

Cancer Epigenetics: A Brief Review

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Abstract

Cancer is a disease that results from the successive accumulation of genetic and epigenetic alterations. Despite intense study, many unanswered questions about the nature of the contribution of epigenetic changes to carcinogenesis remain. In this review, we describe principles of epigenetics as they relate to our current understanding of carcinogenesis. There are a number of *in vivo* models of specific pathways of carcinogenesis that are very useful for the characterization of epigenetic mechanisms that link environmental exposures or genetic susceptibility and cancer progression. Because epigenetic alterations are thought to be reversible, they offer great promise for treatment of cancer. The use of animal models to evaluate the effects of decitabine and zebularine has elucidated the mechanisms of action and indicated the potential for these types of treatment. Ultimately, the greatest challenge lies in the integration of laboratory and epidemiologic data to best prevent and treat this deadly disease.

Key Words: cancer; chromatin; epigenetics; histones; mechanisms; methylation

Introduction

The field of cancer epigenetics has thrived on discoveries from *in vitro*, *in vivo*, and human clinical and epidemiologic studies. Results from these complimentary approaches have challenged the classic view of cancer, which has traditionally been hypothesized as a disease that results from the successive accumulation of genetic alterations in oncogenes and tumor-suppressor genes, which leads to uncontrolled cell growth. It is now appreciated that epigenetic alterations contribute to carcinogenesis and a mechanistic link exists between environmental and dietary exposures and disease. Despite the rapidly developing breadth of

knowledge in this field, many questions remain to understand the contribution of epigenetics to the carcinogenic process. Do environmental toxicants induce epigenetic changes to influence the initiation or promotion of cancer? Can epigenetic changes be initiators in the carcinogenic process, or are they a consequence of cellular transformation and genetic alterations? Most important, because epigenetic changes are largely thought to be reversible, can epigenetic therapy offer an avenue for cancer treatment? In this review, we will describe epigenetic changes in the context of carcinogenesis and offer examples of models of cancer progression and treatment that allow for the elucidation of the role epigenetics plays in cancer progression and treatment. First, we will introduce principles of epigenetic mechanisms in light of carcinogenesis. Then we will discuss how animal models contribute to our understanding of the contribution of epigenetics to understanding distinct pathways of carcinogenesis.

DNA Methylation

One of the most extensively studied epigenetic mechanisms is the methylation of the fifth carbon of a cytosine nucleotide to create 5-methylcytosine (5mC¹). The methyl group of 5mC lies in the major groove of the double helix and can interfere with transcription factor binding to prevent gene expression. Additionally, there is a class of methylated DNA-binding proteins, specifically MECP2 and the MBD family of proteins, which bind to methylated cytosines and repress gene transcription by blocking transcription factors. Cytosine pairs with guanine by means of a phosphate group, and this dinucleotide (CpG) has been a major focus of epigenetic research because of its capacity to directly silence gene expression, particularly with respect to tumor-suppressor genes in carcinogenesis. CpG sites are unevenly distributed throughout the genome, concentrating in repetitive sequences such as tandem and interspersed repeats, distal gene regulatory regions, and CpG islands (Bird 2002; Ehrlich 2009; Ehrlich et al. 1982). DNA methylation is highly dysregulated in cancer. Aberrant patterns of methylation arise, leading to hypomethylation of distal regulatory regions and repetitive elements along with hypermethylation of CpG islands

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¹Abbreviations that appear $\geq 3x$ throughout this article: 5mC, 5-methylcytosine; DNMT, DNA methyltransferase; HDAC, histone deacetylase.

(Bird 2002; Ehrlich 2009). It has been known for some time that tumors from different sites display distinct CpG methylation profiles (Esteller et al. 2001) and exhibit distinct pathways of carcinogenesis within tumor sites (Sartor et al. 2011; Shen et al. 2007). However, how CpG methylation relates to epidemiologic and clinical characteristics is not yet fully understood.

