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.

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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. 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. 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). 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.

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*.

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This article is part of a review series on the molecular pathogenesis of breast cancer.
More recent discoveries include that of acquired mutations in ARID1A (involved in chromatin remodelling), which occur in approximately half of all ovarian cancers. Indeed, acquired mutations in ARID1A have now been found in other types of cancer, including breast cancers. Other examples of mutated epigenetic genes (ie, genes involved in establishing and maintaining epigenetic patterns) include IDH1 mutations in glioblastoma and MLL3 or MLL2 (reclassified as KMT2 genes) mutations in breast cancer. Recently, targeted inactivation of tumour suppressor genes by epigenetic mechanisms has been found to induce cancer under controlled experimental conditions using human mesenchymal cells. In sporadically arising breast and ovarian cancers, the BRCA1 gene is recurrently inactivated by epigenetic mechanisms. 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.

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. 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). 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. 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).

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). 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.

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...
Various types, and further showed that CpG sites located at the non-CpG island 5′ ends best discriminate tissue-specific differences in terms of DNA methylation. Indeed, in that study the different tissue types in the human body were shown to have clear differences in their epigenome-wide DNA methylation patterns. DNA methylation, especially at CpG islands, is generally thought to involve long-term silencing of genes such as those on the inactive X chromosome, imprinted genes, and genes expressed only in germ cells. It is thought that CpG island gene promoter methylation functions to stabilize gene silencing after histone modifications. In this sense, repressive histone marks are applied before CpG island methylation, in what has sometimes been referred to as the locking model of epigenetic gene silencing.

The intragenic regions of transcribed genes (ie, gene bodies) exhibit CpG methylation but are marked with histone H3 trimethylation at K36. The differential methylation between promoter regions and gene bodies supports the emerging view that the position of CpG methylation within the promoter is critical to understanding the relationship of methylation and gene expression activity. For example, CpG island promoter methylation tends to block transcription initiation, whereas gene-body methylation does not. The function of gene-body methylation is not clear, although it may perhaps protect against major sources of mutations such as endogenous reactive oxygen species and suppress intragenic retrotransposons such as LINE1 or Alu elements. Nevertheless, the presence of gene-body methylation comes at the price of the aforementioned deamination of methylated CpGs, which increases the risk of genetic mutations from cytosine to thymine residues. This type of mutation is commonly seen in known cancer genes such as TP53 and is therefore thought to be an important mechanism by which mutations arise and sometimes contribute to the development of cancer. Recent data have demonstrated that the H3K9Me3 repressive histone marker, a marker of constitutive heterochromatin, is associated with increased mutation density in various types of cancers. This finding reinforces the notion that the epigenetic organization of the human genome can influence the rate of acquired mutations relevant to the development of cancer.

### Epigenetic Modifications in Cancer

In the development of cancer, epigenetic mechanisms are important in terms of both silencing of tumor suppressor genes and activation of oncogenes. Both silencing and activation occur through changes in chromatin configuration by which the accessibility of transcription factors is affected, with consequences for gene expression. In breast cancer, tumor suppressor genes such as BRCA1, CDKN2A, and PTEN undergo CpG island promoter methylation, but in normal cells the promoter region is unmethylated. The functional roles of genes inactivated by epigenetic mechanisms in breast cancer and other types of cancers are diverse and reflect various cancer hallmarks (Table 1). Hon et al recently described a novel mechanism of epigenetic gene inactivation through hypomethylation of gene bodies without involving CpG island promoter hypermethylation (discussed below).

