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Seminars in
HEMATOLOGY

Overview of Cancer Epigenetics

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Epigenetic mechanisms including DNA and histone modifications result in silencing of genes without changing the coding sequence of the gene. Even though these events are heritable, they are potentially reversible, thus opening up opportunities for therapeutic intervention. The importance of epigenetic changes in human cancer is only now being recognized in the medical community. A series of discoveries over the last four decades has thrust epigenetics into the forefront of new drug discoveries. Three systems—DNA methylation, RNA-associated silencing, and histone modification—are used to initiate and sustain epigenetic silencing. Current knowledge suggests that agents that intervene in this process by "turning back on" silenced genes may represent a significant advancement in treating many forms of cancer. In addition, changed patterns of methylation can be detected with a high degree of sensitivity thus providing clinicians with prognostic information.
Semin Hematol 42:S3-S8

The term "epigenetics" refers to all meiotically and mitotically heritable changes in gene expression that are not coded in the DNA sequence. Three systems—DNA methylation, RNA-associated silencing, and histone modification—are used to initiate and sustain epigenetic silencing. There are interactions between these three systems and they may act to stabilize one another. A disruption in one of these systems can lead to inappropriate expression or silencing of genes, resulting in epigenetic diseases such as cancer. A distinguishing feature between epigenetic changes and genetic changes is that the former tend to occur in a gradual rather than abrupt fashion. Even though the events are heritable, they are potentially reversible and thus open up opportunities for therapeutic intervention. However, therapeutic interventions must target the multifaceted changes that are associated with epigenetic disease.

Historical Perspective of Epigenetics

The importance of epigenetic changes in human cancer is only now being recognized in the medical community. A series of discoveries over the last four decades has thrust epigenetics into the forefront of new drug discoveries. Srinivasan and Borek[73] first introduced the hypothesis of methylases as oncogenic agents in 1964. Nearly 15 years later, decreased levels of 5-methylcytosine in animal tumors was reported.[52] The first publication describing the use of DNA methylation inhibitors, 5-azacytidine (azacytidine; Vidaza, Pharmion Corp, Boulder, CO) and 5-aza-2′-deoxycytidine (decitabine; Dacogen™, MGI Pharma, Inc, Bloomington, MN) and their role in gene reactivation appeared a year later.[44] Since then, numerous other landmark discoveries have been reported, including decreased genomic and gene-specific methylation in human tumors,[23,28,29] methylation of a CpG island in cancer,[3] hot spots for p53 mutations at methylated CpG sites,[68] allele-specific methylation of the retinoblastoma tumor-suppressor gene,[72] hypermethylation of CpG islands associated with aging,[38,39] fewer tumors developing in mice with decreased methylation,[51] and DNA repair gene (MLH1) methylation in somatic cells.[33] Other important findings related to DNA methylation inhibitors came in 1984 when Frost et al[30] reported that they alter tumorigenic phenotype and in 2002 when synergistic activity with histone deacetylase inhibitors for rapid isolation of tumor-suppressor genes was published. Finally in 2004, the US Food and Drug Administration approved azacytidine for treatment of myelodysplastic syndromes (MDS) and is currently reviewing the application for decitabine for MDS.

Epigenetic Mechanisms

DNA Methylation

CpG Island Methylation

DNA methylation is one of the most common epigenetic events taking place in the human genome. DNA methylation

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Conflict of Interest Statement: The author is a stockholder and consultant for Epigeneomics AG.

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0037-1963/05/$-see front matter
doi:10.1053/j.seminhematol.2005.05.001

is a complex process where DNA methyltransferases (DNMTs) catalyze the addition of a methyl group to the 5-carbon position of the cytosine. DNA methylation takes place only when a guanine base follows the cytosine, so only the dinucleotide CpG is methylated. CpG dinucleotides are underrepresented in DNA and are not uniformly distributed. They occur about once per 80 dinucleotides. Clusters of CpGs are called CpG islands and are found in association with genes, most often in the promoters and first exons but also in regions toward the 3′ end, and are unmethylated in normal cells.[7] Approximately 60% to 90% of CpG sequences are methylated, while unmethylated CpG dinucleotides are located primarily in the CpG islands.[60] If the CpG island remains unmethylated with an open chromatic configuration in association with hyperacetylated histones, then the gene can be transcribed. CpG island methylation is associated with changes in chromatin structure and subsequent repression of gene transcription. Methylation of CpG promoters prevents transcriptional initiation and ensures the silencing of genes on the inactive X chromosome, imprinted genes, and parasitic DNAs. Gene silencing can spread, a finding developed from studies in the field of heterochromatinization in *Drosophila* and in X-inactivation. Gene silencing is a result of the spreading of methylation, thus is not a single discrete event but is a series of events that begins with a drop in transcription potential and ends with its complete cessation.[78] CpG island methylation also may be detected in normal tissue before the onset of cancer, as in, for example, in nondysplastic tissue in patients with Barrett's esophagus and adenocarcinoma.[16,20]

