Chapter 24
Stem Cell Plasticity in Development and Cancer: Epigenetic Origin of Cancer Stem Cells

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Abstract Stem cells are unique cells that can self-renew and differentiate into many cell types. Plasticity is a fundamental characteristic of stem cells and it is regulated by reversible epigenetic modifications. Although gene-restriction programs are established during embryonic development when cell lineages are formed, stem cells retain a degree of flexibility that is essential for tissue regeneration. For instance, quiescent adult stem cells can be induced to proliferate and trans-differentiate in response to injury. The same degree of plasticity is observed in cancer, where cancer cells with stem cell characteristics (or cancer stem cells) are formed by transformation of normal stem cells or de-differentiation of somatic cells. Reprogramming experiments with normal somatic cells and cancer cells show that epigenetic landscapes are more plastic than originally thought and that their manipulation can induce changes in cell fate. Our knowledge of stem cell function is still limited and only by understanding the mechanisms regulating developmental potential together with the definition of epigenetic maps of normal and diseased tissues we can reveal the true extent of their plasticity. In return, the control of plastic epigenetic programs in stem cells will allow us to develop effective treatments for degenerative diseases and cancer.

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24.1 Introduction

How are cells in our body programmed to maintain their identity and function throughout life? The answer to this fundamental question is based on important processes that are initiated during embryo development and maintained in adulthood. This book chapter will describe and discuss the mechanisms that control cell and tissue homeostasis and how these are altered in cancer.

Cell identity is established during embryogenesis when the developmental potential of embryonic cells is restricted by differentiation programs that channel their fate to tissue-specific stem cells and specialised cell types. These dynamic events occur in cells with the same genetic information, thus cell fate depends on the epigenetic regulation of that genetic code. "Epigenetics" can be defined as regulation of gene expression that occurs by modifications imposed on the chromatin without change in the DNA sequence (Bird 2007). It is by changes in chromatin organisation that epigenetic modifications establish heritable transcriptional states responsible for the maintenance of cell function.

Epigenetic regulation includes DNA methylation, modification of histone tails and modulation by non coding RNAs (ncRNAs). Together with chromatin remodelling complexes, these modifications control chromatin organisation and regulate gene transcription (Jaenisch and Bird 2003).

DNA methylation is responsible for gene silencing and occurs at position 5 of cytosine (5mC) within CpG dinucleotides present in repetitive sequences and CpG islands in gene promoters and intragenic regions (Ball et al. 2009; Sharma et al. 2010). DNA methylation is maintained or established *de novo* by the DNA methyltransferases enzymes DNMT1 and DNMT3A/3B/3L, respectively (Bird 2002). Histone modifications comprise a vast range of post-translational modifications, such as acetylation, methylation, phosphorylation, ubiquitylation and ribosylation. These modifications can induce both activation and repression of transcription and their interactions function as a "code" defining cellular states (Turner 2007). Nucleosome remodelling and modulation by ncRNAs are the most important non covalent epigenetic modifications. Non coding RNAs, including microRNAs (miRNAs) and long non coding RNAs (lncRNAs), are single stranded transcripts involved in mRNA degradation and chromatin remodelling (Pauli et al. 2011). While heritable, epigenetic modifications are reversible and their dynamic interplay provides cells with ability to respond to environmental cues. Therefore it is easy to imagine how the epigenetic landscape created by these modifications can regulate phenotype plasticity in different cell types during normal development, but also cause disease if abnormally regulated.

Cancer is a disease characterised by abnormal cell proliferation and it is associated with both genetic lesions and epigenetic abnormalities. Because it can be portrayed as a process of aberrant cell proliferation and differentiation, cancer has been described as "a problem of developmental biology" where a marked resemblance between cancer cells and embryonic cells exists (Pierce and Johnson 1971). Indeed,

cancer cells re-initiate epigenetic programs that favour cell growth and survival at the expense of differentiation, thus behaving like undifferentiated embryonic cells and stem cells. As cancer cells depend on those mechanisms that maintain stem cell plasticity (Garraway and Sellers 2006), it is not a coincidence that many tumour suppressor genes that are epigenetically silenced in cancer are developmental genes involved in the regulation of stem cells (Barrero et al. 2010). It is precisely how stem cell plasticity is programmed in development and cancer that will be the focus of our discussion.

24.2 Epigenetics and Development

Epigenetic modifications regulate the acquisition of totipotency and subsequent progressive restriction of totipotent potential during embryonic development. Acquisition of totipotency is associated with two epigenetic reprogramming events: the formation of the zygote and the germ line (Hemberger et al. 2009). Both developmental stages require resetting of a differentiated epigenetic landscape to establish a new state with augmented developmental potency. Differentiation of somatic cells then requires the establishment of specific epigenetic programs that restrict their potential and maintain lineage memory. This section will describe the epigenetic modifications occurring during embryo development and explain how embryonic developmental potential is programmed in embryonic cells, somatic cells and germ cells.

