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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).

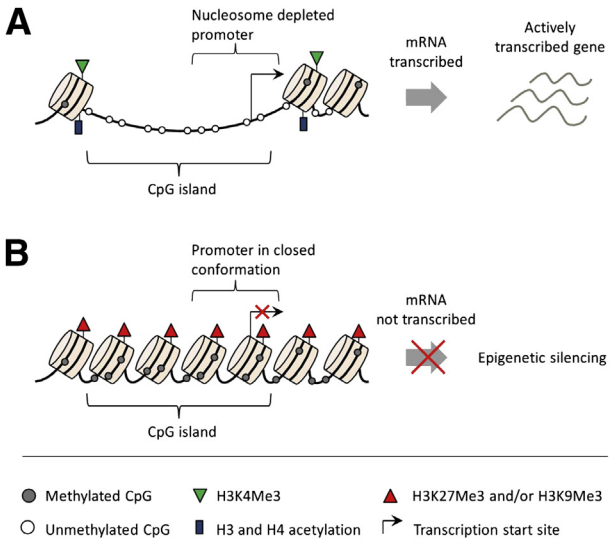

Figure 1 Epigenetic marks influence expression potential by inducing modifications to chromatin configuration at gene promoter regions by affecting the accessibility of transcription factors. **A:** CpG islands of actively transcribed genes are unmethylated and have epigenetic marks attached to histones 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

Table 1 Selected Examples of CpG Island Promoter Hypermethylated Genes in Breast Cancer, Demonstrating Diverse Biological Implications

Gene symbol	Gene name	Known function	References
BRCA1	Breast cancer 1, early onset	DNA damage response	13,14,16
*CDH1**	Cadherin 1, type 1, E-cadherin (epithelial)	Cell-to-cell adhesion	29
RARB2	Retinoic acid receptor, beta	Embryonic morphogenesis, regulation of expression, and negative regulation of cellular proliferation	29,30
CDKN2A	Cyclin-dependent kinase inhibitor 2A	G1 phase of the mitotic cell cycle, cellular senescence and apoptosis	29,31
PTEN	Phosphatase and tensin homolog	PI3K—AKT signaling pathway, negative regulation of cellular proliferation	32,33
RASSF1†	Ras association (RalGDS/AF-6) domain family member 1	Signal transduction, cell cycle arrest, and response to DNA damage stimulus	33
RUNX3	Runt-related transcription factor 3	Transcription factor involved in development and regulation of mitosis	34,35
ESR1	Estrogen receptor 1	Epithelial cell development, sexual development, and reproductive function	36
PITX2	Paired-like homeodomain transcription factor 2	Wnt receptor signaling pathway	37
GSTP1	Glutathione S-transferase pi 1	Detoxification	32,38,39

**CDH1* has been reclassified as fizzy/cell division cycle 20 related 1 (Drosophila) (*FZR1*).
†Alias *RASSF1A*.

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.[26] 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.[22] 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.[22] The function of gene-body methylation is not clear, although it may perhaps protect against major sources of mutagens 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.[27] 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.[28] 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.[4] 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[40] 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.[41] 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.[42] 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

cancers, and other types of cancer.[43] In recent years, noncoding RNAs [44] 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.[45]

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.[26] 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,[46] and some researchers have even found evidence of a CpG methylator phenotype within luminal subtypes,[47] whereas the basal-like subtype seems to have fewer overall gains in CpG methylation.[48] Fang et al[47] 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.[26]

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.[49] 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[49] 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,[40] 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 hypomethylation 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[40] 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[40] 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.[49] In contrast, the hormone receptor-positive subtypes are more prone to CpG island methylation, which occurs predominantly at PcG-regulated genes,[46,47] consistent with the idea that the PcG proteins are involved in establishing DNA methylation.[43]

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.[50] 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.[51,52] CpG island promoter hypermethylation, as a mechanism of gene inactivation, is only infrequently found as the second hit in familial *BRCA1*- or *BRCA2*-mutated breast cancers.[29] Nevertheless, other tumor suppressor genes, including *CDKN2A* (alias *p16*), *FZR1* (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.[29] 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.[53]

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,[54] although this is still debatable.[55,56] Other DNA repair genes, including *PALB2* and *ATM*, have been reported to be epigenetically inactivated by CpG island promoter hypermethylation in breast cancers,[57,58] although these findings are yet to be confirmed by other researchers.[59] 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.[14,57] 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.[60,61]

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.[62] 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.[63] 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.[64,65] 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.[66–68] 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).[69,70] The H3K27Me3 repressive marker was found at the promoter region of *RAD51* after induced expression of the *EZH2* gene,[69] and *EZH2* expression was shown to be associated with activation of RAF1–MEK signaling and expansion of breast cancer stem cells.[70] 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.[71] 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.[72] 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.[73] 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.[74] 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,[75] 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.[76] 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.[77,78] 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