The polycomb group (PcG) protein complex 2 has well-established roles in applying trimethylation to histone H3 at lysine residue 27; this H3K27Me3 mark is recruited to target genes by JARID2, where it induces repressed chromatin configurations. In a study of the landscape of histone modifications in embryonic stem cells, the H3K27Me3 repressive mark was unexpectedly found to co-occur with the H3K4Me3 marker of active transcription. This state, known as a bivalent chromatin state, is thought to be important for maintaining pluripotency by silencing the genes necessary for inducing differentiation while also keeping them poised for activation later in the differentiation process. Interestingly, EZH2 gene products, which are components of the PcG protein complex 2, appear to mark genes that are prone to DNA methylation in some subtypes of gliomas, breast

<table>
<thead>
<tr>
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<th>Known function</th>
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<td><strong>BRCA1</strong></td>
<td>Breast cancer 1, early onset</td>
<td>DNA damage response</td>
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<td><strong>CDH1</strong></td>
<td>Cadherin 1, type 1, E-cadherin (epithelial)</td>
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<td>Retinoic acid receptor, beta</td>
<td>Embryonic morphogenesis, regulation of expression, and negative regulation of cellular proliferation</td>
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<td>Cyclin-dependent kinase inhibitor 2A</td>
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*CDH1 has been reclassified as fuzzy/cell division cycle 20 related 1 (Drosophila) (FZR1).

1Alias RASSF1A.
cancers, and other types of cancer. In recent years, noncoding RNAs have increasingly been recognized as helping to establish global patterns of PcG occupancy [eg, HOTAIR and CDKN2B-AS1 (alias ANRIL)], and it is therefore possible that noncoding RNAs have roles in determining cancer-specific patterns of DNA methylation.

Epigenome-Wide Views of Breast Cancers

In a recent study using DNA methylation profiling of normal and diseased tissue samples, including several types of cancer, our research group found distinct patterns of DNA methylation associated with each cancer type. The patterns persisted even after we subtracted CpG sites associated with distinct types of normal tissues. Similar differences in breast cancer subtypes have been described, and some researchers have even found evidence of a CpG methylator phenotype within luminal subtypes, whereas the basal-like subtype seems to have fewer overall gains in CpG methylation. Fang et al reported a favorable prognosis in relation to the methylator phenotype in different cancer types, including breast cancers. Our research group explored the process of cancer progression, based on the analysis of precancerous lesions together with primary cancers; we found a trend toward progressive gains in CpG methylation within CpG islands, whereas the global loss of CpG methylation became increasingly more prominent at non-CpG islands.

Whole-genome bisulfite sequencing, which provides single-nucleotide resolution of DNA methylation, has revealed that CpGs methylated in pluripotent cells are always completely methylated, whereas in differentiated cells methylated CpGs can be found in partially methylated states. Interestingly, in differentiated cells there are large continuous regions, the so-called partially methylated domains (PMDs), which usually contain repressed genes. PMDs span approximately 40% of the genome, which indicates that the differentiation process is indeed accompanied by substantial modifications of the DNA methylation landscape. Nonetheless, we currently have a poor understanding of the relevance of PMDs and of how they come about during differentiation. Lister et al described an important role for non-CpG methylation in embryonic stem cells, which raises questions about the significance of non-CpG methylation changes in cancers. Hon et al in a study using whole-genome bisulfite sequencing in a breast cancer cell line (HCC1954) and in other cancer types, demonstrated that cancer-specific methylation events are almost entirely confined to the CpG context. Interestingly, their study demonstrated that cancer-specific changes, either gains or losses in CpG methylation, tend to occur in PMDs.

An unexpected finding was that of large-scale hypo-methylation in association with repressed gene expression activity. This change involves aberrant unmethylated CpG states at gene bodies containing repressive histone marks with the homologous alleles that are methylated in the gene body and promoter regions, thereby resulting in transcriptional repression. Thus, by establishing a link between gene body hypomethylation and the formation of heterochromatin, the study of Hon et al has already led to advanced knowledge on the functional consequences of hypomethylation in cancer. According to their results, gene body hypomethylation appears to involve the lack of methylation maintenance after cellular division. The mechanism leading to the formation of heterochromatin, however, remains to be clarified. It should be emphasized here that the HCC1954 cell line in the Hon et al study is classified as basal-like, which is a subtype accounting for only approximately 15% of all breast cancers. Furthermore, basal-like breast cancers undergo relatively few CpG methylation events, compared with other subtypes. In contrast, the hormone receptor-positive subtypes are more prone to CpG island methylation, which occurs predominantly at PcG-regulated genes, consistent with the idea that the PcG proteins are involved in establishing DNA methylation.

