blastocysts. Expression was downregulated as epiblast cells entered the primitive streak and underwent epithelial to mesenchymal transition. After the late-bud stage, expression of Nanog waned, and it was not detectable by day 8. In developing gonads, Nanog expression was detected at embryonic day 11.5 (Hart et al. 2004).

Role for MYC

The MYC (OMIM 190080, Gene map locus 8q24.12-q24.13), a proto-oncogene encodes a DNA-binding factor that can activate and repress transcription. Via this mechanism, MYC regulates expression of numerous target genes that control key cellular functions, including cell growth and cell cycle progression. MYC also has a critical role in DNA replication. Deregulated MYC expression resulting from various types of genetic alterations leads to constitutive MYC activity in a variety of cancers and promotes oncogenesis (Dominguez-Sola et al. 2007). The x-ray structures of the basic/helix-loop-helix/leucine zipper (bHLHZ) domains of MYC-MAX and MAD (OMIM 600021)-MAX heterodimers bound to their common DNA target, the enhancer box (E box) hexanucleotide (5-prime-CACGTG-3-prime) was determined (Lee et al. 1997). E-box recognition by these two structurally similar transcription factor pairs determines whether a cell will divide and proliferate (MYC-MAX) or differentiate and become quiescent (MAD-MAX). Deregulation of MYC has been implicated in the development of many human cancers, including Burkitt lymphoma (OMOM 113970), neuroblastomas, and small cell lung cancers. Induction of MYC promotes cell proliferation and transformation by activating growth-promoting genes, including the ornithine decarboxylase (ODC1) and CDC25A gene (Nair and Burley 2003). MYC transcriptionally represses the expression of the growth arrest gene (GAS1; 139185). A conserved MYC structure, MYC box 2, is required for repression of GAS1 and for MYC induction of proliferation and transformation, but not for activation of ODC1 (Lee et al. 1997). Overexpression of MYC in colorectal cancers is a commonly observed phenomenon. MYC oncogene is a target in adenomatous polyposis coli (APC; 611731)—beta-catenin (CTNNB1; OMIM 116806) signaling pathway (He et al. 1998a). They showed that expression of MYC is repressed by wild-type APC and activated by CTNNB1, and that effects are mediated through T-cell factor 4 (TCF4; OMIM 602228) binding sites in the MYC promoter (He et al. 1998a). Inactivating mutations in the APC gene or DNA-hypermethylation of promoter found in most colorectal cancers, cause aberrant accumulation of CTNNB1 which then binds TCF4 causing increased transcriptional activation of unknown genes. MYC directly activates telomerase by inducing expression of its catalytic subunit; telomerase-reverse transcriptase (TERT) (Wu et al. 1999). MYC activity regulates a pathway linking cell proliferation and chromosome integrity in normal and neoplastic cells. However, TERT-driven cell proliferation is not genoprotective because it is associated with activation of the MYC oncogene (Wu et al. 1999). Human mammary epithelial cells, which normally stop dividing in culture at 55 to 60 population doublings (PDs), were infected with human TERT retrovirus at PD40 and maintained until PD250 (Wang et al. 2000). MYC induces transcription of the E2F1, 2 and 3 genes in mouse embryonic fibroblasts. For S phase arrest and indication of apoptosis by MYC a cell requires distinct E2F activities. The ability of Myc to induce S phase was impaired in the absence of either E2f2 or E2f3 but not E2f1 or E2f4 (Wang et al. 2000).

