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REVIEW

The cancer epigenome—components and functional correlates

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It is increasingly apparent that cancer development not only depends on genetic alterations but on an abnormal cellular memory, or epigenetic changes, which convey heritable gene expression patterns critical for neoplastic initiation and progression. These aberrant epigenetic mechanisms are manifest in both global changes in chromatin packaging and in localized gene promoter changes that influence the transcription of genes important to the cancer process. An exciting emerging theme is that an understanding of stem cell chromatin control of gene expression, including relationships between histone modifications and DNA methylation, may hold a key to understanding the origins of cancer epigenetic changes. This possibility, coupled with the reversible nature of epigenetics, has enormous significance for the prevention and control of cancer.

The fact that virtually all human cancer types have epigenetic abnormalities that collaborate with genetic changes to drive progressive stages of tumor evolution has been the subject of multiple recent reviews (Jones and Baylin 2002; Herman and Baylin 2003; Feinberg and Tycko 2004; Lund and van Lohuizen 2004b; Baylin and Ohm 2006; Feinberg et al. 2006). This recognition intersects with the explosion of knowledge about the role of chromatin assembly and modification in the control of gene expression patterns (Strahl and Allis 2000; Jenuwein and Allis 2001; Bannister et al. 2002; Briggs et al. 2002; Lachner and Jenuwein 2002) to present a rich opportunity for understanding how tumor-related epigenetic changes are initiated and maintained. In mammalian cells, for proper packaging of DNA to ensure the balance between transcriptional activity and repression, there is a dynamic regulation of DNA cytosine methylation at CpG sites, nucleosome remodeling, and a series of deacetylation, methylation, and other modifications at key histone amino acid residues (Bestor 1998; Bird and Wolffe 1999; Strahl and Allis 2000; Jenuwein and Allis 2001; Bannister et al. 2002; Bird 2002; Briggs et al. 2002; Lachner and Jenuwein 2002). Transcriptional repression characterizes the bulk of the heavily DNA methylated mammalian genome and may safeguard against unwanted transcription of normally repressed DNA sequences (Bestor 1998). This DNA compaction also has an important role for the structural maintenance of proper chromosome replication (Okano et al. 1999; Xu et al. 1999; Tuck-Muller et al. 2000; Robertson 2005). In contrast, individual gene promoters or clusters of coordinately regulated genes are maintained in more open transcriptional configurations, which are dependent on states of chromatin balance involving differing ratios of active and repressive histone modifications (Bird and Wolffe 1999; Bernstein et al. 2002, 2005, 2006; Bird 2002; Boyer et al. 2006; Lee et al. 2006). Exciting information, on a genome-wide scale, has recently emerged to describe chromatin marks that accompany and/or allow cell lineage commitment steps (Bernstein et al. 2002, 2005, 2006; Boyer et al. 2006; Lee et al. 2006). This review explores how this information may be critical for understanding the epigenetic abnormalities in cancer and their role in the biology of tumor evolution.

One key component of the cancer epigenome is an altered DNA methylation pattern composed of global demethylation and promoter localized hypermethylation (Fig. 1). These changes fundamentally participate in an altered structure and function of DNA, potentially involving unwanted transcription of repeat elements, abnormal activation of individual genes (Bestor 1998; Bird 2002), predisposition to genomic instability through disruption of chromosome replication control (Narayan et al. 1998; Okano et al. 1999; Xu et al. 1999; Tuck-Muller et al. 2000), and finally, aberrant silencing of genes important to the initiation and progression of tumors (Jones and Laird 1999; Jones and Baylin 2002; Herman and Baylin 2003). The last abnormality is especially being recognized and involves many classic tumor suppressor genes, developmental transcription factors, tissue remodeling genes, DNA repair genes, cell cycle control genes, anti-apoptotic genes, and genes that prevent abnormal activity of developmental pathways in tumors. In fact, any single cancer may simultaneously have all such genes epigenetically silenced (Baylin and Ohm 2006), and the loss of function of genes in tumors may have far more

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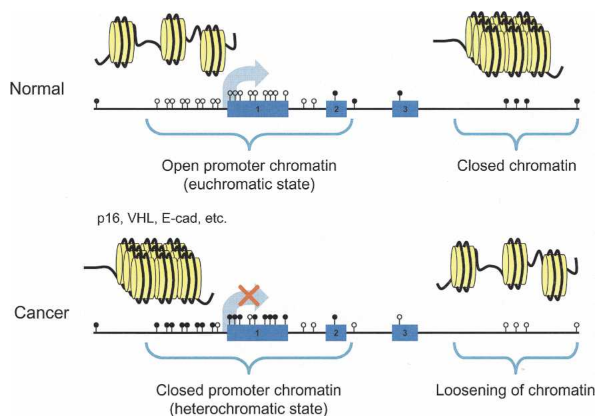

Figure 1. The normal versus cancer epigenome. (*Top*) In normal mammalian cells, CpG islands in proximal gene promoter regions (a three-exon gene is shown, with each exon marked in blue and numbered) are largely protected from DNA methylation (cytosines are shown as open lollipops) and reside in restricted regions of open chromatin (*inset*, upstream of transcription start shows three nucleosomes with wide spacing), or euchromatic states, favorable for gene transcription (large blue arrow). In contrast, for most regions of the genome, such as in the bodies of many genes and areas outside genes, particularly including repeat elements and pericentromeric regions, the cytosines in CpG dinucleotides are methylated (black lollipops). This DNA methylation is characteristic of the bulk of the human genome, which is packaged as closed chromatin (the *inset above* methylated CpGs shows multiple nucleosomes with higher-order, tight compaction) unfavorable for transcription. (*Bottom*) In cancer cells, there tends to be a reversal of this pattern. Proximal promoter CpG islands for many abnormally silenced genes (as represented by the same gene as shown in the *top* panel, and which is depicted as representing the tumor suppressor genes listed) become DNA hypermethylated and reside in a closed chromatin, or more heterochromatic-type state, which is not favorable for transcription (red X). In contrast, cytosines in CpG dinuleotides in other regions of the genome display hypomethylation and are associated with states of aberrantly loosened chromatin. The overall result is abnormal chromatin packaging with the potential for underpinning an abnormal cellular memory for gene expression and for conveying abnormal structural function for chromosomes.

epigenetic causes than genetic ones (Jones and Baylin 2002). In this review, we will particularly stress the emerging theme that epigenetic mechanisms may contribute to the earliest phases of tumor development and that links may exist between stem cell chromatin control and the vulnerability of genes to be epigenetically altered during tumorigenesis.