Epigenetic Changes Contribute to Genetic Mutations in Breast Cancers

Breast cancer genomes usually contain thousands of genetic changes, of which only a small subset might actually drive development of the disease. In some cases, only a few mutations are found, reflecting the slow accumulation of acquired mutations over the lifetime of the individual. Other cases exhibit a large number of mutations, suggesting that DNA repair capacity has been affected, coupled with induction of genetic instability. This occurs in breast cancers arising in BRCA1 and BRCA2 mutation carriers, in whom the loss of the second wild-type allele is generally thought to be an important event leading to breast cancer development. Nevertheless, other tumor suppressor genes, including CDKN2A (alias p16), FZRI (alias CDH1), RARB2, and GSTP1 undergo CpG island hypermethylation at more or less similar frequencies in breast cancers arising in familial cases (including BRCA1 and BRCA2 mutation carriers) and in those arising sporadically. Thus, epigenetic changes are needed even in cancers with DNA repair dysfunction (ie, in breast cancers arising in BRCA mutation carriers). This finding illuminates the important role of epigenetic changes in addition to genetic mutations as contributors to the development of breast cancer. Given the prevalence of mutations in TP53 in breast cancer and other cancer types, many researchers have studied epigenetic changes in this gene, but none have reported inactivation by CpG island promoter hypermethylation or other types of repressive epigenetic modifications.

It is now well established that CpG island hypermethylation of the BRCA1 gene promoter region occurs in approximately 10% to 15% of all sporadic breast cancers.
Most researchers have found that BRCA2 methylation does not occur, although this is still debatable. Other DNA repair genes, including PALB2 and ATM, have been reported to be epigenetically inactivated by CpG island promoter hypermethylation in breast cancers, although these findings are yet to be confirmed by other researchers. In contrast, there is strong support for BRCA1 methylation in sporadic breast cancer development. There are clear phenotypic effects associated with breast cancers arising in BRCA1 mutation carriers, and the effects similarly characterize sporadic breast cancers with acquired BRCA1 methylation. In these cases, the second hit is thought to be acquired genomic deletions of the wild-type and unmethylated allele. With respect to the phenotype, it has been demonstrated that primary breast cancer cells with BRCA1 gene defects, caused by either inherited mutations or acquired promoter methylation, tend to be poorly differentiated. Additionally, BRCA1 dysfunctional breast cancers lack expression of estrogen and progesterone receptors, but express basal-like markers such as CK5/6 and EGFR.

The link between BRCA1 defects and the basal-like phenotype probably reflects an important role of the BRCA1 gene in differentiating somatic stem cells of the breast. Indeed, researchers have demonstrated that loss of BRCA1 gene products preferentially leads to the transformation of luminal progenitor cells with phenotypic similarities to the basal-like phenotype. In addition to the similar expression patterns, sporadic breast cancers with acquired BRCA1 methylation have extensive DNA copy number changes suggestive of instability, similar to those observed in breast cancers arising in BRCA1 mutation carriers. This probably reflects defective DNA repair of DSBs and, consequently, accelerated mutation rates due to unrepaired breaks and the use of error-prone DNA repair processes involving nonhomologous end joining. The characteristic mutational patterns predicted to emerge from nonhomologous end joining include large-scale structural changes (ie, deletions, gains, or translocations), as well as the formation of so-called indels, which are mutations involving a few base pairs erroneously deleted or inserted. Mutations of the indel type have been described in PTEN, RB1, and TP53. All three are well-known tumor suppressor genes, associated with breast cancers arising in BRCA1 mutation carriers and sporadic breast cancers with acquired BRCA1 methylation and those exhibiting the basal-like phenotype. Thus, understanding why acquired mutations arise and lead to cancer inevitably involves the study of epigenetic modifications. It is important to keep epigenetic modifications in mind when considering acquired mutations as predictors of drug response (discussed below).