24.2.1 Epigenetic Reprogramming During Embryo Development

Embryo development initiates with the fusion of the male and female pronuclei after fertilisation. The formation of the zygote is followed by epigenetic reprogramming of the specialised gametic genomes to ensure that the embryonic genome acquires totipotency, defined as the ability of a cell to form an entire organism. Immediately after fertilisation, the paternal nucleus undergoes profound chromatin remodelling. This involves exchange of protamines for histones in the nucleosomes and active DNA demethylation (Oswald et al. 2000; Mayer et al. 2000; Santos et al. 2002). Although a specific DNA demethylase enzyme has not been identified, a process involving DNA repair through the intermediate 5-hydroxymethylcytosine (5hmC) has been proposed (Wossidlo et al. 2010, 2011; Hemberger et al. 2009). After fusion, the progressive decline in DNA methylation up to the morula stage is due to passive loss of methylated cytosine

marks during DNA replication (Howell et al. 2001). Some genomic sequences escape this demetylation, including some repetitive sequences and most imprinted genes (Meissner 2010). Concurrent with DNA demethylation, reprogramming of histone modifications also takes place. The newly incorporated histones in the paternal pronucleus gradually increase active marks, such as acetylation of histone H3 at lysine 9 (H3K9ac), methylation of histone H3 at lysine 4 (H3K4me3), and repressive marks, e.g. methylated histone H3 at lysine 9 (H3K9me1, H3K9me2) and methylated histone H3 at lysine 27 (H3K27me3) (Meissner 2010). Subsequent to the first cleavage divisions, the embryo undergoes segregation of the first two lineages, the inner cell mass (ICM) and the trophectoderm. The cells of the ICM are pluripotent embryonic cells, able to differentiate to all somatic lineages and the germ line (Wray et al. 2010). Epigenetic programming of ICM cells includes *de novo* DNA methylation, acquisition of H3K9ac, H3K27me3, H3K4me3, H3K9me2 and H3K9me3 (Morgan et al. 2005). Re-establishment of DNA methylation is essential for normal embryonic development, as demonstrated by knockout experiments where deletion of DNMTs and other epigenetic modifiers participating in DNA methylation (LSH and G9a) causes embryonic lethality (Okano et al. 1999; Myant et al. 2011). During gastrulation and differentiation of embryonic cells to somatic lineages, a progressive decrease in plasticity is observed and this is accomplished by a program of epigenetic modifications that restricts cell fate, retains cell memory and confers cellular specialisation.

However, epigenetic restrictions imposed during differentiation are reprogrammed in the germ line. Germ cells derive from embryonic precursors of gametes defined as primordial germ cells (PGC), which are responsible for the development of a new organism in the next generation. Epigenetic reprogramming in the germ line is essential for the generation of a cellular state that will allow totipotency in the newly formed embryo. In addition, reprogramming of PGC ensures an equivalent epigenetic state in both sexes prior to differentiation into mature gametes and erasure of acquired epimutations which could be inherited in the next generation (Allegrucci et al. 2005). PGC are specified in the proximal epiblast and then migrate through the hindgut to the developing gonads. It is during migration and after colonisation of the gonads that extensive epigenetic reprogramming occurs in these cells. This involves loss of H3K9me2 and DNA methylation, and an increase in H3K27me3. It is thought that this epigenetic configuration, enriched in H3K27me3, H3K4me2/me3 and H3K9ac confers PGC with the required plasticity to regain pluripotency (Hemberger et al. 2009). In addition, loss of DNA methylation at imprinted genes ensures erasure of epimutations and correct re-establishment of monoallelic expression for gene dosage in the next generation (Allegrucci et al. 2005; Sasaki and Matsui 2008).

The epigenetic reprogramming and programming of cell plasticity during development is orchestrated by a battery of epigenetic modifiers. Their coordinated action ensures a correct program of cell proliferation and differentiation (Table 24.1).

Table 24.1 Key epigenetic modifiers regulating cell proliferation and differentiation

Epigenetic modifier	Modification/Function	Epigenetic mark	Implicated in cancer
EZH1/2	Histone methylation	H3K27me3	√
SUZ12	PcG-PRC2 complex	–	√
EED	PcG-PRC2 complex	–	√
JARID2	Recruits/stabilise PRC2	–	–
RING1	Histone ubiquitylation	H2AK119u	√
BMI-1	PcG-PRC1 complex	–	√
SET/MLL	Histone methylation	H3K4me3	√
	Histone acetylation	H4K16ac	√
UTX	Histone demethylation	H3K27me3	√
JMJD3	Histone demethylation	H3K27me3	√
JARID1A/B	Histone demethylation	H3K4me3	√
JMJD1A	Histone demethylation	H3K9me2	√
JMJD2C	Histone demethylation	H3K9me3	√
G9a	Histone methylation	H3K9me1/2	√
DNMT1	5mC-DNA methylation	5mC	√
DNMT3A	5mC-DNA methylation	5mC	√
DNMT3B	5mC-DNA methylation	5mC	√
DNMT3L	5mC-DNA methylation	5mC	√
GADD45	DNA demethylation	–	√
AID	DNA demethylation	–	–
LSD1	Histone demethylation	H3K9me2	√
		H3K4me1/2/3	√
SUV39	Histone methylation	H3K9me3	√
BRG1	Chromatin remodelling	–	√
BAF250	Chromatin remodelling	–	√
BAF155	Chromatin remodelling	–	√
CHD1/3/4/7	Chromatin remodelling	–	√
HDAC1/2	Histone deacetylation	–	√
MBD3	Chromatin remodelling	–	–
BPTF	Chromatin remodelling	–	√
TIP60-p400	Chromatin remodelling	–	√
CBP/p300	Histone acetylation	–	√
SETDB1	Histone methylation	H3K9me3	√
DICER	ncRNA processing	–	√
TET1/2/3	DNA hydroxymethylation	5hmC	√

