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Biological Perspectives

Epigenetic Dysregulation in Cancer

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One of the great paradoxes in cellular differentiation is how cells with identical DNA sequences differentiate into so many different cell types. The mechanisms underlying this process involve epigenetic regulation mediated by alterations in DNA methylation, histone posttranslational modifications, and nucleosome remodeling. It is becoming increasingly clear that disruption of the "epigenome" as a result of alterations in epigenetic regulators is a fundamental mechanism in cancer. This has major implications for the future of both molecular diagnostics as well as cancer chemotherapy. ***(Am J Pathol 2009, 175:1353–1361; DOI: 10.2353/ajpath.2009.081142)***

Epigenetics is a rapidly evolving field focused on explaining how heritable changes in gene expression occur that do not involve changes in nucleotide sequence.[1] Epigenetic regulation of transcription can be mediated through DNA methylation, histone modifications including histone acetylation, phosphorylation, methylation, ubiquitination, and proteolysis, and alterations in chromatin remodeling. Importantly, increasing evidence shows that epigenetic deregulation is a common mechanism in cancer. The role of DNA methylation in cancer has been extensively studied, and a number of excellent reviews have been published on this topic.[2–4] More recently, it has become clear that histone modifications, as well as disruption of chromatin remodeling machinery, play a fundamental role in cancer, and this is our primary focus in this review. It is important to recognize that these three types of epigenetic regulation are highly interdependent. For example, patterns of histone methylation are important for establishing patterns of DNA methylation. Chromatin remodeling, in turn, is programmed in part by changes in DNA methylation and histone modifications.[3]

DNA Methylation

CpG-rich sequences are generally rare in the mammalian genome except for in so-called CpG islands, which are associated with centromeres, microsatellite sequences, and the proximal promoter regions of approximately half of all genes. CpG-containing sequences are cytosine methylated by a family of DNA methyltransferases or DNMTs (to date, unequivocal evidence for DNA demethylases is lacking). These methyltransferases generally exempt promoter-associated CpG islands, where <20% are CpG methylated.[5]

It was recognized over 25 years ago in studies of colon cancer that patterns of DNA methylation in tumor cells differed considerably from normal cells.[6] These early studies, which detected DNA methylation using methylation-sensitive restriction enzymes and Southern blotting, revealed global hypomethylation of DNA sequences compared with normal cellular counterparts (Figure 1). Subsequently, many high-throughput techniques have been developed for CpG methylation profiling, including restriction landmark genomic screening, bisulfite sequencing, differential methylation hybridization, DNA immunoprecipitation using antibodies directed against 5-methylcytosine, and array or sequence-based detection methods, which have confirmed this finding of global hypomethylation in a variety of cancers.[7] This hypomethylation, whose mechanism remains poorly understood, may play several roles in oncogenesis, including increasing genomic instability as well as contributing to the over expression of genes, such as *MAGE*, *CAGE*, *CYCLIND2*, *S100A4*, *CD30*, as well as loss of imprinting of genes such as *IGF2*.[4] In one well-studied example, loss of imprinting of the maternally inherited *IGF2* allele has been implicated in the pathogenesis of colorectal cancer and Wilm's tumor.[8] Changes in DNA methylation have also been associated with chemotherapy resistance. For example, methylation of the *MLH1* gene is associated with increased resistance to *cis*-platinum and alkylating agents.[9]

Paradoxically, local hypermethylation of specific genes also appears to play an important role in cancer (Figure 1). The first hypermethylated gene identified was calcitonin, which is hypermethylated in a subset of small cell carcinoma cases.[10] This was followed by identifica-

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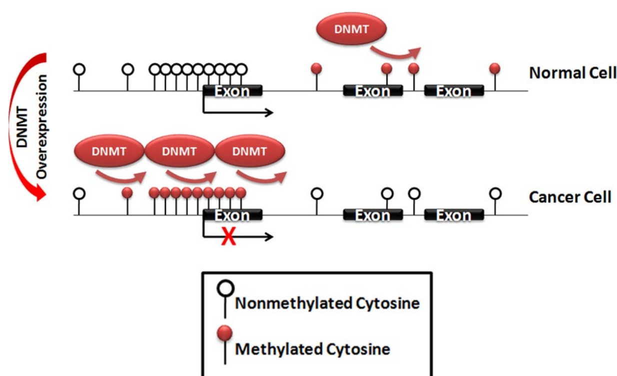

