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Review

Aberrant Epigenetic Landscape in Cancer: How Cellular Identity Goes Awry

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Appropriate patterns of DNA methylation and histone modifications are required to assure cell identity, and their deregulation can contribute to human diseases, such as cancer. Our aim here is to provide an overview of how epigenetic factors, including genomic DNA methylation, histone modifications, and microRNA regulation, contribute to normal development, paying special attention to their role in regulating tissue-specific genes. In addition, we summarize how these epigenetic patterns go awry during human cancer development. The possibility of "resetting" the abnormal cancer epigenome by applying pharmacological or genetic strategies is also discussed.

Introduction

Normal development appears to take place through a unidirectional process characterized by a step-wise decrease in developmental potential and an activation of specific gene programs that trigger differentiation into specialized cell types. Once established, temporal and spatial activation and silencing of specific genes in a cell-type-specific pattern must be stable over many cell generations and long after inductive developmental signals have disappeared. Equally important, a cell must silence expression of genes specific to other cell types to secure its fate. Repression must be maintained throughout the life of the individual in normal development, and epigenetic mechanisms, which are defined as heritable changes in gene function that do not alter the primary DNA sequence, are ideal for regulating such events. The best-studied epigenetic modification is DNA methylation, which consists of the addition of a methyl group to carbon 5 of the cytosine within the dinucleotide CpG. It has been estimated that 3%–6% of cytosines are methylated in normal tissues and that this DNA methylation is necessary for controlling gene expression of tissue-specific, housekeeping or imprinted genes and also for maintaining genomic stability through silencing transposable elements of the genome (Esteller, 2007).

DNA methylation does not work alone and occurs in the context of other epigenetic modifications, such as histone modifications. Histone tails may undergo many posttranslational chemical modifications, including acetylation, methylation, phosphorylation, ubiquitylation, and sumoylation. For instance, the different statuses of acetylation and methylation of specific lysine residues are considered crucial histone marks affecting chromatin structure and gene expression (Kouzarides, 2007). Additionally, recent advances in the rapidly evolving field of epigenetics have demonstrated the extensive role of noncoding RNAs, especially miRNA expression, in maintaining global expression patterns during normal development (Sharma et al., 2010). Although several small-scale studies of specific epigenetic marks have provided limited information about the regulation of genes from different pathways, there is a need for knowledge in a broader perspective. A range of matters remains to be resolved, such as the relationships between the epigenetic players (the "epigenetic code") and how the environment and/or aging modulate the epigenetic marks. Some of this could be achieved by analyzing patterns on a genome-wide scale, an approach that has at last become possible thanks to recent technological advances (Bernstein et al., 2007; Barski et al., 2007; Irizarry et al., 2009).

It is clear that a comprehensive knowledge of the human epigenome will allow a fuller understanding of normal development, aging, abnormal gene control in cancer, and other diseases, as well as the role of the environment in human health. This is the main goal of the International Human Epigenome Consortium (IHEC) and the Roadmap Epigenomics Program at NIH Fund (http://nihroadmap.nih.gov/epigenomics); however, we must bear in mind that there is no single epigenome but, rather, many different ones that are characteristic of normal and diverse pathological states. It is clear that the information extracted from the whole-genome assays will help us understand the role of epigenetic marks in normal development and in diseases such as cancer. The aim of the present review is to provide an overview of how epigenetic factors, including genomic DNA methylation, histone modifications, and microRNA regulation, contribute to normal development, paying special attention to their role in the establishment of cell identity. In the second part, we will focus on how tissue-specific epigenetic patterns go awry during human cancer development.

Epigenetic Changes during Normal Development

It is well described that DNA methylation patterns undergo genome-wide alterations that occur immediately after fertilization and during early preimplantation development (Mayer et al., 2000; Reik et al., 2001) and that enrichment of individual histone modifications (such as H3K9me, H3K4me and H3K27me3) also varies in a specific manner at different stages of development (Reik, 2007). Apart from the extensive chromatin remodeling that occurs during early differentiation, epigenetic factors must guarantee the activation and maintenance of

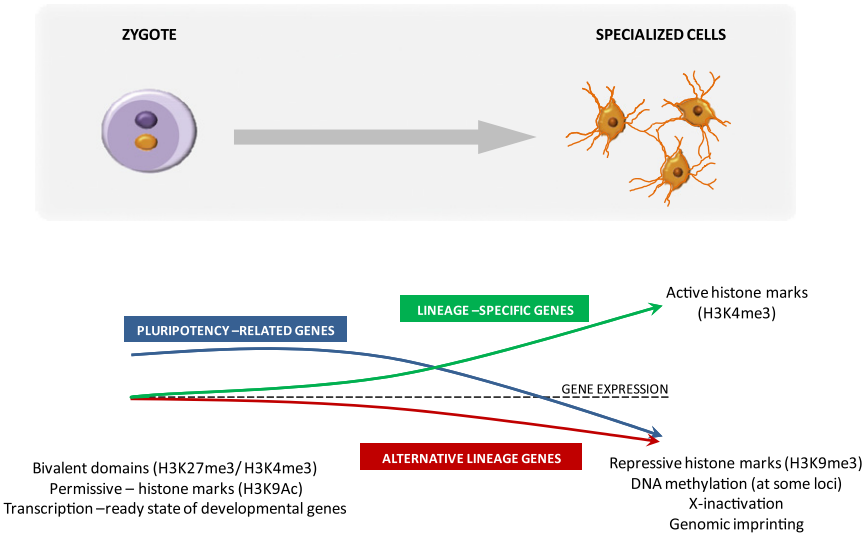

Figure 1. Gene-Specific Changes of the Epigenetic Landscape during Mammalian Development

CpG promoter hypermethylation and histone marks regulate the expression levels of genes that relate to pluripotency, that are required for tissue identity, and that occur in alternative lineages in the transition from a zygote to a highly specialized adult cell. In germ cells and the zygote, key developmental and lineage genes are maintained in a transcriptionally "poised" but inactive state, e.g., by bivalent histone marks. During differentiation, lineage-specific genes must be activated by active epigenetic marks, while those genes required for alternative lineages and pluripotency genes must be permanently silenced. H3K27me3, trimethylated histone H3 at lysine 27; H3K4me3, trimethylated histone H3 at lysine 4; H3K9me3, trimethylated histone H3 at lysine 9, H3K9Ac, acetylated histone H3 at lysine 9.

specific gene programs in specialized cell types. For instance, the expression of pluripotency-related genes must be abolished, while housekeeping genes and appropriate patterns of tissue-specific genes must be guaranteed (Figure 1). In the following sections, we will discuss how DNA methylation, histone modifications, and microRNA regulation could assure cell-type specificity in normal development.