24.3 Epigenetic Regulation of Stem Cells

In the previous section we have reviewed the epigenetic events that regulate development and program cell differentiation in the embryo. Although pluripotent cells exist only for a limited period of time before gastrulation, they can be isolated from the embryo and maintained *in vitro* as embryonic stem cells (ESC). Therefore ESC can be studied as *in vitro* model of naive embryonic cells and differentiated into

many different cell types. Differentiation is not limited to embryonic development, but it continues in the adult as continuous supply of specialised cells is needed for tissue turn-over and repair. This is accomplished by lineage restricted multipotent cells, or adult stem cells (ASC). Correct stem cell function is essential during an individual's life starting at the time when tissues are formed and later on, when they need to be regenerated and repaired. By analysing the epigenetic control of ESC and ASC, this section will describe how developmental plasticity of stem cells is programmed for correct function. Knowledge of how stem cell programs are regulated is important not only to advance stem cell-based therapies but also to understand how we can overcome diseases characteristic of stem cell dysfunction.

24.3.1 Control of Embryonic Stem Cells

ESC can be derived from the blastocyst ICM and their pluripotency maintained *in vitro* for many cell generations. ESC can symmetrically self-renew, hence giving rise to two identical stem cells. ESC ability to self-renew and to respond to developmental cues is controlled by a unique gene expression program. The ground state of pluripotent ESC is defined by the expression of a core network of transcription factors that include OCT4, SOX2 and NANOG. These factors act both as transcription activators and repressors, by activating genes involved in cell proliferation and self-renewal while repressing the expression of lineage-specific genes promoting differentiation (Young 2011). This bivalent state of ESC is essential for pluripotency and it is regulated epigenetically by the interplay of core transcription factors and Trithorax (TrxG) and Polycomb (PcG) epigenetic modifiers. TrxG-related proteins (SET/MLL) catalyse H3K4me3 at promoters of active genes, whereas PcG proteins catalyse histone modifications that are associated with gene silencing. PcG proteins include two complexes, PRC1 and PRC2, responsible for H3K27me3 and ubiquitylation of histone H2A at lysine 119 (H2AK119u), respectively (Meissner 2010). While PRC2 is required for initial gene silencing, recruitment of PRC1 stabilises the established transcriptionally repressive state. It is the presence of both active H3K4me3 and repressive H3K27me3 marks (bivalent domain) at developmentally regulated genes that allows ESC to remain in a poised state, ready for activation upon differentiation. Therefore ESC show a global open chromatin structure, with about 75% of gene promoters enriched for H3K4me3. These promoters can be active or inactive, depending on H3K27me3 co-occupancy.

Among silencing mechanisms DNA methylation plays a fundamental role in ESC. ESC present about 60–80% of methylated CpG nucleotides, with a unique distribution (Meissner 2010). Comprehensive maps of DNA methylation in ESC have demonstrated that the majority of high CpG promoters (HCP) are lacking methylation and are enriched in H3K4me3. These represent housekeeping genes, pluripotency genes and key developmental genes. In contrast, tissue specific gene promoters with low CpG density (LCP) are mostly methylated (Mikkelsen et al. 2007; Meissner et al. 2008). Therefore an epigenetic landscape presenting either
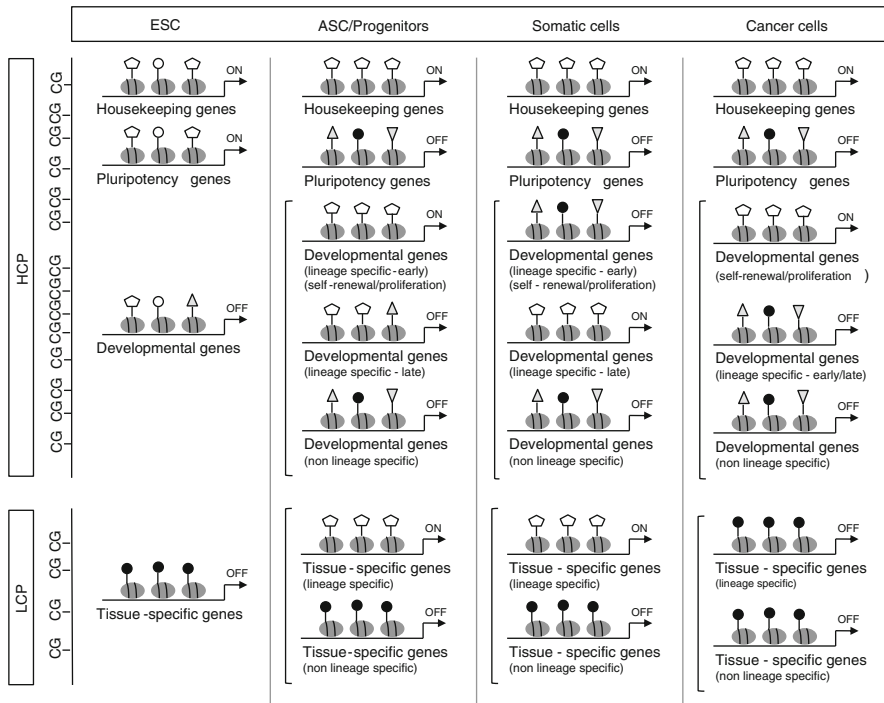
Fig. 24.1 Epigenetic landscapes regulating stem cell plasticity in development and cancer. HCP and LCP gene promoters are enriched for genes with different epigenetic regulation in different cell types. The figure shows how housekeeping genes, pluripotency genes, developmental genes and tissue-specific genes are epigenetically regulated in ESC, ASC, differentiated (somatic) cells and cancer cells. During differentiation there is a decrease in cell plasticity due to loss of bivalent domains and acquisition of repressive chromatin marks that restrict cell fate. Cancer cells reactivate an epigenetic landscape that is more plastic and shifted towards self-renewal and proliferation at the expense of differentiation (△: H3K4me3; ○: 5hmC; △: H3K27me3; ●: 5mC; ▽: H3K9me2/3)