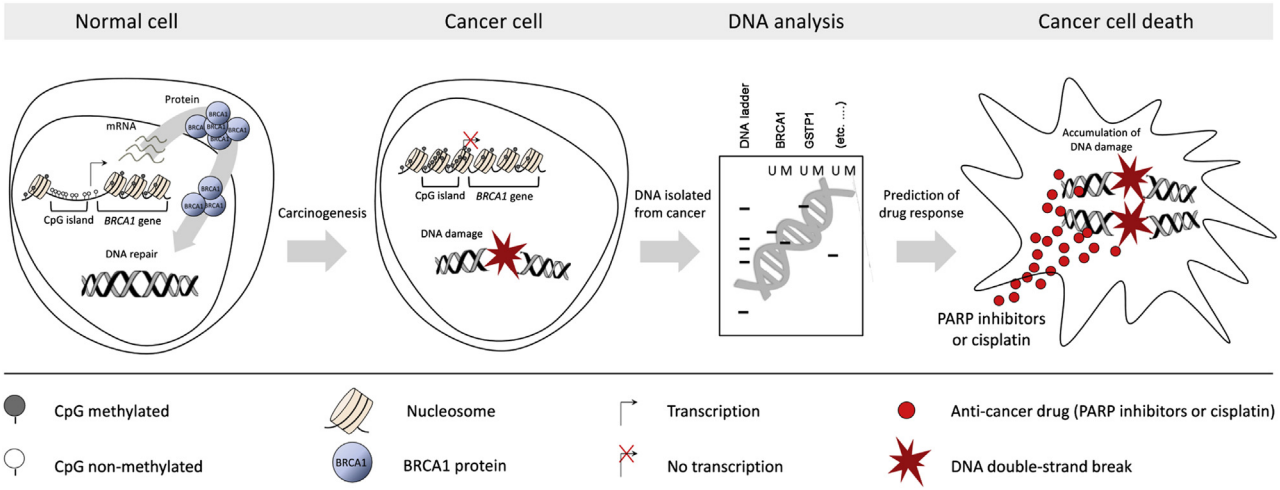

Figure 2 Epigenetic changes contribute to the development of breast cancer and may have implications for personalized medicine. In normal cells, CpG island promoter regions of tumor suppressor genes, such as the *BRCA1* gene, are unmethylated and nucleosome depleted, thereby enabling transcription initiation. 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.[91] 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 reasonable to ask (as Turner and Tutt[101] do) whether clinicians should need any further evidence to change standard clinical practice in the treatment of *BRCA1* mutation carriers. The specific importance of selecting platinum-based drugs as the first-line treatment for patients with *BRCA1* dysfunctional breast cancers has to do not only with increased response rates, but also with resistance to other types of drugs, such as anthracyclines and taxanes.[102,103] Selecting platinum-based drugs, or possibly PARP inhibitors, to treat patients with acquired or inherited defects in the *BRCA1* gene will, we hypothesize, lead to significant improvements in disease outcome for these patients and within the otherwise aggressive TN subtype.

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 mechanisms[96,97] 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* gene[32,33] (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 *RUNX1* 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,[77] 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 *ESR1* estrogen receptor gene is significantly associated with a lack of response to

tamoxifen.[36] 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.[37] 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.[39] 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[33] 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.[40,47] 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 PMDs. Because PMDs are thought to be important in establishing cellular identity during differentiation,[49] 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 al[47] 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 *IDH1* mutations in gliomas[9]). 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 inactivated 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 acquired *BRCA1* methylation as in familial cases with inherited *BRCA1* mutations. Several studies, including two from our research group,[91,97] have supported this hypothesis.

The explosion 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.[77,78] 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