Roles for DNA Methylation in Differentiation, Genomic Stability, X-Inactivation, and Imprinting

Gene expression and DNA methylation are inversely correlated in many genes that must be developmentally controlled following cell-type specification in early development. For example, the pluripotency genes *Oct4* and *Nanog* are expressed in preimplantation embryos and in the germline, and their promoters are hypermethylated and silenced in differentiated cells (Hattori et al., 2004; Hattori et al., 2007). But not only pluripotency-related genes are hypermethylated during differentiation. A number of genes are unmethylated in germline cells but methylated in somatic cell types, such as the *MAGE* (melanoma antigen-encoding genes) family (De Smet et al., 1999). Genome-wide studies characterizing the de novo hypermethylation of promoters during differentiation in mouse models identified 5% of the CpG islands as hypermethylated and, in consequence, silenced in somatic tissues, but not in germline cells (Mohn et al., 2008; Song et al., 2005). A similar result was identified in human tissues, where various methylation studies identify testis- and sperm-specific hypomethylation with respect to several somatic tissues (Schilling and Rehli, 2007; Shen et al., 2007).

DNA hypermethylation of repetitive genomic sequences has also been proposed as a mechanism to prevent chromosomal instability, translocations and gene disruption caused by the activation of transposable DNA sequences or endoparasitic sequences (Xu et al., 1999). In contrast to developmental genes, mobile sequences of the genome need to be silenced completely and stably to prevent them from moving around the genome (Reik, 2007). According to this idea, many transposon families are both methylated and marked by repressive histone modifications, for example H3K9me3. Furthermore, some transposon families, such as the intracisternal A particles, are even resistant to the erasure of DNA methylation in the zygote, possibly resulting in epigenetic inheritance across generations (Reik, 2007).

In addition, DNA methylation is implicated in establishing patterns of monoallelic gene expression (Figure 1). For example, X chromosome inactivation (Xi) involves monoallelic repression of genes (i.e., on one of the two X chromosomes) in female cells to equalize the imbalance of the "extra" X chromosome gene expression as compared to the one X chromosome in males. Random Xi is initiated in the epiblast (Allegrucci et al., 2005), and the inner cell mass (ICM) cells reactivate Xi in the late blastocyst, while extraembryonic cells (trophoectoderm and primitive endoderm) maintain Xi (Kiefer, 2007). Many epigenetic mechanisms promote heterochromatization of Xi. First, the X-inactivated chromosome acquires both K3H9me3 and H3K27me3 but lose histone acetylation and the activating mark H3K4me3 (Plath et al., 2003; Valley et al., 2006). Second, there is a substitution of the core histone H2A with the histone variant MacroH2A (Chadwick and Willard, 2003). Third, there is a hypermethylation of CpG islands (Reik and Lewis, 2005). All of these epigenetic marks guarantee the stability of the Xi for the lifetime of the individual.

Furthermore, genomic imprinting must be included as a second example of monoallelic expression in which epigenetic chromosomal modifications drive differential gene expression according to which parent transmitted the chromosome to the progeny. Expression is exclusively due either to the allele inherited from the mother (e.g., the *H19* and *CDKN1C* genes) or to that inherited from the father (e.g., *IGF2*). This inheritance process is independent of the classical Mendelian model. DNA methylation has been widely described as the main mechanism for controlling genes subjected to imprinting (Kacem and Feil, 2009). One model of this regulation is based on the cluster organization of imprinted genes. The structure within clusters allows them to share common regulatory elements, such as noncoding RNAs and DMRs (Differentially Methylated Regions). DNA methylation of DMRs is thought to interact with histone modifications

and other chromatin proteins to regulate parental allele-specific expression of imprinted genes; for instance, H3K27me3 and H3K9me2 together with CpG promoter hypermethylation are involved in silencing imprinted genes (Henckel et al., 2009). Furthermore, the aforementioned regulatory elements usually control the imprinting of more than one gene, giving rise to imprinting control regions (ICRs). This cluster organization, observed in 80% of imprinted genes and the specific DNA methylation patterns associated with DMRs are two of the main characteristics of imprinted genes. These genes have diverse roles in growth and cellular proliferation, and specific patterns of genomic imprinting are established in somatic and germline cells (Dolinoy et al., 2007; Feil and Berger, 2007). Deletions or aberrations in DNA methylation of ICRs lead to loss of imprinting and inappropriate gene expression (Murrell et al., 2008), and they are usually associated with several syndromes and pathologies such as cancer.

Tissue-Specific DNA Methylation in Somatic Cells

It has long been speculated that cytosine methylation is involved in the establishment and maintenance of cell-type-specific expression of developmentally regulated genes; however, there are no clear examples of such genes, especially in humans. A small but significant proportion of genes have been identified as being differentially methylated between normal tissues and cell types (Imamura et al., 2001; Futscher et al., 2002; Rauch et al., 2009). Expression of the human *MASPIN* (*SERPINB5*) gene, an autosomal gene, is limited to certain types of epithelial cells, but no detectable amounts of the gene are found in skin fibroblasts, lymphocytes, bone marrow, heart, or kidney. The cytosine methylation status of *SERPINB5* shows an inverse correlation with *SERPINB5* expression in normal cells (Futscher et al., 2002). Consistent with this finding, other genes, such as *rSPHK1* and *hSLC6A8*, show promoter hypermethylation associated with gene silencing in specific tissues (Grunau et al., 2000; Imamura et al., 2001). For example, the *rSPHK1* promoter region is hypomethylated in the adult brain, where *Sphk1a* is expressed, whereas it is hypermethylated in the adult heart, where the gene is not expressed (Imamura et al., 2001). Using an array representing over 17,000 CpG islands, Illingworth et al. (2008) established that 6%–8% of CpG islands are methylated in genomic DNA from human blood, brain, muscle, and spleen. Interestingly, some of these genes represent genetic loci that are essential for development, including *HOX* and *PAX* transcription factor family members (Illingworth et al., 2008; Rauch et al., 2009).

These results support the hypothesis posited more than 25 years ago that cell-type-specific patterns of cytosine methylation mediate control of cell-type-specific gene expression and, by extension, cellular differentiation. However, profiling of CpG methylation at chromosomes 6, 20, and 22 identifies several CpG islands that are differentially methylated when eight somatic tissues were compared (Eckhardt et al., 2006). Specifically, authors found that 17% of the analyzed genes (873) were differentially methylated in their 5′ UTRs, but only in about one-third of the differentially methylated 5′ UTRs could an inverse correlation with transcription be established (Eckhardt et al., 2006). Further work is needed to test whether this absence of correlation is due to limitations of the analytical techniques or to the existence of additional methylation-independent regulatory mechanisms.