Loss of DNA methylation was one of the first epigenetic changes described in human cancer. The first study of DNA methylation in human tumor tissue, using methylation-sensitive restriction enzyme digestion paired with Southern blotting, revealed that tumor tissues had a lower proportion of methylated cytosine than normal tissues (Feinberg and Vogelstein 1983). Shortly thereafter, whole genome enzymatic digests paired with high-performance liquid chromatography were used to show that overall 5mC content was inversely associated with tumor progression (Gama-Sosa et al. 1983). Since the publication of these seminal studies, almost every type of cancer has been shown to have an overall deficiency of 5mC compared with normal tissue, occurring specifically in intergenic repetitive regions, which increases genomic instability and promotes the progression of tumorigenesis.

Repetitive Elements

Repetitive elements make up about half of the genome and are normally heavily methylated. In cancer, hypomethylation of these genomic regions make up a large percentage of 5mC loss in cancers (Ehrlich 2009; Lander et al. 2001). Centromeric tandem repeats, adjacent-centromeric (juxtacentromeric) tandem repeats, and short- (Alu) and long-interspersed elements (LINE-1) are the most frequently studied repetitive elements in cancer that are found to be hypomethylated. Tandem repeats at and near the centromere play a role in keeping the DNA packaged into heterochromatin at the point of sister chromatid association, allowing for chromosome stability. Hypomethylation of these regions can lead to chromatin decondensation and chromosome rearrangements through unstable translocations, leading to widespread genomic instability (Eden et al. 2003). For example, *in vitro* experiments conducted to knock out *Dnmt1*, a DNA methyltransferase (DNMT¹), in murine embryonic stem cells showed an increase in chromosomal translocations (Chen et al. 1998). Additionally, loss of heterochromatin can affect the copy number of genes involved in tumorigenesis (Eden et al. 2003; Ehrlich 2009; Kokalj-Vokac et al. 1993). Hypomethylation of tandem repeats at or near centromeric regions contributes to tumorigenesis by unraveling the structure of the genome and amplifying genomic rearrangements (Kokalj-Vokac et al. 1993). However, chromosomal abnormalities are not the only process that occurs in tumor cells, and this is signified by other repetitive elements that are found to be hypomethylated in cancer.

Alu and LINE-1 elements are retrotransposons—that is, genetic elements that have the ability to amplify themselves by means of RNA intermediates. These elements together

make up about 30% of the genome (Chen et al. 1998). There are more than 500,000 LINE-1 elements in the genome, although because of truncations, mutations, and deletions, only about 100 copies are functional. There are more than 1 million copies of Alu (Batzer and Deininger 2002). Both elements contain promoter sequences, which indicates their capacity for gene transcription if unregulated (Cordaux and Batzer 2009; Kazazian and Goodier 2002). In normal tissues, LINE-1 and Alu elements are silenced through DNA methylation; these elements are hypomethylated in cancer (Bird 2002; Thayer 1993). For example, it has been shown that hypomethylation of LINE-1 elements occurs in colorectal cancer early in tumorigenesis, disrupting normal patterns of gene expression (Suter et al. 2004). Hypomethylation of LINE-1 sequences has also been shown in urothelial and hepatocellular cancers (Jurgens et al. 1996; Takai et al. 2000). Alu elements, although less studied, have been shown to be hypomethylated with LINE-1 elements in prostate adenocarcinomas (Cho et al. 2007), pancreatic endocrine tumors, and carcinoid tumors (Choi et al. 2007). Hypomethylation of LINE-1 and Alu elements was found to be strongly linked to genomic instability early in non-small-cell lung cancer, playing a potential role in the formation of lung neoplasias (Daskalos et al. 2009).

Hypomethylation of these elements and their consequent activation has many implications for tumorigenesis. They can cause insertional mutagenesis and potentially disperse processed pseudogenes, which occur when spliced messenger RNA is reverse transcribed by L1 reverse transcriptase and reinserted into the genome. Transduction can occur when LINE-1 elements mobilize their 3' and 5' ends separately and carry them to new genomic locations. Rearrangements also take place when Alu and LINE-1 elements insert to potentially cause deletions or inversions in the genome (Kazazian 2004; Kazazian and Goodier 2002). This results in chromosomal abnormalities, aberrant gene expression, and overall genomic instability.

