

Research Paper

Loss of DNA Methylation and Histone H4 Lysine 20 Trimethylation in Human Breast Cancer Cells is Associated with Aberrant Expression of DNA Methyltransferase 1, Suv4-20h2 Histone Methyltransferase and Methyl-Binding Proteins

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KEY WORDS

DNA hypomethylation, breast cancer, DNA methyltransferases, histone methylation, histone methyltransferases, tumor progression

ABBREVIATIONS

SDS	sodium dodecylsulfate
DTT	dithiothreitol
PMSF	phenylmethylsulfonyl fluoride
DNMT	DNA methyltransferase
HMT	histone methyltransferase
H3-Lys9	histone H3 lysine 9
H4-Lys20	histone H4 lysine 20

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ABSTRACT

Cancer cells are characterized by epigenetic dysregulation, including global genome hypomethylation, regional hypo- and hypermethylation, altered histone modifications, and disturbed genomic imprinting. Despite the long-established fact that global DNA hypomethylation is a common feature of tumors, very little is known about evolution of this and other epigenetic alterations during tumor progression. The present study was undertaken to characterize the status of epigenetic dysregulation in three human breast cancer cell lines (MCF-7, MDA-MB-231 and MDA-MB-231(S30) that represent different stages of human breast cancer. Our data show that breast cancer cells are characterized by significant alterations in cellular epigenetic status compared to non-tumorigenic MCF-10-2A epithelial breast cells. Interestingly, more malignant MDA-MB-231 human breast cancer cells have a more prominent loss of DNA methylation accompanied by altered expression of maintenance DNA methyltransferase DNMT1, methyl-binding proteins MeCP2 and MBD2, decreased trimethylation of lysine 20 of histone H4 and hyperacetylation of histone H4 compared to MCF-7 cells. The decrease in trimethylation of lysine 20 of histone H4 in MDA-MB-231 cells was accompanied by diminished expression of Suv4-20h2 histone methyltransferase. The results of present study demonstrate that MDA-MB-231 cells have more extensive epigenetic alterations than MCF-7. These results demonstrate that human breast cancer cells are characterized by prominent epigenetic alterations which are associated with increased malignant properties of cancer cells. Such epigenetic dysregulation may contribute to and may be indicative of the formation of a more aggressive tumor phenotype during tumor progression.

INTRODUCTION

Cancer cells display a variety of genomic alterations, among which epigenetic alterations play one of the central roles. Epigenetic changes in cancer cells include global DNA hypomethylation, regional hypomethylation and hypermethylation, altered histone modifications, and genomic imprinting.¹ Loss of cytosine DNA methylation at CpG dinucleotides was the first epigenetic abnormality identified in human cancer cells and is considered a hallmark of cancer.²⁻⁶ A significant loss of methyl groups in DNA has been found to occur very early in human colorectal, breast, liver and skin cancers,⁷⁻¹² and aberrant pattern of DNA methylation often took place even at premalignant stages of tumor development.¹²⁻¹⁴ Because of that, genome-wide and locus-specific DNA hypomethylation has been suggested to be an important step in carcinogenesis, and recently, evidence of the causative role of DNA hypomethylation in tumorigenesis has appeared.^{15,16} Genome-wide DNA hypomethylation has been associated with chromosomal and genomic instability.¹⁷⁻²⁰ Because both chromosomal instability and hypomethylation are observed early in the tumorigenic process, it may be speculated that DNA hypomethylation destabilizes the genome and promotes loss of heterozygosity in regions containing tumor suppressor genes.^{1,20} It has also been postulated that DNA hypomethylation plays a role in carcinogenesis by facilitating aberrant gene expression, especially of oncogenes, even in precancerous stages.²⁰

Changes in DNA methylation in cancer cells are not isolated events—they occur in the context of more complex epigenetic deregulation.^{1,21} Recent studies have revealed links between altered DNA methylation and perturbed histone modifications in cancer cells.²¹⁻²⁴ Changes in DNA methylation influence histone modifications^{25,26} and conversely, histone modifications can affect DNA methylation.²¹

Despite the fact that cytosine DNA hypomethylation was the first identified epigenetic alteration in cancer and is the best-studied epigenetic mechanism so far, very little is known about the evolution of this alteration during tumor progression. Recent studies provided evidence that pronounced level of DNA hypomethylation was associated with advanced stages of breast and ovarian cancers.^{10,27} In addition, other studies have shown a causal role of global DNA hypomethylation in metastatic breast cancer²⁸ and the role of regional hypomethylation in the development of resistance of breast cancer cells to chemotherapeutic drugs.^{29,30} However, the changes of histone modifications during tumor progression remain largely unknown.