The position of epigenetic abnormalities in tumor progression

A full understanding of the impact of epigenetic changes in cancer depends on pinpointing at what stages of neoplastic evolution they occur and how they influence the biology of each progression step toward invasive disease. While epigenetic changes, like genetic alterations, may arise at any such steps, it is increasingly apparent that many chromatin-mediated abnormalities appear well before invasive cancer (Baylin and Ohm 2006; Feinberg et al. 2006). This fact potentially alters the view that all tumors begin with genetic mutations and, instead, places epigenetic changes as possible seminal events for tumor initiation (Baylin and Ohm 2006; Feinberg et al. 2006). These points link epigenetic changes in cancer to events that maintain stem/precursor cell phenotypes and how these contribute to tumorigenesis. They also connect epigenetics to cancer predisposition factors, such as aging, and cell responses to stress, such as occur in chronic inflammation, in terms of channeling cells into abnormal clonal expansion.

Cancer, loss of gene imprinting, and the earliest steps in neoplasia

The possibility for epigenetic origins of neoplasia is raised by abnormalities in gene imprinting found in cancer. Normal imprinting, mediated by both DNA methylation and histone modifications, ensures paternally determined, heritable transcriptional expression of one gene allele and repression of the other (Surani 1991, 1993; Bartolomei and Tilghman 1997; Ferguson-Smith and Surani 2001). Several genes undergo loss of imprinting (LOI) in cancers, such that both alleles are expressed in the tumor (Feinberg and Tycko 2004; Feinberg 2005; Holm et al. 2005; Feinberg et al. 2006). The potential for tumorigenesis to ensue in such a setting is evident from development wherein embryos derived strictly from maternal source form ovarian teratomas and those strictly from paternal source form hydatidiform moles/choriocarcinomas. Perhaps the most well studied example in adult cancers is the *IGF2* gene, whose biallelic expression would result in overproduction of a potent growth factor (Feinberg and Tycko 2004; Feinberg 2005; Holm et al. 2005; Feinberg et al. 2006). LOI for *IGF2* is found in normal-appearing colonic epithelium of patients with colorectal cancer and may be associated with increased risk of colon cancer even when found in circulating white cells (Cui et al. 2003; Kaneda and Feinberg 2005; Sakatani et al. 2005). The LOI mechanism is a complicated one. Abnormal increase of promoter DNA methylation in the H19 gene accompanies its transcriptional silencing and transfer of enhancer control for this gene more distally on the same chromosome, resulting in biallelic *IGF2* expression (Bartolomei 2003; Kato and Sasaki 2005). Recent work in an animal model solidifies that such LOI might initiate tumorigenesis. The intestinal epithelium of mice engineered for *Igf2* biallelic expression has a higher proportion of progenitor to differentiated cells as may also occur in humans with constitutive biallelic expression of *IGF2* (Sakatani et al. 2005). Another example wherein LOI of *IGF2* appears to produce abnormal progenitor cell expansion without an associated genetic alteration is in the formation of a subset of Wilm's tumors, a childhood renal cancer. In this setting, the biallelic expression appears to foster abnormal expansion of a renal progenitor cell pool yielding the

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substrate for later cancer progression events (Feinberg et al. 2006).

Finally, a recent elegant mouse model further ups the ante for abnormal imprinting as an initiation event for cancer. The authors developed a mouse model with substantial loss of gene imprinting through transient germline DNA demethylation produced via regulated disruption of the maintenance DNA methyltransferase, Dnmt1 (Holm et al. 2005). Embryonic fibroblasts from these mice formed tumors in immunocompromised mice and have properties of spontaneously immortalized cells in vitro. Furthermore, these cells can be fully transformed in one step with the introduction of the *H-Ras* oncogene. Finally, chimeric animals derived from embryonic stem (ES) cells from the engineered mice developed multiple tumors. The authors concluded from the findings that "LOI alone can predispose cells to tumorgenesis and identify a pathway through which immortality conferred by LOI lowers the threshold for transformation" (Holm et al. 2005). Clearly, this study and all the work discussed above for LOI vividly illustrate the concept that a switch in heritable gene expression patterns, in the absence of mutations, may lead to abnormal expansion of stem/progenitor cells and, thus establish a risk that subsequent events will promote full transformation and evolution of cancer.

Cancer, aberrant transcriptional repression, and the earliest steps in neoplasia

As mentioned earlier, perhaps the most intensely studied epigenetic abnormality in cancer is the aberrant transcriptional silencing of genes associated with DNA hypermethylation of promoter region CpG islands. There are many hints that this epigenetic abnormality, like LOI, could be seminal in neoplastic evolution. For example, one of the most common tumor suppressor genes affected in many tumor types by this loss of function event is $p16^{ink4A}$. The DNA hypermethylation of this gene is observed during progression of tumors such as lung cancer as early as preneoplastic lesions (Belinsky et al. 1998; Nuovo et al. 1999; Belinsky 2004) and recent data in knockout mice indicate that germline loss of this gene can increase stem cell life span (Janzen et al. 2006; Krishnamurthy et al. 2006; Molofsky et al. 2006). Experimentally, loss of $p16^{ink4A}$ appears to facilitate early tumorigenesis by being permissive for subsequent emergence of genomic instability (Foster et al. 1998; Kiyono et al. 1998) and may directly allow for additional epigenetic silencing of other genes (Reynolds et al. 2006). Other evidence for the early tumorigenic role of epigenetic gene silencing comes from additional studies of classic tumor suppressor genes. Germline mutations of these genes cause familial forms of cancer through mechanisms that obviously result in early expansion abnormalities (Hanahan and Weinberg 2000). To a variable extent, these same genes are found to be DNA hypermethylated in subsets of nonfamilial cancers such as *VHL* in renal, *APC* in colon, and *BRCA1* in breast cancers (Herman et al. 1994; Esteller et al. 2000a, b; Hedenfalk et al. 2001; van 't Veer et al. 2002). While it is possible that these epigenetic changes could be late events in the nonfamilial tumor setting, it is just as likely they could affect adult precursor cells in early neoplastic stages. Interestingly, for example, identical patterns of microarray gene expression occur in breast cancers from patients who have germline *BRCA1* mutations and familial breast cancers as in sporadic nonfamilial breast cancers, which harbor hypermethylated *BRCA1* genes (Hedenfalk et al. 2001; van 't Veer et al. 2002).

Most recently, studies of hypermethylated genes identified by emerging techniques to screen cancer cell genomes for epigenetic changes (Ushijima 2005) are revealing a potential network of epigenetic events that, again, stress the theme that epigenetic alterations may have pivotal involvement in abnormal clonal expansion of stem/progenitor cells and predisposition to cancer. One such situation involves the Wnt developmental pathway, which is essential for stem/progenitor cell function, expansion, and maintenance in the normal intestine and elsewhere during embryogenesis and adult cell renewal (Gregorieff and Clevers 2005; Radtke and Clevers 2005). This pathway is canonically overactive in colon cancer via mutations in downstream pathway genes such as *APC* and *β-catenin* (Kinzler and Vogelstein 1996; Morin et al. 1997; Fodde et al. 2001; Gregorieff and Clevers 2005; Radtke and Clevers 2005). A family of secreted frizzled related genes (SFRPs), which encode for proteins that antagonize Wnt activation at the cell membrane (Finch et al. 1997; Rattner et al. 1997; Lacher et al. 2003), are epigenetically silenced early during colon cancer progression and in virtually every established colon cancer (Suzuki et al. 2002, H. Suzuki et al. 2004; Akino et al. 2005). The early silencing of these genes may constitute broaching of an "epigenetic gate keeper" step prior to the downstream mutations in APC or β-catenin, or the loss of "genetic gatekeeper steps," to activate the Wnt pathway, start colon tumorigenesis, and later to collaborate with the mutations to provide for a fully activated Wnt pathway to promote tumor progression (Baylin and Ohm 2006).

