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Breast Cancer Theme Issue

REVIEW

Epigenetic Modifications in Breast Cancer and Their Role in Personalized Medicine

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In cancer, the overall patterns of epigenetic marks are severely distorted from the corresponding normal cell type. It is now well established that these changes can contribute to cancer development through inactivation of tumor suppressor genes and, conversely, through activation of oncogenes. Recent technological advances have enabled epigenome-wide analyses of cancers that are yielding unexpected findings. The study of cancer epigenetics holds great promise for expanding the range of therapeutic opportunities for personalized medicine. Here, we focus on DNA methylation in breast cancer and the potential implications for clinical management of patients. *(Am J Pathol 2013, 183: 1052–1063; http://dx.doi.org/10.1016/j.ajpath.2013.04.033)*

Breast cancer is the most common cancer among women and ranks among the top five leading causes of cancer-related deaths, according to the World Health Organization (*http://www.who.int/mediacentre/factsheets/fs297/en;* reviewed January 1, 2013). Inherited and acquired mutations in genetic material are known to be important contributors to the development of breast cancer. Indeed, family history is the strongest risk factor for developing breast cancer, for which germline mutations in the *BRCA1*, *BRCA2*, and *TP53* (alias *p53*) genes are known to be high-risk factors.[1] Several other inherited mutations and genetic variations have been identified as risk factors confirmed by independent researchers, although their effects are estimated to be either moderate or low.[2] Soon after the discovery that *BRCA1* and *BRCA2* are high-risk breast cancer susceptibility genes, functional studies consistently identified their gene products as components critical to the repair of double-stranded DNA breaks (DSBs).[3] The subsequent hypothesis was that defects in the DNA repair machinery due to mutations in *BRCA1*, *BRCA2*, or other repair genes would accelerate the rate of randomly occurring mutations and that this would, in a step-by-step manner, lead to clonal outgrowth of tumor cells with acquired mutations advantageous to the tumor. This emphasizes the classical view that cancer, including breast cancer, is a genetic disease. However, researchers are increasingly recognizing that epigenetic changes, as well as genetic mutations, are critical contributors to the development of cancer.[4,5]

The body of evidence supporting the involvement of epigenetic changes in promoting cancer development has been growing since the early 1990s, when renal cell carcinomas arising without mutations in the *VHL* gene (alias *VHL1*) were found to have epigenetic inactivation of *VHL*.[6]

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More recent discoveries include that of acquired mutations in *ARID1A* (involved in chromatin remodeling), which occur in approximately half of all ovarian cancers.[7] Indeed, acquired mutations in *ARID1A* have now been found in other types of cancer, including breast cancers.[8] Other examples of mutated epigenetic genes (ie, genes involved in establishing and maintaining epigenetic patterns) include *IDH1* mutations in glioblastoma[9] and *MLL3* or *MLL2* (reclassified as *KMT2* genes) mutations in breast cancer.[10] Recently, targeted inactivation of tumor suppressor genes by epigenetic mechanisms has been found to induce cancer under controlled experimental conditions using human mesenchymal cells.[11] In sporadically arising breast and ovarian cancers, the *BRCA1* gene is recurrently inactivated by epigenetic mechanisms.[12,13] This finding has been confirmed in multiple independent studies and, given that *BRCA1* is a well-known susceptibility gene, the finding strongly supports the hypothesis that epigenetic modifications, as well as genetic mutations, contribute to the development of breast cancer.[14–16] Other examples of epigenetically inactivated DNA repair genes include *MGMT* in glioblastomas,[17] *WRN* in cervical cancer,[18] and *MLH1* in colorectal and endometrial cancers.[19,20] Thus, not only do we now know that somatically acquired mutations arise from the acquired epigenetic repression of DNA repair genes, but also that many epigenetic genes are recurrently and significantly mutated in various cancers. In this review, we focus on DNA methylation in breast cancer and discuss potential implications for clinical practice.

