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Review

Cancer Genetics and Epigenetics: Two Sides of the Same Coin?

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Epigenetic and genetic alterations have long been thought of as two separate mechanisms participating in carcinogenesis. A recent outcome of whole exome sequencing of thousands of human cancers has been the unexpected discovery of many inactivating mutations in genes that control the epigenome. These mutations have the potential to disrupt DNA methylation patterns, histone modifications, and nucleosome positioning and hence, gene expression. Genetic alteration of the epigenome therefore contributes to cancer just as epigenetic process can cause point mutations and disable DNA repair functions. This crosstalk between the genome and the epigenome offers new possibilities for therapy.

Cancer has traditionally been viewed as a set of diseases that are driven by the accumulation of genetic mutations that have been considered the major causes of neoplasia (Hanahan and Weinberg, 2011). However, this paradigm has now been expanded to incorporate the disruption of epigenetic regulatory mechanisms that are prevalent in cancer (Baylin and Jones, 2011; Sandoval and Esteller, 2012).

Both genetic and epigenetic views ultimately involve abnormal gene expression. The expression state of a particular gene is determined by the packaging of its DNA regulatory regions at promoters and/or enhancers and insulators in chromatin and by the presence of TFs and chromatin modifying enzymes. The genetic path to cancer is relatively straightforward: mutation of tumor suppressors and/or oncogenes causes either loss or gain of function and abnormal expression. The epigenetic pathway to cancer is not as simple and is determined by chromatin structure including DNA methylation, histone variants and modifications, nucleosome remodeling as well as small non-coding regulatory RNAs (Sharma et al., 2010). During tumor initiation and progression, the epigenome goes through multiple alterations, including a genome-wide loss of DNA methylation (hypomethylation), frequent increases in promoter methylation of CpG islands, changes in nucleosome occupancy, and modification profiles.

More recently, intriguing evidence has emerged that genetic and epigenetic mechanisms are not separate events in cancer; they intertwine and take advantage of each other during tumorigenesis. Alterations in epigenetic mechanisms can lead to genetic mutations, and genetic mutations in epigenetic regulators lead to an altered epigenome. In this review, we will discuss the collusion between epigenetics and genetics in cancer.

How Epigenetics Affect Genetics

Epigenetic mechanisms help establish cellular identities, and failure of the proper preservation of epigenetic marks can result in inappropriate activation or inhibition of various cellular signaling pathways leading to cancer. It is now generally accepted that human cancer cells harbor global epigenetic abnormalities and that epigenetic alterations may be the key to initiating tumorigenesis (Baylin and Jones, 2011; Sandoval and Esteller, 2012; Sharma et al., 2010). The cancer epigenome is characterized by substantial changes in various epigenetic regulatory layers; herein, we introduce some important examples of epigenetic disruptions that cause mutation of key genes and/or alteration of signaling pathways in cancer development.

Epigenetic Silencing Causes the Loss of Function of Genes and Predisposes to Genetic Mutation

Promoter hypermethylation of classic tumor suppressor genes is commonly observed in cancers, a phenomenon that has been implicated with driving tumorigenesis (Baylin and Jones, 2011). Genes controlling the cell cycle and DNA repair, such as *RB*, *BRCA1/2*, and *PTEN*, have all been reported to be hypermethylated or mutated/deleted in cancer (Hatziapostolou and Iliopoulos, 2011). There are also several genes that are seldom mutated but are silenced in cancer; promoter hypermethylation is the predominant mechanism for the loss of their functions (Baylin and Jones, 2011). *O6-methylguanine-DNA methyltransferase* (*MGMT*), which encodes a DNA repair gene, *Cyclin-dependent kinase inhibitor 2B* (*CDKN2B*), which encodes a cell cycle regulator p15, and *RASSF1A*, which encodes a protein that binds to the RAS oncogene all belong to this category, and they have been implicated with protective roles against tumorigenesis.

Several DNA repair genes are known to be subject to promoter methylation. MGMT removes carcinogen-induced O6-methylguanine adducts from DNA, which result in G to A transition mutations. Cancers with hypermethylated *MGMT* are susceptible to genetic mutation in critical genes such as *p53* or *KRAS* (Baylin and Jones, 2011; Esteller, 2007). The mismatch-repair gene *MLH1* plays an important role in genomic stability, and the loss of function of this gene by promoter hypermethylation causes microsatellite instability, which is a key factor in several cancers, including colorectal and endometrial cancers (Krivtsov and Armstrong, 2007). The *MLH1* promoter is already hypermethylated in normal colonic epithelium of some colorectal cancer patients, suggesting this epigenetic change is an early event of tumorigenesis and precedes downstream genetic mutation (Hitchins et al., 2011). Notably, SNVs of *MLH1* 5′UTR are correlated with the hypermethylation of its promoter, highlighting

a close relationship between genetic and epigenetic disruption in cancer (Hitchins et al., 2011).

Epigenetic Silencing Facilitates the Selection of Mutations in Key Signaling Pathways

Direct evidence for a close epigenetic-genetic cooperation is apparent in the colon cancer cell line HCT116 in which one allele of *MLH1* and *CDKN2A* is genetically mutated, whereas the other allele is silenced by DNA methylation (Baylin and Ohm, 2006). The lack of functional expression of MLH1 and CDKN2A causes defects in DNA mismatch repair and cell cycle regulation. Another example of epigenetic-genetic cooperation is in the WNT signaling pathway (Schepers and Clevers, 2012). In normal cells, secreted frizzled-related proteins (SFRPs) antagonize WNT signaling. Epigenetic silencing of *SFRPs* induces abnormal activation of this signaling pathway, further promoting the expression of several genes whose products are responsible for cell proliferation. As a result of survival and proliferative advantages, these cells accumulate genetic mutations in other components of the WNT signaling pathway. There are also several examples where epigenetic silencing allows abnormal proliferation pathways and increases the likelihood for mutation in genetic gate keepers and increases cancer risk (Baylin and Jones, 2011).