MYC physically interacts with SMAD2 (Mothers against decapentaplegic homolog 2) and SMAD3, two specific signal transducers involved in TGF-beta signaling. Through its direct interaction with SMADs, MYC binds to the SP1-SMAD complex on the promoter of the p15 (INK4B) gene, thereby inhibiting the TGF-beta-induced transcriptional activity of SP1 and SMAD/SP1-dependent transcription of the p15 (INK4B) gene. The oncogenic MYC promotes cell growth and cancer development partly by inhibiting the growth inhibitory functions of SMADs (Feng et al. 2002). Gao et al. (2009) reported that the c-Myc oncogenic transcription factor also regulate microRNAs and stimulate cell proliferation, transcriptionally represses miR23a (OMIM 607962) and miR23b (OMIM 610723), resulting in greater expression of their target protein, mitochondrial glutaminase in human P-493 B lymphoma cells and PC3 prostate cancer cells. (Gao et al. 2009)

Animal model studies implicated the role of MYC in embryonic development, tissue regeneration, and cancer. Baudino et al. (2002) have reported the lethality of c Myc-null embryos by embryonic day 10.5 with defects in growth and in cardiac, neural development, defects in vasculogenesis, and primitive erythropoiesis, and compromised differentiation and growth of yolk sac and embryonic stem (ES) cells. c-Myc expression was required for the expression of Vegf, angiopoietin-1, angiopoietin-2, and thrombospondin-1, and expression of Vegf partially rescued the lethal defects (Baudino et al. 2002). A reversible transgenic mouse model of pancreatic beta-cell oncogenesis, using a switchable form of the MYC protein depicted that activation of MYC in adult, mature beta cells induced uniform beta-cell proliferation but was accompanied by overwhelming apoptosis that rapidly eroded beta-cell mass (Pelengaris et al. 2002). Brief MYC inactivation appears to cause epigenetic changes in tumor cells that render them insensitive to MYC-induced tumorigenesis. The authors

raised the possibility that transient inactivation of MYC may be an effective therapy for certain cancers (Jain et al. 2002). Langenau et al. (2003) described the induction of clonally derived T cell acute lymphoblastic leukemia in transgenic zebrafish expressing mouse c-Myc under the control of the zebrafish Rag2 promoter. This transgenic model provided a platform for drug screens and genetic screens aimed at identifying mutations that suppress or enhance c-MYC-induced carcinogenesis (Langenau et al. 2003). In transgenic mice that conditionally overexpressed Myc in liver cells, upon Myc activation, all transgenic mice developed liver tumors and succumbed to invasive liver cancers. Myc inactivation induced tumor regression and the differentiation of tumor cells into normal liver cells. Their tumorigenic potential remained dormant as long as Myc remained inactive; Myc reactivation immediately restored their neoplastic properties (Shachaf et al. 2004). Ruggero et al. (2004) have generated transgenic mice that overexpressed translation initiation factor-4E, a downstream effector molecule of myc signaling axis, and observed a marked increase in tumorigenesis in the mice compared with their wild type littermates (Ruggero et al. 2004). Sansom et al. (2007) have simultaneously deleted both Apc and Myc in the adult murine small intestine and observed that loss of Myc rescued the phenotypes of perturbed differentiation, migration, proliferation, and apoptosis, which occur on deletion of Apc. Remarkably, this rescue occurred in the presence of high levels of nuclear beta-catenin. Array analysis revealed that Myc is required for the majority of Wnt (OMIM 164820) target gene activation following Apc loss (Sansom et al. 2007). Cells transformed with panel of oncogenes, other than MYC, escaped apoptosis when treated with small-molecule CDK1 (cyclin-dependent kinases 1) inhibitors. The inhibitor of apoptosis protein survivin (BIRC5; 603352), a non-CDK target, was required for the survival of cells overexpressing MYC in MYC-transformed cells. Inhibition of CDK1 had rapidly downregulated survivin expression and induced MYC-dependent apoptosis (Goga et al. 2007). Soucek et al. (2008) used a dominant-interfering Myc mutant to determine both the therapeutic impact and side effects of Myc inhibition in a preclinical mouse model of Ras (OMIM 190020)-induced lung adenocarcinoma. They showed that Myc inhibition triggers rapid regression of incipient and established lung tumors, defining an unexpected role for endogenous Myc function in the maintenance of Ras-dependent tumors in vivo. Systemic Myc inhibition also exerts profound effects on normal regenerating tissues. However, these effects are well tolerated over extended periods and rapidly and completely reversible. These data demonstrated the feasibility of targeting Myc, a common downstream conduit for many oncogenic signals, as an effective, efficient, and tumor-specific cancer therapy (Soucek et al. 2008).