1. Lalloo F, Evans DG: Familial breast cancer. Clin Genet 2012, 82: 105–114
2. Fanale D, Amodeo V, Corsini LR, Rizzo S, Bazan V, Russo A: Breast cancer genome-wide association studies: there is strength in numbers. Oncogene 2012, 31:2121–2128
3. Gudmundsdottir K, Ashworth A: The roles of BRCA1 and BRCA2 and associated proteins in the maintenance of genomic stability. Oncogene 2006, 25:5864–5874
4. Esteller M: Epigenetics in cancer. N Engl J Med 2008, 358: 1148–1159
5. Feinberg AP: Phenotypic plasticity and the epigenetics of human disease. Nature 2007, 447:433–440
6. Herman JG, Latif F, Weng Y, Lerman MI, Zbar B, Liu S, Samid D, Duan DS, Gnarra JR, Linehan WM, Baylin SB: Silencing of the VHL tumor-suppressor gene by DNA methylation in renal carcinoma. Proc Natl Acad Sci USA 1994, 91:9700–9704
7. Jones S, Wang TL, Shih IM, Mao TL, Nakayama K, Roden R, Glas R, Slamon D, Diaz LA Jr., Vogelstein B, Kinzler KW, Velculescu VE, Papadopoulos N: Frequent mutations of chromatin remodeling gene ARID1A in ovarian clear cell carcinoma. Science 2010, 330:228–231
8. Jones S, Li M, Parsons DW, Zhang X, Wesseling J, Kristel P, Schmidt MK, Markowitz S, Yan H, Bigner D, Hruban RH, Eshleman JR, Iacobuzio-Donahue CA, Goggins M, Maitra A, Malek SN, Powell S, Vogelstein B, Kinzler KW, Velculescu VE, Papadopoulos N: Somatic mutations in the chromatin remodeling gene ARID1A occur in several tumor types. Hum Mutat 2012, 33:100–103
9. Noushmehr H, Weisenberger DJ, Diefes K, Phillips HS, Pujara K, Berman BP, Pan F, Pelloski CE, Sulman EP, Bhat KP, Verhaak RG, Hoadley KA, Hayes DN, Perou CM, Schmidt HK, Ding L, Wilson RK, Van Den Berg D, Shen H, Bengtsson H, Neuvial P, Cope LM, Buckley J, Herman JG, Baylin SB, Laird PW, Aldape K; Cancer Genome Atlas Research Network: Identification of a CpG island methylator phenotype that defines a distinct subgroup of glioma. Cancer Cell 2010, 17:510–522
10. Stephens PJ, Tarpey PS, Davies H, Van Loo P, Greenman C, Wedge DC, et al; Oslo Breast Cancer Consortium (OSBREAC): The landscape of cancer genes and mutational processes in breast cancer. Nature 2012, 486:400–404
11. Teng IW, Hou PC, Lee KD, Chu PY, Yeh KT, Jin VX, Tseng MJ, Tsai SJ, Chang YS, Wu CS, Sun HS, Tsai KD, Jeng LB, Nephew KP, Huang TH, Hsiao SH, Leu YW: Targeted methylation of two tumor suppressor genes is sufficient to transform mesenchymal stem cells into cancer stem/initiating cells. Cancer Res 2011, 71:4653–4663
12. Dobrovic A, Simpfendorfer D: Methylation of the BRCA1 gene in sporadic breast cancer. Cancer Res 1997, 57:3347–3350
13. Rice JC, Ozcelik H, Maxeiner P, Andrulis I, Futscher BW: Methylation of the BRCA1 promoter is associated with decreased BRCA1 mRNA levels in clinical breast cancer specimens. Carcinogenesis 2000, 21:1761–1765
14. Esteller M, Silva JM, Dominguez G, Bonilla F, Matias-Guiu X, Lerma E, Bussaglia E, Prat J, Harkes IC, Repasky EA, Gabrielson E, Schutte M, Baylin SB, Herman JG: Promoter hypermethylation and BRCA1 inactivation in sporadic breast and ovarian tumors. J Natl Cancer Inst 2000, 92:564–569
15. Catteau A, Harris WH, Xu CF, Solomon E: Methylation of the BRCA1 promoter region in sporadic breast and ovarian cancer: correlation with disease characteristics. Oncogene 1999, 18:1957–1965
16. Birgisdottir V, Stefansson OA, Bodvarsdottir SK, Hilmarsdottir H, Jonasson JG, Eyfjord JE: Epigenetic silencing and deletion of the BRCA1 gene in sporadic breast cancer. Breast Cancer Res 2006, 8:R38
17. Esteller M, Garcia-Foncillas J, Andion E, Goodman SN, Hidalgo OF, Vanaclocha V, Baylin SB, Herman JG: Inactivation of the DNA-repair gene MGMT and the clinical response of gliomas to alkylating agents, [Erratum appeared in N Engl J Med 2000, 343:1740]. N Engl J Med 2000, 343:1350–1354
18. Masuda K, Banno K, Yanokura M, Tsuji K, Kobayashi Y, Kisu I, Ueki A, Yamagami W, Nomura H, Tominaga E, Susumu N, Aoki D: Association of epigenetic inactivation of the WRN gene with anticancer drug sensitivity in cervical cancer cells. Oncol Rep 2012, 28:1146–1152
19. Sinicrope FA, Sargent DJ: Molecular pathways: microsatellite instability in colorectal cancer: prognostic, predictive, and therapeutic implications. Clin Cancer Res 2012, 18:1506–1512
20. Esteller M, Levine R, Baylin SB, Ellenson LH, Herman JG: MLH1 promoter hypermethylation is associated with the microsatellite instability phenotype in sporadic endometrial carcinomas. Oncogene 1998, 17:2413–2417
21. Baylin SB, Jones PA: A decade of exploring the cancer epigenome–biological and translational implications. Nat Rev Cancer 2011, 11: 726–734
22. Jones PA: Functions of DNA methylation: islands, start sites, gene bodies and beyond. Nat Rev Genet 2012, 13:484–492
23. Antequera F, Bird A: Number of CpG islands and genes in human and mouse. Proc Natl Acad Sci USA 1993, 90:11995–11999
24. Williams K, Christensen J, Helin K: DNA methylation: TET proteins–guardians of CpG islands? EMBO Rep 2011, 13:28–35
25. Delhommeau F, Dupont S, Della Valle V, James C, Trannoy S, Massé A, Kosmider O, Le Couedic JP, Robert F, Alberdi A, Lécluse Y, Plo I, Dreyfus FJ, Marzac C, Casadevall N, Lacombe C, Romana SP, Dessen P, Soulier J, Viguié F, Fontenay M, Vainchenker W, Bernard OA: Mutation in TET2 in myeloid cancers. N Engl J Med 2009, 360:2289–2301
26. Fernandez AF, Assenov Y, Martin-Subero JI, Balint B, Siebert R, Taniguchi H, et al: A DNA methylation fingerprint of 1628 human samples. Genome Res 2012, 22:407–419
27. Nik-Zainal S, Alexandrov LB, Wedge DC, Van Loo P, Greenman CD, Raine K, et al; Breast Cancer Working Group of the International Cancer Genome Consortium: Mutational processes molding the genomes of 21 breast cancers. Cell 2012, 149:979–993
28. Schuster-Böckler B, Lehner B: Chromatin organization is a major influence on regional mutation rates in human cancer cells. Nature 2012, 488:504–507