Another fascinating possibility for fostering early abnormal clonal expansion involves a network of epigenetic events linking sustained increases in SIRT1 to the tumor suppressor, p53, and to generalized silencing of cancer genes. SIRT1 is a multitasking, stress-sensing protein and is a member of the class III histone deacetylases (HDACs) or sirtuins. SIRT1 deacetylase activity can post-translationally modify p53 to down-modulate its transcriptional activity (Luo et al. 2001; Vaziri et al. 2001; Langley et al. 2002). Normal tissue responses to cell stress and injury are transient and must coordinate cell renewal, tissue repair, and apoptotic responses. The latter, dependent upon cell cycle checkpoints mediated by p53 (Sharpless and DePinho 2002), prevent cell survival following cytokine exposure and increases in reactive oxygen species (ROS) that can generate DNA damage (Nelson et al. 2004; Bartsch and Nair 2005; Nagata 2005; Lu et al. 2006). These normal responses to acute inflammation and wound healing then use feedback cir-

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cuits to modulate resistance to stress such that cell repopulation occurs and then properly ceases (Coussens and Werb 2002; Balkwill and Coussens 2004; Nelson et al. 2004; Lu et al. 2006). Abnormally proplonged survival responses during chronic exposure to such stress may often be at the heart of abnormal clonal expansion, which promotes tumor risk.

The first focal point of an epigenetic circuit that links SIRT1 to such abnormal survival responses (Fig. 2) involves silencing of *HIC1*, which occurs early in progression of major tumors (Wales et al. 1995; Eguchi et al. 1997; Fujii et al. 1998; Hayashi et al. 2001). *HIC1*, a member of the zinc finger containing POZ family transcriptional repressors (Bardwell and Treisman 1994; Zollman et al. 1994), was discovered in a random search for hypermethylated genes in a frequent deletion area of chromosome region 17p13.3, and is itself a transcriptional activation target of p53 (Wales et al. 1995; Guerardel et al. 2001). HIC1 and SIRT1 form a complex that localizes to the SIRT1 promoter and suppresses its transcription (Chen et al. 2005). *Hic1* homozygous knockout mice have multiple lethal epithelial defects (Carter et al. 2000), eightfold increased levels of Sirt1 in embryonic fibroblasts (Chen et al. 2005), and the heterozygotes are tumor prone (Chen et al. 2003). When crossed with p53 heterozygotes, a new spectrum of virulent tumors is induced (Chen et al. 2004). Thus, the epigenetic loss of HIC1 function (Fig. 2) can then potentially blunt normal

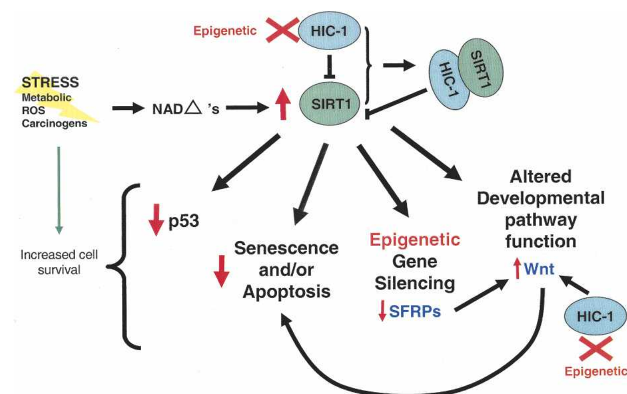

Figure 2. Epigenetic silencing of HIC1—a potential model for how a network of epigenetic abnormalities may facilitate abnormal cellular expansion. Under normal cellular conditions, cellular stress response signals to genotoxic stresses, such as a build-up of ROS, lead to changes in the balance between NAD and NADH and induction of SIRT1 expression. This response in normal cells aids cell survival to allow repair in a transient manner. This response is then suppressed through feedback steps involving HIC1–SIRT1 complex formation, transcriptional repression of *SIRT1* expression, and p53 activation of HIC1 as described in the text. However, during chronic cell renewal, epigenetic inactivation of HIC1 (red Xs), a frequent early event in tumorigenesis, can lead to abnormal cell survival and clonal expansion. This involves chronic SIRT1 increases, and participation of this protein in suppression of p53 function, epigenetic silencing of additional genes, and increases in WNT pathway activity through loss of expression of *SFRPs* and direct loss of HIC1 modulation of WNT pathway transcription.

cellular responses to p53 through SIRT1 increases and, potentially, increase cell life span through this route. Even one copy increases of the SIRT1 homolog Sir2 can prolong the life span of cells in organisms from *Caenorhabditis elegans* to mammals (Kaeberlein et al. 1999; Lin et al. 2000; Tissenbaum and Guarente 2001; Howitz et al. 2003). Hic1-deficient mouse and human cancer cells have a p53- and SIRT1-dependent reduction in apoptotic responses to DNA damage (Chen et al. 2005). Taken together (Fig. 2), early heritable loss of *HIC1* expression could trigger blunting of apoptosis and prolongation of cellular life span to facilitate early abnormal clonal expansion in settings, such as chronic inflammation, that predispose to cancer.

The next step in the potential network of SIRT1 events would suggest epigenetic events that beget epigenetic events. Among the targets for SIRT1 deacetylase activity are histone amino acids such as Lys 16 of histone H4 (H4K16) and H3K9. These modifications are key for a role of the yeast SIRT1 ortholog Sir2 in maintaining transcriptional silencing for genes in telomeric and mating loci regions (Guarente 2000; Kimura et al. 2002; Suka et al. 2002). Additionally, in *Drosophila*, Sir2 participates in long-term maintenance of gene silencing fundamental for development and stem cell function (Furuyama et al. 2004). SIRT1 appears to play a similar role in the transcriptional silencing of DNA hypermethylated cancer genes by localizing to the promoters of such genes to deacetylate H4K16 and H3K9. Induced decreases in SIRT1 activity, thus, results in re-expression of the silenced genes (Pruitt et al. 2006). For example, this experimental depletion of SIRT1 in cancer cells results in reactivation of the silenced Wnt antagonist genes, the SFRPs, and thus reverses the overactivity of Wnt pathway in breast and colon cancer cells (Fig. 2; Pruitt et al. 2006). A final nodal point for epigenetic silencing of HIC1 involves other routes by which silencing of this gene results in overactivity of the Wnt pathway. HIC1 can associate with TCF-4 and thus recruit β-catenin to localized nuclear structures, termed HIC1 bodies (Fig. 2). This HIC1 activity then normally helps prevent formation of nuclear β-catenin–TCF complexes that would otherwise drive canonical Wnt pathway activity (Valenta et al. 2006).