Data are emerging to support the involvement of the histone methyltransferase EZH2 in inducing epigenetic silencing of RAD51 (a well-known DNA repair gene). The H3K27Me3 repressive marker was found at the promoter region of RAD51 after induced expression of the EZH2 gene, and EZH2 expression was shown to be associated with activation of RAF1—MEK signaling and expansion of breast cancer stem cells. Consistent with an important early event and carcinogenic potential, the targeted overexpression of EZH2 in mammary glands of mouse models leads to disruption of ductal morphogenesis and precancerous lesions. The link to expansion of cancer stem cells in the breast suggests a mechanistic link to aggressive behavior in breast cancer patients. Indeed, a high level of expression of the EZH2 gene has been described in association with basal-like and luminal-B subtypes, both of which are known to be poorly differentiated subtypes associated with unfavorable disease outcome. Additionally, the EZH2 gene product is known to be recruited to sites of DSB damage, in conjunction with other PcG proteins, such as BMI1, and the NuRD complex. This recruitment occurs very early, and may function to establish the repressed chromatin configuration at the damaged sites to prevent transcription. Interestingly, CBX1 (alias HP1-β) gene products, which are chromodomain-containing proteins functionally implicated in reading the histone code, are rapidly mobilized from DSB sites shortly after damage occurs. This is important for subsequent repair, because phosphorylation of H2AX (an essential signal for inducing the recruitment of DNA repair proteins) does not occur at sites occupied by HP1-β. Thus, sensing of DSBs is critically dependent on the dynamic reorganization of chromatin structure to facilitate the repair process.

Finally, the pattern of epigenetic marks surrounding the lesion needs to be re-established. This implies that the epigenetic machinery has a direct role in maintaining genomic stability, which suggests a therapeutic potential in breast cancers with dysfunctional DSB repair processes or, alternatively, the sensitization of cancer cells to DNA-damaging agents. Of interest in this respect are the findings of Puppe et al., who used murine breast cancer cell line models to demonstrate that targeted disruption of the BRCA1 gene leads to cellular sensitivity to inhibitors of EZH2 gene products.

The Central Role of BRCA1 Methylation in Predicting Treatment Response and in Personalized Medicine

In personalized medicine, decisions about drug treatment are tailored to each patient on the basis of which cancer genes are affected in the primary cancer. This concept is based on the idea that some cancer genes harboring genetic changes are predictive of patient response to specific anticancer drugs, such as BRCA1 or BRCA2 mutations in relation to PARP inhibitors [eg, olaparib (AZD-2281)] or PIK3CA mutations in relation to PI3K inhibitors. Resequencing of a large number of cancer cell line models has recently been conducted and analyzed with respect to drug sensitivity. The resulting reports, although supporting the validity of using mutated cancer genes as predictors for drug response, describe many previously unexpected
relationships between genomic predictors and drug response that are worthy of further investigation. Given that most cancer genes are only infrequently mutated, it is likely that personalized medicine could be applied to a larger number of patients if epigenetic changes as well as mutations were considered. For example, breast cancers arising in *BRCA1* and *BRCA2* mutation carriers affect a minority of patients (fewer than 5%), whereas sporadically arising breast cancers with acquired *BRCA1* methylation account for 10% to 15% of all patients.14,16 By taking into account epigenetic changes, more patients than just the mutation carriers could derive benefits from treatments based on targeting deficiencies for either *BRCA1* or *BRCA2*. In this context, the use of specific anticancer drugs predicted to be effective against cancers with defective DSB repair mechanisms is highly relevant.79,80 Anticancer drugs leading to the formation of DSBs, such as platinum-based drugs, have been tested for breast cancer, because this treatment is predicted to be effective in killing *BRCA1*- or *BRCA2*-defective cells.