Cell Identity and Histone Modifications

The *β-globin* locus offers one of the best-studied examples of the regulatory relationship between histone modifications and developmental expression of genes, in part because vertebrates express different *globin* genes at different stages of development. Initial small-scale studies of the murine *β-globin* locus revealed acetylated histones associated with *globin* gene promoters in a tissue-specific and developmentally regulated manner (Bulger et al., 2003). Furthermore, H3K4me2 generally follows the acetylation process (Kiefer et al., 2008). We now know a considerable amount about the characterization of global levels of histone modifications in differentiated mammalian tissues, mainly thanks to the development of large-scale techniques (ChIP-Seq, ChIP on chip, among others) (Barski et al., 2007; Bernstein et al., 2007).

T cells, especially with respect to their methylation and acetylation marks, are one of the most widely employed biological systems in the study of how histone modifications influence cell fate. Differentiation of $CD4^+$ T cells into their corresponding subtypes appears to be controlled by various layers of modifications. First, the cytokine genes that define lineage identity (IFN-γ, IL-4, and IL-17 for Th1, Th2, and Th17 cells, respectively) are enriched in the activation mark H3K4me3 in the correct lineage (Schoenborn et al., 2007; Akimzhanov et al., 2007). It is important to note that the repressive mark H3K27me3 does not correlate with the genes that are not expressed in the appropriate lineage (Wei et al., 2009), indicating that some additional repressive marks could be involved or that some degree of cellular plasticity may persist. A similar scenario was found for the transcription regulators of lineage specificity, including *Foxp3*, *RORγt*, *T-bet*, and *GATA-3* (Wei et al., 2009; Cuddapah et al., 2010).

In addition, genome-wide studies have indicated that the genomes of differentiated T cells (Roh et al., 2006; Barski et al., 2007) also possess bivalent domains, marked with both H3K27me3 and H3K4me3, similar to those described for embryonic stem cells (ESCs) (Bernstein et al., 2006; Azuara et al., 2006). Similarly, the bivalent domains were also described in genome-wide approaches for the study of histone modifications during differentiation of multipotent human primary hematopoietic stem cells/progenitor cells into erythrocyte precursors (Cui et al., 2009) or in the acquisition of oligodendrocyte identity (Liu and Casaccia, 2010). As a conclusion to these reports, it seems that certain histone modifications can preserve a poised but inactive gene state in embryonic stem cells (Bernstein et al., 2006, Barski et al., 2007) in more lineage-restricted but nonetheless multipotent progenitors (Cui et al., 2009; Orford et al., 2008) and in terminally differentiated cells that can be reprogrammed in the presence of external stimuli (Roh et al., 2006).

Epigenetic Control of MicroRNAs in Normal Development

MicroRNAS (miRNAs) are small, approximately 22 nucleotide-long, non-coding RNAs that function as endogenous posttranscriptional silencers of target genes (Ambros, 2004). miRNAs are expressed in a tissue-dependent manner and are important regulators of several cellular processes, including proliferation, differentiation, apoptosis, and development (He and Hannon, 2004). The list of miRNAs identified in the human genome and their corresponding target genes is rapidly lengthening, validating their role in the maintenance of global gene expression

patterns (Zhang et al., 2007). Crosstalk between epigenetic regulation of miRNA and lineage commitment has recently been established (Nomura et al., 2008; Szulwach et al., 2010). First, the coding transcript of the brain-specific miR-184, imprinted and exclusively expressed from the paternal allele, may be induced by methyl-binding protein MeCP2 release (Nomura et al., 2008). Likewise, mir-137, an intrinsic modulator of adult neurogenesis (Silber et al., 2008), is also subjected to epigenetic control mediated by MeCP2 (Szulwach et al., 2010). *Sox2*, a gene with key functions in stem cell self-renewal (Ferri et al., 2004), acts directly in tandem with MeCP2 for proper transcriptional regulation of mir-137 in adult neurogenesis (Szulwach et al., 2010). To understand the pathways by which mir-137 modulates adult neurogenesis, the authors looked for putative gene targets of mir-137 regulation. Interestingly, they found that one of miR-137 targets is EzH2, a H3K27 methyltransferase and component of the Polycomb protein complexes. In consequence, mir-137 mediated repression of EzH2 is associated with a global decrease in H3K27me3.

Epigenetic Changes in Cancer Cells

Initially, cancer was thought to be solely a consequence of genetic changes in key tumor-suppressor genes and oncogenes that regulate cell proliferation, DNA repair, cell differentiation, and other homeostatic functions. However, the study of epigenetic mechanisms in cancer during the last decade, such as DNA methylation, histone modification, nucleosome positioning, and micro-RNA expression, has provided extensive information about the mechanisms that contribute to the neoplastic phenotype through the regulation of expression of genes critical to transformation pathways. Cancer cells have a specific epigenome. Regarding DNA methylation, the low level of CpG methylation in tumors compared with that in their normal-tissue counterparts was one of the first epigenetic alterations to be found in human cancer (Feinberg and Vogelstein, 1983; Goelz et al., 1985). From a functional point of view, hypomethylation in cancer cells is associated with a number of adverse outcomes, including chromosome instability, activation of transposable elements, and loss of genomic imprinting. Decreased methylation of repetitive sequences in the satellite DNA of the pericentric region of chromosomes is associated with increased chromosomal rearrangements, mitotic recombination, and aneuploidy (Eden et al., 2003; Karpf and Matsui, 2005). Intragenomic endoparasitic DNA, such as L1 (long interspersed nuclear elements) (Schulz, 2006) and Alu (recombinogenic sequence) repeats, are silenced in somatic cells and become reactivated in human cancer. Furthermore, the effect on the loss of imprinting must also be considered. Wilms' tumor, a nephroblastoma that typically occurs in children, is the best-characterized imprinting defect associated with increased susceptibility to cancer (Bjornsson et al., 2007). At a specific level, aberrations in DNA methylation patterns of the CpG islands in the promoter regions of tumor-suppressor genes are accepted as being a common feature of human cancer (Esteller, 2008). CpG island promoter hypermethylation affects genes from a wide range of cellular pathways, such as cell cycle, DNA repair, toxic catabolism, cell adherence, apoptosis, and angiogenesis, among others (Esteller, 2008), and may occur at various stages in the development of cancer.