29. Esteller M, Fraga MF, Guo M, Garcia-Foncillas J, Hedenfalk I, Godwin AK, Trojan J, Vaurs-Barrière C, Bignon YJ, Ramus S, Benitez J, Caldes T, Akiyama Y, Yuasa Y, Launonen V, Canal MJ, Rodriguez R, Capella G, Peinado MA, Borg A, Aaltonen LA, Ponder BA, Baylin SB, Herman JG: DNA methylation patterns in hereditary human cancers mimic sporadic tumorigenesis. Hum Mol Genet 2001, 10:3001−3007
30. Hoque MO, Topaloglu O, Begum S, Henrique R, Rosenbaum E, Van Criekinge W, Westra WH, Sidransky D: Quantitative methylation-specific polymerase chain reaction gene patterns in urine sediment distinguish prostate cancer patients from control subjects. J Clin Oncol 2005, 23:6569−6575
31. Herman JG, Graff JR, Myöhänen S, Nelkin BD, Baylin SB: Methylation-specific PCR: a novel PCR assay for methylation status of CpG islands. Proc Natl Acad Sci USA 1996, 93:9821−9826
32. Dejeux E, Rønneberg JA, Solvang H, Bukholm I, Geisler S, Aas T, Gut IG, Børresen-Dale AL, Lønning PE, Kristensen VN, Tost J: DNA methylation profiling in doxorubicin treated primary locally advanced breast tumours identifies novel genes associated with survival and treatment response. Mol Cancer 2010, 9:68
33. Muggerud AA, Rønneberg JA, Wärnberg F, Botling J, Busato F, Jovanovic J, Solvang H, Bukholm I, Børresen-Dale AL, Kristensen VN, Sørlie T, Tost J: Frequent aberrant DNA methylation of ABCB1, FOXC1, PPP2R2B and PTEN in ductal carcinoma in situ and early invasive breast cancer. Breast Cancer Res 2010, 12:R3
34. Lau QC, Raja E, Salto-Tellez M, Liu Q, Ito K, Inoue M, Putti TC, Loh M, Ko TK, Huang C, Bhalla KN, Zhu T, Ito Y, Sukumar S: RUNX3 is frequently inactivated by dual mechanisms of protein mislocalization and promoter hypermethylation in breast cancer. Cancer Res 2006, 66:6512−6520
35. Subramaniam MM, Chan JY, Soong R, Ito K, Ito Y, Yeoh KG, Salto-Tellez M, Putti TC: RUNX3 inactivation by frequent promoter hypermethylation and protein mislocalization constitute an early event in breast cancer progression. Breast Cancer Res Treat 2009, 113:113−121
36. Widschwendter M, Siegmund KD, Müller HM, Fiegl H, Marth C, Müller-Holzner E, Jones PA, Laird PW: Association of breast cancer DNA methylation profiles with hormone receptor status and response to tamoxifen. Cancer Res 2004, 64:3807−3813
37. Maier S, Nimmrich I, Koenig T, Eppenberger-Castori S, Bohlmann I, Paradiso A, Spyratos F, Thomssen C, Mueller V, Nährig J, Schittulli F, Kates R, Lesche R, Schwope I, Kluth A, Marx A, Martens JW, Foekens JA, Schmitt M, Harbeck N; European Organisation for Research and Treatment of Cancer (EORTC) PathoBiology group: DNA-methylation of the homeodomain transcription factor PITX2 reliably predicts risk of distant disease recurrence in tamoxifen-treated, node-negative breast cancer patients−technical and clinical validation in a multi-centre setting in collaboration with the European Organisation for Research and Treatment of Cancer (EORTC) PathoBiology group. Eur J Cancer 2007, 43:1679−1686
38. Esteller M, Corn PG, Urena JM, Gabrielson E, Baylin SB, Herman JG: Inactivation of glutathione S-transferase P1 gene by promoter hypermethylation in human neoplasia. Cancer Res 1998, 58:4515−4518
39. Hoque MO, Feng Q, Toure P, Dem A, Critchlow CW, Hawes SE, Wood T, Jeronimo C, Rosenbaum E, Stern J, Yu M, Trink B, Kiviat NB, Sidransky D: Detection of aberrant methylation of four genes in plasma DNA for the detection of breast cancer. J Clin Oncol 2006, 24:4262−4269
40. Hon GC, Hawkins RD, Caballero OL, Lo C, Lister R, Pelizzola M, Valsesia A, Ye Z, Kuan S, Edsall LE, Camargo AA, Stevenson BJ, Ecker JR, Bafna V, Strausberg RL, Simpson AJ, Ren B: Global DNA hypomethylation coupled to repressive chromatin domain formation and gene silencing in breast cancer. Genome Res 2012, 22:246−258
41. Pasini D, Cloos PA, Walfridsson J, Olsson L, Bukowski JP, Johansen JV, Bak M, Tommerup N, Rappsilber J, Helin K: JARID2 regulates binding of the polycomb repressive complex 2 to target genes in ES cells. Nature 2010, 464:306−310