Figure 1. Aberrant DNA methylation patterns in cancer cells as a result of DNA methyltransferase overexpression. CpG dinucleotides are methylated in normal cells, whereas CpG islands, consisting of overrepresented CpG clusters near gene regulatory regions, are unmethylated. In contrast, cancer cells generally show global hypomethylation with hypermethylation at CpG islands, resulting in gene silencing at a subset of genes, including tumor suppressor genes.

tion of a number of bona fide tumor suppressors, including *RB*, *VHL*, and *BRCA1*.[4] Recently, expression of potentially important micro-RNAs has also been shown to be regulated by DNA.[11] Interestingly, many genes that are mutated in familial cancers, such as *BRCA1*, are also hypermethylated or deleted in sporadic cancer cases. In addition, hypermethylation of some genes can promote genomic instability. For example, the mismatch repair gene *MLH1* is hypermethylated in colorectal cancers associated with microsatellite instability.[12]

DNA methylation is intimately associated with histone modifications. Methyl CpG proteins such as MECP2, MBD1, and MBD2, which specifically bind to CpG methylated DNA, are associated with histone deacetylase (HDAC)-containing complexes so that they "erase" the transcription-activating histone acetyl marks. Increasing evidence indicates that patterns of repressive histone methylation, specifically histone H3 lysine 27 methylation established in stem cells, correlates with genes that are commonly hypermethylated in cancer.[13]

Histone Modifications

Histone Acetylation

Various mechanisms of histone modification may contribute to epigenetic gene regulation. Histone tail acetylation at lysine residues on histones H3 and H4 is associated with transcriptional activation. Acetylation neutralizes the negative charge of DNA and generally renders DNA more accessible to transcription factors. In addition, histone acetylation creates marks that are "read" by chromatin-associated proteins, many of which have evolutionarily conserved domains, termed bromodomains, that selectively interact with acetylated lysines. Experimentally, histone acetylation (and other modifications such as methylation) are readily detected by chromatin immunoprecipitation.

A wide variety of histone acetyltransferases (HATs) have been identified, a number of which have been implicated with aberrant transcriptional activation in cancer

Table 1. Epigenetic Regulators Altered in Cancer

Histone regulator class	Epigenetic regulator	Function	Histone modification	Associated cancer	Alteration in cancer
Writer	DNMT1	DNMT	Methyl CpG	Various types	Overexpressed
Writer	DNMT3a	DNMT	Methyl CpG	Various types	Overexpressed
Writer	DNMT3b	DNMT	Methyl CpG	Various types	Overexpressed
Writer	p300	HAT	Multiple lysines	Leukemia, myelodysplasia	Translocation/inactivating mutation
Writer	CBP	HAT	Multiple lysines	Leukemia, myelodysplasia	Translocation/inactivating mutation
Writer	MOZ	HAT	Multiple lysines	Leukemia	Translocation
Writer	MORF	HAT	Multiple lysines	Leukemia	Translocation
Writer	HDAC1-3, 6	HDAC	General	Various types	Overexpressed
Writer	RIZ1	HMT	H3K9	Various types	Down-regulation/mutation
Writer	EZH2	HMT	H3K27	Various types	Overexpressed
Writer	MLL1	HMT	H3K4	Leukemia, lymphoma	Translocation
Writer	SMYD3	HMT	H3K4	Colorectal, hepatocellular carcinoma	Overexpressed
Writer	DOT1L	HMT	H3K79	Leukemia	Deregulated recruitment
Writer	NSD1	HMT	H3K36	Leukemia, hepatocellular carcinoma	Translocation/inactivating mutations
Writer	NSD2/MMSET	HMT	H3K36	Multiple myeloma	Translocation
Writer	NSD3	HMT	H3K36	Breast cancer	Translocation/overexpressed
Eraser	JMJD2C/GASC1	Histone demethylase	H3K9	Various types	Overexpressed
Reader	HP1	Methylated histone-binding protein	H3K9	Breast cancer, melanoma	Down-regulation
Reader	ING1-5	Methylated histone-binding protein	H3K4	Various types	Down-regulation/mutation
Reader	MBD1-4	Methyl-CpG-binding protein	Methyl CpG	Various types	Overexpressed
Reader	MeCP2	Methyl-CpG-binding protein	Methyl CpG	Various types	Overexpressed
Remodeler	INI1	SWI/SNF complex		Malignant rhabdoid tumor	Inactivating mutations
Remodeler	BRM	SWI/SNF complex		Various types	Inactivating mutations
Remodeler	BRG1	SWI/SNF complex		Various types	Inactivating mutations
Remodeler	ATRX	SWI/SNF complex		Myelodysplasia	Inactivating mutations