It has been suggested that, in addition to the role of epigenetic dysregulation in cancer initiation, epigenetic alterations may play an important role in tumor progression.³ For example, there could be variations in epigenetic dysregulation in an initial tumor cell population, which could contribute to tumor cell heterogeneity and, possibly, to the emergence of more aggressive “metastatic” tumor cell clones.³ To investigate this hypothesis, we examined and compared changes in DNA methylation, histone modifications, expression of DNA and histone methyltransferases, and methyl binding proteins in non-tumorigenic human MCF-10-2A epithelial breast cells and three breast cancer cell lines—MCF-7, MDA-MB-231 and MDA-MB-231(S30)—that represent different stages of human breast cancer with different hormonal responsiveness status. Here we provide the first experimental evidence of the profound and cancer cell aggressiveness-dependent changes in DNA and histone methylation status in human breast cancer cells. The observed changes were paralleled by the alteration in the expression of DNA methyltransferase DNMT1, Suv39h1 and Suv4-20h2 histone methyltransferases, and MeCP2 and MBD4 methyl-binding proteins.

MATERIALS AND METHODS

Cells and cell culture. The MCF-10-2A, MCF-7 and MDA-MB-231 human breast cell lines were obtained from the American Type Culture Collection (ATCC, Manassas, VA) and were maintained according to ATCC's recommendation. MDA-MB-231(S30), a stably transfected MDA-MB-231 cell line with wild-type ER gene³¹ were obtained from Dr. V.C. Jordan (Fox Chase Cancer Center, Philadelphia, PA). Cells were seeded at density 0.5×10^6 viable cells per 100 mm plate, and the media was changed every other day for 6 days. Trypsinized cells were washed in PBS and immediately frozen at -80°C for subsequent analyses. The experiments were repeated twice, and each cell line tested in triplicate.

Determination of DNA methylation. Genomic DNA was isolated from harvested cells by using QIAamp DNA Mini Kits (Qiagen, Valencia, CA) according to manufacturer's protocol. A radiolabeled [^3H]-dCTP extension assay was used to evaluate the level of global DNA methylation as described previously.³² Briefly, 1 μg of genomic DNA was digested with 20 U of methylation-sensitive HpaII restriction endonuclease (New England Biolabs, Beverly, MA) for 16-18 h at 37°C . A second DNA aliquot (1 μg) was digested with methylation-insensitive isoschizomer MspI, which cleaves CCGG sites in DNA regardless of methylation status. Undigested DNA served as background control. The single nucleotide extension reaction was performed in a 25 μl reaction mixture containing 1.0 μg DNA, 1X PCR

buffer II, 1.0 mM MgCl_2 , 0.25 U AmpliTaq DNA polymerase, and 0.1 μl of [^3H]dCTP (57.4 Ci/mmol), and incubated at 56°C for 1 h. Samples were applied to DE-81 ion-exchange filters and washed three times with 0.5 M Na-phosphate buffer (pH 7.0) at room temperature. The filters were dried and processed for scintillation counting. The [^3H]dCTP incorporation into DNA was expressed as mean disintegrations per minute (dpm) per μg of DNA after subtraction of the dpm incorporation in undigested samples (background).

Histone extraction. The acid cell extracts were prepared from $3-5 \times 10^6$ cells using lysis buffer containing 10 mM HEPES, pH 7.9, 1.5 mM MgCl_2 , 10 mM KCl, 0.5 mM DTT, and 1.5 mM PMSE, followed by addition of HCl to a final concentration of 200 mM according to manufacturer's protocol (Upstate, Charlottesville, VA). Cell lysates were incubated on ice for 1 h, centrifuged at $14000 \times g$ for 10 min at 4°C , and the acid-insoluble pellets were discarded. The supernatant fractions, which contain the acid soluble proteins, were mixed with ten volumes of acetone. After the precipitates had coagulated overnight at -20°C , they were collected by centrifugation and air-dried. The histones were dissolved in water and aliquots of total histones were stored at -80°C . Protein concentrations were determined by Bradford assay (Pierce, Rockford, IL).