Other targets of hypomethylation are the CpG sites found in promoter regions that are outside CpG islands. These are found in promoter regions of normally repressed genes and are methylated in normal cells (Bird 2002; Ehrlich 2002, 2009). In cancer cells, these regions are found to be hypomethylated, affecting repression of normally silenced genes. For example, imprinted genes are normally monoallelically expressed; however, hypomethylation of CpG sites in promoter regions of these genes leads to their biallelic expression and is linked to carcinogenesis (Holm et al. 2005). Hypomethylation of promoter regions leads to activation of otherwise silenced genes, promoting aberrant gene expression, disruption of normal cellular processes, and overall genomic instability.

DNA Methyltransferases

Although cancer genomes are globally hypomethylated, some regions of the genome are found to be hypermethylated. The

mechanism through which this occurs is DNMT overexpression. DNA methylation is regulated by DNMTs that act as the methyl donors to the cytosine residue. Although five members of the DNMT family have been discovered, only DNMT1, DNMT3a, and DNMT3b are known to contribute to the global pattern of cytosine methylation (Kulis and Esteller 2010; Okano et al. 1999). DNMT1 is classified as a maintenance protein and appears to be involved in methylation of CpG sites in newly synthesized daughter DNA strands to match the methylation pattern of the parental strand. It also directly binds histone deacetylases to promote heterochromatin formation and silence gene activity (Bird 2002; Kulis and Esteller 2010; Li et al. 1992). DNMT3a and DNMT3b are classified as de novo enzymes that are essential for establishment of mammalian development methylation patterns during embryogenesis and germ-cell development (Kulis and Esteller 2010).

DNMT overexpression seems to be a common characteristic of tumors, although only DNMT1 and DNMT3a/b are implicated in tumorigenesis (Issa et al. 1993). It has been proposed that these enzymes cooperate to initiate and maintain de novo methylation in cancer cells (Rhee et al. 2002). DNMT1 and DNMT3b have been shown to form a complex with oncogenic transcription factors to induce de novo methylation of CpG islands in promoter regions (Di Croce et al. 2002). Patients with DNMT3a mutations had significantly worse prognosis in acute myeloid leukemia (Ley et al. 2010). Therefore, DNMTs in cancer have a crucial role in the hypermethylation that is found on CpG islands and its subsequent downstream effects.

The genomic regions that are targeted for hypermethylation tend to be CpG islands. Contrary to individual CpG sites throughout the genome in intergenic regions, CpG islands are usually unmethylated in normal cells, regardless of gene expression (Jones and Laird 1999). However, there are very specific instances in which CpG islands are methylated in normal cells, such as in imprinted genes and X-chromosome inactivation.

CpG Islands and Gene Expression

CpG islands occupy approximately 60% of human gene promoters, most of which are constitutively expressed genes (Vu et al. 2000). A CpG island is generally defined as a 1000-kb stretch of DNA with GC content greater than 50%. The normal hypomethylated pattern of CpG islands is found to be consistent across various types of somatic tissues despite tissue-specific differences, illustrating that DNA methylation of these islands is not used as a regulatory mechanism of gene expression (Cotton et al. 2011). Therefore, when a CpG island becomes aberrantly methylated, it can have detrimental effects by stably silencing the associated gene (Cotton et al. 2011). The cancer cell genome is characterized by hypermethylation of CpG islands in promoter regions (Edwards and Ferguson-Smith 2007; Jones and Laird 1999; Meehan et al. 1992; Riggs and Pfeifer 1992). In contrast with hypomethylation of intergenic

CpG sites in cancer that lead to genomic instability, hypermethylation of CpG islands promotes the progression of tumorigenesis by silencing tumor-suppressor genes. For example, PTEN, a protein that prevents rapid proliferation, is commonly hypermethylated in brain and thyroid cancers, whereas APC, a protein involved in cell-cycle regulation, cell-cell adhesion, and cell mobility, is inactivated by hypermethylation in many lung, breast, and colorectal cancers (Fan and Zhang 2009; Hatzia Apostolou and Iliopoulos 2011; Illingworth and Bird 2009). Suppression of p16, a cell-cycle regulator, occurs in essentially all common human cancers (Liggett and Sidransky 1998). Inactivating these tumor suppressors directly promotes tumorigenesis due to lack of control over cellular processes.