unmethylated promoters (HCP with H3K4me3 or bivalent domain with H3K4me3/ H3K27me3), or methylated promoters (LCP) define ESC (Fig. 24.1). In addition to methylation of CpG dinucleotides, other modifications of the DNA have been discovered in ESC. These include cytosine methylation in a non CG context (Lister et al. 2009) and cytosine hydromethylation (5hmC). Hydroxylation of 5mC to 5hmC is catalysed by the TET family of enzymes (Koh et al. 2011) and it is believed to be involved in the demethylation of 5mC and prevention of DNMTs activity (Xu et al. 2011). A genome-wide study of 5hmC in ESC revealed that this mark is enriched in gene bodies, transcription start sites of HCP promoters and enhancers. Bivalent or PcG only marked promoters are also particularly enriched for 5hmC (Xu et al. 2011; Pastor et al. 2011). Although a clear function for 5hmC in transcription regulation is still elusive, its distribution suggests a role in preparing genomic loci for activation upon differentiation. Indeed, 5hmC has been shown to be present in ESC, but

declines after differentiation (Tahiliani et al. 2009; Ficz et al. 2011). Finally, ncRNAs also participate in the epigenetic regulation of ESC plasticity. MicroRNAs regulate stability and translation of mRNAs involved in stem cell self-renewal and differentiation. ESC express a unique set of miRNA whose transcription is regulated by the core pluripotency factors, and these miRNA are involved both in sustaining self-renewal (e.g mir-290-295/302) and inducing rapid degradation of ESC transcription factors during differentiation (e.g mir-145) (Marson et al. 2008; Tay et al. 2008; Xu et al. 2009). In addition, many lineage-specific miRNA gene promoters are co-occupied by OCT4/NANOG/SOX2 and PcG and these are repressed in ESC, but become active upon differentiation (e.g let-7, mir-155, mir-124, mir-9) (Young 2011; Pauli et al. 2011). Balance between self-renewal and differentiation is a key characteristic of ESC. Altogether bivalent histone modifications, DNA methylation and miRNAs contribute to the establishment of an open chromatin state that allows undifferentiated cell function and the ability to respond to developmental signals in a timely fashion.

24.3.2   Control of Adult Stem Cells and Somatic Cells

Differentiation of pluripotent cells is associated with a loss of developmental potency that ensures cellular specialisation and committed identity. During lineage specification, ASC are formed and it is from these committed multipotent stem cells that specialised cells are derived. The role of stem cells in the adult is to maintain tissue homeostasis by regenerating aged or damaged cells. ASC reside in tissue-specific niches that control their asymmetrical self-renewal, defined as the ability to form a stem cell and a differentiated progenitor at cell division. ASC are more restricted in their differentiation potential compared to ESC as they can only give rise to multiple cell types within a tissue under physiological conditions. Genome-wide maps of epigenetic modifications in ASC and differentiated cells show that restriction in developmental potential is associated with a resolution of ESC open chromatin into a more restricted configuration (Fig. 24.1). Silencing of pluripotency genes is readily observed during differentiation, by loss of H3K4me3, gain of H3K27me3, H3K9me3, DNA methylation (Barrero et al. 2010) and expression of specific miRNAs (e.g. mir-134, mir-296, mir-470) (Tay et al. 2008). Differentiation into a specific lineage involves expression of genes specific to that cell type and silencing of genes expressed in other tissues. In this way, differentiation into the neural stem/progenitor cells (NSC) is accompanied by a decrease of H3K27me3 at neural genes silenced by bivalent marks, which correspond to increased gene expression. Genes poised or weakly induced retain bivalent marks, while H3K27me3 silencing is increased in non-neural lineage genes, together with H3K9me3 (Hawkins et al. 2010; Bernstein et al. 2006; Mikkelsen et al. 2007; Bracken et al. 2006). The same pattern is also observed in muscle and germ cell differentiation (Caretti et al. 2004; Chen et al. 2005; Asp et al. 2011), suggesting a PcG-mediated regulation of cell fate decisions.