Western blot analysis of histone modification. Equal amount of total histones (40 μg) were mixed with two volumes of gel loading buffer (250 mM Tris-HCl, pH 8.0, 20% β -mercaptoethanol, 40% glycerol, 8% SDS, and 1.2 mg/ml bromophenol blue), heated for five min at 95°C , and resolved on 15% polyacrylamide gel. Proteins were transferred onto PVDF membranes. The membranes were blocked for 4 h in TBS buffer containing 5% non-fat dry milk. Anti-dimethyl histone H3 lysine 9 (H3-Lys9), anti-trimethyl histone H4 lysine 20 (H4-Lys20), anti-acetyl histone H3 and anti-acetyl histone H4 primary antibodies were diluted 1:2000, 1:6000, 1:2000 and 1:1000, respectively, according to manufacturer's recommendations (Upstate). Primary antibody binding was performed at 4°C overnight with constant shaking. Secondary donkey anti-rabbit antibodies labeled with alkaline phosphatase (Santa Cruz Biotechnology, Santa Cruz, CA) were applied at 1:5000 and 1:10000 dilutions, and binding was carried out at room temperature for 1.5 h. Chemifluorescence detection was performed with the ECF Substrate for Western Blotting (Amersham Biosciences, Piscataway, NJ) and detected directly by a Storm imaging system (Molecular Dynamics, Sunnyvale, CA). Images are representative of three independent immunoblots, and were analyzed by ImageQuant software. All membranes were stained with Coomassie Blue to confirm equal protein loading.

Western blot analysis of DNA methyltransferase 1(DNMT1), DNMT3a, DNMT3b, methyl-binding proteins, and of Suv39h1 and Suv4-20h2 histone methyltransferases (HMTs) Expression. Cell lysates were prepared by homogenization of $3-5 \times 10^6$ cells in 500 μl of lysis buffer (50 mM Tris-HCl, pH 7.4; 1% NP-40; 0.25% sodium deoxycholate; 150 mM NaCl; 1 mM EDTA; 1 mM PMSF; 1 $\mu\text{g}/\text{ml}$ each aprotinin, leupeptin, pepstatin; 1 mM Na_3VO_4 , and 1 mM NaF), sonication, and incubation at 4°C for 30 minutes followed by centrifugation at $10000 \times g$ at 4°C for 20 minutes. Extracts containing equal quantities of proteins were separated on 8–12% polyacrylamide gels and transferred to PVDF membranes. Membranes were probed at 4°C overnight with primary antibodies specific for DNMT1 (1:1000, Abcam, Cambridge, MA), DNMT3a, DNMT3b (1:500, Abgent, San Diego, CA), MeCP2 (1:1000, Abcam), MBD2 (1:500, Abcam), Suv39h1 (1:1000, Upstate) and Suv4-20h2 (1:1500, Abcam). Antibody binding was revealed by incubation with horseradish peroxidase-conjugated or alkaline phosphatase-coupled donkey anti-rabbit secondary antibodies and the ECL Plus Immunoblotting Detection System (Amersham Biosciences). Signals were quantified using NIH ImageJ 1.63 Software and normalized relative to GAPDH or β -actin. All membranes were stained with Coomassie Blue to confirm equal protein loading.

Statistical analysis. Results are presented as mean \pm S.D. Statistical analyses were conducted by one-way ANOVA using the Sigmapstat software (Jandel Scientific, San Rafael, CA).

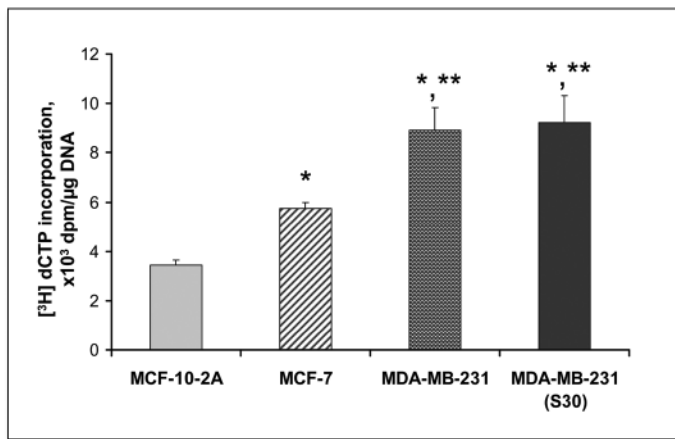


Figure 1. Loss of global DNA methylation in human breast cancer cells. *Significantly different from MCF-10-2A cells, $p < 0.05$, $n = 4$. **Significantly different from MCF-7 cells, $p < 0.05$, $n = 4$.