(Table 1). The HATs are, in turn, modulated by a number of HDACs, which fall into three general classes: class I HDACs, which are homologous to yeast Rpd3, class II HDACs, which are homologous to yeast Hda1, and class III HDACs, which are distinguished by their dependence on NAD^+. HDAC inhibitors are finding increasing clinical applications. As is a general theme with histone-modifying enzymes, a number of nonhistone substrates have been identified, including proteins important for carcinogenesis, such as p53, GATA-1, and E2F1.[14–16]

Histone Methylation

Whereas histone acetylation is highly dynamic, modification of histones by mono-, di-, and trimethylation of lysine residues, both in the histone tail as well as core nucleosomes, is thought to be a more lasting modification that comprises a form of "cellular memory." Studies of *Drosophila* have identified two groups of proteins that are associated with either transcriptional maintenance or repression that are termed *Trithorax* or *Polycomb* group proteins, respectively. Several *Trithorax* proteins, most notably the mixed lineage leukemia protein MLL, are histone H3 lysine 4 methyltransferases. In contrast, *Polycomb* group proteins such as EZH2 have histone H3 lysine 27 methyltransferase activity, which plays important roles in silencing at euchromatic regions as well as in maintenance of heterochromatic regions in association with histone H3 lysine 9 methylation.

Recently, a number of "readers" of methylated histone tails have been identified. One group of these proteins contains chromodomains, which recognize histones methylated on lysine 9 or 27. One possible role for these chromodomain- containing proteins is to target Polycomb repression complexes to sites of transcriptional regulation and DNA replication. The latter has been implicated as a possible means of perpetuating the lysine 27 methyl mark during DNA replication.[17]

Although it is clear that histone methylation is involved in establishing long-term patterns of gene expression, it is increasingly apparent that this modification is also dynamic. Indeed, a variety of histone demethylases, including LSD1 and the jumonji family of proteins,[18,19] have been identified.

Other Histone Modifications

Replacement of modified histones with unmodified or variant histones is another possible mechanism of changing expression patterns.[20] Interestingly, proteolytic cleavage of histone tails has also been recognized as a mechanism for "erasing" histone modifications.[21] Like HATs, histone methyltransferases (HMTs) have activity on a variety of nonhistone substrates, such as p53, which may have important cancer implications.[22]

Further adding to the complexity of posttranslational modifications, histones undergo a variety of other modifications, including phosphorylation, sumoylation, and ubiquitination. Histone monoubiquitination (as opposed to polyubiquitination, which is associated with proteosomal degradation) occurs not on tails but on the core histones H2A at lysine 119 (mediated by RING1A) and H2B on lysine 123 (mediated by BRE1/RAD6). These modifications are required for the subsequent methylation at lysine 27 and lysines 4 and 79, respectively.[23]

Disruption of "Writers," "Readers," and "Erasers" of the Histone Code in Cancer

Ample evidence suggests that the "histone code" is deranged in cancer. For example, cancer cells commonly show loss of lysine 16 acetylation and lysine 20 methylation.[24] Furthermore, global changes in histone acetylation and methylation are seen in cancer cells when compared with normal cells, and these changes can be used to predict disease outcome in tumors such as prostate cancer.[25] For example, differentiation of embryonic stem cells is accompanied by the appearance of large regions of H3K9-dimethylated chromatin (>4 Mb) termed LOCKs. LOCKs are common in differentiated tissues such as liver and brain and have been found to be dramatically reduced in cancer cell lines.[26]