Role for hedgehog and wnt

The regulation of stem cells and in particular their dysregulation in cancer is thought to occur through a relatively small number of signaling pathways such as Hedgehog and Wnt (Joshua et al. 2008). The name Wnt was coined as a combination of Wg (wingless) and Int and can be pronounced as “wint”. These pathways are all likely to be co-regulated to maintain stem-cell homeostasis and their dysregulation may be crucial to the emergence of a TICs phenotype or morphological characteristics of more advanced disease. The “Hedgehog” proteins are secreted hydrophobic proteins that are made up of three signaling genes Sonic hedgehog (Shh), Indian hedgehog, and Desert hedgehog. Shh binds to the specific receptor, Patched, on the cell surface. It ultimately activates an intracellular signal transduction pathway activating the Gli (GLIoma-associated oncogene homolog) family of transcription factors. This family of transcription factors has multiple oncogenic effects: (1) acceleration of proliferation rate by activation of regulators of G1/S and G2/M phase progression, (2) induction of Bcl-2 (B-cell lymphoma 2) and direct inhibition of apoptosis, and (3) activation of epithelial to mesenchymal transition-promoting factors such as Snail, and enhancement of invasiveness and metastasis. Furthermore, there are multiple control mechanisms at the membrane level with a second transmembrane protein, Smo (Smoothened), a cell-surface hedgehog ligand sequestration protein, Hip (also known as Hedgehog interacting protein), transcription repression through Gli3 and a cytoplasmic network of proteins including Fused and SuFu (Suppressor of Fused). The expression of these Hedgehog proteins is high in the fetal human prostate and decreases to low levels in adult prostate tissue where it is thought to regulate the prostatic epithelial homeostasis by inhibiting proliferation and promoting terminal differentiation of ducts (Joshua et al. 2008; Hooper and Scott 2005; Wang et al. 2003; Karhadkar et al. 2004; Shaw and Bushman 2007). Similar to the Hedgehog pathway, the Wnt pathway is implicated in directing embryonic growth, and governing processes such as cell fate specificity, proliferation, polarity, and migration. The *wingless* gene had originally been identified as a recessive mutation affecting wing and haltere development in *Drosophila melanogaster*. It was subsequently characterized as segment polarity gene in *D. melanogaster* that functions during embryogenesis and also during adult limb formation during metamorphosis. The INT genes were originally identified as vertebrate genes near several integration sites of mouse mammary tumor virus. The following is a list of human genes that encode WNT signaling proteins: WNT1, WNT2, WNT2B, WNT3, WNT3A, WNT4, WNT5A, WNT5B WNT6 WNT7A, WNT7B WNT8A, WNT8B, WNT9A, WNT9B, WNT10A, WNT10B, WNT11, and WNT16. The Int-1 gene and the

wingless genes were found to be homologous, with a common evolutionary origin evidenced by similar amino acid sequences of their encoded proteins. The canonical Wnt pathway is characterized by binding of Wnt proteins, through transmembrane receptors, to ultimately form a complex with axin to induce its dephosphorylation. Axin acts as a scaffold protein for a complex involving the APC gene and beta-catenin, thereby facilitating phosphorylation of both APC and beta-catenin by glycogen synthetase kinase 3B. As a consequence, cytoplasmic beta-catenin is translocated to the nucleus, where it associates with the T-cell factor (Tcf) and lymphoid enhancer (LEF) family of transcription factors. The beta-catenin/Tcf/LEF complex activates transcription of target genes with relevance to carcinogenesis including those that regulate cellular proliferation (c-MYC, c-Jun, cyclinD1, cellular migration (uPA, CD44, MMP-7) and cellular differentiation (FGF2, PPAR-gamma; Joshua et al. 2008; Ikeda et al. 1998; Kobayashi et al. 2000; Roose and Clevers 1999; He et al. 1998b; Gounari et al. 2002; de la Taille et al. 2003).