Molecular mechanisms for the significance of this promoter hypermethylation have been investigated. The presence of the 5′ methyl-cytosine in the DNA helix may prevent binding of transcription factors[40,47] or may bind with less affinity.[40,55,75] Preferential binding of proteins to methylated promoters may prevent binding of transcriptional factors to the sequence. An example of such proteins are the methylcytosine-binding proteins, which bind to DNA at methylated sites and recruit histone deacetylases to methylated DNA in regions of transcriptional silencing.[8,14,43,60] Therefore, DNA methylation enables the conversion of histones and other proteins into a nonacetylated state, which changes the configuration of chromatin to where it is refractory to transcription.

DNA Methyltransferases

The mechanisms by which DNA methylation is regulated or how patterns are established are poorly understood. To date, three DNMTs (DNMT1, DNMT3a, and DNMT3b) have been identified for "de novo" and "maintenance" methylation.[62] No defects in these enzymes have been found in human tumors; however, upregulation of DNMT1 and DNMT3b has been described.[25,57,70] The first DNMT to be discovered was DNMT1, which acts to maintain pre-existing methylation and preferentially acts on hemimethylated DNA, thus copying methylation patterns in newly synthesized DNA strands. DNMT1 can also form a transcriptional repressive complex with histone deacetylase 2 at replication foci.[69] The DNMT3 family of methyltransferases is tissue-specific and responsible for the de novo methylation that occurs during embryonic development.[62] The DNMT3 enzymes add a methyl group to unmethylated CpG base pairs, leading to a hemimethylated then fully methylated CpG. The role of DNMTs may be more complex than originally appreciated. In cell culture models, each DNMT can directly repress transcription in reporter gene systems by interacting with histone deacetylases and by binding with other proteins with transcriptional-repression activities[2,31,32,69,71] (Fig 1). Therefore DNMTs may participate in gene silencing with or without DNA methylation, which raises questions as to what comes first, methylation or gene silencing?

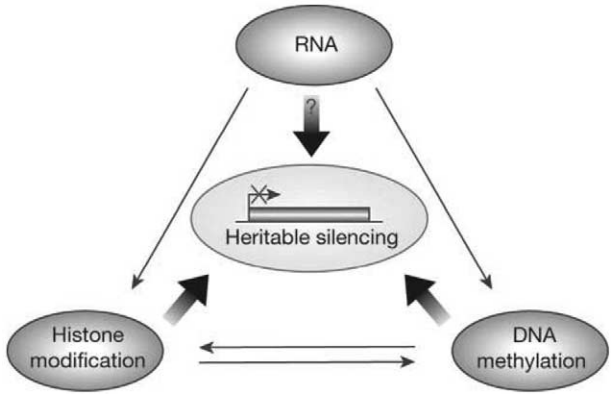

Figure 1 Interaction between RNA, histone modification, and DNA methylation in gene silencing. Histone deacetylation and other modifications cause chromatin condensation and block transcriptional initiation. Histone modification can also attract DNA methyltransferases to initiate cytosine methylation, which can reinforce histone modification patterns conducive to silencing. Experiments in yeast and plants have shown the involvement of RNA interference in the establishment of heterochromatic states and silencing. RNA triggering of heritable quiescence might therefore also be involved in higher organisms. Adapted with permission.[21] © Nature Publishing Group (http://www.nature.com/).

RNA-Associated Silencing

The role of RNA in post-transcriptional silencing has also been studied and can lead to mitotically heritable transcriptional silencing by the formation of heterochromatin. This can occur in the form of antisense transcripts, noncoding RNAs (Xist), or RNA interference (RNAi). RNAi-directed silencing has not been described in mammals; however, antisense RNAs that are involved in silencing of some genes have been reported in mammals.[64] An example is in a case of α-thalassaemia where antisense transcription led to DNA methylation and silencing of a globin gene.[77] RNA modifications may be a trigger for histone modifications and DNA methylation to specific loci.

Histone Modification

Post-transcriptional modifications of histones including acetylation and methylation of conserved lysine residues on the amino-terminal tail domains also have been implicated in