More recent results from The Cancer Genome Atlas project provide an integrative view of ovarian carcinoma based on integrated genomic analyses (Network, 2011). The mutation spectrum is unexpectedly simple, showing the predominance of *p53* mutations and other low frequency mutations in nine genes including *BRCA1*, *BRCA2*, and *RB*. On the other hand, promoter hypermethylation is observed in 168 genes, and those genes are epigenetically silenced and correlated with reduced expression. It is noteworthy that clustering of variable DNA methylation across tumors can identify subtypes. Indeed, the CpG island methylator phenotype (CIMP) is reported in colorectal cancer and glioblastoma, and this subgroup shows distinctive characters such as genetic and clinical features (Hinoue et al., 2012; Noushmehr et al., 2010). A CIMP-high subgroup is strongly associated with *MLH1* DNA hypermethylation and *BRAF* mutation, while a CIPM-low subgroup is related to *KRAS* mutation (Hinoue et al., 2012).

Role of 5-methylcytosine in Generating Disease-Causing Mutations

The methylation of cytosine residues in the germline has led to an approximately 75% decrease in the frequency of CpG methyl acceptor sites. This is thought to be due to the spontaneous hydrolytic deamination of 5-methylcytosine (5mC) to thymine rather than uracil, which is formed by deamination of cytosine. The resulting T:G mismatch is more difficult to repair, and about a third of all disease causing familial mutations and single nucleotide polymorphisms or variants (SNPs or SNVs) occur at methylated CpG sites. What is often overlooked is that the presence of 5mC in the gene bodies and coding regions of genes such as *p53* is responsible for generating inactivating C to T transition mutations, causing hotspots in somatic cells (Rideout et al., 1990). For example, as many as 50% of p53 point mutations in colon cancer occur at such sites, clearly demonstrating that an epigenetic mark (5mC) directly causes somatic mutations.

More interestingly, a somatic *DNMT3A* hotspot mutation in acute myeloid leukemia (AML) is caused by C to T transitions at a CpG site, possibly due to the methylation of its own exon by the enzyme (epigenetic alteration) and the subsequent deamination of 5mC (genetic mutation) (Ley et al., 2010) (Figure 1). The effect of the point mutation is not yet fully understood since methylation changes are not observed in the tumor. It is possible that this mutation alters DNMT3A function and/or activity and may further disrupt whole epigenetic regulation mechanism (Figure 1).

Role of MicroRNA in Tumorigenesis

MicroRNAs (miRNAs) are a class of small noncoding RNAs that play key roles in epigenetic regulation by controlling the translation and/or stability of mRNAs. There are over 1,000 human miRNAs and, interestingly, these miRNAs frequently target regions related to cancer development (Ryan et al., 2010). They have been classified as oncogenic, tumor-suppressive, or context-dependent miRNAs (Kasinski and Slack, 2011). Indeed, oncogenic miRNAs such as miR-155 or miR-21 are frequently overexpressed, and tumor suppressive miRNAs such as miR-146 or miR-15∼16 are deleted in cancers (Kasinski and Slack, 2011). Mutation in the miRNA can disrupt its recognition of binding targets and further result in oncogene activation and/or tumor suppressor repression. Additionally miRNAs including miR-101 and miR-29 target epigenetic modifiers such as EZH2 (Friedman et al., 2009; Varambally et al., 2008) and DNMT3A/B (Fabbri et al., 2007), respectively. This can result in further widespread epigenetic alterations (Fabbri and Calin, 2010; Kasinski and Slack, 2011) and might lead to the methylation of promoters of other miRNAs that target oncogenes. miR-127, which targets BCL6, is abnormally methylated and silenced in cancer (Saito et al., 2006), highlighting the reciprocal regulation of miRNAs, epigenetic modifiers, and genetic defects in cancer.

Given the importance of epigenetic silencing in the development of cancer, distinguishing "drivers" and "passengers" is becoming an important priority for the field. Driver genes must be essential for cancer causation, whereas passenger genes are not necessary (Kelly et al., 2010). With the improvement of technology, it may eventually be possible to specifically distinguish epigenetic disruptions of the driver genes (De Carvalho et al., 2012; Kalari and Pfeifer, 2010). Current evidence shows that epigenetic disruption plays a key role at every stage of tumorigenesis and has a significant impact on the underlying mechanisms of tumorigenesis and development of cancer therapy.

How Genetics Affect Epigenetics

While epigenetics and genetics can cooperate in cancer initiation and progression, the interconnectedness between of these two processes is becoming increasingly apparent with the realization that several epigenetic modifiers are mutated in human cancers (Kasinski and Slack, 2011; Rodríguez-Paredes and Esteller, 2011; Schuettengruber et al., 2011; Wilson and Roberts, 2011). Some examples of genetic mutations of epigenetic modifiers are shown in Table 1 and Figure 2. The mutation of epigenetic modifiers presumably leads to profound epigenetic changes, including aberrant DNA methylation, histone modifications, and nucleosome positioning, although this remains to be demonstrated. These epigenetic alterations can lead to abnormal gene expression and genomic instability, which may predispose to cancer (Rodríguez-Paredes and Esteller, 2011; Wilson and Roberts, 2011).