In addition to tumor-suppressor genes, hypermethylation of other classes of genes such as DNA repair genes and transcription factors can indirectly lead to tumorigenesis through silencing of further downstream targets or accumulation of genetic errors. For example, GATA-4 and GATA-5 are transcription factors silenced in colorectal and gastric cancers (Alvarez-Nunez et al. 2006). Inactivation of DNA repair genes, such as O-6-methylguanine-DNMT, is commonly found in primary neoplasias (Esteller et al. 2000). Therefore, hypermethylation of CpG islands in cancers can affect multiple pathways to promote carcinogenesis.

Promoter hypermethylation is often an early event in tumorigenesis. Several mechanisms have been proposed for targeting CpG islands for hypermethylation. One explanation is that the location of these islands in genomic regions that have potentially undergone massive epigenetic reprogramming leads to hypermethylation as a byproduct or for prevention of error (Bird 2002). Another explanation is that some gene promoters are targeted specifically by DNMTs complexed to oncogenic transcription factors (Okano et al. 1999). Finally, it has been proposed that hypermethylation is a result of histone marks created in a tumor-specific manner (Hatzia Apostolou and Iliopoulos 2011).

Although it may appear that hypomethylation and hypermethylation in cancer are opposing forces, the patterns usually coexist within the same tumor, although they occur in different genomic regions. Further, the epigenetic abnormalities that occur because of hypo- and hypermethylation can interact in various ways to produce distinct subtypes of cancer. However, these patterns are stable but not irreversible and remain flexible as the cellular environment changes, contributing to the complexity of the cancer cell epigenome.

The dysregulation of DNA methylation patterns observed in cancer does not occur independent of other epigenetic changes. Methylated DNA-binding proteins, which are attracted to methylated cytosine residues and contribute in gene silencing, have been shown to interact with a number of other partners involved in epigenetic regulation. In particular, methylated DNA-binding proteins have been shown to interact with proteins that are involved in controlling the interaction between DNA and histones, the proteins involved in DNA packaging.

Chromatin Remodeling in Cancer

The estimated 1.8 linear meters of DNA in the human cell are organized into a 3-dimensional structure and compacted within the cell nucleus by means of associations with histones, the major DNA packaging proteins. These DNA–histone complexes are the primary components of chromatin, which makes up the bulk of the material in the nucleus. The basic chromatin unit is the nucleosome, which consists of a protein octamer containing pairs of each of the four core histone proteins (H2A, H2B, H3, H4). Nucleosome structures are highly conserved and repetitive throughout the genome, forming a “beads-on-a-string” structure. Nucleosomes are organized by histone protein H1, a linker protein found outside the main histone octamer complex that binds to linker DNA at the entry and exit points of the nucleosome (Allan et al. 1980).

There are two common higher levels of nucleosome organization that are defined by the level of compaction of the nucleosome structures euchromatin and heterochromatin. Euchromatin is loosely packed and typically represents transcriptionally active genic regions due to the increased accessibility of the DNA in the nucleosome structure. Heterochromatin is densely packed, with intense cytological nuclear staining due to the high density of nuclear proteins. Heterochromatin is further classified into constitutive heterochromatin, or permanently silenced chromatin, and facultative heterochromatin, which is silenced chromatin that can become reactivated in response to appropriate genetic or environmental cues. Thus, throughout an organism’s lifetime, chromatin conformation is a fluid, cell type–specific process, and it is prone to restructuring in response to environmental or physiologic signals. Altered or abnormal chromatin conformation has also now been recognized as an epigenetic hallmark of many cancers.

Chromatin conformation is controlled by chemical modifications, mainly covalent modifications, of the *N*-terminus tails of the histone proteins that form the core of the nucleosome. Histone modifications can affect the interaction between histone proteins and DNA as well as between adjacent histone proteins. Histone modification is a dynamic process, with enzymes catalyzing the addition of covalent modifications (“writers”), their removal (“erasers”), and recognition of marks previously laid down (“readers”) (Wang et al. 2007). Dysregulation of each of these classes of enzymes has been associated with a variety of cancer types. Here, we will detail the functional consequences of aberrant control of these enzymes during the carcinogenic process for histone methylation and acetylation, the two best-characterized histone modifications.

Histone Methylation

Histone methylation has been widely shown to regulate transcription; methylation at specific histone tail residues is associated with both transcriptional activation and repression.