Heterochomatin Protein 1

In recent years, a number of readers, writers and erasers of the histone code have been implicated in carcinogenesis. Heterochromatin protein 1 (HP1) is a good example of a histone reader that is disrupted in cancer. The HP1 family, which is composed of HP1α (CBX5), HP1β, (CBX1) and HP1γ (CBX3), plays an integral role in maintaining silenced heterochromatin. All three family members specifically bind histone H3 that is methylated on lysine 9 (H3K9), a transcriptionally repressive modification associated with heterochromatin and silenced euchromatin.[27] HP1 is targeted to methylated H3K9 via the chromodomain.[28] This mark is deposited by several proteins, including the Suppressor of variegation, Enhancer of Zeste and Trithorax (SET) domain HMT SUV39H1.[29] HP1 dimerization results in recruitment of other H3K9 HMTs, leading to additional HP1 recruitment.[30] HP1 recruitment of Suv4-20 HMTs and the Dnmt3a/3b DNA methyltransferases establishes a complete transcriptionally repressed state.[27]

HP1γ is localized to silenced euchromatic sites, and HP1α and HP1β are localized to pericentric chromatin. Loss of HP1 function results in kinetochore defects, defective chromosome condensation and segregation, and impaired telomere function. HP1 down-regulation has been noted in metastatic breast cancer, papillary thyroid carcinoma, and medulloblastoma.[31,32] Strikingly, overexpression of HP1 in metastatic breast cancer cells decreased invasiveness, whereas knockdown of HP1 in nonmetastatic cells increased invasiveness, suggesting HP1 functions as a metastasis suppressor.[33] Frame shift, missense mutations, and epigenetic silencing also contribute to HP1 down-regulation, allowing for cancer progression.[31,32] HP1 is also recruited to the cell cycle control gene, *cyclin E*, indicating a direct link to cell proliferation following HP1 inhibition.[34] As noted above, chromosome