Both H3K4me3 and bivalent HPC remain mostly unmethylated during differentiation. In contrast, resolution to univalent H3K27me3 mark results in an increase in DNA methylation and a complete loss of the bivalent marks results in DNA hypermethylation. A different epigenetic regulation is observed at LCP associated with tissue specific genes, as methylated LCP associated with neural genes gain H3K4me3 and non lineage specific genes retain DNA methylation (Meissner et al. 2008). Although overall DNA methylation levels are similar in pluripotent and differentiated cells, a small subset of genes displays tissue specific methylation. A recent study demonstrated 491 differentially methylated regions (DMR) being more methylated in fibroblasts compared to ESC (Lister et al. 2009), with DMR representing only 6–8% of CpG islands in different tissues (Berdasco and Esteller 2010). Important DNA methylation differences can be observed in ASC compared to differentiated cells of the same lineage. For instance, breast self-renewal and proliferation genes are hypomethylated in CD44$^+$/CD24$^-$ stem cells compared to differentiated luminal CD24$^+$ cells (Bloushtain-Qimron et al. 2008).

Many studies indicate DNA methylation as a mechanism providing long term gene silencing and epigenetic memory in differentiated cells, however experiments of conditional deletion of DNMT1 suggest that DNA methylation plays also an important role in maintaining ASC self-renewal and suppressing differentiation (Sen et al. 2010). Indeed, loss of methylation causes differentiation alterations in epithelial progenitor cells (EPC) (Sen et al. 2010) and hematopoietic stem cells (HSC) (Trowbridge et al. 2009; Broske et al. 2009). Other epigenetic mechanisms are involved in regulation ASC self-renewal, but their relation to DNA methylation is still unknown. For instance, the PCR1 PcG protein BMI-1 is required for NSC, HSC, mammary and intestinal stem cell proliferation (Molofsky et al. 2003; Lessard and Sauvageau 2003; Pietersen et al. 2008; Sangiorgi and Capecchi 2008), while overexpression of PCR2 PcG protein EZH2 blocks differentiation of myoblasts and EPC (Caretti et al. 2004; Sen 2011) and prevents HSC exhaustion (Kamminga et al. 2006). Because cell memory is set during development and inherent to each tissue-type, it was long assumed that differentiation of ASC is strictly specific to their lineage. However, recent studies demonstrate that under certain conditions, particularly after injury, ASC can trans-differentiate into cells of different tissues (Lotem and Sachs 2006). Therefore ASC, like ESC, show a differentiation plasticity that is conferred by epigenetic programs that can reversibly regulate transcription of genes expressed in different tissue according to physiological and pathological signals. The contribution of ASC plasticity to cancer will be described in the next section.

24.4 Cancer Stem Cells

The idea that cancer is caused by transformed cells with stem cell properties is not novel, but it has received renewed interest among scientists in recent years. The observation that tumours are formed by cells with functional heterogeneity has led to the postulation of two mutually exclusive models for the cellular origin of

cancer: the stochastic model and the cancer stem cell hierarchy. The stochastic (or clonal evolution) model predicts that every cell can become tumorigenic under the influence of endogenous (transcription factors) and exogenous (microenvironment) factors that can generate their own heterogeneous sub clones (Nowell 1976). In the stochastic model, cancer cells fluctuate between several states, owing to their plasticity (Wang and Dick 2005). In contrast, the cancer stem cell (CSC) model considers that tumours originate from transformed stem cells and they are organised in a hierarchical manner, whereby CSC lies at the apex and the proliferating progenitors and terminally differentiated cancer cells reside at the bottom of the hierarchy (Bonnet and Dick 1997). Recent studies show that both models can act together depending on microenvironmental signals and that tumour initiating cells can originate both from transformation of normal ASC or epigenetic reprogramming of more differentiated cells (Campbell and Polyak 2007) (Fig. 24.2). Both theories converge on the idea that cancer arises from transformed cells that acquire growth and survival advantage, which is a landmark of stem cells. The theory that cancer could arise from embryo-like cells was proposed about 150 years ago (Virchow 1855) and was later developed by Cohnheim and Durante with the concept of "maturation arrest", according to which cancer could develop from embryonic rudiments remaining in adult organs (Cohnheim 1867; Durante 1974). These theories were proven years later by studies on germ cell tumours demonstrating that teratocarcinomas contain CSC with very similar characteristics to ESC, with self-renewal and differentiation potential (Sell and Pierce 1994; Sperger et al. 2003). For a long time it was known that only a small population of cancer cells is tumorigenic and can propagate the tumour. Single-cell analysis of leukaemia revealed two different populations of cancer cells in terms of proliferative kinetics: the frequent large, fast-cycling cells and the rare, smaller slow-cycling cells with the same properties to that of normal HSC (Clarkson 1974). Through elegant studies, Dick and colleagues proved the existence of CSC by showing that in acute myeloid leukaemia (AML), a rare population of CSC with $CD34^+/CD38^-$ cell surface expression were able to recapitulate the original disease over repeated transplantation into NOD/SCID (non-obese diabetic/severe combined immunodeficiency) mice (Lapidot et al. 1994). Since then, CSC have been identified and isolated in solid tumours including breast (Al-Hajj et al. 2003), brain (Singh et al. 2003), melanoma (Fang et al. 2005), pancreatic (Hermann et al. 2007), prostate (Tang et al. 2007) and ovarian cancers (Bapat et al. 2005) (Table 24.2). CSC are a rare population of cells that resemble normal stem cells. They can self-renew, are long-lasting, remain relatively quiescent, and can generate all heterogeneous cell types comprising the tumour. CSC can lay dormant within their niche and therefore escape chemotherapy, which only targets highly proliferating cells. Their resistance to current cancer therapies (chemotherapy and radiotherapy) is also due to expression of ATP-binding cassette (ABC) transporters (pumping out harmful drugs), increased free radical scavenging and high expression of anti-apoptotic proteins (Visvader 2011). Since CSC retain many features of normal stem cells, their identification often relies on the expression of tissue specific stem cell markers. For example, leukemic stem cells can be