Although the ultimate causes of aberrant DNA methylation remain to be determined, several studies showed that alterations in the DNA methylome could be directly affected by diet, xenobiotic chemicals, and exogenous stimuli, such as inflammation or viral/bacterial infection. Diets that are deficient in folate and methionine, which are necessary for normal biosynthesis of S-adenosylmethionine (SAM), the methyl donor for methylcytosine, lead to DNA hypomethylation and aberrant imprinting of insulin-like growth factor 2 (IGF2) (Waterland et al., 2006). Furthermore, particular genetic variants in the enzymes involved in the metabolism of folate and methionine are associated with different DNA methylation levels (Paz et al., 2002). Exposure to metals, such as arsenic (Benbrahim-Tallaa et al., 2005), cadmium (Poirier and Vlasova, 2002), lead (Silbergeld et al., 2000), nickel (Salnikow and Costa, (2000)), and chromium (Wei et al., 2004), is linked to changes in the expression of epigenetically controlled genes via interactions with DNA-methylation-associated enzymes, histone acetyltransferase, and histone deacetylase enzymes. Between exogenous stimuli, it has been described that altered patterns of DNA methylation could be associated with *Helicobacter pylori* infection of gastric epithelial cells and contribute to gastric cancer risk (Niwa et al., 2010). In addition, chronic inflammation has been proposed as possible inducer of aberrant methylation in liver cancers or ulcerative colitis-associated colon cancers (Kondo et al., 2000; Issa et al., 2001). However, a causal role of most of these inductors in DNA methylation patterns remains to be established. In this regard, studies performed with genetically identical individuals, such as mice clones (Rideout et al., 2001) and human monozygotic twins (Fraga et al., 2005a), could be an excellent method for understanding the environment-epigenome interaction.

Because of the complexity of permutations and combinations, less is known about the patterns of histone modification disruption in human tumors. Results have shown that the CpG promoter hypermethylation event in tumor-suppressor genes in cancer cells is associated with a particular combination of histone markers and the opposite of that observed in normal cells: deacetylation of histones H3 and H4, loss of H3K4 trimethylation, and gain of H3K9 methylation and H3K27 trimethylation (Jones and Baylin, 2007). The association between DNA methylation and histone modification aberrations in cancer also occurs at the global level. In human and mouse tumors, histone H4 loses monoacetylated and trimethylated lysines 16 and 20, respectively, especially in repetitive DNA sequences (Fraga et al., 2005b). Furthermore, histone acetylation and dimethylation of five residues in histones H3 and H4 also define two disease subtypes with distinct risks of tumor recurrence in patients with low-grade prostate cancer (Seligson et al., 2005).

Finally, as DNA methylation and histone modifications act as mechanisms for controlling cellular differentiation, allowing the expression only of tissue-specific and housekeeping genes in somatic differentiated cells, it is possible that the inappropriate (re)activation of tissue-specific genes also plays a role in cancer (Fan et al., 2008). The epigenetic alterations affecting genes expressed in a tissue-specific manner and their involvement in tumor development are briefly reviewed below.

Tumor-Type-Specific CpG Island Hypermethylation

Aberrations in DNA methylation patterns of the CpG islands in the promoter regions of tumor suppressor genes are accepted

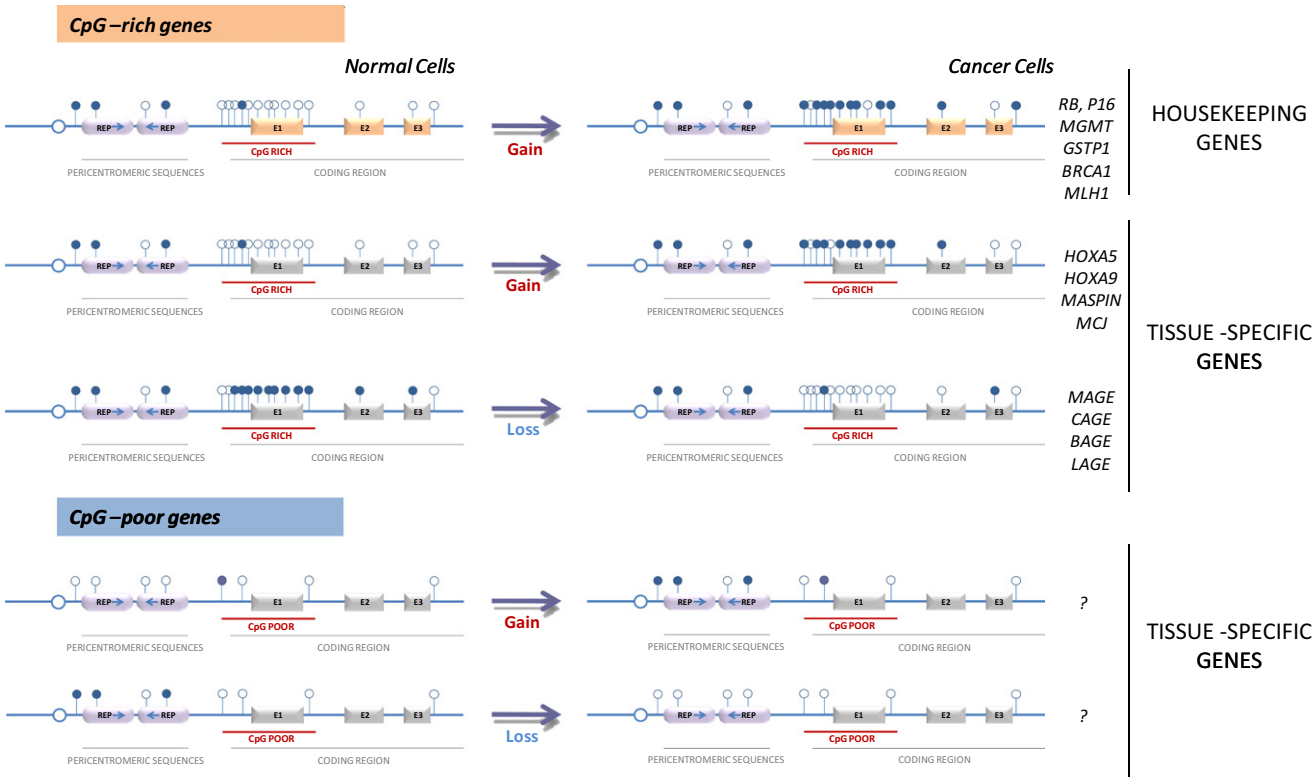

Figure 2. Distribution of CpG Islands in Promoters of Housekeeping and Tissue-Specific Genes