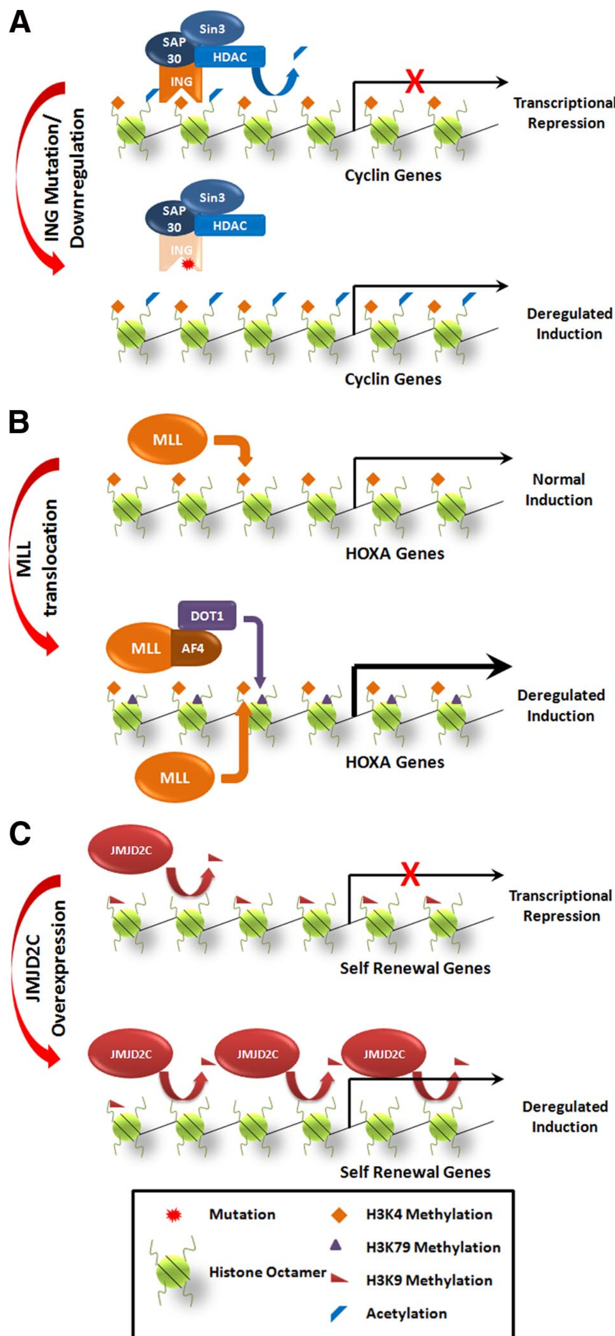

Figure 2. Disruption of histone readers, writers, and erasers in cancer. **A:** ING proteins use PHD fingers to recognize the trimethylated histone H3K4. Cell cycle arrest is mediated by the ING protein binding to proliferative genes, such as cyclins, and recruitment of HDAC complexes that deacetylate histone tails, resulting in gene silencing. Inactivating mutations or down-regulation of ING proteins results in deregulated cyclin expression and proliferation. **B:** MLL is a histone H3K4 methyltransferase that is required for maintenance of HOX gene expression. MLL translocations interact with various partners, including AF4, leading to the aberrant recruitment of the histone H3K79 methyltransferase Dot1. Dot1-mediated methylation at H3K79 results in deregulated expression of HOX genes, which are critical for transformation. Other proteins recruited by MLL fusion proteins that may also play a role in transcriptional activity are not shown. **C:** JMJD2C normally functions to demethylate H3K9, leading to transcriptional activation. In cancers that overexpress JMJD2C, a global reduction in H3K9 is observed, resulting in demethylation and increased expression of target genes such as self renewal genes, which likely contribute to tumorigenesis.

instability, including aneuploidy and telomere fusion, results from reduction or overexpression of HP1, respectively.[31] Thus, down-regulation of HP1 in cancer likely contributes to tumor progression by leading to aneuploidy of other chromosomal abnormalities.

Inhibitor of Growth 1

The inhibitor of growth (ING) protein ING1 is another reader of the histone code that was discovered through a candidate tumor suppressor screen using cDNA from normal and breast cancer cell lines (Figure 2A).[35] Subsequent phylogenetic analysis revealed four more members of the ING family, ING2–5.[36] Consistent with their role as putative tumor suppressors, ING proteins interact with p53 to induce apoptosis, cellular senescence, and growth arrest.[37] ING proteins also associate with a large chromatin-remodeling complex that includes HDAC1, Sin3a, and SAP30,[38,39] which functions as a general repressor of transcription.[40] The domain required for interaction with Sin3-HDAC is essential for ING-dependent cell cycle arrest.[38]

All ING proteins share a plant homeodomain (PHD), which preferentially binds di- and tri-methylated H3K4.[41,42] The PHD finger of ING2 also interacts with phosphatidylinositol-5-phosphate, aiding in ING2 localization to chromatin.[43] Mutations detected within the PHD finger result in premature stop codons or disrupted Zn^{2+} coordination and improper folding of the domain.[44] This likely disrupts ING binding to methylated H3K4, resulting in improper regulation of target genes such as p21 and cyclin B1, which are thought to be important for tumorigenesis in cells with ING disruption.[45,46] These mutations have been described in breast cancer, melanoma, head and neck and esophageal squamous cell carcinoma.[47] Similarly, nuclear localization signal mutations lead to ING exclusion from the nucleus and have been found in brain and breast tumors, as well as melanoma and lymphoblastic leukemia.[47] Additionally, ING proteins have been found to be down-regulated by either loss of heterozygosity or promoter hypermethylation in a variety of tumors, including breast, gastric, esophageal, blood, lung, and brain cancers.[37,47,48] Reduction of expression of *ING1* has been noted in >50% of cases of head and neck cancer and esophageal squamous cell carcinoma and 25% of ovarian cancers.[49,50]

Mixed Lineage Leukemia

The *Mixed Lineage Leukemia* (*MLL*) gene is rearranged in human lymphoid and myeloid acute leukemias (Figure 2B).[51] MLL is an example of a histone code writer; MLL has HMT activity specific for histone H3K4 that is mediated by its carboxyl terminal SET domain[52,53] *Mll* knockout mice are embryonic lethal at day E10.5 and show defects of the axial skeleton and hematopoietic system that are accompanied by defects in *HOX* gene expression and histone H3K4 methylation.[54] Translocations involving *MLL* result in fusion of N-terminal sequences of MLL up to and including a DNA methyltransferase homology (CXXC) domain to one of >60 translocation partners.