Fig. 24.2 Cancer stem cell-of-origin model. Different cell types from the lineage hierarchy can undergo oncogenic events to transform into tumour cells. CSC can either originate from a transformed normal stem cell, transit-amplifying cell, progenitor cell, or a terminally differentiated cell to give rise to a heterogeneous population of cancer cells
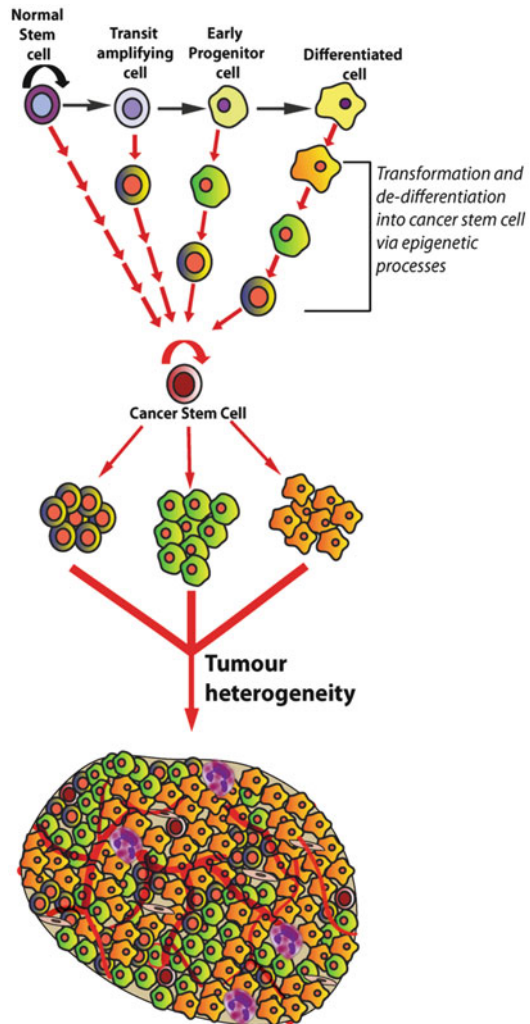
identified by CD34 and CD38 which are expressed on the cells in the HSC hierarchy (Bonnet and Dick 1997). Other universal CSC markers are instead based on their ability to pump out toxicants, which defines them as "side population" cells able to extrude a Hoechst dye, expressing ABC transporters and the detoxifying enzyme ALDH1 (Visvader and Lindeman 2008). Functional assays for CSC identification include xenografts into immunocompromised mice and formation of spheroids in culture. The xenograft assay involves transplanting cancer cells into NOD/SCID mice for tumour formation. Isolated CSC are generally more tumorigenic than differentiated tumour cells and their serial transplantation shows that they can reproduce the original disease through every passage. Sphere forming assays, which involve culturing CSC under stem cell conditions, preserve survival

Table 24.2 CSC markers in human tumours

Cancer	CSC markers	Cell-of-origin	References
Acute myeloid leukemia (AML)	CD34$^+$CD38$^-$	HSC	Bonnet and Dick (1997)
	ALDH1		Ran et al. (2009)
Acute lymphoblastic leukemia (ALL)	CD34$^+$CD19$^-$CD10$^-$	HSC	Cox et al. (2004)
Breast	CD44$^+$CD24$^{low/-}$	Mammary stem cells	Al-Hajj et al. (2003)
	ALDH1		Ginestier et al. (2007)
Colon	CD133$^+$	Intestinal stem cells	O'Brien et al. (2007)
	EpCAM^{hi} CD44$^+$		Dalerba et al. (2007)
Melanoma	JARID1B	Skin stem cell	Roesch et al. (2010)
	ABCB5$^+$		Schatton et al. (2008)
Prostate	CD133$^+$ TRA-160	Basal progenitor cells	Goldstein et al. (2010)
	TRA-160$^+$CD151$^+$CD166$^+$		Rajasekhar et al. (2011)
Pancreas	CD24$^+$CD44$^+$EpCAM$^+$	_	Li et al. (2007)
Brain	CD133$^+$	NSC	Singh et al. (2003)
Head and neck	CD44$^+$	_	Prince et al. (2007)
Lung	CD133$^+$	Bronchioalveolar stem cells	Eramo et al. (2008)
	ALDH1		Jiang et al. (2009)
Liver	CD44$^+$ CD90$^+$	_	Yang et al. (2008)
Ovary	CD44$^+$ CD177$^+$	_	Zhang et al. (2008)
Stomach	CD44$^+$	_	Takaishi et al. (2009)
Osteosarcoma	CD133$^+$	Mesenchymal stem cells	Tirino et al. (2008)