Epigenetic Modifications

The genetic material is organized within the nucleus by the DNA helix wrapping around histone proteins. The structural organization of this DNA–histone complex, known as chromatin, is regulated by epigenetic factors involving DNA methylation and various types of histone marks and noncoding RNAs.[21] The term epigenetics refers to heritable states of gene expression that are not attributed to the DNA sequence. DNA methylation, a well-known epigenetic mark, occurs at cytosine residues where cytosine (C) precedes a guanine (G) residue, known as CpG dinucleotides (where p stands for the phosphodiester bond connecting cytosine and guanine).[22] The distribution of CpGs is not random; genomic regions enriched in CpGs, known as CpG islands, are often found at gene promoter sequences. CpG islands characterize the promoter region of more than half of all genes in the human genome.[23] It is thought that the overall reduction in genomic CpGs has occurred over evolutionary time and that it relates to the spontaneous or enzymatic deamination of methylated cytosine residues in the germline and thereby conversion to thymine. Transcriptionally active genes are depleted in DNA methylation at their gene promoter CpG islands and, in this case, the flanking nucleosomes are often marked with trimethylation at histone H3 on lysine residue K4, known as the H3K4Me3 mark, while also containing the histone variant H2A.Z and acetylated lysine residues on histones H3 and H4. These features are thought to reduce the formation of nucleosomes, thereby leading to a stable nucleosome-depleted region that is characteristic of actively transcribed genes (Figure 1).

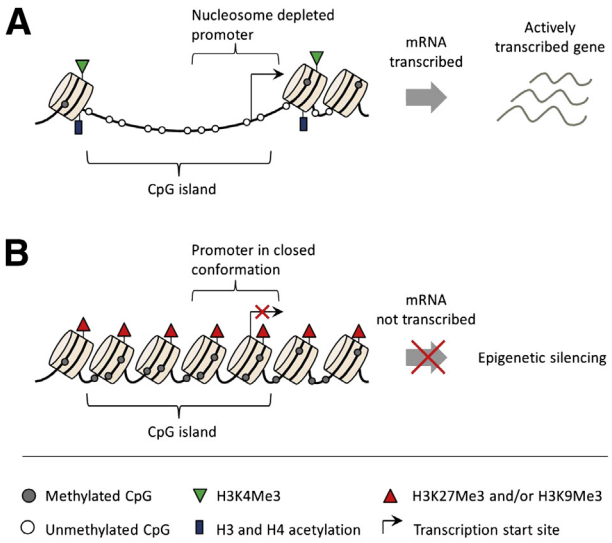

Figure 1 Epigenetic marks influence expression potential by inducing modifications to chromatin configuration at gene promoter regions by affecting the accessibility of transcription factors. **A:** CpG islands of actively transcribed genes are unmethylated and have epigenetic marks attached to histones flanking the promoter region to induce states of open chromatin configuration, which prevent nucleosomes from occupying the promoter region. These marks include histone tail modifications involving trimethylation at histone H3 lysine residue K4 (H3K4Me3) and extensive acetylation on lysine residues at histones H3 and H4. **B:** Epigenetic gene inactivation by CpG island promoter methylation is frequently associated with histone modifications involving trimethylation of lysine residue K27 or trimethylation of lysine residue K9 at histone H3, coupled with loss of the active marks H3K4Me3 and acetylated H3 and H4. Importantly, nucleosomes spread over the promoter region and across the transcriptional start site, leading to closed chromatin configuration and repressed transcription due to reduced accessibility of transcription factors.

It is now becoming clear that DNA demethylation can be achieved through the activity of the TET enzymes (ten-eleven methylcytosine dioxygenases) which convert methylated cytosines into hydroxymethylated cytosines (5hmC).[24] In one model, the 5hmCs are not maintained during cellular division, but are instead interpreted by the replication machinery as unmethylated cytosines and are propagated as such leading to passive demethylation. Another model, however, holds that 5hmCs are one type of many intermediates catalyzed by the TET enzymes and that these intermediates can be replaced with unmethylated cytosines without the need for cellular division through the activity of a DNA repair pathway known as base-excision repair. This mechanism of active DNA demethylation is of potential relevance in maintaining CpG islands in unmethylated states. The discovery of mutations in the *TET2* gene in acute myeloid leukemia highlights the importance of these genes and their functionality in cancer biology.[25]

In normal cells, CpG island methylation occurs infrequently and affects only a small number of autosomal genes, of which most are involved in developmental processes. Our research group confirmed CpG island methylation by DNA methylation profiling of 424 normal human tissue samples of