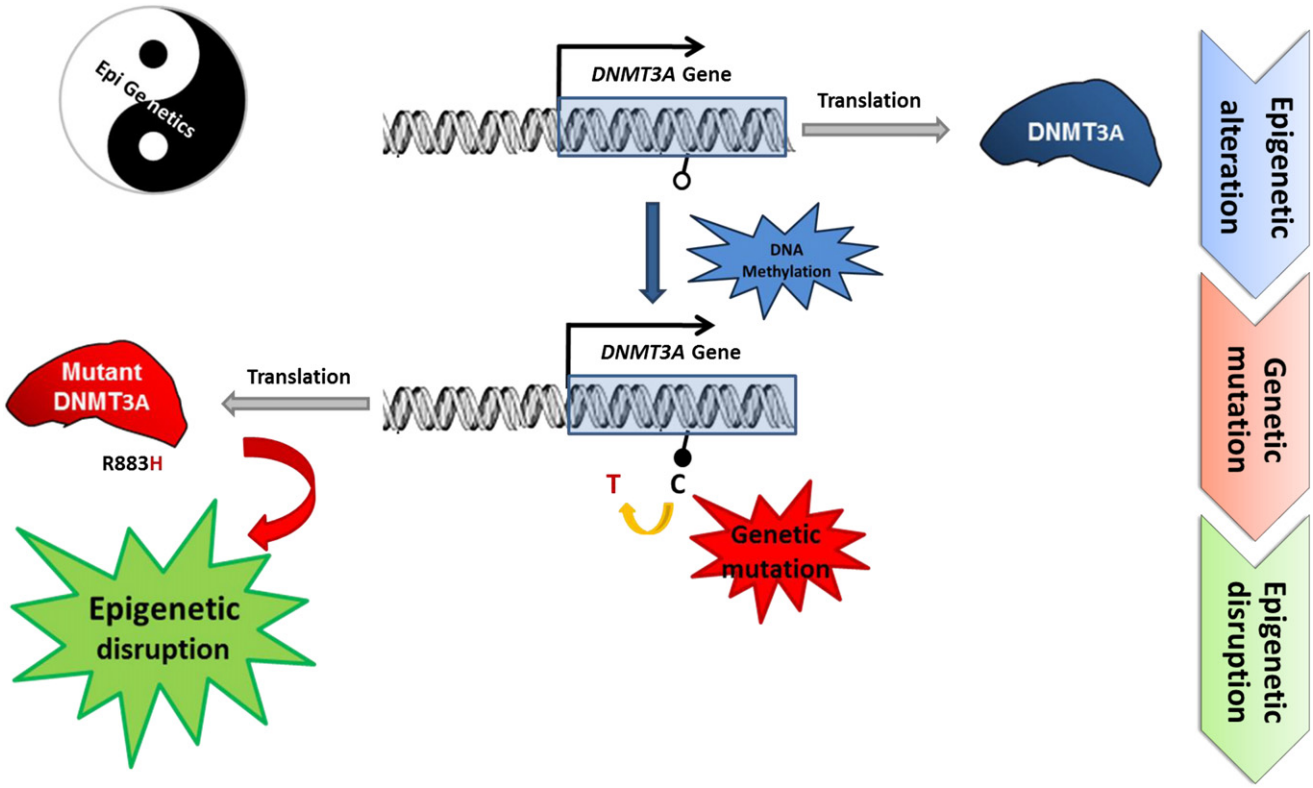

Figure 1. The Crosstalk between Cancer Genetics and Epigenetics

The methylation of CpG sites located in *DNMT3A* exons (epigenetic alteration, represented as a black circle) potentially leads to genetic mutation in somatic cells by the hydrolytic deamination of 5mC to form a C to T transition mutation. Although it is not known whether DNMT3A directly methylates its own exon and the effect of this genetic mutation is not yet fully understood, it is possible that the C to T transition alters DNMT3A function and/or activity and thereby disrupts the epigenetic landscape. The Yin-Yang diagram emphasizes how epigenetic and genetic interactions are required to achieve perfect balance and suggests that disruption of the balance can lead to disease.

DNA Methylation Machinery

While non-CpG methylation has been reported in pluripotent cells (Hawkins et al., 2010; Meissner et al., 2008), DNA methylation in mammals occurs predominantly at CpG dinucleotides, and methylation of CpG islands acts as a relatively stable gene silencing mechanism (Jones and Liang, 2009). The majority of the CpG islands, which represent over 50% of promoters, remain mostly unmethylated in somatic cells. DNA methylation is important for the regulation of non-CpG island as well as CpG island promoters and in repetitive sequences (LINE and/or SINE) to maintain genomic stability (De Carvalho et al., 2010; Jones and Liang, 2009). DNA methylation in mammalian cells is regulated by a family of DNA methyltransferases (DNMTs) that catalyze the transfer of methyl groups from S-adenosyl-L-methionine to the 5′ position of cytosine bases in the CpG dinucleotide. DNMT3A and DNMT3B, which are expressed throughout the cell cycle (Kinney and Pradhan, 2011), establish new DNA methylation patterns early in development. During replication, the original DNA methylation pattern is maintained largely by DNMT1 activity, which prefers hemi-methylated DNA over nonmethylated DNA as a substrate and which is also supported by recent structure study (Song et al., 2012) and is therefore responsible for the maintenance of methylation patterns during cell division, with some participation by DNMT3A and DNMT3B (Jones and Liang, 2009; Sharma et al., 2010).

DNMT1 mutations have been described in colorectal cancer (Kanai et al., 2003), and as previously noted, *DNMT3A* mutations are frequent in myelodysplastic syndromes (MDS) and AML (Ley et al., 2010; Yamashita et al., 2010; Yan et al., 2011). Germline mutations in *DNMT3B* underlie immunodeficiency-centromeric instability-facial anomalies (ICF) syndrome and chromosome instability (Wijmenga et al., 2000), and SNPs in *DNMT3B* have been suggested to be associated with risk of several cancers including breast and lung adenocarcinoma (Shen et al., 2002). In addition to the example in Figure 1, other mutations of *DNMT3A* occur at several positions and generally represent a loss of function, similar to *DNMT3B* mutations that are associated with ICF syndrome (Ley et al., 2010). Recent studies uncovered a role of DNMT3A in silencing self-renewal genes in hematopoietic stem cells (HSCs) to permit efficient hematopoietic differentiation, and its loss progressively impairs HSC differentiation (Challen et al., 2012; Trowbridge and Orkin, 2012). All known *DNMT3A* mutations are related to poor survival in AML (Ley et al., 2010; Yan et al., 2011), suggesting that these mutations prevent differentiation and have an important role in the progression of disease.