of CSC while inducing cell death by apoptosis in non-CSC (Visvader and Lindeman 2008). However, regardless of the assay, a major challenge for studying CSC is their inherent developmental plasticity, which involves the co-existence of different epigenetic states during cancer progression. For instance, CD44$^+$/CD24$^-$ breast CSC exist in a metastable state oscillating between differentiation and de-differentiation, with CSC giving rise to luminal CD24$^+$ cells and luminal cells de-differentiating back into CSC (Meyer et al. 2009). The same has been observed in melanoma, where JARID1B$^+$ CSC generate JARID1B$^-$ cells and vice versa (Roesch et al. 2010). In addition, CSC plasticity can often extend beyond their lineage and they can express genes normally expressed in different tissues. Consistent with the trans-differentiation potential of ASC after injury, CSC show the same plasticity resulting in abnormal tissue regeneration (Lotem and Sachs 2006). CSC plasticity is influenced by embryonic developmental programmes and a similar gene expression signature between highly malignant, poorly differentiated solid tumours and ESC has been reported (Ben-Porath et al. 2008). This is due to the ability of cancer to take control of normal developmental programs for selective advantage, albeit in part related to an upregulated Myc-regulatory network (Kim et al. 2010). For example, the epithelial-to-mesenchymal (EMT) transition, a reversible embryonic programme that allows transition between cellular phenotypes during gastrulation, contributes to CSC plasticity. EMT is recapitulated during

tumour progression and metastasis by a transition from an epithelial to a mesenchymal phenotype with acquired cell motility. This is induced by activation of key signalling pathways (TGF-β, Notch, FGF) that drive epigenetic silencing of the adhesion molecule E-cadherin (Thiery et al. 2009). EMT is also important for maintenance of stem cell properties and CSC can hijack this program to regulate their plasticity. In addition to this, CSC establish their own niche by recruiting cells to recreate a similar microenvironment to that of a normal stem cell niche. The niche can induce and expand CSC by enhancing "stemness" features in non tumourigenic cells by overexpressing signals that are important for stem cell renewal and promote EMT through epigenetic alterations (Mani et al. 2008).

24.4.1 Epigenetic Origin of Cancer Stem Cells

Epigenetic alterations are generally observed at early stages of tumorigenesis and are likely candidates for a mechanism of tumour initiation. ASC are long-lived and during their aging process they may undergo epigenetic insult which can induce survival programs and predispose to the onset of cancer after further genetic and epigenetic alterations (Feinberg et al. 2006; Baylin and Ohm 2006). Numerous evidences indicate a role for epigenetic defects in the development of CSC. Normal stem cells are vulnerable to epigenetic alteration when induced to sustained self-renewal. Extensive DNA methylation alterations have been reported in ESC after long term in culture, with changes which are inherited after differentiation and associated with cancer (Allegrucci et al. 2007). Similar alterations have been observed in NSC (Shen et al. 2006), with a recent study demonstrating hypermethylation of HPC after many generations and inherited after differentiation to astrocytes (Meissner et al. 2008). Hypermethylation of bivalent domain genes in stem cells is particularly important for tumorigenesis as tumour suppressor genes have bivalent promoters in ESC and ASC (Barrero et al. 2010) and hypermethylation of tumour suppressor genes is a hallmark of cancer (Jones and Baylin 2007). As bivalent genes are developmental genes and transcription factors that regulate stem cell fate, it seems apparent how their epigenetic silencing in ASC could generate stem cells locked in a self-renewal state with impaired or limited differentiation potential (Fig. 24.1). Several studies have demonstrated that PcG target genes are much more likely to become hypermethylated in cancer (Widschwendter et al. 2007; Ohm et al. 2007; Schlesinger et al. 2007) and a mechanism by which the PcG-H3K27me3 mark could direct DNA methylation has been proposed (Keshet et al. 2006; Vire et al. 2006). In addition, overexpression of PcG proteins BMI-1 and EZH2 are also often found in cancer and they both play a fundamental role in regulating stem cell function (Bracken and Helin 2009). DNA methylation alterations at other genomic regions can also participate in the development of CSC. Hypomethylation of the genome can induce chromosome instability together with aberrant activation of proto-oncogenes associated with stem cell self-renewal and proliferation (Sharma et al. 2010). Chromosome translocations producing MLL fusion proteins are involved in AML, with more than

50 different fusion partners being identified. Importantly, MLL-ENF fusion is able to transform HSC and committed progenitors, thus creating CSC with acquired self-renewal and de-differentiated phenotype (Milne et al. 2005).