42. Bernstein BE, Mikkelsen TS, Xie X, Kamal M, Huebert DJ, Cuff J, Fry B, Meissner A, Wernig M, Plath K, Jaenisch R, Wagschal A, Feil R, Schreiber SL, Lander ES: A bivalent chromatin structure marks key developmental genes in embryonic stem cells. Cell 2006, 125:315−326
43. Viré E, Brenner C, Deplus R, Blanchon L, Fraga M, Didelot C, Morey L, Van Eynde A, Bernard D, Vanderwinden JM, Bollen M, Esteller M, Di Croce L, de Launoit Y, Fuks F: The polycomb group protein EZH2 directly controls DNA methylation, [Erratum appeared in Nature 2007, 446:824]. Nature 2006, 439:871−874
44. Esteller M: Non-coding RNAs in human disease. Nat Rev Genet 2011, 12:861−874
45. Aguilo F, Zhou MM, Walsh MJ: Long noncoding RNA, polycomb, and the ghosts haunting INK4b-ARF-INK4a expression. Cancer Res 2011, 71:5365−5369
46. Holm K, Hegardt C, Staaf J, Vallon-Christersson J, Jönsson G, Olsson H, Borg A, Ringnér M: Molecular subtypes of breast cancer are associated with characteristic DNA methylation patterns. Breast Cancer Res 2010, 12:R36
47. Fang F, Turcan S, Rimner A, Kaufman A, Giri D, Morris LG, Shen R, Seshan V, Mo Q, Heguy A, Baylin SB, Ahuja N, Viale A, Massague J, Norton L, Vahdat LT, Moynahan ME, Chan TA: Breast cancer methylomes establish an epigenomic foundation for metastasis. Sci Transl Med 2011, 3:75ra25
48. Cancer Genome Atlas Network: Comprehensive molecular portraits of human breast tumours. Nature 2012, 490:61−70
49. Lister R, Pelizzola M, Dowen RH, Hawkins RD, Hon G, Tonti-Filippini J, Nery JR, Lee L, Ye Z, Ngo QM, Edsall L, Antosiewicz-Bourget J, Stewart R, Ruotti V, Millar AH, Thomson JA, Ren B, Ecker JR: Human DNA methylomes at base resolution show widespread epigenomic differences. Nature 2009, 462:315−322
50. Stratton MR, Campbell PJ, Futreal PA: The cancer genome. Nature 2009, 458:719−724
51. Collins N, McManus R, Wooster R, Mangion J, Seal S, Lakhani SR, Ormiston W, Daly PA, Ford D, Easton DF, Stratton MR: Consistent loss of the wild type allele in breast cancers from a family linked to the BRCA2 gene on chromosome 13q12−13. Oncogene 1995, 10:1673−1675
52. Smith SA, Easton DF, Evans DG, Ponder BA: Allele losses in the region 17q12−21 in familial breast and ovarian cancer involve the wild-type chromosome. Nat Genet 1992, 2:128−131
53. Barekati Z, Radpour R, Kohler C, Zhang B, Toniolo P, Lenner P, Lv Q, Zheng H, Zhong XY: Methylation profile of TP53 regulatory pathway and mtDNA alterations in breast cancer patients lacking TP53 mutations. Hum Mol Genet 2010, 19:2936−2946
54. Collins N, Wooster R, Stratton MR: Absence of methylation of CpG dinucleotides within the promoter of the breast cancer susceptibility gene BRCA2 in normal tissues and in breast and ovarian cancers. Br J Cancer 1997, 76:1150−1156
55. Cucer N, Taheri S, Ok E, Ozkul Y: Methylation status of CpG islands at sites -59 to +96 in exon 1 of the BRCA2 gene varies in mammary tissue among women with sporadic breast cancer. J Genet 2008, 87:155−158
56. Pal R, Srivastava N, Chopra R, Gochhait S, Gupta P, Prakash N, Agarwal G, Bamezai RN: Investigation of DNA damage response and apoptotic gene methylation pattern in sporadic breast tumors using high throughput quantitative DNA methylation analysis technology. Mol Cancer 2010, 9:303
57. Potapova A, Hoffman AM, Godwin AK, Al-Saleem T, Cairns P: Promoter hypermethylation of the PALB2 susceptibility gene in inherited and sporadic breast and ovarian cancer. Cancer Res 2008, 68:998−1002
58. Vo QN, Kim WJ, Cvitanovic L, Boudreau DA, Ginzinger DG, Brown KD: The ATM gene is a target for epigenetic silencing in locally advanced breast cancer, [Erratum appeared in Oncogene 2005, 24:1964]. Oncogene 2004, 23:9432−9437
59. Brandes JC, Carraway H, Herman JG: Optimal primer design using the novel primer design program: MSPprimer provides accurate