In addition to various mutations, DNMT1, DNMT3A, and DNMT3B are often overexpressed in various cancers and possibly contribute to ectopic hypermethylation (Wu et al., 2007). However, careful studies should be done to understand

Table 1. Epigenetic Modifiers in Cancer

	Gene	Function	Tumor Type	Alteration
DNA methylation	*DNMT1*	DNA methyltransferase	Colorectal, non-small cell lung, pancreatic, gastric, breast cancer	Mutation (Kanai et al., 2003)
				Overexpression (Wu et al., 2007)
	DNMT3A	DNA methyltransferase	MDS, AML	Mutation (Ley et al., 2010; Yamashita et al., 2010; Yan et al., 2011)
	DNMT3B	DNA methyltransferase	ICF syndrome, SNPs in breast and lung adenoma	Mutation (Wijmenga et al., 2000)
				Mutation (Shen et al., 2002)
	MBD1/2	Methyl binding protein	Lung and breast cancer	Mutation (Sansom et al., 2007)
	TET1	5′methylcytosine hydroxylase	AML	Chromosome translocation (De Carvalho et al., 2010; Wu and Zhang, 2010)
	TET2	5′methylcytosine hydroxylase	MDS, myeloid malignancies (AML), gliomas	Mutation/silencing (Tan and Manley, 2009)
	IDH1/2	Isocitrate dehydrogenase	Glioma, AML	Mutation (Figueroa et al., 2010; Lu et al., 2012; Turcan et al., 2012)
	AID	5′cytidine deaminase	CML	Aberrant expression (De Carvalho et al., 2010)
Histone modification	*MLL1/2/3*	Histone methyltransferase H3K4	Bladder TCC, ALL and AML, non-Hodgkin lymphoma, B cell lymphoma, prostate (primary)	Translocation, mutation, aberrant expression (Gui et al., 2011; Morin et al., 2011)
	BRD4	Bromodomain containing 4	Nuclear protein in testis, midline carcinoma, breast, colon, and AML	Translocation (fusion protein), aberrant expression (Filippakopoulos et al., 2010; Zuber et al., 2011)
	EZH2	Histone methyltransferase H3K27	Breast, prostate, bladder, colon, pancreas, liver, gastric, uterine tumors, melanoma, lymphoma, myeloma, and Ewing's sarcoma	Mutation, aberrant expression (Chase and Cross, 2011; Tsang and Cheng, 2011)
	ASXL	Enhancer of trithorax and polycomb group (EAP) Additional sex combs like 1	MDS and AML, Bohring-Opitz syndrome	Mutation (Gelsi-Boyer et al., 2012; Hoischen et al., 2011)
	BMI-1	PRC1 subunit	Ovarian, mantle cell lymphomas and Merkel cell carcinomas	Overexpression (Jiang et al., 2009; Lukacs et al., 2010)
	G9a	Histone methyltransferase H3K9	HCC, cervical, uterine, ovarian, and breast cancer	Aberrant expression (Varier and Timmers, 2011)
	PRMT1/5	Protein arginine methyltransferase	Breast/gastric	Aberrant expression (Miremadi et al., 2007)
	LSD1	Histone demethylase H3K4/H3K9	Prostate	Mutation (Rotili and Mai, 2011)
	UTX (*KDM6A*)	Histone demethylase H3K27	Bladder, breast, kidney, lung, pancreas, esophagus, colon, uterus, brain	Mutation (Rotili and Mai, 2011)
	JARID1B/C	Histone demethylase H3K4/H3K9	Testicular and breast, RCCC	Overexpression (Rotili and Mai, 2011)
	EP300	Histone deacetyltransferase	Breast, colorectal, pancreatic cancer	Mutation (Miremadi et al., 2007)
	CREBBP	Histone acetyltransferase	Gastric and colorectal, epithelial, ovarian, lung, esophageal cancer	Mutation, overexpression (Miremadi et al., 2007)
	PCAF	Histone acetyltransferase	Epithelial	Mutation (Miremadi et al., 2007)
	HDAC2	Histone deacetyltransferase	Colonic, gastric, endometrial cancer	Mutation (Ropero et al., 2006)
	SIRT1, *HDAC5/7A*	Histone deacetyltransferase	Breast, colorectal, prostate cancer	Mutation, aberrant expression (Miremadi et al., 2007)

Table 1. *Continued*

	Gene	Function	Tumor Type	Alteration
Chromatin remodeling	*SNF5* (*SMARCB1*, *INI1*)	BAF subunit	Kidney malignant rhabdoid tumors, atypical rhabdoid/teratoid tumors (extra-renal), epithelioid sarcomas, small cell hepatoblastomas, extraskeletal myxoid chondrosarcomas, and undifferentiated sarcomas	Mutation, silencing, loss of expression (Wilson and Roberts, 2011)
	BRG1 (*SMARCA4*)	ATPase of BAF	Lung, rhabdoid, medulloblastoma	Mutation, low expression (Wilson and Roberts, 2011)
	BRM (*SMARCA2*)	ATPase of BAF	Prostate, basal cell carcinoma	Mutation, low expression (de Zwaan and Haass, 2010; Sun et al., 2007)
	ARID1A (*BAF250A*)	BAF subunit	Ovarian clear cell carcinomas, 30% of endometrioid carcinomas, endometrial carcinomas	Mutation, genomic rearrangement, low expression (Guan et al., 2011; Jones et al., 2010)
	ARID2 (*BAF200*)	PBAF subunit	Primary pancreatic adenocarcinomas	Mutation (Li et al., 2011)
	BRD7	PBAF subunit	Bladder TCC	Mutation (Drost et al., 2010)
	PBRM1 (*BAF180*)	PBAF subunit	Breast tumors	Mutation (Varela et al., 2011)
	SRCAP	ATPase of SWR1	Prostate	Aberrant expression (Balakrishnan et al., 2007)
	P400/Tip60	ATPase of SWR1, acetylase of SWR1	Colon, lymphomas, head-and-neck, breast	Mutation, aberrant expression (Mattera et al., 2009)
	CHD4/5	ATPase of NURD	Colorectal and gastric cancer, ovarian, prostate, neuroblastoma	Mutation (Bagchi et al., 2007; Kim et al., 2011; Wang et al., 2011a)
	CHD7	ATP-dependent helicase	Gastric and colorectal	Mutation (Wessels et al., 2010)

MDS, myelodysplastic syndromes; AML, acute myeloid leukemia; TCC, transitional cell carcinoma; RCCC, renal clear cell carcinoma.

the relationship between the expression of DNMTs and methylation disruption since the overexpression of DNMTs may be a reflection of increased cell proliferation.