Promoters associated with CpG islands are found in all known housekeeping genes and half of all tissue-specific genes. Generally, housekeeping genes are unmethylated in normal cells, while tissue-specific genes may be unmethylated or methylated, depending on their requirement for lineage commitment. In a cancer cell, some of the housekeeping genes become aberrantly hypermethylated. Gains and losses of CpG hypermethylation can be observed for tissue-specific genes that are aberrantly expressed in cancer. CpG-poor regions are always found in tissue-specific genes, where expression is independent of CpG methylation status. Some examples of genes relevant to each of these circumstances are illustrated.

as being a common feature of human cancer. The initial discovery of silencing was performed in the promoter of the retinoblastoma (*Rb*) tumor suppressor gene (Greger et al., 1989; Sakai et al., 1991), but hypermethylation of genes like *VHL* (associated with von Hippel-Lindau disease), *p16INK4a*, *hMLH1* (a homolog of *Escherichia coli* MutL), and *BRCA1* (breast-cancer susceptibility gene 1) were subsequently described (Herman and Baylin, 2003). It is expected that improvements in genome-wide epigenomic studies will increase the number of hypermethylated tumor suppressor genes in a broad spectrum of tumors; by 2001, 100–400 instances of gene-specific methylation had already been noted in particular tumors (Esteller et al., 2001a).

A CpG island hypermethylation profile of human primary tumors has emerged, which shows that the CpG island hypermethylation profiles of tumor-suppressor genes are specific to the cancer type (Costello et al., 2000; Esteller et al., 2001a). Each tumor type can be assigned a specific, defining DNA "hypermethylome," rather like a physiological or cytogenetic marker. These marks of epigenetic inactivation occur not only in sporadic tumors but also in inherited cancer syndromes, in which hypermethylation may be the second lesion in Knudson's two-hit model of cancer development (Esteller et al., 2001b). Although initial results have been obtained in cancer cell lines (Paz et al., 2003; Ehrich et al., 2008), CpG hypermethylation "maps" have been identified for primary tumors, including acute myeloid leukemia (Figueroa et al., 2010), glioblastomas (Martinez et al., 2009), astrocytomas (Wu et al., 2010a), and ovarian epithelial carcinoma (Houshdaran et al., 2010), among others.

Aberrant Promoter Hypermethylation of Housekeeping Genes in Cancer

In contrast to tissue-specific genes that are expressed at a much higher level in a single tissue type than in others, some genes are constitutively expressed in all tissues. These genes have been referred to as housekeeping genes and include a wide spectrum of genes essential for cell metabolism, such as cell-cycle, ribosomal RNA, or stress defense-related genes. The regulatory mechanisms underlying the differential expression patterns of housekeeping genes compared with tissue-specific genes are also poorly characterized. For a long time, it was accepted that all known housekeeping genes and half of the tissue-specific genes have associated CpG islands (Figure 2) (Antequera, 2003). In agreement with this, a recent report systematically and quantitatively demonstrated the enrichment of CpG islands in housekeeping genes, although the pattern tends to be more variable for tissue-specific genes (with a prevailing depletion of CpG islands) (She et al., 2009). Normally, housekeeping genes have a nonmethylated CpG island tightly associated with their promoter (Caiafa and Zampieri, 2005). Because such genes tend to be expressed ubiquitously and because these autosomal CpG islands are by and large poorly methylated, housekeeping

genes were thought not to be regulated by DNA methylation. It seems that this is an oversimplification and several genes that are considered as constitutively expressed in all cells are known to be inactivated by CpG promoter hypermethylation in cancer cells, affecting a wide range of cellular pathways, such as cell cycle (Rb, $p16^{INK4a}$, $p15^{INK4b}$), DNA repair (BRCA1, MGMT, MLH1, WRN), transcription factors (BMAL1, GATA-4, GATA-5, ID4, VHL), epigenetic enzymes (NSD1, RIZ1), receptors (AR, CRBP1, ESR1, PR, TSHR), signal transduction (APC, RASSF1A, LKB1, SFRP, TLE1, WIF1), toxic catabolism and drug resistance (GSTP1), metastasis and cell invasion (CDH1, CDH13, FAT, TIMP3), apoptosis (DAPK, TMS1, CASP8), and angiogenesis (THBS1), among others (reviewed in Costello and Plass, 2001; Esteller, 2007; Jones and Baylin, 2007; Esteller, 2008). Interestingly, it has been proposed that CpG hypermethylation events in cancer were significantly more likely to occur in the promoters of those genes with enriched Polycomb occupancy and the presence of bivalent histone domains (3mK4H3 + 3mK27H3) in embryonic stem cells (Schlesinger et al., 2007; Ohm et al., 2007; Widschwendter et al., 2007).

Increased CpG Methylation in Tissue-Specific Genes during Tumorigenesis

We have described how CpG methylation also constitutes a mechanism of epigenetic control of differentiation genes, allowing the expression in a time- and tissue-dependent manner. These same genes can also be deregulated in cancer by aberrant CpG promoter hypermethylation. For example, the tissue-specific expression of *maspin*, which encodes the mammary serine protease inhibitor protein and is expressed only in cells of epithelial origin, is epigenetically regulated by DNA methylation (Futscher et al., 2002). Additionally, the aberrant CpG hypermethylation of *maspin* leads to gene silencing in cancers, such as breast, thyroid, skin, and colon (Boltze et al., 2003; Bettstetter et al., 2005; Khalkhali-Ellis, 2006; Wu et al., 2010b). A second example is that of the *MCJ* (*methylation-controlled DNAJ*) gene, which showed restricted expression associated with high levels of methylation in epithelial cells, but not in lymphoid and mesenchymal-derived cells (Strathdee et al., 2004). The methylation of the *MCJ* CpG island is associated with chemotherapy resistance in ovarian cancer (Strathdee et al., 2004), and aberrant epigenetic inactivation of *MCJ* may play a role in the development of a range of pediatric brain tumor types (Lindsey et al., 2006). The homeobox gene *HOXA5* is another example of a tissue-specific gene in which CpG methylation plays a dual role in establishing patterns of expression in normal tissues and contributing to human malignancies. Hypermethylation of *HOXA5* could be detected in adult hematopoietic cells, but not in epithelial cells and, additionally, in acute myeloid leukemia (Strathdee et al., 2007).