These translocations consistently delete the more C terminal PHD fingers, which have been shown to inhibit transformation.[55–57]

Despite deletion of the SET domain, MLL fusion proteins potently up-regulate target genes, including *HOXA7*, *HOXA9*, and the HOX cofactor *MEIS1*, which are essential for MLL fusion protein-mediated transformation.[58] This transcriptional activation appears to be mediated via recruitment of a complex containing multiple MLL translocation partners, including AF4, AF5q31, and LAF4, in addition to two proteins with enzymatic activity that stimulate transcriptional elongation: CDK9, which together with cyclin T1 or 2 comprises the pTEFb complex, and DOT1L, a histone H3 lysine 79-specific HMT[59] that has previously been shown to interact with the MLL translocation partner AF10 (Figure 2B).[60] Histone H3 K79 methylation is associated with active transcription.[61] Interestingly, MLL rearranged leukemias show abnormally high lysine 79 methylation that is broadly distributed across the *HOXA* and *MEIS1* loci.[62] Preliminary experiments suggest that inhibition of DOT1 methyltransferase activity inhibits *HOX* expression and the growth of cells with MLL rearrangements. Although DOT1 specificity for MLL-rearranged leukemias remains to be established, these data suggest that DOT1 may be a promising therapeutic target.[63]

Enhancer of Zeste 2

Enhancer of Zeste (EZH2), a member of the *Polycomb* group of proteins, is another histone code writer that is disrupted in cancer. EZH2 has intrinsic histone H3K27 methyltransferase activity and assembles into a multiprotein complex termed Polycomb repressive complex 2 (PRC2), which consists of EZH2, the WD40 repeat protein EED, and the zinc finger protein SUZ12.[64,65] The methylation of histone H3 lysine 27 catalyzed by this complex is recognized by a second Polycomb complex, PRC1, which is primarily composed of HPC, HPH, RING1, and BMI1; PRC1 binds and maintains a state of transcriptional repression.[66,67] Collectively, these complexes inhibit expression of a variety of proteins including the *HOX* genes and thereby antagonize the activity of *Trithorax* group proteins such as MLL.[68] It has been reported that DNMTs and HDACs, such as SIRT1, are recruited by the PRC2 complex and contribute to gene silencing,[69] thereby linking two major silencing pathways: histone H3 lysine 27 methylation and DNA methylation. However, the recent report[70] that EZH2 down-regulation restores expression of H3K27-targeted genes without affecting DNA methylation raises questions about the significance of DNA methylation in Polycomb-mediated repression.

Up-regulation of *EZH2* is seen in a number of tumor types, including lymphomas, prostate cancer, and breast cancer, where the expression level appears to correlate with disease progression.[68,71–73] EZH2 may contribute to cancer progression by maintaining a stem cell-like phenotype. Overexpression of EZH2 has been shown to prevent exhaustion of hematopoietic stem cells in serially transplanted mice, and ES cell lines cannot be established from $Ezh2^{-/-}$ blastocysts.[74,75] Importantly, a causal link between EZH2 and cancer was established when it was shown that overexpression of EZH2 in the B-cell-derived Ramos cell line or multiple myeloma cells caused increased proliferation.[71,76] Conversely, differentiation of the promyelocytic HL60 cell line results in down-regulation of *EZH2.* Furthermore, RNA interference-mediated knockdown of *EZH2* causes growth arrest at the G_2-M phase in prostate cells and suppression of DNA synthesis in HL60 cells.[72,77] Importantly, the HMT activity of EZH2 and the deacetylase activity of EED-recruited HDACs are necessary for EZH2-mediated cell proliferation and target gene repression.[72]