In summarizing this first section, there is compelling evidence, from deregulation of gene imprinting to aberrant transcriptional silencing of genes, that epigenetic abnormalities may play an influential role in allowing abnormal clonal expansion and initiating the neoplastic cascade. How these heritable abnormalities in gene expression patterns come to exist in neoplastic cells and how the mechanisms involved relate to normal epigenetic control of gene expression are explored in the remainder of this review.

Components of control for DNA methylation and chromatin, packaging of the genome, and cancer

If epigenetic abnormalities of gene expression are especially important to stem/precursor cell contribution to

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the earliest stages of neoplasia, how do we begin to understand the molecular underpinnings for their appearance? The answers lie in dissecting the growing appreciation of how chromatin construction and DNA methylation recruitment are joined, and how these events differ between normal stem/precursor cells and neoplastic cells.

The DNA methylation machinery

As discussed, epigenetic abnormalities in cancer include both losses and gains of DNA methylation (Feinberg and Vogelstein 1983; Feinberg et al. 1988; Jones and Laird 1999; Jones and Baylin 2002; Feinberg and Tycko 2004; Baylin and Ohm 2006) and we understand more about the latter. DNA methyltransferases (DNMTs), which catalyze the covalent addition of methyl groups to cytosines in the CpG dinucleotide context, have been incriminated in the DNA methylation abnormalities in cancer. Overexpression of DNMT1 induces transformation of NIH 3T3 cells (Wu et al. 1993) and contributes to cell transformation by the *Fos* oncogene (Bakin and Curran 1999). Inhibition of DNMT activities by 5-aza-2′-deoxycytidine, a cytosine analog, delays large T antigen (TAg)-induced prostate cancer transformation in the TRAMP mouse model (Czermin et al. 2002) and lung cancer development in a rodent carcinogenesis model (Belinsky et al. 2003). Elevated levels of DNMT proteins and activities occur in various cancer types, including gastric, bladder, leukemia, colon, and lung (Issa et al. 1993; Belinsky et al. 1996; Melki et al. 1998; De Marzo et al. 1999; Robertson et al. 1999; Ramsahoye et al. 2000; Wagner et al. 2003; Etoh et al. 2004; Agoston et al. 2005).

Most of what we know about how mammalian DNA methylation patterns are established comes from studies of mouse normal development. Homozygous deletion of any of the three *Dnmt* loci encoding catalytically active Dnmts, Dnmt1, Dnmt3a, and Dnmt3b, is lethal (Li et al. 1992; Lei et al. 1996; Okano et al. 1999). In this regard, deletion of *Dnmt3a* and *Dnmt3b* abolishes de novo methylation, while *Dnmt1* deletion produces bulk DNA demethylation, reflecting a maintenance methyltransferase role for this enzyme (Okano et al. 1999) consistent with in vitro enzymatic studies (Gruenbaum et al. 1982; Pedrali-Noy and Weissbach 1986). The above paradigm for embryonic separation of maintenance versus de novo Dnmt activities has been challenged in the cancer setting. Using both genetic deletion and RNA interference (RNAi) approaches in human colon and other cancer cells (Rhee et al. 2000, 2002; Ting et al. 2004, 2006), severe depletion of DNMT1 has produced only minor decreases in overall DNA methylation, minimal loss of promoter hypermethylation, and undetectable re-expression of silenced tumor suppressor genes. Undeniably, there have been controversies regarding such lack of requirement for DNMT1 in maintaining promoter hypermethylation and gene silencing in cancer cells, and some investigators have found changes in the above parameters in cancer cells with DNMT1 depletion (Robert et al. 2003; Yan et al. 2003; M. Suzuki et al. 2004). Most recently, small amounts of a cryptic splice form of DNMT1 has been found in HCT116 DNMT1$^{-/-}$ colon cancer cells, which may be sustaining them (Egger et al. 2006). One resolution to some of the above differences may be that threshold requirements differ for DNMT1 in various cancers. In our own studies of RNAi-induced depletion of DNMT1, we identified one cell line, T47D breast cancer cells, that displayed an essential requirement for retention of substantial DNMT1 levels for maintaining DNA methylation and cell survival (Ting et al. 2006). Yan et al. (2003) also found a specific requirement for DNMT1 in MDA-MB-231 and Hs578t breast cancer cell lines for maintaining Estrogen Receptor methylation and repression.

The biology behind this differential requirement for DNMT1 may involve cooperativity between DNMTs for maintenance of DNA methylation in human cancer cells. In HCT116 colon cancer cells, knockout of *DNMT3b*, the conventional de novo methyltransferase, from *DNMT1*$^{-/-}$ HCT116 cells (double knockout or DKO cells) results in a >95% loss in genomic 5-methyl cytosines and complete promoter demethylation and re-expression of aberrantly silenced genes (Rhee et al. 2002; Akiyama et al. 2003; Paz et al. 2003; Satoh et al. 2003; H. Suzuki et al. 2004). Multiple DNMTs are being colocalized to promoters of hypermethylated genes and defined as components of transcriptional repression complexes (Di Croce et al. 2002; Kim et al. 2002; Datta et al. 2003). However, the precise interactions between these proteins require further investigation.

The above discussion relates primarily to the maintenance of DNA methylation in cancer, but what establishes the patterns to start with? For example, does DNMT1 contribute to de novo methylation and to aberrant promoter CpG island methylation and abnormal gene silencing? DNMT1 has low intrinsic de novo activity against unmethylated substrates, and there is a lack of in vivo evidence for Dnmt1 de novo methylation in the murine ES cell system (Lei et al. 1996) and in studies of its ectopic expression of DNMT1 in *Drosophila* (Lyko et al. 1999). However, overexpression of the protein results in detectable de novo methylation of CpG island sequences in human fibroblasts (Vertino et al. 1996). Unmethylated human CpG islands are robust substrates for DNMT1 de novo DNA methylation activity, and overexpression of DNMT1 in cultured *Drosophila* cells can specifically establish methylation on these substrates when they are stably incorporated into the fly genome (Jair et al. 2006). DNMT1 in HCT116 colon cancer cells may account for 50% of the de novo methyltransferase activity against these substrates in vitro (Jair et al. 2006). DNMT1 may, then, be capable of initiating aberrant CpG island hypermethylation at least in cancer cells.