Histone methylation occurs at both arginine and lysine residues on the tails of histone proteins H3 and H4. A summary of enzymes that modify or read histone methylation marks that have been shown to be dysregulated in cancer is shown in Table 1. Lysine methylation is catalyzed by histone-lysine-*N*-methyltransferases, also known as K-methyltransferases, and involves the transfer of methyl groups from the cofactor S-adenosyl methionine. A key protein involved in control of stem cell maintenance and differentiation, EZH2 (enhancer of Zeste 2), is a K-methyltransferase that catalyzes the trimethylation of H3K27 (Cao et al. 2002). EZH2 is a member of the polycomb repressive complex 2, a protein complex that involves both a K-methyltransferase and “reader” proteins that recognize H3K27me3. The H3K27me3 mark is normally involved in silencing genes related to development and stem cell differentiation, including the Hox gene cluster (Lewis 1978). In many cancers, however, EZH2 is overexpressed both at the transcriptional and protein levels. EZH2 overexpression has been described as important in prostate cancer, where an increase in EZH2 protein staining in the cell nucleus was observed with a progression from benign to metastatic disease (Varambally et al. 2002). Further studies have identified overexpression of EZH2 as a key feature in breast cancer, lymphomas, and glioblastomas, among other cancers (Kleer et al. 2003; Suvà et al. 2009; van Kemenade et al. 2001). In cancer cells, H3K27me3 has also been shown to repress gene expression independent of gene-promoter DNA methylation (Kondo et al. 2008), whereas in normal cells, EZH2 has been shown to control DNA methylation by interacting with DNMTs (Vire et al. 2006). Additionally, dysregulation of other members of the polycomb repressive complex, including proteins that interact with polycomb repressive complex 2 proteins following the transfer of the H3K27me3 mark by EZH2, have also been recently described. In contrast with the silencing histone modification H3K27me3, histone methylation can also be a mark associated with transcriptional activation. The mixed lineage leukemia (MLL) is a K-methyltransferase that catalyzes the methylation of H3K4. MLL acts in opposition to polycomb repressive complex proteins, activating genes involved in development and differentiation (Milne et al. 2002). MLL genetic events, particularly gene fusions and overamplification, have been shown to be an important characteristic of leukemia. An experimental mouse model with an *MLL–AF9* gene fusion introduced by homologous recombination led to the development of acute leukemia in all chimeric mice (Corral et al. 1996). A study of acute lymphoblastic leukemia patients with MLL translocations found a unique gene expression profile when compared with patients with conventional B-precursor acute lymphoblastic leukemia (Armstrong et al. 2002). Specifically, patients with MLL translocations were found to have multilineage gene expression, aberrantly overexpressing genes associated with early-stage hematopoiesis.

Histone methylation marks are removed by a variety of enzymes, with marks at specific histone tail residues interacting with distinct histone lysine demethylases, or K-demethylases. JMJD2C is a K-demethylase that catalyzes

Table 1 Examples of histone methylation dysregulation in cancer

Histone-modifying enzyme	Target modification	Cellular function/related cancers	References
Lysine methyltransferases (KMTs)			
MLL	H3K4	Transcriptional activation; gene fusions identified in leukemia	Armstrong et al. 2002; Corral et al. 1996
SETDB1	H3K9	Transcriptional repression; amplified in melanoma	Ceol et al. 2011
EZH2	H3K27	Transcriptional repression; associated with tumor aggressiveness Upregulated in breast cancer, prostate cancer, lymphoma, glioblastoma	Kleer et al. 2003; Suvà et al. 2009; van Kemenade et al. 2001; Varambally et al. 2002
NSD1	H3K36, H4K20	Transcriptional activation; gene fusions in leukemia, multiple myeloma	Taketani et al. 2009; Wang et al. 2007
DOT1	H3K79	DNA damage repair; involved in leukemia	Chang et al. 2010; Okada et al. 2005; Tatum and Li 2011
Lysine demethylases (KDMs)			
LSD1	H3K4, H3K9	Transcriptional repression; dysregulated in breast cancer, upregulated in aggressive prostate cancer	Kahl et al. 2006; Lim et al. 2010; Wang et al. 2009
JMJD2C	H3K9	Transcriptional activation; rearranged in lymphoma, amplified in breast cancer and esophageal cancer	Liu et al. 2009; Vinatzer et al. 2008; Yang et al. 2000
JMJD3	H3K27	Transcriptional activation; upregulated in aggressive prostate cancer	Xiang et al. 2007
Lysine methylation readers			
ING4	H3K4	Tumor suppressor; deleted in head and neck cancer and breast cancer, reduced expression in glioma	Garkavtsev et al. 2004; Gunduz et al. 2005; Kim, Chin, et al. 2004; Tapia et al. 2011
BMI-1	H3K27	Oncogene; overexpressed in lymphoma, leukemia, colorectal and breast cancer	Beà et al. 2001; Kim, Yoon, Kim, et al. 2004; Kim, Yoon, Jeong, et al. 2004; Lessard et al. 2003; Pietersen et al. 2008