EZH2 mRNA and protein levels are low in benign prostate and increase progressively from localized to metastatic tumors, suggesting EZH2 could be a useful prognostic indicator as well as a potential therapeutic target.[72] Interestingly, EZH2 expression is regulated by micro-RNA-101, which is encoded by a locus that is commonly deleted in prostate cancer. The *miR-101* locus is deleted at one or both loci in 37.5% of clinically localized prostate cancer and 66.7% of metastatic prostate tumors, suggesting loss of micro-RNA-101 leads to EZH2 overexpression and cancer progression mediated by deregulated epigenetic mechanisms.[78]

Jumonji Domain Containing 2C

Jumonji domain containing 2C (JMJD2C), also known as GASC1, is one of a family of three histone demethylases (including JMJD2A and JMJD2B) that is amplified in a variety of cancers and functions as erasers of the histone code (Figure 2C).[79] The jumonji domain family is charac-

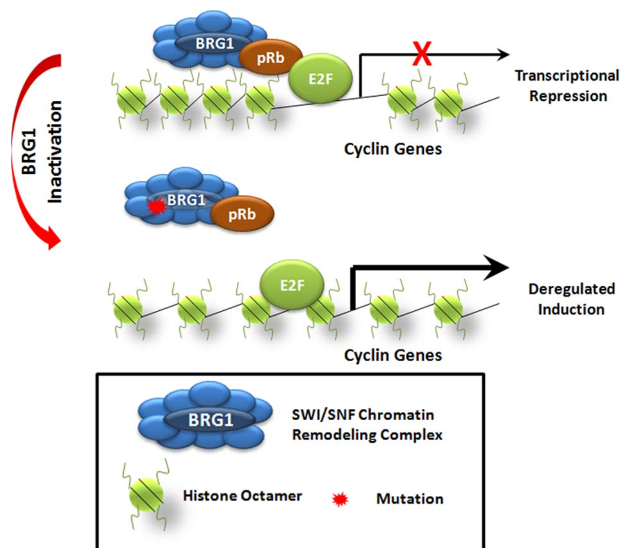

Figure 3. SWI/SNF-mediated nucleosomal remodeling and transcription in cancer. BRG1 is a DNA-dependent protein that functions in a SWI/SNF complex, which can remodel histones in several ways to make transcriptional start sites more accessible to transcription machinery. For example, SWI/SNF complexes allow histones to "slide" along DNA to expose DNA sequences as well as allow DNA looping away from histones to increase accessibility. BRG1 can also recruit pRb to regulate E2F target genes. Mutations in BRG1 in cancer inhibit the function of SWI/SNF complexes resulting to deregulated transcription.

terized by the presence of the jumonji domain, which is the catalytically active histone demethylase. The jumonji domain in JMJD2C is specific for di- and trimethylated histone H3 lysine 9.[80] JMJD2C overexpression dramatically reduces histone H3 lysine 9 methylation, resulting in delocalization of HP-1 and thereby impairing heterochromatin formation.[80] Considerable insights into JMJD2 function have come from studies of embryonic stem cell differentiation, which is accompanied by widespread increases in histone H3 lysine 9 methylation. Oct4, one of the key transcription factors involved in maintaining embryonic stem cell self-renewal, regulates JMJD2 expression, which in turn regulates expression of Nanog, another transcription factor critical for stem cell maintenance. Furthermore, depletion of JMJD2C or JMJD2A results in embryonic stem cell differentiation, suggesting that JMJD2 promotes stem cell self-renewal.[81]

JMJD2 overexpression has been reported in a number of human tumors, including esophageal squamous cell carcinoma, desmoplastic medulloblastoma, and occasional cases of MALT lymphoma, the latter as a result of chromosomal translocation with the *IgH* locus.[82–84] Consistent with its role as an oncoprotein, inhibition of JMJD2C expression in esophageal carcinoma or U2OS osteosarcoma cells results in decreased proliferation.[80]

Chromatin Remodeling

The nucleosome presents a barrier to transcription factor binding as well as transcriptional elongation. A variety of evolutionarily conserved mechanisms exist to overcome this, most notably the SWItch/Sucrose NonFermentable (SWI/SNF) ATP-dependent chromatin remodeling complex.[85] Perhaps through promoting chromatin accessibility to both coactivators and repressors through nucleosome displacement or through DNA displacement from nucleosomes, the SWI/SNF complex has both positive or repressive effects on transcription depending on chromatin context.[86] Determining the precise role of chromatin remodeling in cancer has been somewhat hampered by the relatively difficult and insensitive testing methods available, which use DNase hypersensitivity and Southern blot analysis for assessing nucleosomal position (phasing) in tumor tissues.