Conclusion and perspectives

Recent research in cancer biology, including prostate cancer has provided support for the cancer stem-cell hypothesis (Blum et al. 2009). Two important components of this hypothesis are that tumors originate in stem or progenitor cells as a result of dysregulation of the normally tightly regulated process of self-renewal. As a result, tumors contain and are driven by a cellular subcomponent that retains key stem-cell properties including self-renewal, which drives tumorigenesis and differentiation that contributes to cellular heterogeneity. Advances in stem-cell technology have led to the identification of stem cells in normal and malignant tissues. The study of these stem cells has helped to elucidate the origin of the molecular complexity of human cancers. The cancer stem-cell hypothesis has important implications for early detection, prevention, and treatment of prostate and other cancers. Notably, both hereditary and sporadic prostate cancers may develop through dysregulation of stem-cell self-renewal pathways. These aberrant stem cells may provide targets for the development of cancer prevention strategies. Furthermore, because prostate cancer stem cells may be highly resistant to radiation and chemotherapy, the development of more effective therapies for this disease may require the effective targeting of this cell population.

The origin of cancer stem cells is still debated, but the most probable hypothesis is that they arise from normal stem cells over time, in a process that parallels, and in fact underlies, the slow and multi-step development of cancer from normal tissues (Miller et al. 2005). The rationale for this theory is that stem cells, through their longer life span, are the only cells able to accumulate all the mutations necessary to initiate cancer. Furthermore, tissue-specific stem cells and CSCs are notably similar, sharing fundamental abilities of self-renewal and differentiation (Martínez-Climent et al. 2006). DNA methylation and PRCs were analyzed by a new experimental and analytical strategy using customized high-density tiling arrays to investigate coordinated patterns of gene expression (Gal-Yam et al. 2008). Both DNA methylation and polycomb marks differentiate cancer cells from their normal counterparts. Disruption of bivalent chromatin profile in ES cell may also be responsible for cancer formation. Control of gene expression by key regulatory genes (Nanog, Oct4, Sox2, Esrrb, Tbx3, Tcl1, and Dppa4), passive or active DNA methylation/demethylation and histone modification maintain pluripotency and self-renewal character in ES cell. They also help in embryo development and cell differention. Three major changes in the epigenomic landscape distinguished the two cell types. Developmentally, significant genes containing CpG islands which are silenced by PRCs in the normal cells acquire DNA methylation silencing and lose their PRC marks (epigenetic switching). Because these genes are normally silent this switch does not cause de novo repression but might significantly reduce epigenetic plasticity. Two other groups of genes are silenced by either de novo DNA methylation without PRC occupancy (5mC reprogramming) or by de novo PRC occupancy without DNA methylation (PRC reprogramming). These data suggested that the two silencing mechanisms act in parallel to reprogram the cancer epigenome and that DNA hypermethylation may replace polycomb-based repression near key regulatory genes, possibly reducing their regulatory plasticity. Any small mistake in these vital control systems may cause cancer. Unlike genetic alterations, epigenetic changes are potentially reversible. The large-scale development of small-molecule inhibitors of DNA and histone-modifying enzymes is now in full swing. Clear information about epigenetic altaration makes a glorious path in cancer biology research. In the clinic, the success of HDAC inhibitors and DNA demethylating agents like aza cytidine as anti-cancer drugs demonstrates “proof of principle” of this approach and provides great hope for the development of a more comprehensive portfolio of “epigenetic drugs” in the future.

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