the removal of methylation marks from H3K9, a mark typically associated with gene repression (Snowden et al. 2002). Amplification of JMJD2C has been observed in a variety of cancers, including breast and esophageal cancer (Liu et al. 2009; Yang et al. 2000). Lysine specific demethylase 1, a K-demethylase that targets H3K9 and H3K4 methylation, has recently shown to be overexpressed in estrogen receptor-negative breast cancer (Lim et al. 2010), mesenchymal tumors (Schildhaus et al. 2011), and bladder cancers (Hayami et al. 2011). Although more research is necessary to fully understand the functional consequences of dysregulation of histone methylation, it is clear that K-demethylases and K-methyltransferases are important in the carcinogenic process and represent novel targets for therapy.

Histone Acetylation

Unlike histone methylation, which can be associated with transcriptional activation or repression based on the specific residue methylated, histone acetylation is strongly associated with transcriptional activation. Histone acetylation occurs on lysine residues and is thought to enhance transcription by charge neutralization of the positively charged histones, decreasing their interaction with the negatively charged DNA phosphate backbone. Maintenance of histone acetylation marks and the dynamic state of chromatin conformation are controlled by histone acetyltransferases (HATs), also known as K-acetyltransferases, and histone deacetylases (HDACs¹). HATs catalyze the addition of acetyl groups to histone lysines

using acetyl coenzyme A as a cofactor and induce an open or permissive chromatin state, whereas HDACs remove acetyl groups and induce a closed or repressive state (Roth et al. 2001). The normal *in vivo* role of HATs and HDACs is often obfuscated in cancer, leading to an abnormal chromatin phenotype.

There are three distinct families of HATs: The Gcn5 family, the p300/CBP family, and the MYST family (Lee and Workman 2007). HATs from each of these families have been shown to play a role in carcinogenesis, from either inappropriate activation or repression of target gene activity. The Wnt signaling pathway, previously shown to be commonly dysregulated in cancers, particularly those with a stem cell phenotype, has been shown to be augmented by the HAT Gcn5 in breast cancer (Chen et al. 2010). CBP (cyclic AMP response element-binding [CREB] protein) and p300, have been shown to be capable of acetylation of all four core histones as well as a number of other nonhistone proteins, including p53, Rb, E2F, and myb (Iyer et al. 2004). Loss of heterozygosity at either p300 or CBP has been detected in a large proportion of cancer cell lines examined, with 51% of cell lines experiencing loss at p300 and 35% experiencing loss at CBP (Tillinghast et al. 2003). These findings suggest that both p300 and CBP are important tumor-suppressor genes that may be lost through loss of heterozygosity in a number of different cancers. MYST family HATs have been identified as important in hematopoiesis and, as such, also identified as dysregulated in acute myeloid leukemia (Yang and Ullah 2007). In the M4/M5 subset of leukemia cases, a stable and recurrent translocation t(8;16)(p11;p13) causes a fusion between MOZ, a MYST family acetyltransferase, and CBP, leading to aberrant chromatin acetylation (Borrow et al. 1996). Similarly, MOZ is found fused to p300 following a t(8;22)(p11;q13) translocation observed in a subset of acute monocytic leukemia cases (Chaffanet et al. 2000).