PARP inhibitors have shown their effectiveness against cancer cells with defects in DNA repair of DSBs.81,82 These inhibitors block the activity of PARP enzymes, of which PARP-1 is an important target involved in signaling the presence of single-strand breaks. The inhibition of PARP-1 therefore leads to more unrepaired single-strand breaks. The presence of single-strand breaks at replication forks leads to the formation of DSBs that cannot be repaired in *BRCA1*- or *BRCA2*-defective cells, thereby leading to the accumulation of DSBs and to cellular death. This is known as synthetic lethality, in which cellular death results from the blocking of two or more pathways, but blocking of only one does not affect cellular survival (although phenotypic effects may arise).83

Initial testing of PARP inhibitors in clinical trials involved the use of olaparib, which successfully induced pathological responses in breast cancer patients with inherited mutations in either *BRCA1* or *BRCA2*.84,85 The effectiveness of the drug in patients with inherited *BRCA1* or *BRCA2* mutations raised the question of whether this drug could also be useful in some sporadically arising breast cancers. Based on the phenotypic similarities between *BRCA1*-mutated breast cancers and basal-like breast cancers,86 PARP inhibitors were tested in patients with triple-negative (TN) sporadic breast cancers. The TN phenotype [ie, breast cancers negative for the expression of estrogen and progesterone receptors and lacking *ERBB2* (alias *HER2*) amplification] significantly coincides with that of the basal-like phenotype; TN breast cancers are basal-like, and vice versa.87 Because inherited mutations in the *BRCA1* gene preferentially result in the development of basal-like breast cancers, acquired defects in the *BRCA1* gene, or in other genes within the same pathway, might also be found in sporadic cases of basal-like or TN breast cancers. Testing PARP inhibitors in TN breast cancers initially gave promising results,88 but subsequent trials failed to confirm these findings. The PARP inhibitor used in that study, iniparib, was later found to be ineffective in blocking the activity of the PARP-1 enzyme.89 Thus, it is still not known whether PARP inhibitors could be useful in this subset of sporadic breast cancer patients or only in the small group of patients with inherited mutations in *BRCA1* or *BRCA2*.

Nonetheless, the genetic heterogeneity of basal-like breast cancers (and TN breast cancers) was recently brought into sharp focus,90 and only a subset of these tumors show signs of *BRCA1* deficiency.48 In terms of epigenetic aberrations, our research group showed that approximately half of all TN breast cancers have acquired *BRCA1* methylation,60 and that

![Figure 2](ajp.amjpathol.org/1057)

**Figure 2** Epigenetic changes contribute to the development of breast cancer and may have implications for personalized medicine. In normal cells, CpG island promoter regions of tumor suppressor genes, such as the *BRCA1* gene, are unmethylated and nucleosome depleted, thereby enabling transcription initiation. This enables translation, which in turn facilitates its normal biological activity, such as *BRCA1* gene products performing DNA repair to maintain genomic integrity. During carcinogenesis, epigenetic changes can contribute to the inactivation of tumor suppressor genes. In this example, *BRCA1* CpG island promoter methylation in conjunction with the accelerated formation of acquired mutations leads to cancer. In personalized medicine, treatment decisions are based on the individual patient's specific gene aberrations. In this case, CpG island promoter hypermethylation of the *BRCA1* can be detected with simple, fast, and low-cost epigenetic techniques such as methylation-specific PCR. The presence of acquired CpG island promoter hypermethylation of *BRCA1* enables clinical oncologists to predict that the patient will respond to DNA-damaging drugs such as PARP inhibitors, or platinum-based drugs. M, methylated; U, unmethylated.
this epigenetic marker is a good predictor of response to PARP inhibitors in breast cancer cell line models.101 The use of BRCA1 methylation as a predictor of therapeutic response to PARP inhibitors was subsequently confirmed in xenograft tumor models.92 These preclinical data, along with contributing to a clear mechanistic understanding, provide a strong rationale for including BRCA1 methylation in clinical trials as a candidate predictor of response to PARP inhibitors (Figure 2).