Methyl-binding domain (MBD) proteins, including MeCP2, MBD1, MBD2, and MBD4, bind to methylated CpG sites and might be involved in mediating transcriptional repression (Bogdanović and Veenstra, 2009). Genetic mutations in *MBD1* and *MBD2* increase the risk of lung and breast cancer, respectively (Sansom et al., 2007). MeCP2 and other MBD protein alterations have been reported in several cancers, however the mechanism is yet to be uncovered.

The field of DNA demethylation has been controversial (Ooi and Bestor, 2008), but recent evidence suggests that this demethylation can occur through two processes: active and passive (De Carvalho et al., 2010; Wu and Zhang, 2010). Passive DNA demethylation occurs when maintenance DNA methylation is impaired during DNA replication, resulting in loss of methylation of the newly synthesized DNA strand. In contrast, active DNA demethylation is dependent on the ability of one or more enzymes to hydroxylate, further oxidize, or deaminate 5mC and can occur independent of DNA replication (Bhutani et al., 2011; Wu and Zhang, 2010). Recently, several proteins have been implicated to be erasers of DNA methylation including *TET* (*ten-eleven-translocation*) and *AID* (*activation-induced cytidine deaminase*) (De Carvalho et al., 2010; Ko et al., 2010; Wu and Zhang, 2010). Active DNA demethylation is currently thought of as being a stepwise process—hydroxylation of 5mC (5hmC) by TET proteins followed by deamination by AID/APOBEC protein or carboxylation and entry in to the subsequent base excision repair pathway (Bhutani et al., 2011). Alternatively, 5hmC is not recognized by DNMT1 (Lao et al., 2010); replication of DNA containing this base would lead to loss of the 5mC mark in the subsequent S phase.

Three TET family members (TET1, TET2, and TET3) have been reported so far, and each protein seems to have a distinct function in different cellular contexts (Cimmino et al., 2011). Mutations in *TET2*, including frame shift, nonsense, and missense mutations, have been found in MDS and in myeloproliferative neoplasms (Tan and Manley, 2009). Notably, *TET2* loss-of-function mutations were mutually exclusive of mutations in *IDH1* (*isocitrate dehydrogenase1*) and *IDH2*, which are known to induce DNA hypermethylation and impair differentiation in hematopoietic cells (Figueroa et al., 2010). *IDH1/2* mutations in glioma and AML cause accumulation of 2-hydoxyglutarate which is called an "oncometabolite" and further impairs the DNA demethylation process and causes hypermethylation in glioma (Turcan et al., 2012). Remarkably, *IDH1/2* mutations also disrupt histone demethylation and block cell differentiation in nontransformed cells (Lu et al., 2012).

Considering that DNMTs/MBD proteins and enzymes involved in DNA demethylation contribute directly to the level of DNA methylation but also to nucleosome occupancy patterns, the alteration of these machineries in cancer development could be broader than previously realized.

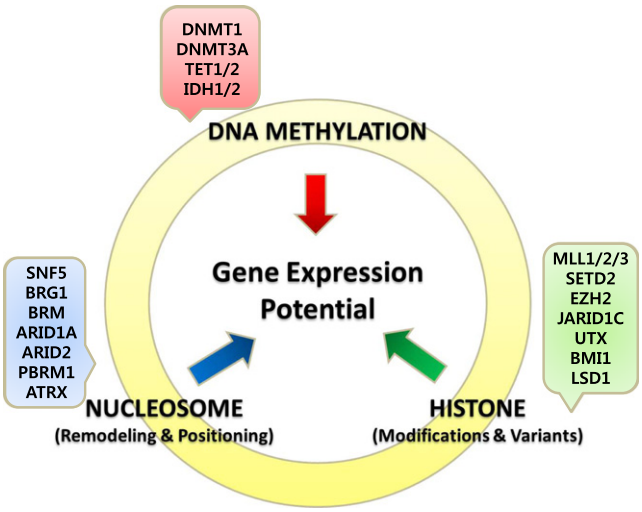

Figure 2. Genetic Mutations in Epigenetic Modifiers in Cancer
The drawing shows the interaction between epigenetic processes in specifying gene expression patterns. Recent whole exome sequencing studies show that mutations in the three classes of epigenetic modifiers is frequently observed in various types of cancers, further highlighting the crosstalk between genetics and epigenetics. Examples of some but not all of these mutations that are discussed in this review are shown. The mutations of epigenetic modifiers probably cause genome-wide epigenetic alterations in cancer, but these have yet to be demonstrated in a genome-wide scale. Understanding the relationship of genetic and the epigenetic changes in cancer will offer novel insights for cancer therapies.