HDACs are enzymes that catalyze the removal of histone acetyl marks and are involved in transcriptional repression. HDACs, like HATs, also have nonhistone proteins as potential substrates and are involved in the deacetylation of a number of proteins identified as important in carcinogenesis, including p53, YY1, and STAT3 (Glozak et al. 2005). The 18 human proteins identified with HDAC activity suggest that there is likely some redundancy in function between HDACs as well as the potential for different histone tail residues or other nonhistone proteins as targets.

Studies of multistage models of carcinogenesis have identified histone deacetylation as an early step in the process (Fraga et al. 2005). Specifically, early loss of monoacetylation of histone H4K16 was observed in a mouse model of multistage skin carcinogenesis. Additionally, a number of cancer cell lines, as well as primary lymphomas and colorectal adenomas, were also found to be hypoacetylated compared with normal cells, suggesting that histone deacetylation is a widespread event in cancer. HDACs are often overexpressed in many different tumor types, including breast (Krusche et al. 2005), prostate (Weichert, Röske, Niesporek, et al. 2008), and colorectal cancer (Weichert, Röske, Gekeler, et al.

2008). A study of the function of HDAC3, a class 1 HDAC, in cancer cells, found that long term knockdown by means of RNA interference led to inhibition of β -catenin's translocation to the nucleus (Godman et al. 2008). In addition to disrupting Wnt signaling, HDAC3 inhibition also increased expression of the vitamin D receptor, rendering those cells more sensitive to the effects of vitamin D. The common pattern of HDAC deregulation in cancer cells has provided a novel target for chemotherapeutic intervention—the HDAC inhibitor. HDAC inhibitors, both natural and synthetic, have been widely used in the treatment of a number of diseases, including psychiatric diseases and cancer. There are two HDAC inhibitors currently approved by the US Food and Drug Administration for the treatment of cutaneous T-cell lymphoma—suberoylanilide hydroxamic acid (vorinostat) and romidepsin. Additionally, there are a number of other HDAC inhibitors under investigation in early- and late-stage clinical trials, which may provide novel epigenetic therapies for cancer treatment.

Animal Models of Carcinogenesis

Findings from *in vivo* models of carcinogenesis can be used to predict how the most susceptible humans in the population may respond to genetic lesions or exposure to environmental carcinogens. Additionally, these studies can identify epigenetic biomarkers and provide insight into the specific mechanisms of tumor progression.

There are a number of *in vivo* models of carcinogenesis that allow for the characterization of epigenetic mechanisms that link environmental exposures or genetic susceptibility and cancer progression. These models typically involve the induction of tissue-specific cancer through toxicant exposure or transgenic manipulation. A carefully designed animal model can specifically characterize molecular pathways of carcinogenesis, providing evidence for a sequential series of epigenetic and genetic effects as a malignancy progresses from carcinoma *in situ* to metastatic disease. Often these models are particularly useful for elucidating the contribution of epigenetic dysregulation of specific pathways in carcinogenesis in a temporal fashion. Lung cancer is an example of a cancer where epidemiologic studies have identified relevant exposures, but the early events in carcinogenesis are not well characterized (Betancourt et al. 2010; Jenkins et al. 2009). Exposure to 3-methylcholanthrene and diethylnitrosamine has been known for at least two decades to induce lung tumors in animal models (Henry et al. 1981; Schuller et al. 1988). These models have proven useful to understand the basic processes that underlie neoplastic lung adenocarcinoma initiation and progression. More recently, researchers have extended the use of these lung carcinogenesis models to understand the specific epigenetic mechanisms involved in lung cancer progression, including increases in promoter methylation of the cell-cycle regulator genes *p27* and *p57* (Liu et al. 2010). Epidemiologic studies have consistently identified inflammation as an important initiator and promoter