Loss of CpG Methylation in Tissue-Specific Genes during Tumorigenesis

DNA hypomethylation in tumors has been associated with the activation of expression of a discrete number of genes (Baylin et al., 1998). The major contribution of DNA hypomethylation to tumor development is its association with genomic instability, through the deregulation of transposable elements, pericentromeric regions, or activation of endoparasitic sequences. However, the range of affected loci also includes growth regulatory genes, imprinted genes, developmentally critical genes, and tissue-specific genes. Of the latter, the best characterized are the germ-cell-specific tumor antigen genes such as the *MAGE*, *BAGE*, *LAGE*, and *GAGE* gene families. Repression of these germline-specific genes (CG genes) in normal somatic tissues is because of DNA methylation (De Smet et al., 1999), but these CG genes are expressed in histologically distinct types of malignant human tumors and transcriptional activation is correlated with hypomethylation (Weber et al., 2007; Almeida et al., 2009). The main consequence of the activation of CG genes in cancer is the production of tumor-specific antigens leading to immune rejection. Examples of CG genes hypomethylated in cancer are widely reported in the literature, including the *MAGE* gene family in testicular and hepatocellular cancer (De Smet et al., 2004; Qiu et al., 2006) and the *BAGE* loci in breast, ovarian, and myeloma cancer (Grunau et al., 2005).

Although other genes—such as *synuclein gamma* (associated with metastasis; Gupta et al., 2003), *S100P*, and *claudin-4* (Sato et al., 2004)—are known to be hypomethylated in cancers, the correlation between promoter hypermethylation and gene repression seems less straightforward in these cases (Dokun et al., 2008).

Deregulation of miRNAs in Cancer Cells

The deregulation of miRNA expression has also been linked to human developmental defects and to tumor progression. miRNAs can function as tumor suppressors or oncogenes, depending upon their target genes. Changes in miRNA expression can be achieved through various mechanisms, including chromosomal abnormalities, transcription factor binding, and genetic alterations (Deng et al., 2008). For example, miRNA expression and activity can be influenced by impairment of miRNA processing machinery, such as the recently identified mutations of *TRBP2* (an essential functional partner of the *DICER1* complex) in sporadic and hereditary carcinomas with microsatellite instability (Melo et al., 2009).

In addition, the expression of miRNAs may be affected by epigenetic changes, such as the methylation of CpG islands and accompanying changes in histone modifications. The initial report by Saito et al. (2006) demonstrated that mir-127, which has tumor-suppressor properties, was suppressed by promoter hypermethylation in cancer cells from different tissue types. Furthermore, this epigenetic repression of mir-127 was associated with changes in histone modifications and may be reversed by adding chromatin-modifying drugs (Saito et al., 2006) in T24 bladder cancer cells. Subsequently, using DNA methyltransferase DNMT1 and 3b mutant cells, mir-124a was identified as an epigenetically regulated modulator of the expression of the CDK6 tumor-suppressor, which phosphorylates Rb. mir-124a CpG hypermethylation recruits MeCp2 and MBD2 transcriptional repressors and is associated with a reduction in active histone marks, such as acetylation of histones H3 and H4 and trimethylation of histone H3 (lysine 4) in human cancer cells. DNA demethylation drugs could revert this phenotype (Lujambio et al., 2007). Indeed, several groups have now used strategies based on the treatment of cancer cell lines with DNA-demethylating reagents and/or histone deacetylase inhibitors in vitro to study changes in miRNA expression (Lujambio et al., 2008; Grady et al., 2008; Kozaki et al., 2008; Furuta et al., 2010). These works enabled a wide range of tumor-suppressor miRNAs with aberrant hypermethylation to be identified in several types of cancer,

such as mir-9-1 (Lehmann et al., 2008), mir-193a (Kozaki et al., 2008), mir-137 (Kozaki et al., 2008), mir-342 (Grady et al., 2008), mir-203 (Bueno et al., 2008), mir-34b/c (Toyota et al., 2008), and mir-1 (Datta et al., 2008). These miRNAs are, in turn, associated with the deregulation of key genes such as *CDK6*, *TGIF2* (Lujambio et al., 2008), or central mediators of *p53* function (Bommer et al., 2007). Likewise, the *SOX4* oncogene is the target of mir-129-2 and is overexpressed in endometrial cancer when mir-129-2 is aberrantly hypermethylated (Huang et al., 2009). These findings suggest that the profile of miRNA methylation could be a useful tool in cancer diagnosis and prognosis.

Interestingly, as seen during normal development (q.v. mir-137 and EzH2 discussed above), cancers can also use miRNAs reciprocally to target the epigenetic machinery. For example, in prostate and bladder tumors (Varambally et al., 2008), EzH2 is regulated by mir-101 instead of mir-137. Prostate cancer cells (Noonan et al., 2009) control HDAC1 via mir-449a. And mir-29b directly represses expression of DNMT3A and DNMT3B, resulting in DNA hypomethylation in acute myeloid leukemia (Garzon et al., 2009). Re-expression of mir-143, which also controls DNMT3A expression, reduces tumor cell proliferation in in vitro colon cancer cells (Ng et al., 2009). All of these reports strongly suggest important mechanisms of crosstalk between miRNAs, DNA methylation, and histone modifications. Indeed, there is increasing information about the specific epigenetic control of miRNA clusters, defined as a set of miRNAs grouped by their close proximity or sequence conservation (Tsai et al., 2009). A comprehensive analysis showed that approximately 37% of all known human miRNAs appear in clusters (Altuvia et al., 2005). Expression of a large miRNA cluster located on human chromosome 19 (C19MC) was observed in normal placental tissue but was repressed in cervical cancer cell lines and gastric cancer cells. These differential patterns of expression correlate well with the hypomethylated or hypermethylated status in normal or cancer cells, respectively (Tsai et al., 2009).

Tissue-Specific Aberrations of Histone Modifications in Cancer

Although alterations in the levels of DNA methylation are commonly observed in cancer, no clear genetic lesions have been described in the DNA-methylation machinery in cancer cells. However, histone-modifier genes have tissue-type-specific patterns of expression in cancer, which could explain the differences in specific histone modifications between tumor types (Ozdağ et al., 2006). Interestingly, genetic alterations of histone modifiers enzymes are detected in a tissue-specific manner, and the landscape varies if solid tumors or hematological malignancies are compared (Table 1).

For instance, inappropriate silencing of the *Nuclear receptor SET domain protein 1* (*NSD1*) gene by CpG hypermethylation of its promoter results in decreases of H3K36me3 and H4K20me3; this phenomenon plays an important role in the Sotos overgrowth syndrome and in tumors of the nervous system (neuroblastomas and gliomas) but is conspicuously absent from leukemia, lymphoma, and colon or breast cancers (Berdasco et al., 2009). CpG island hypermethylation has been also described for the histone methyltransferase RIZ1 in several types of human cancer (Du et al., 2001). For more information on the wide variety of genetic alterations in histone modifiers that have now been observed, see Table 1.