RESULTS

Global genome hypomethylation in breast cancer cells. To determine the status of DNA methylation, we used the HpaII-based cytosine extension assay that measures the proportion of CCGG that had lost methyl groups on both strands. HpaII is a methylation-sensitive restriction endonuclease that cleaves CCGG sequences when internal cytosine residues are unmethylated on both strands and leaves a 5'-guanine overhang after cleavage that can be used for subsequent single nucleotide extension with [³H]dCTP. The extent of [³H]dCTP incorporation opposite to the exposed guanine is directly proportional to the number of cleaved (unmethylated) sites. Because the vast majority of the frequently-occurring HpaII tetranucleotide recognition sequences are constitutively methylated in vivo, an increase in cleavage at these sites is an indicator of genome-wide hypomethylation. The incorporation of [³H]dCTP into HpaII-digested DNA isolated from MCF-7, MDA-MB-231 and MDA-MB-231(S30) breast cancer cells was 65%, 120% and 110% higher compared to MCF-10-2A non-tumorigenic breast cells (Fig. 1). Interestingly, [³H]dCTP incorporation into HpaII-digested DNA isolated from MDA-MB-231 and MDA-MB-231(S30) breast cancer cells was ~55% higher than into DNA isolated from MCF-7 cells (Fig. 1). The higher extent of [³H]dCTP incorporation indicates that the MDA-MB-231 cells have a more prominent loss of DNA methylation compared to MCF-7 cells.

Mechanisms of global DNA hypomethylation in breast cancer cells—Roles of DNA methyltransferases and methyl-binding proteins. In order to uncover the mechanisms leading to the loss of global DNA methylation in MCF-7, MDA-MB-231 and MDA-MB-231(S30) breast cancer cells, we analyzed the expression of DNA methyltransferases and methyl-CpG binding proteins. DNA methyltransferases—maintenance methyltransferases DNMT1 and de novo methyltransferases DNMT3a and DNMT3b—are the three main functional enzymes that are responsible for setting and maintaining DNA methylation patterns in mammalian cells. These enzymes have diverse abilities to catalyze maintenance and de novo methylation, thus dysregulation of their expression and/or activity may lead to alterations in DNA methylation. We found that expression of DNMT1 in breast cancer cells was significantly elevated in comparison to normal MCF-10-2A breast epithelial cells ($p < 0.05$) (Fig. 2). Furthermore, expression of DNMT1 was the highest in the most malignant MDA-MB-231 cells. It was previously shown that cancer cells, including breast cancer cells, frequently exhibit paradoxical increase of DNMT1 alongside with global genome hypomethylation.^{9,33,34} Increase of DNMT1 expression was paralleled by increased expression of methyl-binding proteins MeCP2 and MBD2 in breast cancer cells. Overall, the expression of DNMT1, MeCP2 and MBD2 in MCF-7 and MDA-MB-231 cells increased in a cancer cell aggressiveness-dependent