methylation analysis of the ATM promoter, [Erratum appeared in Oncogene 2007 Oct 11;26(46):6684]. Oncogene 2007, 26:6229–6237
60. Stefansson OA, Jonasson JG, Olafsdottir K, Hilmarsdottir H, Olafsdottir G, Esteller M, Johannsson OT, Eyfjord JE: CpG island hypermethylation of BRCA1 and loss of pRb as co-occurring events in basal/triple-negative breast cancer. Epigenetics 2011, 6:638–649
61. Foulkes WD, Stefansson IM, Chappuis PO, Bégin LR, Goffin JR, Wong N, Trudel M, Akslen LA: Germline BRCA1 mutations and a basal epithelial phenotype in breast cancer. J Natl Cancer Inst 2003, 95:1482–1485
62. Furuta S, Jiang X, Gu B, Cheng E, Chen PL, Lee WH: Depletion of BRCA1 impairs differentiation but enhances proliferation of mammary epithelial cells. Proc Natl Acad Sci USA 2005, 102: 9176–9181
63. Lim E, Vaillant F, Wu D, Forrest NC, Pal B, Hart AH, Asselin-Labat ML, Gyorki DE, Ward T, Partanen A, Feleppa F, Huschtscha LI, Thorne HJ, kConFab, Fox SB, Yan M, French JD, Brown MA, Smyth GK, Visvader JE, Lindeman GJ: Aberrant luminal progenitors as the candidate target population for basal tumor development in BRCA1 mutation carriers. Nat Med 2009, 15:907–913
64. Stefansson OA, Jonasson JG, Johannsson OT, Olafsdottir K, Steinarsdottir M, Valgeirsdottir S, Eyfjord JE: Genomic profiling of breast tumours in relation to BRCA abnormalities and phenotypes, [Erratum appeared in Breast Cancer Res 2009, 11:40]. Breast Cancer Res 2009, 11:R47
65. Joosse SA, Brandwijk KI, Mulder L, Wesseling J, Hannemann J, Nederlof PM: Genomic signature of BRCA1 deficiency in sporadic basal-like breast tumors. Genes Chromosomes Cancer 2011, 50: 71–81
66. Saal LH, Gruvberger-Saal SK, Persson C, Lövgren K, Jumppanen M, Staaf J, Jönsson G, Pires MM, Maurer M, Holm K, Koujak S, Subramaniyam S, Vallon-Christersson J, Olsson H, Su T, Memeo L, Ludwig T, Ethier SP, Krogh M, Szabolcs M, Murty VV, Isola J, Hibshoosh H, Parsons R, Borg A: Recurrent gross mutations of the PTEN tumor suppressor gene in breast cancers with deficient DSB repair. Nat Genet 2008, 40:102–107
67. Jönsson G, Staaf J, Vallon-Christersson J, Ringnér M, Gruvberger-Saal SK, Saal LH, Holm K, Hegardt C, Arason A, Fagerholm R, Persson C, Grabau D, Johnsson E, Lövgren K, Magnusson L, Heikkilä P, Agnarsson BA, Johannsson OT, Malmström P, Fernö M, Olsson H, Loman N, Nevanlinna H, Barkardottir RB, Borg Å: The retinoblastoma gene undergoes rearrangements in BRCA1-deficient basal-like breast cancer. Cancer Res 2012, 72:4028–4036
68. Holstege H, Joosse SA, van Oostrom CT, Nederlof PM, de Vries A, Jonkers J: High incidence of protein-truncating TP53 mutations in BRCA1-related breast cancer. Cancer Res 2009, 69:3625–3633
69. Zeidler M, Varambally S, Cao Q, Chinnaiyan AM, Ferguson DO, Merajver SD, Kleer CG: The polycomb group protein EZH2 impairs DNA repair in breast epithelial cells. Neoplasia 2005, 7:1011–1019
70. Chang CJ, Yang JY, Xia W, Chen CT, Xie X, Chao CH, Woodward WA, Hsu JM, Hortobagyi GN, Hung MC: EZH2 promotes expansion of breast tumor initiating cells through activation of RAF1-β-catenin signaling. Cancer Cell 2011, 19:86–100
71. Li X, Gonzalez ME, Toy K, Filzen T, Merajver SD, Kleer CG: Targeted overexpression of EZH2 in the mammary gland disrupts ductal morphogenesis and causes epithelial hyperplasia. Am J Pathol 2009, 175:1246–1254
72. Perou CM, Børresen-Dale AL: Systems biology and genomics of breast cancer. Cold Spring Harb Perspect Biol 2011, 3:a003293
73. Chou DM, Adamson B, Dephoure NE, Tan X, Nottke AC, Hurov KE, Gygi SP, Colaiácovo MP, Elledge SJ: A chromatin localization screen reveals poly (ADP ribose)-regulated recruitment of the repressive polycomb and NuRD complexes to sites of DNA damage. Proc Natl Acad Sci USA 2010, 107:18475–18480
74. Ayoub N, Jeyasekharan AD, Bernal JA, Venkitaraman AR: HP1-beta mobilization promotes chromatin changes that initiate the DNA damage response. Nature 2008, 453:682–686