It is intriguing to note that solid tumors are more commonly associated with point mutations, deletions, gene amplification, and alterations in expression levels; however, occasional chromosomal translocations are observed. One example is the zinc finger gene *SUZ12* (*suppressor of zeste 12 homolog*), which has been identified at the breakpoints of a recurrent chromosomal translocation reported in endometrial stromal sarcoma (Panagopoulos et al., 2008). The protein is a component of the PRC2/EED/EZH2 complex, which methylates H3K9 and H3K27 and is also a recruiting platform for DNA methyltransferases, leading to the transcriptional repression of affected target genes (such as *HOXC8*, *HOXA9*, *MYT1*, and *CDKN2A*). Recombination of SUZ12 results in fusion with the zinc finger domain protein JAZF1, affecting cell proliferation and survival through a pathway that depends on H3K27me3 levels (Li et al., 2007).

Such chromosomal translocations are much more common in hematological malignancies than in solid tumors. The mixed-lineage leukemia 1 (*MLL1/ALL-1/HRX*) histone methyltransferase is commonly found fused by translocation to one of more than 50 known partners in aggressive myeloid and lymphoid leukemias (Canaani et al., 2004). MLL1 regulates transcription at least in part by means of H3K4 methylation, and it controls the expression of *HOX* genes (Mishra et al., 2009) and NF-κB pathway genes (Robert et al., 2009). Other histone methyltransferases are also affected by chromosomal translocations, such as NSD1 (Cerveira et al., 2003) or DOT1L (Okada et al., 2005). The human DOT1L interacts with AF10, an MLL fusion partner, causing leukemic transformation in an hDOT1L methyltransferase activity-dependent manner (Okada et al., 2005), resulting in the upregulation of several leukemia-relevant genes, such as *HoxA9* (Okada et al., 2005). Interestingly, DOT1L is not recruited to the promoters that are DNA promoter demethylated after 5-aza-deoxycytidine treatment or genetic deletion of DNA methyltransferases (Jacinto et al., 2009), suggesting that DNA demethylating interventions alone are not able to restore complete euchromatic status and full transcriptional reactivation of the epigenetically silenced tumor-suppressor genes. Regarding aberrant lysine acetylation, the genes encoding p300, CBP (CREB binding protein), MOZ, and MORF histone acetyltransferases are frequently rearranged in recurrent leukemia-associated chromosomal abnormalities (Yang, 2004). Mistargeting of such translocations contributes to global alterations in histone acetylation patterns. In addition, at a specific level, this leukemia-associated MOZ-CBP fusion protein inhibits p53-mediated transcription (Rokudai et al., 2009). So far, no roles for MLL1 and MOZ disruptions have been proposed in solid tumors. A more detailed list of chromosomal translocations affecting histone modifiers could be found in Table 1.

Induced-Cell Differentiation Mediated by Epigenetic Therapy

Unlike genetic alterations, epigenetic changes are potentially reversible. Reactivation of epigenetically silenced genes has been possible for years by treatment with DNA demethylation drugs, such as zebularine and 5-aza-2′- deoxycytidine (5-ADC), or with histone deacetylase (HDACs) inhibitors, including SAHA (suberoylanilide hydroxamic acid), valproic acid (VPA), and trichostatin A (TSA). Indeed, some of these drugs have shown significant antitumor activity, and the US Food and Drug

Table 1. A Representative List of Histone Modifiers Disrupted in Cancers

Gene Name	Substrate Specificity	Genetic Defect	Gain or Loss of Function	Tumor Type	References	PubMed ID Numbers
Histone acetyltransferases (HATs)						
*CBP (KAT3A)	H2AK5, H2BK12, H2BK15, H3K14, H3K18, H4K5, H4K8	deletion	loss	ALL; lung	Shigeno et al. (2004); Kishimoto et al. (2005)	15312679; 15701835
CBP (KAT3A)	H2AK5, H2BK12, H2BK15, H3KI14, H3K18, H4K5, H4K8	mutation	loss	lung; MSI+	Kishimoto et al. (2005); Ionov et al. (2004)	15701835; 14732695
*CBP (KAT3A)	H2AK5, H2BK12, H2BK15, H3KI14, H3K18, H4K5, H4K8	translocation	loss	AML	Panagopoulos et al. (2001, 2003)	11157802; 12461753
*p300 (KAT3B)	H2AK5, H2BK12, H2BK15	deletion	loss	cervix; ALL	Ohshima et al. (2001); Shigeno et al. (2004)	11181085; 15312679
p300 (KAT3B)	H2AK5, H2BK12, H2BK15	mutation	loss	breast; CRC	Gayther et al. (2000)	10700188
*p300 (KAT3B)	H2AK5, H2BK12, H2BK15	translocation	loss	AML	Ida et al. (1997); Chaffanet et al. (2000)	9389684; 10824998
pCAF (KAT2B)	H3K9, H3K14, H3K18; H2B	mutation	loss	epithelial cancer	Ozdağ et al. (2002); Zhu et al. (2009)	12402157; 19525977
*MORF (KAT6B)	H3K14; H4K16	translocation	loss	AML	Panagopoulos et al. (2001)	11157802
*MOZ (KAT6A)	H3K14; H4K16	translocation	loss	AML	Chaffanet et al. (2000); Panagopoulos et al. (2003)	10824998; 12461753
Histone Methyltransferases (HMTs)						
*DOT1L (KMT4)	H3K79	translocation	loss	AML	Okada et al. (2005)	15851025
EZH2 (KMT6)	H3K27	amplification	gain	prostate	Bracken et al. (2003)	14532106
*EZH2 (KMT6)	H3K27	mutation	loss	lymphoma	Morin et al. (2010)	20081860
G9a (KMT1C)	H3K9	overexpression	gain	HCC	Kondo et al. (2000)	11050047
*MLL1 (KMT2A)	H3K4	translocation	loss	AML, ALL	reviewed in Miremadi et al. (2007)	17613546
*MLL3 (KMT2C)	H3K4	deletion	loss	leukemia	Tan and Chow (2001)	11718452
NSD1 (KMT3B)	H3K36, H4K20	CpG hypermethylation	loss	neuroblastoma, glioma	Berdasco et al. (2009)	20018718
*NSD1 (KMT3B)	H3K36, H4K20	translocation	loss	AML	Jaju et al. (2001)	11493482
NSD3	H3K4, H3K27	amplification	gain	breast	Angrand et al. (2001)	11374904
RIZ1 (KMT8)	H3K9	CpG hypermethylation	loss	breast, liver	Du et al. (2001)	11719434
SMYD2 (KMT3C)	H3K36	amplification	gain	ESCS	Komatsu et al. (2009)	19423649
SUZ12 (HMT complex)	H3K9, H3K27	translocation	loss	ESS	Li et al. (2007); Panagopoulos et al. (2008)	18077430, 18722875
Histone deacetylases (HDACs)						
HDAC2	Many acetyl residues (except H4K16)	mutation	loss	MSI+	Ropero et al. (2006); Hanigan et al. (2008)	16642021; 18834886
Histone demethylase (HDMTs)						
GASC1 (KDM4C)	H3K9, H3K36	amplification	gain	ESCS; lung; breast	Cloos et al. (2006); Italiano et al. (2006); Liu et al. (2009)	16732293; 16737911; 19784073
LSD1 ((KDM1)	H3K4, H3K9	amplification	gain	prostate; bladder; lung; CRC	Kahl et al. (2006); Hayami et al. (2010)	17145880; 20333681
UTX (KDM6A)	H3K27	mutation	loss	multiple types	van Haaften et al. (2009)	19330029