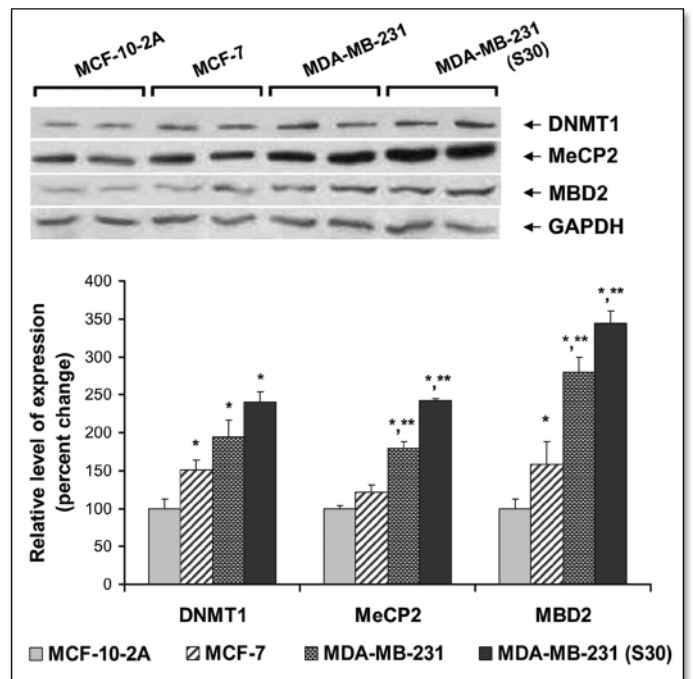


Figure 2. Human breast cancer cell lines exhibit elevated levels of DNA methyltransferase DNMT1 and methyl-binding proteins MeCP2 and MBD2. Cell lysates were subjected to immunoblotting using primary antibodies against DNMT1, MeCP2 and MBD2 as described in Materials and Methods. Expression levels of proteins in breast cancer cells are shown as relative to expression in non-tumorigenic MCF-10-2A breast epithelial cells after normalization to GAPDH levels. All samples loading were normalized to protein content. Representative western blots from two independent experiments are shown. Each lane represents a cell extract of an individual plate. *Significantly different from MCF-10-2A cells, $p < 0.05$, $n = 4$. **Significantly different from MCF-7 cells, $p < 0.05$, $n = 4$.

manner—the more advanced MDA-MB-231 breast cancer cells displayed higher expression of DNMT1, MeCP2 and MBD2 compared to MCF-7 cells. We have also seen a slight (~20%) yet statistically insignificant decrease of the expression of de novo DNA methyltransferases DNMT3a and 3b in the MDA-MB-231 and MDA-MB-231(S30) cells compared to MCF-10 cells.

Status of histone H3 and histone H4 modification. Considering the tight interaction between the status of DNA methylation and modifications of histones²¹⁻²⁶ and the fact that changes in DNA methylation occur in the context of global chromatin dysregulation, we measured the level of acetylation and methylation of histones H3 and H4 (Fig. 3). The extent of acetylation of histone H3 and dimethylation of histone H3-Lys 9 did not differ between the all four cell lines. At the same time, acetylation of histone H4 and trimethylation of histone H4-Lys 20 changed in two opposite directions. MCF-7, MDA-MB-231 and MDA-MB-231(S30) breast cancer cells displayed significant 85%, 130% and 160% ($p < 0.05$) increases in histone H4 acetylation, respectively, as compared to non-tumorigenic MCF-10-2A cells. In contrast, trimethylation of histone H4-Lys 20 in cancer cells substantially and statistically significantly ($p < 0.05$) decreased compared to MCF-10-2A cells. The significant difference in acetylation of histone H4 and trimethylation of histone H4-Lys 20 between MCF-7 and MDA-MB-231 cells indicates severe disorganization of chromatin structure in MDA-MB-231 cells representing advanced stages of breast cancer.

Expression of Suv39h1 and Suv4-20h2 HMTs. Status of histone H4 Lys20 trimethylation was significantly altered in breast cancer cells lines. The degree of changes of histone H4 Lys20 trimethylation correlated with the carcinogenic potential of the cells and was the most pronounced in the most malignant MDA-MB-231 cells. In order to determine the possible

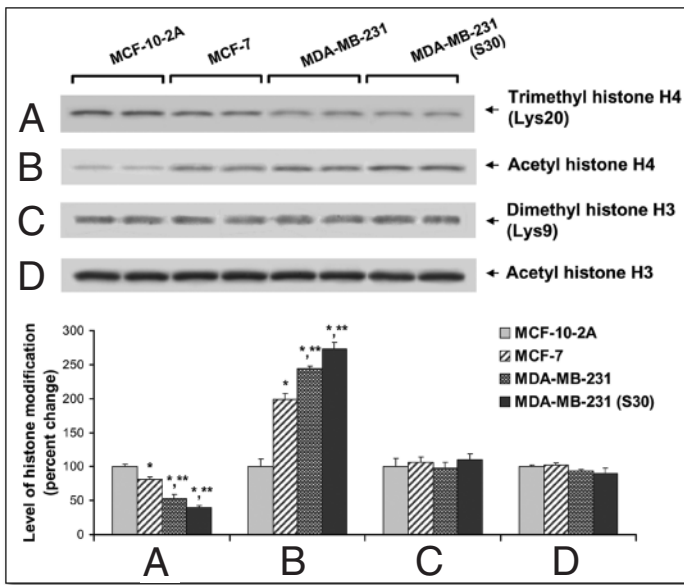


Figure 3. Human breast cancer cell lines exhibit altered histone modification status. Acidic cell lysates were subjected to immunoblotting using primary antibodies against anti-trimethyl-histone H4 (lys20) (A); anti-acetyl histone H4 (B); anti-dimethyl-histone H3 (lys9) (C); anti-acetyl histone H3 (D). Equal protein loading was confirmed by staining with Coomassie Blue. The results are presented as percent change in breast cancer cells relative to non-tumorigenic MCF-10-2A breast epithelial cells. Representative western blots from two independent experiments are shown. Each lane represents a cell extract of an individual plate. *Significantly different from MCF-10-2A cells, $p < 0.05$, $n = 4$. **Significantly different from MCF-7 cells, $p < 0.05$, $n = 4$.