Loss of imprinting (LOI) via DNA demethylation can also be associated with growth advantage in stem cells and biallelic expression of IGF2 accounts for half of Wilms tumours and predisposition to colon cancer. Other LOI involved in cancer include PEG1/MEST involved in lung cancer, CDK1C in pancreatic cancer, TP73 in gastric cancer and DIRAS3 in breast cancer (Feinberg et al. 2006). Finally, DNA methylation at promoters of miRNA genes involved in stem cell differentiation can lead to CSC. For instance, silencing of the miRNA *let-7* contributes to breast, colon and lung cancer (Zimmerman and Wu 2011) and silencing of the mir-200 gene family induces EMT and CSC phenotype in breast, lung and ovarian cancer (Brabletz and Brabletz 2010). As epigenetic alterations can result in CSC and plasticity is a fundamental property of stem cells, we should not be surprised that CSC share this characteristic. What becomes apparent is that the effect of the environment is of primary importance for cancer initiation and progression while the behaviour of stem cells is completely dependent on physiological and pathological signals. As controlling environmental conditions is not an easy endeavour, we need to develop treatments that are able to completely eradicate CSC as their plasticity is a likely prospect of tumour recurrence.

24.5   Resetting Cancer by Epigenetic Re-programming

The developmental plasticity demonstrated by CSC and the reversible nature of epigenetic alterations has led to the development of epigenetic therapies as a new treatment option for cancer patients. Several epigenetic drugs that aim at halting tumour progression and restoring normal cell function are being tested in human clinical trials. Because epigenetic drugs can induce differentiation of ESC and ASC, their use for resumption of normal tissue differentiation is also been tested (Berdasco and Esteller 2010). The concept of differentiation therapy as an alternative or adjuvant treatment to chemotherapy has been inspired by many years of research. Landmark experiments have shown that embryonic microenvironments that program cell fate during development are able to reverse malignancy by resetting normal pathways of cell differentiation. For instance, teratocarcinoma cells can be induced to differentiate when transplanted into chimeric embryos, and malignancy can be reverted by injecting cancer cells into zebrafish, chicken and mouse embryos (Telerman and Amson 2009). Nuclear transfer experiments have shown that the epigenotype of cancer cells can be reprogrammed by oocyte molecules (Blelloch et al. 2004; Hochedlinger et al. 2004) and recent experiments with oocyte extracts have shown that this effect is mediated by chromatin remodelling and reactivation of silenced tumour suppressor genes resulting in reduction of tumour growth in mouse xenografts (Allegrucci et al. 2011). New insights have also come from studies of reprogramming to pluripotency. Yamanaka's work demonstrated that the ectopic expression

of core pluripotency factors (OCT4, SOX2, MYC, KLF4) in somatic cells can reprogram the epigenetic state of somatic cells to that of pluripotent cells giving rise to induced pluripotent stem cells (iPSC) (Takahashi and Yamanaka 2006). With a similar approach, cancer cells are reprogrammed to induced pluripotent cancer cells (iPC) with re-acquired differentiation potential (Sun and Liu 2011). Reprogramming studies suggest that even if finely regulated, epigenetic landscapes are plastic and reversible and that cellular transformation and de-differentiation share similar epigenetic mechanisms. This view is sustained by the evidence that many factors able to reprogram somatic/cancer cells to induced pluripotent cells act as oncogenes. This is the case for OCT4 and SOX2 (aberrant expression is tumorigenic in epithelial tissues), NANOG (overexpressed in germ cell tumours), KLF4 (associated with colorectal cancer), LIN28 (associated with hepatic cancer) and MYC (potent oncogene involved in many cancer types) (Daley 2008). Therefore, differentiation therapy could be the way to reset CSC to normal function and epigenetic programs that regulate stem cells are of particular interest as novel treatment targets.

24.6   Conclusions

More than 50 years have passed since Waddington proposed the idea that signals from embryonic environments can influence cell fate and behaviour according to an "epigenetic landscape" (Waddington 1940). He proposed that embryonic development can be visualised as a landscape delimited by hills and valley, where cells can take different directions depending on the signals received along the path. However, possible paths in this landscape are restricted by barriers dictated by hills and only defined valleys are available when cells are restricted to a defined initial trajectory. With a modern view, we identify these landscapes as epigenetic modifications regulating expression of developmental genes during differentiation in a coordinated fashion. Although cell fate is developmentally established, a degree of plasticity is retained for tissue turnover or acquired in pathological conditions. Waddington recognised that cancer cells escape the effect of developmental forces (Waddington 1935), introducing the concept of cancer as a defect in the mechanisms that control cell differentiation. We now know that epigenetic alterations are hallmark of cancer and they can transform normal cells to tumour cells with altered proliferation and differentiation. Our knowledge of cell plasticity has greatly increased over the last few years as stem cell technologies have developed and genome-wide mapping of epigenetic modifications of stem cells and somatic cells are being established. Defining the epigenome of cellular states in normal and cancer tissues is therefore a new challenge, but a most beneficial one as it holds the promise to eradicate or control cancer, an expected disease of an aging population.

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