75. Puppe J, Drost R, Liu X, Joosse SA, Evers B, Cornelissen-Steijger P, Nederlof P, Yu Q, Jonkers J, van Lohuizen M, Pietersen AM: BRCA1-deficient mammary tumor cells are dependent on EZH2 expression and sensitive to polycomb repressive complex 2-inhibitor 3-deazaneplanocin A. Breast Cancer Res 2009, 11:R63
76. Cho SH, Jeon J, Kim SI: Personalized medicine in breast cancer: a systematic review. J Breast Cancer 2012, 15:265–272
77. Garnett MJ, Edelman EJ, Heidorn SJ, Greenman CD, Dastur A, Lau KW, et al: Systematic identification of genomic markers of drug sensitivity in cancer cells. Nature 2012, 483:570–575
78. Barretina J, Caponigro G, Stransky N, Venkatesan K, Margolin AA, Kim S, et al: The Cancer Cell Line Encyclopedia enables predictive modelling of anticancer drug sensitivity, [Erratum appeared in Nature 2012, 492:290]. Nature 2012, 483:603–607
79. Turner N, Tutt A, Ashworth A: Hallmarks of 'BRCAness' in sporadic cancers. Nat Rev Cancer 2004, 4:814–819
80. Lord CJ, Ashworth A: The DNA damage response and cancer therapy. Nature 2012, 481:287–294
81. Farmer H, McCabe N, Lord CJ, Tutt AN, Johnson DA, Richardson TB, Santarosa M, Dillon KJ, Hickson I, Knights C, Martin NM, Jackson SP, Smith GC, Ashworth A: Targeting the DNA repair defect in BRCA mutant cells as a therapeutic strategy. Nature 2005, 434:917–921
82. Bryant HE, Schultz N, Thomas HD, Parker KM, Flower D, Lopez E, Kyle S, Meuth M, Curtin NJ, Helleday T: Specific killing of BRCA2-deficient tumours with inhibitors of poly(ADP-ribose) polymerase, [Erratum appeared in Nature 2007, 447:346]. Nature 2005, 434: 913–917
83. Rehman FL, Lord CJ, Ashworth A: Synthetic lethal approaches to breast cancer therapy. Nat Rev Clin Oncol 2010, 7:718–724
84. Fong PC, Boss DS, Yap TA, Tutt A, Wu P, Mergui-Roelvink M, Mortimer P, Swaisland H, Lau A, O'Connor MJ, Ashworth A, Carmichael J, Kaye SB, Schellens JH, de Bono JS: Inhibition of poly(ADP-ribose) polymerase in tumors from BRCA mutation carriers. N Engl J Med 2009, 361:123–134
85. Tutt A, Robson M, Garber JE, Domchek SM, Audeh MW, Weitzel JN, Friedlander M, Arun B, Loman N, Schmutzler RK, Wardley A, Mitchell G, Earl H, Wickens M, Carmichael J: Oral poly(ADP-ribose) polymerase inhibitor olaparib in patients with BRCA1 or BRCA2 mutations and advanced breast cancer: a proof-of-concept trial. Lancet 2010, 376:235–244
86. Nielsen TO, Hsu FD, Jensen K, Cheang M, Karaca G, Hu Z, Hernandez-Boussard T, Livasy C, Cowan D, Dressler L, Akslen LA, Ragaz J, Gown AM, Gilks CB, van de Rijn M, Perou CM: Immunohistochemical and clinical characterization of the basal-like subtype of invasive breast carcinoma. Clin Cancer Res 2004, 10:5367–5374
87. O'Shaughnessy J, Osborne C, Pippen JE, Yoffe M, Patt D, Rocha C, Koo IC, Sherman BM, Bradley C: Iniparib plus chemotherapy in metastatic triple-negative breast cancer. N Engl J Med 2011, 364: 205–214
88. O'Shaughnessy J, Schwartzberg LS, Danso MA, Rugo HS, Miller K, Yardley DA, Carlson RW, Finn RS, Charpentier E, Freese M, Gupta S, Blackwood-Chirchir A, Winer EP: A randomized phase III study of iniparib (BSI-201) in combination with gemcitabine/carboplatin (G/C) in metastatic triple-negative breast cancer (TNBC). J Clin Oncol 2011, 29(15 Suppl):1007
89. Patel AG, De Lorenzo SB, Flatten KS, Poirier GG, Kaufmann SH: Failure of iniparib to inhibit poly(ADP-ribose) polymerase in vitro. Clin Cancer Res 2012, 18:1655–1662
90. Marotta LL, Almendro V, Marusyk A, Shipitsin M, Schemme J, Walker SR, Bloushtain-Qimron N, Kim JJ, Choudhury SA, Maruyama R, Wu Z, Gönen M, Mulvey LA, Bessarabova MO, Huh SJ, Silver SJ, Kim SY, Park SY, Lee HE, Anderson KS, Richardson AL, Nikolskaya T, Nikolsky Y, Liu XS, Root DE, Hahn WC, Frank DA, Polyak K: The JAK2/STAT3 signaling