Histone Modification Machinery

Nucleosomes, which are the basic building blocks of chromatin, contain DNA wrapped around histones (Luger et al., 1997). Histones are regulators of chromatin dynamics either by changing chromatic structure by altering electrostatic charge or providing protein recognition sites by specific modifications (Mills, 2010; Suganuma and Workman, 2011). Histone modifications at specific residues characterize genomic regulatory regions, such as active promoter regions which are enriched in trimethylated H3 at lysine 4 (H3K4me3), inactive promoters which are enriched in trimethylated H3 at lysine 27 (H3K27me3) or trimethylated H3 at lysine 9 (H3K9me3), and regulatory enhancers that are enriched in monomethylated H3 at lysine 4 (H3K4me1) and/or acetylated H3 at lysine 27 (H3K27ac) (Hawkins et al., 2011; Hon et al., 2009; Mills, 2010). These histone modification patterns are regulated by enzymes including histone acetyltransferases (HATs) and deacetylases (HDACs), which introduce and remove acetyl groups, respectively. Histone methyltransferases (HMTs) and demethylases (HDMs), on the other hand, introduce and remove methyl groups. During tumorigenesis, cells undergo global changes in histone modifications and in the distribution of histone variants such as H2A.Z (Conerly et al., 2010), which may affect the recruitment of TFs and often components of the transcription machinery, thereby contributing to aberrant gene expression (Mills, 2010; Sharma et al., 2010).

The acetylation of lysine residues on histones is generally associated with active gene transcription. HATs can be grouped into three categories based on their sequence similarities: Gcn5/PCAF, p300/CBP, and the MYST families (Yang, 2004). Mutations or translocations of these genes are observed in colon, uterine, and lung tumors and in leukemias (Esteller, 2007). Further, these HATs (p300, CBP, and MYST4) are commonly involved in chromosomal translocations in hematological cancers rather than in solid tumors (Iyer et al., 2004). For example, AML1-ETO, the fusion protein generated by the t(8;21) translocation, which is also the most common fusion protein in AML, requires its acetylation mediated by p300 for oncogenic activity (Wang et al., 2011b). HDACs remove acetyl groups from histone tails, and at least 18 HDAC genes have been identified in the human genome. HDACs as well as HATs function as part of large multi-protein complexes (Marks et al., 2001). HDACs have been implicated in cancer due to their aberrant binding and consequent silencing of tumor suppressor genes. For example, hypoacetylation of the *p21waf1/cif1* (*CDKN1A*) promoter results in its silencing and can be reversed by HDAC inhibitors (Ocker and Schneider-Stock, 2007). Germline mutations of HDACs increase the risk of breast and lung cancers, and abnormal HDAC overexpression has also been observed in various cancers (Miremadi et al., 2007). As a result, HDAC inhibitors have been developed as anti-cancer drugs (Shankar and Srivastava, 2008). Several independent reports have identified truncation mutations in HDAC2 in epithelial, colonic, gastric, and endometrial cancers, and these mutations confer resistance to HDAC inhibitors (Smith and Workman, 2009). Screening for these mutations may improve the efficacy of HDAC inhibitors. Conversely, there is evidence that HDACs may function as tumor suppressors by maintaining proper chromatin structure and further stabilizing the genome (Bhaskara et al., 2010). Potentially, either loss or gain of function mutations of HDACs could contribute to tumorigenesis.

In addition to chromatin modifying enzymes, chromatin binding proteins or so-called epigenetic ''readers'', such as the bromodomain proteins which read lysine acetylation marks, can also play an important during tumorigenesis. For example, the fusion of the bromodomain protein Brd4 with nuclear protein in testis (NUT) results in the development of aggressive NUT midline carcinoma (Filippakopoulos et al., 2010). Aberrant regulation of Brd4 has also been reported in other cancers such as colon and breast, suggesting that the selective inhibitors which target these kinds of epigenetic readers may give us a novel clue for cancer therapy (Filippakopoulos et al., 2010; Zuber et al., 2011).

Methylation of arginine and lysine residues on histones or nonhistone proteins such as TFs regulate chromatin structure and therefore gene expression (Greer and Shi, 2012). The best-known example of alterations in HMTs during tumorigenesis may be in the mixed lineage leukemia (MLL) protein, which introduces the active H3K4me3 mark and plays important roles in development. *MLL* is located on chromosome 11q23, which is a common region of chromosomal translocation in AML and ALL (Slany, 2009). Translocations of MLL with multiple different partners can result in the generation of fusion proteins that are frequently associated with tumorigenesis and poor prognosis by generating abnormal patterns of H3K4me3 and/or recruiting other epigenetic modifiers (Balgobind et al., 2011). These MLL fusion proteins have close relationships with other epigenetic modifiers and cause altered epigenetic programs in cancer. For example, the aberrant H3K79 methylation pattern mediated by DOT1L is required for the maintenance of the MLL

translocation-associated oncogenic program (Bernt et al., 2011). Inhibition of DOT1L activities decreases expression of MLL fusion-driven transcriptional programs and might have profound therapeutic implications (see the detailed discussion in Therapeutic Perspective). In addition, alternative splicing and mutations in *MLL1*, *MLL2*, and *MLL3* genes have been identified in bladder, breast, and pancreatic cancers and in glioblastoma (Gui et al., 2011; Morin et al., 2011).

The Polycomb group (PcG) of repressor proteins controls the accessibility of gene regulatory elements to the transcription machinery (Mills, 2010), This group is crucial for early development and often becomes deregulated in cancer. EZH2, together with SUZ12 and EED, form the polycomb repressive complex 2, which methylates H3K27. Overexpression of EZH2 has been reported in several cancers such as prostate, breast, lung, and bladder and seems to result in an increase in H3K27me3 (Chase and Cross, 2011). However, other studies show that there is no association between EZH2 and H3K27me3 in ovarian and pancreatic cancers (Füllgrabe et al., 2011). Downregulation of microRNA-101, a negative regulator of EZH2, has been described as a cause of overexpression of EZH2 in bladder and prostate cancers (Friedman et al., 2009; Varambally et al., 2008), and *EZH2* mutations have been reported in lymphoma and myeloid neoplasm (Chase and Cross, 2011). In lymphoma, a heterozygous missense mutation at amino acid Y641, within the SET domain, results in a gain of function, showing enhanced catalytic activity. The *EZH2* mutations in myeloid neoplasms are associated with poor prognosis, and the mutations frequently result in loss of function of HMT. Although the mechanism of action of EZH2 in cancer is not yet clear, it appears to play a role in growth control (Tsang and Cheng, 2011).