Perhaps the best studied example of chromatin-remodeling enzyme disruptions in cancer is the loss of expression of the *INI1* (*SNF5/SMARCB1/BAF47*), a core component of the SWI/SNF complex, in malignant rhabdoid tumors as well as other primitive undifferentiated pediatric sarcomas. This aggressive tumor of childhood appears to be the result of deregulation of multiple oncogenic pathways, including *cyclin D1* up-regulation.[87,88] Mutations have also been identified in the BRG1 ATPase of the SWI/SNF complex in a variety of solid tumors, including lung, prostate, pancreas, colorectal, and breast carcinoma, among others.[87] BRG1 interacts with Rb, so it has been postulated that BRG1 mutations disrupt the ability of Rb to act as a tumor suppressor (Figure 3).[87,89] In addition, constitutional mutations of ATRX, a SNF2 family chromatin remodeling protein, are associated with a variety of developmental abnormalities, including facial dysmorphism, mental retardation, and α thalassemia.[90] Acquired mutations of ATRX are associated with the α thalassemia myelodysplastic syndrome. As further evidence of the interplay between different epigenetic modifications, ATRX mutations are also associated with abnormal patterns of DNA methylation.[91]

Implications for Therapy

Success with the development of kinase inhibitors such as imatinib in CML therapy raises hopes that epigenetic regulators may also be attractive therapeutic targets. Currently, two types of epigenetic-based therapies have made their way into clinical use, HDAC inhibitors (HDACi) and inhibitors of DNA methyltransferases.[92] Tumor cells generally show higher sensitivity to HDACi than normal cells.[93] HDACi have shown particular efficacy against cutaneous T cell lymphomas and one, suberoylanilide hydroxamic acid (vorinostat), has been Food and Drug Administration approved for this application.[94] The mechanisms through which HDACi inhibit growth or kill tumor cells, however, remains unclear. Although HDACi can inhibit tumor growth through the up-regulation of the cyclin-dependent kinase inhibitor p21, many other mechanisms of HDACi action have been identified, including inhibiting DNA repair mechanisms and acetylating nonhistone proteins.[92] A number of HDAC inhibitors, including valproic acid, vorinostat, depsipeptide, and many others are currently in clinical trials for solid tumors with generally mixed results.

The inhibitors of DNMT in most wide clinical use are nucleoside analogs that get converted to dNTPs and become incorporated into DNA in place of cytosine during DNA replication. Of these, 5-azacytidine received Food and Drug Administration approval for myelodysplastic disorders and leukemia in 2004[95] and decitabine (5-Aza-2′-deoxcytidine) in 2006.[96] These inhibitors, and many others such as zebularine, 5-fluoro-2′-deoxycytidine, and 5,6-dihydro-5-azacytidine, are currently in clinical trials for a wide range of hematological malignancies and for solid tumors, where their efficacy in general has been less.[92] In addition, some studies suggest the combination of HDAC and DNA methylation inhibitors for cancer therapy are more effective than either agent alone.[97]

One of the biggest concerns with current epigenetic regulators is their nonspecific effects. Induction of global hypomethylation, for example, has the potential to activate other oncogenes as well as induce additional genomic instability. HDAC inhibitors have many "off target" effects that contribute to their toxicity. It is likely that additional inhibitors will be developed that target specific HMTs such as EZH2 or DOT1. Alternatively, inhibitors might be developed to inhibit DNA or histone recognition modules such as CXXC or PHD domains.

Another exciting possibility is the use of epigenetic modifiers to induce tumor antigens that can then be used as targets for cancer immunotherapy. Cancer testis antigens as an example are normally expressed in male germ cells. Cancer testis antigens are generally highly

immunogenic and, when reactivated by DNA demethylating agents, show promise when combined with cancer immunotherapy.[98] Similar approaches are being explored to restore hormone sensitivity such as reinducing immunotherapy, thereby targeting reactivated estrogen receptor re-expression in conjunction with tamoxifen therapy.[99]

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