Other drugs of potential relevance include platinum-based agents, such as cisplatin. These drugs exert their effectiveness by inducing the formation of cross-links. These types of lesions are ineffectively repaired in BRCA1- and BRCA2-defective cells, because they involve the formation of DSBs.93 Consistent with this notion, ovarian cancer patients with inherited mutations in either BRCA1 or BRCA2 respond better than noncarriers to platinum drugs.94,95 Mutations in the TP53 gene, however, appear to be associated with resistance to cisplatin treatment.96 In contrast, we demonstrated that this type of treatment is effective in breast cancer cells and xenografts with acquired BRCA1 methylation.97 As already noted, TN breast cancer patients have a higher incidence of BRCA1 gene dysfunctions, because of BRCA1 methylation in sporadic cases or BRCA1 mutations in familial cases. This probably explains the generally good response to platinum-based treatment in TN breast cancer patients.98 In support of this hypothesis, BRCA1 methylation has been found to predict significantly higher response rates to cisplatin treatment in patients with TN breast cancer.99 Finally, a prospective phase II clinical study of 20 patients with metastatic breast cancer and inherited BRCA1 mutation demonstrated high activity of cisplatin treatment.100

The use of platinum-based treatment has already been approved for use in breast cancer and, based on the high pathological response rates reported by Byrski et al,100 it is approved for use in breast cancer and, based on the high pathological response rates reported by Byrski et al,100 it is approved for use in breast cancer and, based on the high pathological response rates reported by Byrski et al,100 it is approved for use in breast cancer and, based on the high pathological response rates reported by Byrski et al,100 it is approved for use in breast cancer and, based on the high pathological response rates reported by Byrski et al,100 it is approved for use in breast cancer and, based on the high pathological response rates reported by Byrski et al,100 it is approved for use in breast cancer and, based on the high pathological response rates reported by Byrski et al,100 it is approved for use in breast cancer and, based on the high pathological response rates reported by Byrski et al,100 it is approved for use in breast cancer and, based on the high pathological response rates reported by Byrski et al,100 it is approved for use in breast cancer and, based on the high pathological response rates reported by Byrski et al,100 it is approved for use in breast cancer and, based on the high pathological response rates reported by Byrski et al,100 it is approved for use in breast cancer and, based on the high pathological response rates reported by Byrski et al,100 it is approved for use in breast cancer and, based on the high pathological response rates reported by Byrski et al,100 it is approved for use in breast cancer.101 The use of PARP inhibitors in breast cancer cell line models.91 The use of PARP inhibitors in breast cancer cell line models.91 The use of PARP inhibitors in breast cancer cell line models.91 The use of PARP inhibitors in breast cancer cell line models.91 The use of PARP inhibitors in breast cancer cell line models.91 The use of PARP inhibitors in breast cancer cell line models.91 The use of PARP inhibitors in breast cancer cell line models.91

Will There Be a Use for Epigenetic Markers in Routine Clinical Management of Breast Cancer Patients?

The resequencing of cancer genomes has led to the discovery of several previously unknown cancer genes.10,48 These efforts have identified more than 50 cancer genes that, when mutated, can contribute to breast cancer development. It is clear, however, that the large majority of these genes are mutated in fewer than 5% of all breast cancers. Thus, the question arises whether any of these cancer genes, when not mutated, could be affected by epigenetic mechanisms. Based on current knowledge, the catalog of mutated cancer genes has a rather limited overlap with the catalog of genes found to be epigenetically inactivated. One example is of course the BRCA1 gene; other examples include PTEN and CDKN2A.