BMI-1, a component of PRC1, is indispensable for the regulation of self-renewal of normal and leukemic stem cells and for the differentiation of T cells (Nakayama and Yamashita, 2009; Sauvageau and Sauvageau, 2010). BMI-1 has been considered a key regulator of self-renewal in cancer stem cells (Jiang et al., 2009). More recently, overexpression of BMI-1 has been observed in solid tumors such as prostate cancer (Lukacs et al., 2010; Yang et al., 2010).

Subgroups of genes that are normally repressed by H3K27me3 in early development often acquire abnormal DNA methylation in cancers, a process which we have called ''epigenetic switching'' (Sharma et al., 2010). The differentiation of stem cells begins by turning off master regulators that define ''stemness'' (e.g., OCT4 in embryonic stem cells), followed by the expression of lineage specific genes, resulting in the acquisition of particular phenotypes (e.g., MYOD1 in muscle and NEUROG1 in neurons) (Young, 2011). Progress through these steps is often, but not always, controlled by PcG and does not involve DNA methylation. Once these key regulators become methylated, they become locked in a repressed state, and this prevents switching from one phenotype to another. The outcome of the ''epigenetic switch'' may therefore be an increase in the number of cancer initiating cells (Baylin and Jones, 2011). Full understanding of this mechanism remains to be clarified.

Other lysine HMTs (NSD1, SMYD3, and G9a) are aberrantly expressed in several cancers (Varier and Timmers, 2011). Evidence for the role of arginine HMTs (PRMTs) in tumorigenesis has not been as well established as that of lysine HMTs, although alteration of expression of PRMT1 in breast cancer and PRMT5 in gastric cancer has been reported (Lee and Stallcup, 2009).

Two distinct classes of HDMs have been defined based on their mechanism of action (Mosammaparast and Shi, 2010). Lysine-specific histone demethylase 1 (LSD1), lysine-specific demethylase 6A (KDM6A/UTX), and jumonji C-domain containing proteins (JARID1A-D) have all been implicated in tumorigenesis. Mutations in *LSD1* (prostate cancer) and *KDM6A/UTX* (various cancers including bladder, breast, kidney, and colon) have been reported (Rotili and Mai, 2011). Reintroduction of KDM6A/UTX in the UTX mutant cancer cells results in the slowing of proliferation, suggesting that genetic mutations of these enzymes reinforce the epigenetic deregulation in cancers.

The exact mechanism by which these histone modifying enzymes affect tumorigenesis remains to be elucidated; altered expression of histone modifiers caused by mutations may disrupt whole epigenetic regulation mechanisms and result in aberrant gene expression patterns. Indeed, the disruption of histone modifications has been linked to all the hallmarks of cancer, and it is important to be aware that a precise balance between the enzymes that write, read, and erase histone marks is crucial in preventing tumorigenesis.

Chromatin Remodeling Complexes

Nucleosome occupancy is a key mechanism for gene expression, and it has been known for some time that chromatin remodelers are responsible for regulating this process (Clapier and Cairns, 2009; Segal and Widom, 2009; Valouev et al., 2011). ATP dependent chromatin remodelers are generally divided into four main families: switch/sucrose non-fermenting (SWI/SNF), imitation SWI, inositol requiring 80, and nucleosome remodeling and deacetylation chromatin helicase DNA binding (NURD/Mi2/CHD) complexes (Ho and Crabtree, 2010). Although the ATPase domains are highly similar, the distinct chromatin interacting domains carry out specific roles and can be selectively targeted. These ATPase dependent remodelers act in the context of multisubunit complexes and have dual roles as activators and repressors of gene expression. The importance of chromatin remodeling machines is becoming apparent with the realization that many of them are mutated in several types of cancer (Hargreaves and Crabtree, 2011; Wilson and Roberts, 2011).

SWI/SNF is a large complex with 9 to 12 subunits including ATPases (BRG1 or BRM), core subunits (SNF5, BAF155, and BAF 170), and other accessory subunits (Ho and Crabtree, 2010). The variety of subunits allows for combinatorial assembly that leads to functional diversity as evidenced by the cellular stage-specific composition of SWI/SNF complexes (Hargreaves and Crabtree, 2011). SWI/SNF complexes remodel chromatin by changing nucleosome occupancy pattern, thereby contributing to either transcriptional activation or repression (Reisman et al., 2009; Wilson and Roberts, 2011).

SNF5 of the SWI/SNF core subunit is at the nexus of the link between chromatin remodeling and tumorigenesis, and many rhabdoid tumors contain inactivating mutations in this gene. Loss of SNF5 is also observed in renal carcinomas and melanomas, where it is correlated with poor survival rates (Lin et al., 2009). SNF5 loss affects expression of genes associated with cell proliferation and cell cycle, such as RB or p53 and Hedgehog-Gli, a key signaling pathway in early development

and cancer. Antagonism between EZH2 and SNF5 has also been reported during tumorigenesis (Jagani et al., 2010; Wilson and Roberts, 2011), and there is accumulating evident that SNF5 deletion plays a role in tumorigenesis, but the exact mechanism of SNF5 loss in tumorigenesis remains to be elucidated.

ARID1A/BAF250a mutations have been frequently observed in ovarian clear cell carcinoma (50%) and endometrioid carcinomas (30%) (Guan et al., 2011; Jones et al., 2010; Wiegand et al., 2010). More recently, *ARID1A/BAF250a* mutations have been observed in primary pancreatic adenocarcinomas, and transitional cell carcinoma and low ARID1A expression was found to be significantly associated with a specific subgroup of breast cancers (ER-/PR-/HER2-) (Zhang et al., 2011). In mice, ARID1B/ BAF250b-containing complexes, which include components of an E3 ubiquitin ligase and are mutually exclusive of ARID1A, have also been shown to play a role in the control of cell cycle and differentiation. Mutations in human *ARID1B/ BAF250b* have been reported very recently as a cause of Coffin-Siris syndrome (Santen et al., 2012; Tsurusaki et al., 2012).