Chromatin conformation, remodeling, histone modifications, and the cancer epigenome

To understand the origins of epigenetic alterations in cancer, we must tap the explosion of knowledge about molecular control for organizing and maintaining the

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chromatin structure of the normal nucleus and how histone modifications, including lysine acetylation, lysine and arginine methylation, serine and threonine phosphorylation, glutamic acid ADP-ribosylation, and lysine ubiquitination and sumoylation participate in this process. Positioning of the nucleosome with its 147 base pairs of DNA wrapped around the octamer of the core histones, H2A, H2B, H3, and H4, in conjunction with the above modifications of histones, modulates the normal epigenome in terms of maintaining gene expression patterns and normal chromosome structure and function (Jenuwein and Allis 2001). These components are delicately balanced, and small changes in a given parameter can have major consequences for cell phenotype and transcriptional patterns. In yeast, a single copy change in the histone deactylase Sir2 can completely alter zones of gene silencing with respect to telomeric versus more proximal chromosome distribution (Kimura et al. 2002; Suka et al. 2002). It is not hard to imagine, then, that in cancer cells, global and local shifts of DNA methylation and chromatin parameters would have great impact. Cancers have not only altered DNA methylation but also global changes in the levels of proteins that participate in chromatin modifications, such as polycomb complex constituents, and in histone modifications, such as acetylation and methylation of lysine residues on histones H3 and H4 (Varambally et al. 2002; Kirmizis et al. 2003; Kleer et al. 2003; Fraga et al. 2005; Seligson et al. 2005).

DNA methylation and chromatin connections

There is tight interdependence between DNA methylation and chromatin modifications for DNA packaging. Select type I and II HDACs, which mediate removal of acetyl groups from histone lysine residues, are associated both with complexes involving each of the DNMTs and with a family of methyl cytosine-binding proteins, MBDs, which interpret and mediate the transcriptional repressive activities of DNA methylation (Bird and Wolffe 1999; Robertson et al. 2000; Rountree et al. 2000; Bachman et al. 2001; Fuks et al. 2001; Bird 2002; Burgers et al. 2002). The MBDs have potential for gene promoter specificity and have been localized to DNA hypermethylated and aberrantly silenced cancer genes (Nguyen et al. 2001; Bakker et al. 2002; El-Osta et al. 2002; Koizume et al. 2002; Darwanto et al. 2003; Muller et al. 2003; Ballestar and Esteller 2005). Typically, actively transcribed genes are surrounded by acetylated histones but by deacetylated histones when aberrantly silenced in cancer in association with DNA hypermethylation (Cameron et al. 1999; Fahrner et al. 2002; Nguyen et al. 2002; Kondo et al. 2003; McGarvey et al. 2006). In this apparent collaboration between DNA methylation and lysine deacetylation, the methylation appears dominant in that it must be diminished by the demethylating agent, 5-aza-2′-deoxycytidine, before cellular inhibition of the type I and II HDACs can effectively achieve transcriptional reactivation of the genes (Cameron et al. 1999; Suzuki et al. 2002). This demethylation also causes release of the MBDs, and presumably of the HDACs, from the promoters (Nguyen et al. 2001; Bakker et al. 2002; El-Osta et al. 2002; Koizume et al. 2002; Darwanto et al. 2003; Muller et al. 2003).

Contrary to the above scenario, SIRT1, a class III HDAC, participates in the aberrant silencing of cancer genes but acts differently with respect to promoter DNA hypermethylation. For SIRT1-target genes, concomitant increases in acetylation of H4K16, and to a lesser extent H3K9, induced by inhibition of SIRT1 occur without any loss of the promoter DNA methylation and may, then, modulate the transcriptional repression downstream from DNA methylation (Pruitt et al. 2006). The exact mechanisms involved, and the holistic role of SIRT1 in both establishing and maintaining sites of DNA methylation, becomes important with the recent association of SIRT1 with protein complexes fundamental to long-term gene silencing in stem/precursor cells (Kuzmichev et al. 2005).

While acetylation of histone lysines is associated with active transcription, methylation of these residues associates with either active or repression states depending upon the modified site (Strahl and Allis 2000; Jenuwein and Allis 2001; Briggs et al. 2002). The acetylation dynamics are balanced by actions of histone acetyltransferases (HATs) and HDACs, and histone methylation was recently recognized as also being dynamically regulated by histone methyltransferases (HMTs) and histone demethylases (Shi et al. 2004, 2005; Forneris et al. 2005; Metzger et al. 2005; Fodor et al. 2006; Tsukada et al. 2006; Yamane et al. 2006). The complex nature of the "histone code" is further expanded by the presence of mono, di, and tri forms of lysine methylation, each form being catalyzed by a different HMT (Rea et al. 2000; Lachner and Jenuwein 2002; Rice et al. 2003; Lachner et al. 2004). For example, trimethylation of histone H3 Lys 9 (H3K9me3) is associated with the compaction and transcriptionally repressive characteristics of pericentromeric heterochromatin, while H3K9me2 is more associated with euchromatic gene silencing (Lachner et al. 2003; Gibbons 2005). Histone acetylation and methylation patterns are translated by effector proteins, as is DNA methylation through the MBDs. Proteins containing bromodomains, which recognize acetylated lysine residues, and chromodomains, which bind to methylated lysine residues, are targeted to the histones and cause changes in gene transcription and genome organization (Jacobs et al. 2001; Plath et al. 2003).

Cancer-associated DNA hypermethylated and silenced genes can be models to examine chromatin control of gene expression. When such genes are not DNA methylated, and basally expressed, their promoters have a virtually identical distribution of the active marks, H3K9acetyl and H3K4me (Fahrner et al. 2002; Ghoshal et al. 2002; Nguyen et al. 2002; Kondo et al. 2003; Kondo and Issa 2004; McGarvey et al. 2006). In contrast, when silenced in cancer cells and associated with DNA methylation, these active marks are severely diminished, and virtually every histone methylation mark, including mono-, di-, and trimethylation of H3K9 and H3K27, that

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has best been associated with transcriptional repression is enriched (McGarvey et al. 2006). As previously noted, H3K9me3 is associated with the tightly closed configuration of pericentromeric heterochromatin, H3K9me2 is more associated with euchromatic gene silencing, and H3K27me is associated with facultative heterochromatin, such as that for silenced genes on the inactive X-chromosome of mammalian female cells (Lachner et al. 2003). H3K27me is placed by the polycomb protein complexes (PcG) that, from *Drosophila* to man, mediate long-term gene silencing (Orlando 2003; Lund and van Lohuizen 2004a,b; Ringrose and Paro 2004; Schwartz et al. 2004; Pirrotta and Gross 2005) and, as noted below, has a potentially critical role in the origins of cancer gene silencing.

