DNA Methylation and Demethylation as Targets for Anticancer Therapy

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Abstract—Cancer growth and metastasis require the coordinate change in gene expression of different sets of genes. While genetic alterations can account for some of these changes, it is becoming evident that many of the changes in gene expression observed are caused by epigenetic modifications. The epigenome consists of the chromatin and its modifications, the “histone code” as well as the pattern of distribution of covalent modifications of cytosines residing in the dinucleotide sequence CG by methylation. Although hypermethylation of tumor suppressor genes has attracted a significant amount of attention and inhibitors of DNA methylation were shown to activate methylated tumor suppressor genes and inhibit tumor growth, demethylation of critical genes plays a critical role in cancer as well. This review discusses the emerging role of demethylation in activation of pro-metastatic genes and the potential therapeutic implications of the demethylation machinery in metastasis.

Key words: DNA methylation, DNA demethylation, DNA methyltransferase (DNMT), DNA demethylase, MeCP2, MBD2, histone acetylation, epigenetics, epigenome, histone deacetylase (HDAC), TSA, HDAC inhibitors, metastasis

The development of cancer involves the concurrent disruption of regulation of expression of multiple genes. This switch in gene expression first allows the cancer cell to override normal breaks on uncontrolled growth and at a later stage enables cell invasion and motility, which allows the cancer to metastasize to distal organs. Such a concerted change in regulation of expression of numerous genes must involve some basic processes responsible for the normal programming of gene expression. Since the genome contains all the potential information required to manage a living organism but only a fraction of this information is active at different time points and in different cell types and individual cells, this information must be regulated.

The epigenome is responsible for regulation of the genome. In contrast to the genome, which is identical in different cell types and through life, the epigenome is dynamic and varies from cell type to cell type and from time point to time point in life. It is responsive to developmental, physiological, environmental, and pathological signals and confers cell type and temporal identities of gene expression programs. The epigenome consists of the chromatin and its modifications [1, 2] as well as a covalent modification of cytosine rings found at the dinucleotide sequence CG [3].

The basic building block of chromatin is the nucleosome, which is formed of an octamer of histone proteins containing a H3-H4 tetramer flanked on either side with a H2A-H2B dimer [4]. The N-terminal tails of these histones are extensively modified by methylation [5, 6], phosphorylation, and acetylation [7, 8]. Different histone variants also play a regulatory role [9]. The pattern of histone modification creates a “histone code”, which dictates, which parts of the genome are expressed at a given point in time [2].

Specific transcription factors and transcription repressors recruit histone-modifying enzymes to specific genes and thus define the profile of histone modification around genes [10]. The best-studied examples are histone acetyltransferases (HAT), which acetylate histone H3 and H4 tails at the K9 residue, and histone deacetylases (HDAC), which deacetylate histone tails [11].

Histone acetylation is believed to be a predominant signal for an active chromatin configuration. Deacetylated histones signal inactive chromatin. Many repressors and repressor complexes recruit HDACs to genes, thus causing their inactivation. Histone tail acetylation is believed to enhance the accessibility of a gene to the transcription machinery, whereas deacetylated tails are tightly interacting with DNA and limit its accessibili-
Histone methylation at K9 of their N-terminal tail signals inactivity and is also determined by the recruitment of histone methyltransferases such as SUV39 to genes [12]. The heterochromatin protein HP-1, which binds methylated histones and precipitates an inactive chromatin structure [12], recognizes the methylated histones. Chromatin remodeling complexes, which are ATP dependent, alter the position of nucleosomes around the transcription initiation site and define its accessibility to the transcription machinery [13]. The different combinations of these changes determine the diverse programs of gene expression. Chromatin is dynamic and plastic and could respond by altered recruitment of the different histone modification enzymes in response to different signals. Nevertheless, the majority of epigenomic programs is laid down during development and remains relatively stable for the entire life span of an organism. Some parts of the epigenome such as heterochromatin are particularly stably repressed [6].

In addition to chromatin, which is associated with DNA, the DNA molecule itself is chemically modified by methyl residues at the 5’ position of the cytosine rings in the sequence CG in vertebrates [14]. What distinguishes DNA methylation in vertebrate genomes is the fact that not all CGs are methylated in any given cell type [3]. Different CGs are methylated in different cell types, generating cell type specific patterns of methylation.

Thus, the DNA methylation pattern confers upon a genome itself its cell type identity. Since DNA methylation is part of the chemical structure of the DNA itself, it remains long after all other proteins and epigenomic marks are degraded, and thus it has extremely important diagnostic potential [15, 16].

It was originally believed that the DNA methylation pattern is established during development and is then maintained faithfully through life by the maintenance DNA methyltransferase [3]. The DNA methylation reaction was believed to be irreversible, thus the only way methyl residues could be lost was believed to be through replication in the absence of DNA methyltransferase (DNMT) by passive demethylation [14]. This mechanism is not applicable to postmitotic tissue such as neurons in the brain. However, I will propose here based on our own data and data of others that the DNA methylation pattern is dynamic and is an equilibrium of methylation and demethylation reactions [17].

We have proposed that DNA methylation is a reversible signal like any other biological signal and could therefore potentially change in response to environmental and physiological signals [18]. A hallmark of DNA methylation patterns is the correlation between chromatin and the DNA methylation pattern. Active chromatin is usually associated with unmethylated DNA while inactive chromatin is associated with methylated DNA [19]. This linkage between DNA methylation and chromatin structure has important implications for our understanding of the function of DNA methylation as well as the processes responsible for generating, maintaining, and altering DNA methylation patterns under physiological and pathological conditions. It was originally believed that DNA methylation precedes and is dominant over chromatin structure changes. Methylation was believed to be generated independently of chromatin structure. Methylated DNA attracts methylated DNA binding proteins, which recruit repressor complexes containing histone deacetylases, which results in inactive chromatin [20, 21]. This model, positioning DNA methylation as driving chromatin inactivation is widespread and has profoundly influenced our understanding of how altered DNA methylation is involved in cancer. Nevertheless, there is currently data suggesting that the state of chromatin structure could also determine DNA methylation and that chromatin can affect DNA methylation in both directions triggering either de novo DNA methylation or demethylation as will be discussed extensively below. This data forces us to revisit the classic model of a DNA methylation pattern which is predetermined during development and is then maintained through life and adopt a more dynamic view of the DNA methylation pattern. This has obviously implications for our understanding of how DNA methylation patterns are altered in cancer and on how to approach the DNA methylation pattern therapeutically. This review will focus on the implication of the new understanding of the dynamic epigenome on our therapeutic approach to cancer.

Since the epigenome is responsible for coordinating gene expression programs and DNA methylation is so tightly linked to chromatin structure and since cancer progression involves concurrent change in expression of numerous genes, it is not surprising that DNA methylation patterns are altered in cancers and that changes in the DNA methylation enzymes are noted as well [22]. Hypermethylation of tumor suppressor genes received considerable attention and there are a number of clinical trials with inhibitors of DNMT aiming at demethylating these genes resulting in their activation and arrest of cell growth [23]. Nevertheless, a hallmark of cancer cells is also global hypomethylation [24], which has received very limited attention. However, recent studies point to a role for hypomethylation in activating genes required for metastasis [25].

This review will discuss the notion that demethylase(s), which catalyze replication-independent demethylation, are responsible for demethylation of pro-metastatic genes and that they can be targeted in antimetastasis therapy [25]. An additional implication of the emerging role of hypomethylation is that although DNA methylation inhibitors might be excellent agents to inhibit growth through demethylation of tumor suppressor genes, they might nevertheless also induce pro-metastatic genes.