The PI3K–AKT signaling pathway is commonly enhanced in breast cancers,104 mainly through PIK3CA mutation or ERBB2 amplification, but to a lesser extent by mutations in either PTEN or AKT.10,48 Clinical trials are currently underway for testing various PI3K inhibitors developed to target different components of the pathway.105 In the PI3K pathway, PTEN inactivation by epigenetic mechanisms96,97 is likely to extend the use of drug inhibitors effective for PTEN-defective cancer cells. Another way of studying this overlap is to look at cellular pathways, such as the CpG island promoter methylation of the PPP2R2B gene32,33 (a phosphatase and negative regulator of AKT, which is an important component within the PI3K pathway) that occurs in breast cancers. In this way, epigenetic modifications may target the same pathway even though the genes affected are different from those identified by cancer genome resequencing. In other instances, the overlap between epigenetic and genetic changes arises within gene families, such as in the runt-related transcription factor family genes, in which RUNX1 is mutated by homozygous deletions,106 but only RUNX3 (but not RUNXI or RUNX2) is known to be inactivated by epigenetic mechanisms.34,35 The RUNX gene products are known to be transcription factors involved in differentiation, but were recently found to localize and interact with other proteins at the centromeres, suggesting roles in the regulation of mitosis.107 These functional analyses are potentially relevant to the findings emerging from the systematic screening of drug sensitivity in cancer cell lines,57 which demonstrate increased sensitivity of RUNX1-mutated cancer cell lines to serine/threonine kinase inhibitors of the mitosis regulators aurora kinase B, Wee1-like protein kinase, and serine/threonine-protein kinase Chk1 (encoded by AURKB, WEE1, and CHEK1, respectively). It might therefore be worthwhile to determine whether the subset of cancer cell lines without RUNX1 mutations but still showing sensitivity to these inhibitors have acquired CpG island promoter methylation of the RUNX3 gene.

In some cases, genes previously implicated in cancer development have not been found to be mutated but are frequently found inactivated in cancer by epigenetic mechanisms [eg, RASSF1 (alias RASSF1A), GSTP1, MGMT, and BRMS1]. Some of these markers are potential candidates for predicting response to drug treatment. For example, CpG island hypermethylation of the ESRI estrogen receptor gene is significantly associated with a lack of response to
In breast cancer, expression states of the estrogen and progesterone hormone receptors are both prognostic in terms of disease outcome and predictive of response to tamoxifen. Tamoxifen is used when hormone-receptor expression is positive, and it has been shown to be beneficial for reducing the risk of disease relapse. The analysis of ESR1 methylation could be helpful in a clinical setting for identifying patients who will not benefit from tamoxifen, thereby sparing them from ineffective treatment. More recently, PITX2 methylation was identified as a marker associated with tamoxifen resistance.3 In such cases, the use of epigenetic drugs such as 5-azacytidine (Vidaza) or decitabine (5-aza-2'-deoxycytidine; Dacogen), along with histone deacetylase inhibitors such as vorinostat or romidepsin could be useful for overcoming tamoxifen resistance.

CpG island promoter hypermethylation of the GSTP1 gene, first described in 1998,38 has recently been linked to therapeutic response to doxorubicin.32 The GSTP1 gene encodes an enzyme involved in detoxification, and repression of GSTP1 by epigenetic mechanisms probably leads to an accumulation of xenobiotics and carcinogens that contributes to cancer development while enhancing the effects of various anticancer drugs.

In this context, GSTP1 methylation was found to be highly prevalent in prostate cancer,30 and has now been validated by other researchers as a promising marker for the early detection of prostate cancer based on noninvasive analysis of circulating DNA from urine samples.30,108 The principle behind the use of bodily fluids for detection of DNA derived from solid cancers is based on the direct release of DNA after necrosis or cell lysis at the primary site of origin and, in some cases, the capacity of this DNA to migrate to the blood stream. In terms of breast cancer diagnosis in women, the use of several markers (ie, RASSF1, RARB2, and APC) along with GSTP1 can increase the sensitivity of methylation-based diagnosis without loss of specificity in serum samples.30 In fact, other marker panels have shown promising results in this regard, using ductal lavage samples.109 The high frequency of RASSF1 methylation events in breast cancers makes RASSF1 an ideal diagnostic marker, and RASSF1 has also shown promise in monitoring adjuvant therapeutic efficacy.110 DNA methylation markers can be analyzed in blood samples using powerful bisulfite-based PCR techniques (eg, methylation-specific PCR), enabling highly sensitive detection of methylated alleles derived from cancer.31 This is clinically significant, because currently no biomarkers are available for detecting the presence of breast cancer cells using blood-based analyses.