HMTs and PcG

The promoters of DNA hypermethylated cancer genes are enriched for enzymes known to catalyze the above silencing histone methylation marks including G9a and EuHMTase for H3K9me2 and EZH2 for H3K27me (McGarvey et al. 2006). The origins of the H3K9me3 mark is not known, although at pericentromeric heterochromatin, this modification is established by SuVar HMT family members (Melcher et al. 2000; Peters et al. 2001; Maison et al. 2002; Lehnertz et al. 2003; Ebert et al. 2004; Krouwels et al. 2005). H3Kme2 and H3K9me3 are recognized by chromodomain proteins, including HP1 family proteins (Aagaard et al. 1999; Melcher et al. 2000; Rea et al. 2000; Schotta et al. 2002, 2003; Rice et al. 2003; Krouwels et al. 2005; Chin et al. 2006), and HP1α localizes to the promoters of DNA hypermethylated and silenced cancer genes (McGarvey et al. 2006). Interestingly, the tumor suppressor Rb may participate in targeting H3 methylation and binding of HP1α to target genes, suggesting a role for SUV39H1 in euchromatic gene repression and heterochromatic silencing (Aagaard et al. 2000; Nielsen et al. 2001).

One approach to further understand the roles of these components and events in the maintenance, and even the origins, of aberrant gene silencing in cancer is to exploit the reversible nature of epigenetics and monitor the chromatin responses. Several groups have induced re-expression of DNA hypermethylated and silenced cancer genes through 5-aza-2′-deoxycytidine-induced DNA demethylation, or examined demethylated genes in the HCT116 DKO colon cancer and noted reappearance of the active marks H3K9acetyl and H3K4me (Fahrner et al. 2002; Ghoshal et al. 2002; Nguyen et al. 2002; Kondo et al. 2003; McGarvey et al. 2006). However, while these active marks are enriched, only one silencing mark, H3K9me2, is strikingly decreased, while each of the other repressive marks, H3K9me3, H3K27me2, and H3K27me3, are retained (McGarvey et al. 2006). This may incriminate H3K9me2 in the maintenance and/or the origins of the silencing and suggest that the polycomb complex-induced H3K27me mark may have more to do with origins of the gene silencing than with its maintenance.

Conclusive proof for the precise roles of each of the defined transcriptional repressive marks in the maintenance and the origins of aberrant gene silencing in cancer remains to be garnered. In terms of a hierarchy of events, debate continues as to whether DNA methylation initiates the silencing or is superimposed upon it. Data supporting a role for DNA methylation affecting histone methylation exist for *Arabidopsis*, wherein removal of the maintenance DNA methyltransferase results in loss of H3K9 methylation in heterochromatin (Tariq et al. 2003). Further evidence in human cells shows that loss of DNMT1 results in a decrease of H3K9me2 and H3K9me3 (Espada et al. 2004). However, it seems that evidence is building to suggest a primary role for histone modifications in starting aberrant gene silencing. First, in *Neurospora crassa*, the HMT dim-5, which catalyzes H3K9me3, is required for DNA methylation while in *Arabodopsis*, the HMT KRYPTONITE is necessary for CpNpG methylation by CHROMOMETHYLASE3 (Tamaru and Selker 2001; Jackson et al. 2002, 2004; Johnson et al. 2002; Tamaru et al. 2003). Second, mouse ES cells null for the HMT Suv39h display altered DNA methylation at pericentromeric satellite repeats while Dnmt1 single- or Dnmt3a/3b double-deficient mouse ES cells do not impair H3K9 methylation (Lehnertz et al. 2003). Also, studies on mouse ES cells lacking G9a, one of the enzymes responsible for the H3K9me2 mark, suggest a role for histone methylation in the maintenance of imprinted regulatory regions (Xin et al. 2003). In human cancer cells, histone deacetylation and H3K9 methylation precede resilencing and re-DNA-methylation of the $p16^{ink4a}$ gene, which recur after initial DNA demethylation and re-expression in the DKO cells (Bachman et al. 2003).

Finally, ever increasing evidence suggests a particularly critical role for PcG proteins in epigenetic cancer gene dysfunction (Lund and van Lohuizen 2004b; Muyrers-Chen et al. 2004; Valk-Lingbeek et al. 2004). The PcG complexes play an essential role in development through establishment of long-term gene silencing in *Drosophila* (Orlando 2003; Lund and van Lohuizen 2004a; Ringrose and Paro 2004; Schwartz et al. 2004; Pirrotta and Gross 2005). These complexes exist in at least four groups, including the maintenance complex, PRC1, consisting of RNF2, HPC, ECR, and BMI1, and different initiation complexes, PRC2 through PRC4, which contain EZH2, SUZ12, and different isoforms of EED (Orlando 2003; Kirmizis et al. 2004; Kuzmichev et al. 2004, 2005; Lund and van Lohuizen 2004a; Ringrose and Paro 2004; Schwartz et al. 2004; Pirrotta and Gross 2005). The SET domain of EZH2 is responsible for the contribution of the PRC2 complex to methylation of both H3K27 and H1K26. As noted, H3K27me is found at the promoters of all the DNA hypermethylated and silenced cancer genes examined (McGarvey et al. 2006). Interestingly, the exact composition of the complex may direct which histone residue is methylated by EZH2 (Kuzmichev et al. 2004, 2005). Increased levels of EZH2 have been implicated in several types of cancer, and the expression level correlates with prognosis in both prostate and breast can-

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cer (Varambally et al. 2002; Bracken et al. 2003; Kleer et al. 2003; Bachmann et al. 2006; Matsukawa et al. 2006). SUZ12, another key PRC2 constituent, is also up-regulated in several tumor types, including colon, breast, and liver (Bracken et al. 2003; Kirmizis et al. 2004; Reynolds et al. 2006). The PRC1 component Bmi1 has also been reported as elevated in cancer and can initiate aspects of abnormal clonal expansion fundamental to development of hematopoietic and brain tumors (Lessard et al. 1999; Leung et al. 2004; Lund and van Lohuizen 2004b; Bruggeman et al. 2005). Most recently, EZH2 has been linked with gene targeting for all three mammalian DNMTs and suggested to have roles in both the triggering of DNA methylation and its maintenance in gene silencing (Vire et al. 2006). Bmi1 is also reported to colocalize with DNMT1 (Hernandez-Munoz et al. 2005). These initial findings need to be verified and extrapolated to other normal and neoplastic DNA methylation events.