Enzymes are grouped according to their catalytic activity, including histone acetyltransferases (HATs), histone methyltransferases (HMTs), histone deacetylases (HDACs) and histone demethylases (HDMTs). Hematological malignancies commonly exhibit chromosomal translocations; while solid tumors are more often affected by different genetic and epigenetic alterations, such as CpG promoter hypermethylation, deletions, point mutations or gene amplification. ALL, acute lymphoblastic leukemia; AML, acute myeloid leukemia; CRC, colorectal carcinoma; ESCS, esophageal squamous cell carcinoma; ESS, endometrial stromal sarcomas; HCC, hepatocellular carcinoma; MSI+, colorectal carcinoma with microsatellite instability. Genes affected in leukemia are marked with an asterisk (*).

Administration (FDA) has approved the use of some of them to treat patients (Esteller, 2005; Kaminskas et al., 2005; Fiskus et al., 2008; Scuto et al., 2008). However, as we have discussed above, removing DNA methylation is often insufficient to reactivate gene expression; further study of the interplay with other chromatin modifications is, therefore, essential. Although this process is far from trivial, the investment in high-throughput genetic and biochemical analyses to identify the enzymatic pathways involved in the epigenetic machinery is further warranted by evidence that epigenetic therapy may be applicable to other disorders as well. As an example, the binding of histone acetyltransferase CBP (CREB-binding protein) promotes the activation of neuronal, astrocytic, and oligodendroglial differentiation genes (Wang et al., 2010). A recent study demonstrated that CBP-knockdown mice exhibit a range of cognitive defects because of impartial cortical precursor differentiation and, most important, that this phenotype could be reversed by inhibition of HDACs with TSA (Wang et al., 2010). In addition, in vitro treatment with chromatin-modifying drugs can alter the potential of pluripotent and multipotent stem cells to differentiate in vitro into several lineages. As just one example, the use of DNA demethylation treatment (5-ADC) promotes differentiation of multipotent cells into cardiac myogenic cells (Choi et al., 2004) and drives the osteogenic differentiation of mesenchymal stem cells (Zhou et al., 2009).

Bearing in mind this capacity for "resetting," there are several examples of how epigenetically deregulated tissue-specific genes in cancer could restore their differentiated phenotype. Two different strategies for correcting the aberrant patterns may be employed: first, by treatment with an epigenetic drug that directly restores the epigenetic alteration and, second, if an epigenetic modifier is altered, it is possible to restore the expression of the gene by genetic techniques. As an example of the first strategy, an effective use of zebularine in the treatment of T cell lymphoma has been proposed (Herranz et al., 2006). In this study, all the animals presented large thymic T lymphomas but only zebularine-treated mice were able to survive. Interestingly, after treatment, the animals presented a thymus structure and volume similar to those of normal (non-tumor-prone) mice (Herranz et al., 2006). As an example of the second strategy, the in vitro restoration of histone methyltransferase NSD1 activity in neuroblastoma cells that do not express the enzyme was associated with an increase of glial differentiation (Berdasco et al., 2009). These results highlight the advantages of epigenetic therapy in cancer treatment by restoring normal expression of differentiation-related genes.

Concluding Remarks

It has become increasingly clear that epigenetic mechanisms play a central role in cellular differentiation and that their deregulation can contribute to cancer development. In addition to the epigenetic inactivation of classical tumor-suppressor genes, there is new evidence that tissue-specific genes may also be targets for epigenetic deregulation in cancer. Furthermore, aberrant profiles of CpG promoter hypermethylation and histone marks in tumors are not exclusively observed in promoters of tumor-suppressor genes because promoter hypermethylation of miRNAs or even the epigenetic machinery itself (NSD1, RIZ1) could be affected in a tissue-dependent manner. These findings underline the necessity of evaluating the potential of epigenetic drugs to erase the pre-established patterns of epigenetic marks in cancer therapy. Epigenetic factors are highly dynamic, which means that they are stable, but not static, and can be modulated by the environment. How dynamic are the epigenetic profiles of adult differentiated cells? In differentiated cells, epigenetic marks with short-term flexibility (which could be removed after a few divisions) coexist with long-term and more stable marks (maintained for many divisions). According to Waddington's epigenetic landscape, it has been accepted that, during differentiation, there is an increased presence of stable repressive marks in those genes that are no longer required, such as pluripotency or nonessential tissue-specific genes. Indeed, for some multipotent progenitors, one of the hallmarks of their plasticity lies in their ability to maintain simultaneous expression of diverse competing transcription factors that would otherwise be tissue-specific and, indeed, drive divergent modes of downstream differentiation (Chang et al., 2008).

Recent investigations have revealed a new complexity to the concept of cell flexibility and differentiation in animals. Under certain experimental conditions, differentiated cells can revert to a less differentiated state in a process known as "reprogramming." A mature cell can be converted into a pluripotent state by the in vitro application of a defined set of transcription factors, creating induced pluripotent stem cells (iPSCs). Generation of iPSCs has been possible in many cell types derived from all three germ layers, implying the operation of "epigenetic reprogramming." In fact, there is evidence that HDAC inhibitors and G9a inhibitors are useful in this regard (Huangfu et al., 2008; Shi et al., 2008). These studies have suggested that the roles of such factors may be analogous in normal development and in diseases associated with loss of differentiation, such as cancer. Consequently, they provide new opportunities for therapy strategies. Further understanding of the epigenetic regulation of tissue-specific genes along with the development of more specific epigenetic drugs may hold the key to our ability to successfully reset the abnormal cancer epigenome.

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