pathway is required for growth of CD44+CD24- stem cell-like breast cancer cells in human tumors. J Clin Invest 2011, 121:2723–2735
91. Veeck J, Ropero S, Setien F, Gonzalez-Suarez E, Osorio A, Benitez J, Herman JG, Esteller M: BRCA1 CpG island hypermethylation predicts sensitivity to poly(adenosine diphosphate)-ribose polymerase inhibitors. J Clin Oncol 2010, 28:e563–e564
92. Drew Y, Mulligan EA, Vong WT, Thomas HD, Kahn S, Kyle S, Mukhopadhyay A, Los G, Hostomsky Z, Plummer ER, Edmondson RJ, Curtin NJ: Therapeutic potential of poly(ADP-ribose) polymerase inhibitor AG014699 in human cancers with mutated or methylated BRCA1 or BRCA2. J Natl Cancer Inst 2011, 103:334–346
93. Kim H, D'Andrea AD: Regulation of DNA cross-link repair by the Fanconi anemia/BRCA pathway. Genes Dev 2012, 26: 1393–1408
94. Bolton KL, Chenevix-Trench G, Goh C, Sadetzki S, Ramus SJ, Karlan BY, et al; EMBRACE; kConFab Investigators; Cancer Genome Atlas Research Network: Association between BRCA1 and BRCA2 mutations and survival in women with invasive epithelial ovarian cancer. JAMA 2012, 307:382–390
95. Yang D, Khan S, Sun Y, Hess K, Shmulevich I, Sood AK, Zhang W: Association of BRCA1 and BRCA2 mutations with survival, chemotherapy sensitivity, and gene mutator phenotype in patients with ovarian cancer, [Erratum appeared in JAMA 2012, 307:363]. JAMA 2011, 306:1557–1565
96. Reles A, Wen WH, Schmider A, Gee C, Runnebaum IB, Kilian U, Jones LA, El-Naggar A, Minguillon C, Schönborn I, Reich O, Kreienberg R, Lichtenegger W, Press MF: Correlation of p53 mutations with resistance to platinum-based chemotherapy and shortened survival in ovarian cancer. Clin Cancer Res 2001, 7:2984–2997
97. Stefansson OA, Villanueva A, Vidal A, Martí L, Esteller M: BRCA1 epigenetic inactivation predicts sensitivity to platinum-based chemotherapy in breast and ovarian cancer. Epigenetics 2012, 7: 1225–1229
98. Chang HR, Glaspy J, Allison MA, Kass FC, Elashoff R, Chung DU, Gornbein J: Differential response of triple-negative breast cancer to a docetaxel and carboplatin-based neoadjuvant treatment. Cancer 2010, 116:4227–4237
99. Silver DP, Richardson AL, Eklund AC, Wang ZC, Szallasi Z, Li Q, Juul N, Leong CO, Calogrias D, Buraimoh A, Fatima A, Gelman RS, Ryan PD, Tung NM, De Nicolo A, Ganesan S, Miron A, Colin C, Sgroi DC, Ellisen LW, Winer EP, Garber JE: Efficacy of neoadjuvant cisplatin in triple-negative breast cancer. J Clin Oncol 2010, 28: 1145–1153
100. Byrski T, Dent R, Blecharz P, Foszczynska-Kloda M, Gronwald J, Huzarski T, Cybulski C, Marczyk E, Chrzan R, Eisen A, Lubinski J, Narod SA: Results of a phase II open-label, non-randomized trial of cisplatin chemotherapy in patients with BRCA1-positive metastatic breast cancer. Breast Cancer Res 2012, 14:R110
101. Turner NC, Tutt AN: Platinum chemotherapy for BRCA1-related breast cancer: do we need more evidence? Breast Cancer Res 2012, 14:115
102. Rhiem K, Wappenschmidt B, Bosse K, Köppler H, Tutt AN, Schmutzler RK: Platinum sensitivity in a BRCA1 mutation carrier with advanced breast cancer. Clin Oncol (R Coll Radiol) 2009, 21:448–450
103. Fan Y, Xu BH, Yuan P, Ma F, Wang JY, Ding XY, Zhang P, Li Q, Cai RG: Docetaxel-cisplatin might be superior to docetaxel-capecitabine in the first-line treatment of metastatic triple-negative breast cancer. Ann Oncol 2013, 24:1219–1225
104. Adams JR, Schachter NF, Liu JC, Zacksenhaus E, Egan SE: Elevated PI3K signaling drives multiple breast cancer subtypes. Oncotarget 2011, 2:435–447
105. Bendell JC, Rodon J, Burris HA, de Jonge M, Verweij J, Birle D, Demanse D, De Buck SS, Ru QC, Peters M, Goldbrunner M, Baselga J: Phase I, dose-escalation study of BKM120, an oral pan-class I PI3K inhibitor, in patients with advanced solid tumors. J Clin Oncol 2012, 30:282–290
106. Banerji S, Cibulskis K, Rangel-Escareno C, Brown KK, Carter SL, Frederick AM, et al: Sequence analysis of mutations and translocations across breast cancer subtypes. Nature 2012, 486:405–409
107. Chuang LS, Lai SK, Murata-Hori M, Yamada A, Li HY, Gunaratne J, Ito Y: RUNX3 interactome reveals novel centrosomal targeting of RUNX family of transcription factors. Cell Cycle 2012, 11:1938–1947
108. Van Neste L, Herman JG, Otto G, Bigley JW, Epstein JI, Van Criekinge W: The epigenetic promise for prostate cancer diagnosis. Prostate 2012, 72:1248–1261
109. Fackler MJ, Malone K, Zhang Z, Schilling E, Garrett-Mayer E, Swift-Scanlan T, Lange J, Nayar R, Davidson NE, Khan SA, Sukumar S: Quantitative multiplex methylation-specific PCR analysis doubles detection of tumor cells in breast ductal fluid. Clin Cancer Res 2006, 12:3306–3310
110. Fiegl H, Millinger S, Mueller-Holzner E, Marth C, Ensinger C, Berger A, Klocker H, Goebel G, Widschwendter M: Circulating tumor-specific DNA: a marker for monitoring efficacy of adjuvant therapy in cancer patients. Cancer Res 2005, 65:1141–1145
111. Vissers JH, van Lohuizen M, Citterio E: The emerging role of polycomb repressors in the response to DNA damage. J Cell Sci 2012, 125:3939–3948