Importantly, the role of PcG complexes in gene silencing intersects with recent findings of a role for the deacetylase SIRT1 at silenced cancer gene promoters (Pruitt et al. 2006). In flies, overexpression of EZH2 promotes formation of a newly defined polycomb complex, PRC4, which appears to contain SIRT1 and a specific isoform of Eed termed Eed2 (Furuyama et al. 2004; Kuzmichev et al. 2004; Chopra and Mishra 2005). In this complex, SIRT1 deacetylates H1K26 and EZH2 preferentially methylates this residue. Interestingly, cancer cells display elevated levels of PcG proteins, including all four isoforms of EED as well as SIRT1 while these proteins are barely detectable in nontumor cells (Kuzmichev et al. 2005). A mouse model for human prostate cancer additionally demonstrated increased EZH2 and SIRT1 levels in cancer-derived tissues versus normal prostate (Kuzmichev et al. 2005). Thus, the role of SIRT1, in concert with PcG complexes, may be critical for maintenance of aberrant gene silencing in cancer, emphasizing again the remarkable potential network of cellular events outlined in Figure 2.

Stem cells, cancer stem cells, yeast models, and the cancer epigenome

How can we weave all of the data discussed in this review into a working model for understanding further the origins of the cancer epigenome and particularly the most intensely studied component, epigenetic gene silencing? Key clues may relate to the very large numbers of such silenced genes in cancer cells. Random screening of the cancer genome for aberrantly silenced genes is predicting that any given cancer may harbor hundreds of these latter genes (Suzuki et al. 2002). In most cases, the silencing appears to involve individual genes interspersed throughout a wide range of chromosome regions, but large areas of involvement for a chromosome arm have recently been reported for colon cancers (Frigola et al. 2006). In colon and other cancers, Issa and colleagues (Issa 2004) have identified what they have termed a CpG island methylator phenotype, or "CIMP." While this notion has been challenged by some (Yamashita et al. 2003), most recent data appear to validate the concept (Weisenberger et al. 2006). These large-scale gene-silencing events certainly could represent stochastic changes accruing during tumor progression. However, it seems more likely a program might exist to predispose groups of genes to the chromatin alterations associated with such a large amount of genomic transcriptional repression. We speculate that recent findings for chromatin control of gene expression for stem cell biology may be providing a major clue to how groups of cancer genes would undergo such coordinated transcriptional repression.

It is an old concept that many cancers may arise through a series of progression steps, with resultant increase in cellular heterogeneity, in a clone of abnormally expanding adult stem cells (Reya et al. 2001; Lund and van Lohuizen 2004b; Muyrers-Chen et al. 2004; Valk-Lingbeek et al. 2004; Bjerkvig et al. 2005). New views of such "cancer stem cells" emphasize they may be responsible for continued population of the cancer rather than their progeny cells. Certainly, recent experimental evidence suggests that molecular events that lock in a degree of "stemness" in neoplastic cells can drive tumor progression. Thus, in a mouse model, conditional germline overexpression of the stem cell gene *Oct4* can drive rapid tumorigenesis in epithelial cells of the intestine and other organs (Hochedlinger et al. 2005).

What could be the targets for such a stem cell-driven neoplasia in terms of the cancer epigenome? We suggest that recent observations by several groups concerning how ES and progenitor cells use chromatin organization to maintain their status may hold the answer. In both murine and human ES cells, transcription factors specifying for cell stemness, including *OCT4*, *NANOG*, and *SOX2*, are localized to promoter regions of a restricted group of some 1000 genes for each factor, and some 350 target genes for all three (Boyer et al. 2006; Lee et al. 2006). These target genes, including transcription factors, genes guiding cell proliferation control, morphogenesis regulating genes, etc., have one central theme in that all are generally related to the pluripotency of stem cells (Beach et al. 2005; Bernstein et al. 2006; Boyer et al. 2006; Lee et al. 2006). Importantly, the target genes are largely maintained at low expression states in ES cells by having a zone spanning their proximal promoter regions characterized by PcG proteins and the pivotal repressive mark, H3K27me3 (Bernstein et al. 2006; Boyer et al. 2006; Lee et al. 2006). Also, such genes usually have proximal promoter CpG islands and sequences outside gene coding regions that are conserved between mouse and man (Bernstein et al. 2006).

The PcG occupancy of the above promoters appears to function for preventing their full expression until ES and other precursor cells are signaled to undergo commitment steps toward cell lineages (Bernstein et al. 2006; Boyer et al. 2006; Lee et al. 2006). Importantly, this dictates that the maintained ES expression state must preserve gene expression plasticity such that PcG influences can be reversed or diminished when gene expression is required (Bernstein et al. 2006). In subsequent

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maturation steps, or in adult cell differentiation, the role of PcG complexes may come back into play in that their maintenance of long-term gene silencing is seen in mature stem/precursor cells as well (Orlando 2003; Lund and van Lohuizen 2004a; Ringrose and Paro 2004).

Another defining chromatin feature of the above PcG target genes in ES cells has been observed that may also have a special link to aberrant silencing of genes in cancer. Surprisingly, within the broad zone of PcG localization surrounding their promoters, as marked by the distribution of H3K27me3, there is a narrower zone distinctly marked by the presence of the activating mark, H3K4me3. This has been termed as "bivalent chromatin," which is hypothesized as essential to maintain certain ES genes at a low expression level, poised for eventual up-regulation as needed for cell lineage commitment (Bernstein et al. 2006). Indeed, when ES cells are pushed toward neural differentiation in vitro, the expression of several examined genes is increased, the PcG mark is notably reduced, and the active H3K4me3 mark is maintained and/or increased (Bernstein et al. 2006). Such a change is also observed for genes compared between the ES cells and naturally committed cell states (Bernstein et al. 2006). Therefore, a carefully orchestrated, plastic state of gene expression is maintained by PcG proteins and their induced chromatin mark to allow balance between maintenance of stem cell phenotype and cell differentiation during embryonic development.

The chromatin of DNA hypermethylated and silenced cancer may be remarkably similar to the above bivalent chromatin. These genes start with a heritable, silenced state associated with chromatin consisting of highly repressive marks, including mono-, di-, and tri-H3K9me and H3K27me and the absence of H3K14 acetylation (McGarvey et al. 2006). As previously discussed, after the induction of DNA demethylation, the chromatin of the re-expressed genes does not fully return to the fully activated state but, rather, active marks are indeed restored while repressive marks, including the PcG related mark, H3K27me, are highly retained (McGarvey et al. 2006).