Conclusions

The introduction of next-generation sequencing and array-based technologies for analyzing epigenetic states in normal and cancerous cells holds great promise for furthering our understanding of the roles of epigenetic changes in cancer. Next-generation sequencing and array-based methods for analyzing DNA methylation have confirmed classical CpG island promoter hypermethylation as an important mechanism for inactivating tumor suppressor genes in various types of cancers, including breast cancer.30,47 Moreover, findings emerging from next-generation sequencing of a breast cancer methylome, described by Hon et al,40 have led to new discoveries wherein loss of gene-body methylation (without involving changes at promoter regions), coupled with gains in repressive histone marks H3K27Me3 and H3K9Me3, represents a prominent mechanism by which gene inactivation occurs in breast cancer.40 This occurs predominantly at PDMs. Because PDMs are thought to be important in establishing cellular identity during differentiation,39 this mechanism could yield further insights into the epigenetic processes underlying the origin of cancer stem cells.

For breast cancer, there are now convincing data reported by Fang et al17 to show that, in some cases, the epigenetic landscape is characterized by a hypermethylated CpG island methylator phenotype (CIMP). In this instance, the genes affected in CIMP-associated cancers are mostly those regulated by the PcG proteins in embryonic stem cells, marked for repression by H3K27Me3, and the majority of these are genes with functional roles associated with development and cellular differentiation. The cause of the CIMP in breast cancer is currently unknown, although candidates have been identified in other cancer types (eg, acquired IDHI mutations in gliomas32). Thus, these are two mechanistically different processes by which widespread epigenetic changes involving gene inactivation can arise during breast carcinogenesis. In both cases, the affected genes tend to have functional roles in differentiation, suggesting effects on stem cell properties and possibly also on disease aggressiveness.

After the discovery that acquired mutations in epigenetic genes can drive events in many types of cancer, including breast cancer, the notion that epigenetic changes and genetic mutations act cooperatively in driving disease development has been widely discussed in the literature. We therefore stress the finding that BRCA1, a DNA repair gene, is activated by epigenetic mechanisms in at least 10% to 15% of breast cancers, thus probably contributing to the accumulation of genetic mutations and thereby facilitating cancer development. Additionally, structural rearrangements may well arise as a consequence of global DNA hypomethylation at CpG-poor regions, in which normally repressed regions are exposed, including those covering repetitive and transposable elements. Furthermore, there is growing evidence of a direct role for PcG proteins in maintaining genomic stability by mediating chromatin changes at sites of DNA damage involving DSBs.111

These findings point toward a link between defects in the epigenetic machinery and the onset of genetic instability. Much work remains to be done to clarify this relationship. For example, what is the role of mutations in epigenetic genes, how do they affect the epigenetic machinery, and are
there phenotypic consequences with respect to genetic instability? Additionally, the use of anticancer drugs with DNA-damaging properties in cancers arising in BRCA1 or BRCA2 mutation carriers has attracted considerable attention, with testing of PARP inhibitors and platinum-based drugs in clinical trials. As we have noted, the consequences of BRCA1 inactivation by epigenetic mechanisms in sporadically arising breast cancers are the same as for BRCA1 mutations in familial cases. It is therefore reasonable to hypothesize that these drugs will be equally effective in sporadic cases with inherited BRCA1 mutations. Several studies, including two from our research group, have supported this hypothesis.

The exploration of knowledge in breast cancer genetics enabled by the resequencing of cancer genomes and genome-wide association studies has already begun to open up new possibilities for treatment. However, mutation is rare in almost all known cancer genes. Thus, identification and understanding of epigenetic changes in cancers hold great promise for bringing personalized medicine to a larger number of patients—a concept that is excellently illustrated by BRCA1 methylation and its potential as a predictive marker in the clinical management of patients.

References

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