of lung carcinogenesis, but human studies are limited in studying the sequential molecular events that characterize this pathway. Blanco and colleagues (2007) used a silica exposure-based inflammatory in vivo model of lung carcinogenesis to study the role of inflammation in lung carcinogenesis. They identified multiple epigenetic alterations, particularly methylation of the cell-cycle control proteins *p16*, *APC*, and *Cdh13*, during tumor progression that characterize lung cancers arising from this pathway. Similarly, chronic inflammation is hypothesized to drive somatic mutation and neoplastic transformation in prostate cancer. A study of prostate carcinogenesis using transgenic adenocarcinoma of mouse prostate (TRAMP) mice identified that expression of the oxidative stress-sensing enzyme Nrf2 is suppressed by DNA methylation and chromatin silencing in prostate cancer (Yu et al. 2010). There are also a number of in vivo models of carcinogenesis that focus on the progression of hematological malignancies, such as leukemias and lymphomas. A mouse model of acute lymphoblastic leukemia, *Il15* transgenic FVB/NJ mice, was used to identify epigenetic alterations specific to leukemia progression, finding that the putative tumor-suppressor gene *Irb4* was epigenetically silenced in both mouse and human leukemias but not in solid tumors (Yu et al. 2005). Epigenetic alterations, particularly the DNA methylation silencing of tumor-suppressor genes *Pten* and *p53*, after overexpression of the oncogene *MYC* were found using a bitransgenic mouse model of T cell lymphoma (Opavsky et al. 2007).

The elucidation of epigenetic mechanisms through animal models has given rise to therapeutic interventions in the treatment of carcinogenesis. Because methylation of tumor-suppressor genes is a common characteristic of tumorigenesis, demethylating agents such as 5-azacytidine, decitabine, and zebularine have been studied in various animal models. Mice induced to develop oral cavity carcinogenesis were treated with 5-azacytidine and exhibited reduced lesions compared with untreated mice (Tang et al. 2009). Zebularine administered to BALB/c nu/nu mice with human bladder carcinoma xenografts significantly reduced tumor size through demethylation of the p16 promoter (Cheng et al. 2003). Aside from methylation, histone acetylation also occurs early in carcinogenesis, and as such, HDAC inhibitors have been considered as treatment options. Bachmann and colleagues (2010) used the acute lymphoblastic leukemia xenograft SCID mouse model to find that vorinostat, an HDAC inhibitor, reinstated gene expression of *BIM*, a tumor suppressor, which is silenced in lymphoid malignancies. Valproic acid, another HDAC inhibitor, was found to induce histone hyperacetylation and inhibit angiogenesis, resulting in prolonged survival of orthotopic xenograft mouse models of medulloblastoma (Zhang et al. 2011). Although therapeutic effects have been ascertained from administering demethylating agents and HDAC inhibitors alone, combination treatments of epigenetic therapies with either chemotherapeutic drugs or each other have been found to be most effective in stimulating synergistic antitumor activity (Fang et al. 2010; Gagnon et al. 2003; Gollob et al. 2006; Lemaire et al. 2009;

Plumb et al. 2000; Venturelli et al. 2007). Drug-resistant ovarian and colon tumor xenograft mouse models treated with decitabine increased hMLH1 expression through promoter demethylation. Although this did not affect tumor growth, the treatment sensitized the xenografts to cisplatin and other chemotherapeutic drugs, increasing their efficacy (Plumb et al. 2000). Xenograft hepatoma models were used to examine the effects of azacytidine and an HDAC inhibitor when administered alone or in combination. It was found that the combination therapy generated significant antitumor effects compared with each agent administered alone (Venturelli et al. 2007). The potential of combination therapy with epigenetic agents has been established through the use of in vivo models, and many demethylating agents and HDAC inhibitors are currently in or have completed clinical trials for human use (Amatori et al. 2010; Fang et al. 2010; Gollob et al. 2006).

Conclusions

The greatest challenge to cancer researchers is the integration of human and animal data to realize the translational potential of findings. Although epidemiologic studies have identified dietary and environmental factors as associated with risk of cancer, animal models can identify the mechanisms and temporal relationship between these factors and carcinogenesis. Translational research that integrates results from human and animal studies will provide insight into the temporal nature of epigenetic dysregulation during tumor initiation and progression and into how environmental and dietary exposures influence the epigenetic phenotype of a tumor. Additionally, these studies will determine which cancer subtypes are susceptible to chemotherapy that influences epigenetic state, including DNA demethylating agents, HDAC inhibitors, or a number of the other promising epigenetic therapies currently in preclinical and clinical trials. Molecular characterization of tumor progression, including genetic and epigenetic profiles, is a key step in the development of individualized treatment modalities and personalized cancer therapy.

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