All of the above perhaps suggest a working model (Fig. 3), wherein during chronic hyperproliferative states that predispose to cancer, such as prolonged inflammation, stem/precursor cells may normally harbor genes marked

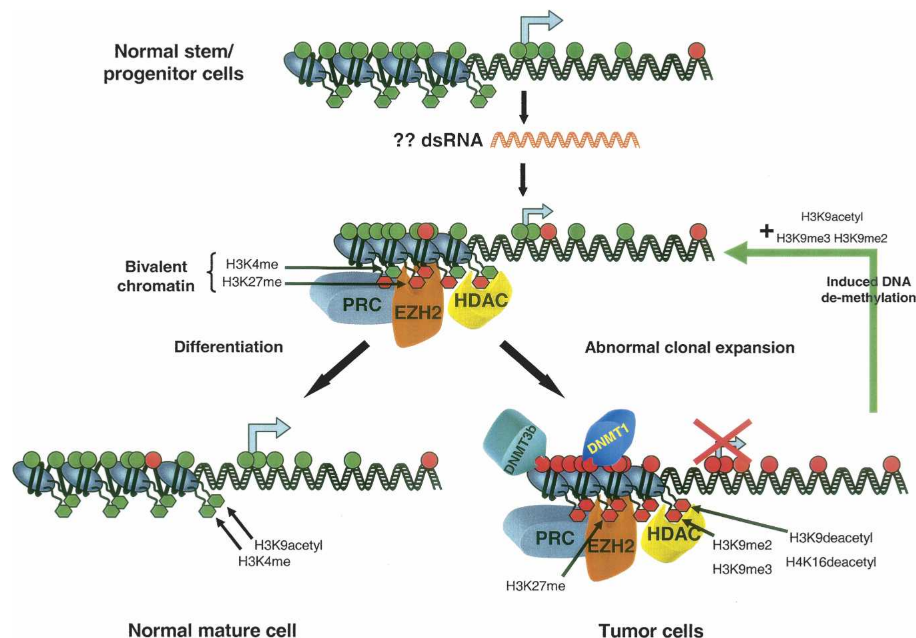

Figure 3. A model for the potential contribution of stem cell chromatin to the initiation and maintenance of aberrant epigenetic gene silencing in cancers. During normal ES cell formation, a bivalent chromatin is recruited to the promoters of a subset of genes that need to be held in a low expression state to prevent lineage commitment. The involvement of small interfering RNA (siRNA) species could be a trigger to this process, and the chromatin is comprised of histone modifications associated with active transcription (H3K4me) and inactive transcription (H3K27me). The PRC are responsible for the H3K27me3 mark through the HMT, EZH2, and deacetylation of key histone lysine residues is catalyzed by HDACs that are recruited by multiple transcriptional repressive complexes. At such genes, DNA is largely unmethylated (green circles), and histones may be maintained in a mixture of acetylated (green hexagons) and deacetylated (red hexagons) states. (*Bottom left*) With normal cell differentiation and lineage commitment, the genes become transcriptionally active, and the silencing marks are reduced while active histone marks are retained. DNA remains unmethylated. However, as shown in the *bottom right*, during cancer-predisposing events, abnormal pressure for stem/progenitor cell proliferation with retained bivalent chromatin may allow polycomb proteins and/or marks to recruit other silencing marks such as H3K9me2 and H3K9me3 and DNMTs. The promoter evolves abnormal DNA methylation (red circles) and a tight heritable gene silencing (large red X), which results in loss of function for genes. Tumors may arise in such clones with subsequent progression steps. Experimentally, the potential underlying bivalent chromatin for such tumor genes, plus retained H3K9me3, can be revealed by induced DNA demethylation (large green arrow) and resultant gene re-expression.

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by the above bivalent chromatin state. Indeed, many genes on the list of PcG targets in ES cells, such as *GATA-4*, *TIMP 3*, and others (Lee et al. 2006), are frequently DNA hypermethylated and silenced in human adult cancers. The repressive marks, particularly H3K27me3 and H3K9me2 and/or H3K9me3, and residence of the PcG proteins may make these promoter regions vulnerable to recruitment of the DNA methylation machinery through mechanisms discussed during this review. Imposition of the DNA methylation on these gene promoters would then convert a low expression state, with a plasticity that would normally allow transcriptional activation during cell maturation, to an epigenetically heritable repressive state. This loss of expression for many developmental and cell maturation genes, including some that would otherwise prevent cell proliferation and would trigger cell senescence or apoptosis, gives the early progenitor cells selective advantage and they would comprise, in essence, tumor stem cells that may participate in neoplastic evolution if the initial clones undergo subsequent tumor progression events. Some of these latter events will clearly be genetic while others may arise through continued evolution of epigenetic abnormalities.

There are certainly more questions inherent in this preliminary model than answers. Nevertheless, the model may help to map out the areas in which profound clues are emerging and help guide where research may be directed. One question is: What initiates the above PcG marking in normal stem/precursor states? Although this will have to be identified, one exciting possibility might be a potential participation by noncoding RNA species (Fig. 3). Participation of such species in triggering transcriptional repression is being incrementally defined especially, from work in plants and yeast (Wassenegger and Pelissier 1998; Pelissier and Wassenegger 2000; Jones et al. 2001; Volpe et al. 2002; Cam and Grewal 2004). Such species, in yeast, can target histone-modifying enzymes, especially those for catalysis of H3K9 and H3K27, to centrimeric repeat regions (Volpe et al. 2002, 2003; Cam and Grewal 2004). The type I–III HDACs we have discussed could be targeted to remove acetyl groups from histone tails, allowing the HMTs to catalyze the mono-, di-, and trimethyl additions to select lysine residues. HDACs have recently been implicated as critical players in yeast for initial establishment of transcriptional silenced regions (Yamada et al. 2005). Recently, several groups have discovered that small double-stranded RNA (dsRNA) species targeted to gene promoter regions can reproducibly induce transcriptional gene silencing in mammalian cells (Kawasaki and Taira 2004; Morris et al. 2004; Castanotto et al. 2005; Janowski et al. 2005; Ting et al. 2005). This type of silencing is observed with a distinctive increase in histone repressive marks at H3K9 and can be alleviated with a coadministration of TSA and 5-aza-2′-deoxycytidine (Morris et al. 2004). Whether this dsRNA-dependent transcriptional silencing (RdTS) is directly connected to DNA methylation remains to be verified. However, the strong link between RdTS and histone modifications raises the interesting prospect that RdTS may be an endogenous mechanism by which cells establish epigenetic gene regulation. Recently, a report in *Drosophila* implicates dcr-2, aub, and piwi to be involved in PcG-mediated gene silencing (Grimaud et al. 2006). This point, taken together with the data discussed above, further hints at a possibility that noncoding RNA species and/or RNAi pathways could be important in the process of chromatin-based gene silencing in cancer.

Summary

While our knowledge of the cancer epigenome is advancing rapidly, much remains to be discovered, and many surprises will surely emerge to enrich our knowledge of basic chromatin function, relationships of adult stem/precursor cells to normal cell renewal, and of the origins and progression of human cancer. Studies of the cancer epigenome are already beginning to diversify our approaches to cancer control and care and are providing potential molecular marker strategies to assess cancer risk, provide early cancer detection, improve monitoring of cancer prognosis, and predict therapy responses (Herman and Baylin 2003; Laird 2003; Belinsky 2004). Reversal of tumor-associated silencing of tumor suppressor genes is increasingly being targeted for cancer treatment and prevention strategies (Silverman et al. 2002; Issa 2005; Gore et al. 2006). From both basic and clinical research standpoints regarding epigenetics, we have clearly entered a remarkable era of basic and translational studies linking chromatin and cancer research.

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