The PBRM1/BAF180, BAF200, and BRD7 subunits belong to polybromo BRG1 associated factor (PBAF) complexes and facilitate transcriptional activation by nuclear receptors (Wilson and Roberts, 2011). Mutation of *PBRM/BAF180* has been identified in 41% renal cell carcinomas and in breast cancers, and this mutation affects senescence in human cells. Mutation in another PBAF specific subunit, *BRD7*, has been reported in breast cancers. Since BRD7 has a variety of binding partners including p53 and BRCA1, mutations in it may be important in tumorigenesis.

Mutations in SWI/SNF ATPase subunits *BRG1* or *BRM* have been reported in several cancers including lung, medulloblastoma, rhabdoid, and prostate tumors (Wilson and Roberts, 2011). Although BRG1 and BRM show some redundancy in vivo and in vitro, they seem to be mutually exclusive and have distinctive roles based on their expression changes during early development. Tumor suppressor properties of BRG1 and BRM have been reported in lung, breast, and prostate cancer cell lines (Roberts and Orkin, 2004) and, in vitro, BRG1 and BRM have been observed to interact with several tumor suppressors including BRCA1(Wang et al., 2007).

Mutation of BAF complexes is a frequent event in various cancers; however, the dependency between the subunits and whether mutation of one subunit results in a modification of the activity of the complex is not clear. In addition, mutations of BAF complex components frequently coexist with those of canonical oncogenes or tumor suppressors such as *KRAS*, *CDKN2A*, or *p53*, suggesting a synergistic effect on tumorigenesis (Wilson and Roberts, 2011).

In addition to SWI/SNF complexes, mutations of other ATP dependent chromatin remodelers are beginning to be identified in several cancers (Clapier and Cairns, 2009; Hargreaves and Crabtree, 2011; Ho and Crabtree, 2010). Despite emerging evidence that closely connects these ATPase remodelers in tumorigenesis, the direct causality and/or mechanism still remains to be explicated.

The Role of SNPs on Epigenetic Regulation and Cancer

Genome-wide association studies have identified a large number of SNPs associated with an increased risk of a variety of diseases including several cancers. Surprisingly, cancer-associated SNPs are significantly enriched at regions defined as functional enhancers in ES cells (Teng et al., 2011) and might confer cancer susceptibility by altering the chromatin landscape. Further, several genome-wide expression quantitative trait loci studies in humans have demonstrated a link between genetic variation and changes in gene regulation (Nica et al., 2010; Nicolae et al., 2010). More recently, these genetic variants were shown to modify the chromatin accessibility of TF binding sites, thereby leading to gene expression differences (Degner et al., 2012). Although allele-specific DNA methylation and allele-specific gene expression have been well studied in imprinting and X chromosome inactivation, recent studies show that these allele-specific phenomena are more pervasive to other cellular activities (Tycko, 2010). Notably, most of the allele-specific DNA methylation outside of imprinted genes shows a strong correlation with SNP genotypes that affect TF binding insulators and long-range chromosome structure. Conversely, SNPs can create or delete CpGs (termed as CpG SNPs), thereby influencing the binding of specific TFs (Tycko, 2010). Future studies aimed at understanding functional associations among epigenetic variation (epigenotype), genetic variation (genotype), and trait or disease (phenotype) may help us to determine the causality of diseases.

Therapeutic Perspective

An increasing number of nucleoside analogs/small molecules are being studied as anti-cancer drugs. Inhibitiors of DNMTs 5-azacytidine (5-Aza-CR; Vidaza; azacitidine) and 5-Aza-2-deoxycytidine (5-Aza-CdR; Dacogen; decitabine) or HDACs by SAHA or Rhomidepsin have been approved for cancer treatment by the FDA and proven to have therapeutic efficacy in a variety of malignancies (Kelly et al., 2010). Recently, several novel compounds have been reported to target epigenetic components and have therapeutic effects in the presence of specific genetic defects. The DOT1L inhibitor (EPZ004777) inhibits H3K79 methylation, prevents transcription of genes that are involved in leukemogenesis, and kills cancer cells bearing MLL translocations (Daigle et al., 2011). Selective bromodomain inhibitors (JQ1 or GSK525762) (Filippakopoulos et al., 2010; Nicodeme et al., 2010) inhibit transcription by MYC, which is overexpressed in a majority of cancers (Delmore et al., 2011). The presence of multiple genetic and epigenetic aberrations within a cancer suggests that effective cancer therapies will be most beneficial when combined with epigenetic and/or other anti-cancer strategies such as standard chemotherapy (Juergens et al., 2011; Matei and Nephew, 2010).

Conclusions

Recent whole exome sequencing of thousands of human cancers have come up with the unexpected results that mutations in genes that control the epigenome are surprisingly common in human cancers. The presence of these mutations was unknown and overlooked, which is surprising in view of the fact that were almost 1,000 cell lines recently analyzed by whole exome sequencing contain a large number of potential mutations in epigenetic modifiers (Barretina et al., 2012). The fact that the epigenome acts at the pinnacle of the hierarchy of gene control mechanisms means that the mutations probably

have effects on multiple pathways relevant to the cancer phenotype, and a single mutation could cause wide scale misregulation. This realization opens the door to further drug development since it might be possible to correct several pathways by altering or inhibiting one enzyme. These data also show a much closer Yin-Yang relationship between the genome and the epigenome, as indicated in this review. This has heralded the dawn of a new era in cancer research in which the way the genes are organized and controlled is being recognized as a major relevant factor for human carcinogenesis. Traditionally, cancer is diagnosed by pathologists using light microscopes to analyze the morphology of the nucleus among other cellular features. Understanding how epigenetic modifiers communicate with each other and alter nuclear architecture, and therefore gene expression, is a major challenge for the future but one which should yield better options for patients.

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