mechanism that may be involved in alteration of DNA and histone methylation in breast cancer cells, we studied the expression of proteins responsible for this methylation reaction. The expression of Suv39h1 HMT was lower in breast cancer cells and did not differ between cancer cell lines (Fig. 4). The expression of Suv4-20h2 HMT in MCF-7 cells was not different from its level in MCF-10-2A. At the same time, the expression of Suv4-20h2 was almost 2 fold higher than in MDA-MB 231 and MDA-MB-231(S20) cells and strongly correlated with the changes in histone H4 Lys20 trimethylation ($r^2 > 0.85$; $p < 0.05$).

DISCUSSION

The present study was undertaken to investigate the status of epigenetic dysregulation during the progression of breast cancer by using non-tumorigenic human epithelial breast cells and breast cancer cell lines—MCF-7, MDA-MB-231 and MDA-MB-231(S30)—that represent different stages of human breast cancer. MCF-7 cells, a estrogen receptor (ER)-positive, hormone-sensitive and low invasive cell line, represent early stage of human breast cancer, whereas MDA-MB-231 cells, which are ER-negative, hormone-insensitive, highly invasive and metastatic, represent a late stage of breast cancer.³³ MDA-MB-231(S30), a stably transfected MDA-MB-231 cell line with wild-type ER gene,³¹ was used to investigate the dependence between epigenetic alterations and hormonal responsiveness status at advanced stages of breast cancer. Despite the long-established fact that global DNA hypomethylation is the common feature of tumors and the recently demonstrated causative role of DNA hypomethylation in tumor development,^{15,16} very little is known about evolution of this epigenetic alteration during tumor progression, especially during breast cancer progression. Our data show substantial loss of global

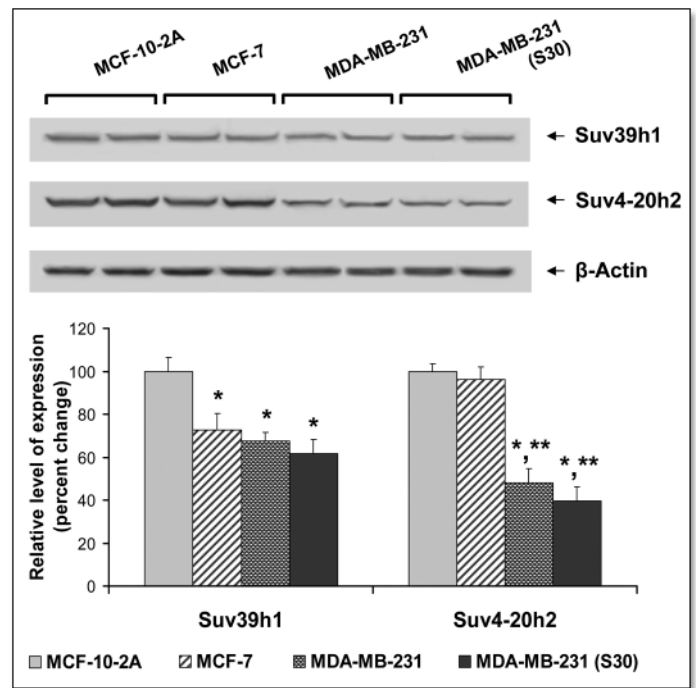


Figure 4. Changes in expression of histone methyltransferases Suv39h1 and Suv4-20h2 in human breast cancer cell lines. Cell lysates were subjected to immunoblotting using antibodies against Suv39h1 and Suv4-20h2 histone methyltransferases as described in Materials and Methods. Expression levels of proteins in breast cancer cells are shown as relative to expression in non-tumorigenic MCF-10-2A breast epithelial cells after normalization to β -actin levels. All samples loading were normalized to protein content. Representative western blots from two independent experiments are shown. Each lane represents a cell extract of an individual plate. *Significantly different from MCF-10-2A cells, $p < 0.05$, $n=4$. **Significantly different from MCF-7 cells, $p < 0.05$, $n=4$.

DNA methylation in human breast cancer cells compared to non-tumorigenic breast epithelial cells. Interestingly, MDA-MB-231 human breast cancer cells are characterized by more prominent loss of DNA methylation compared to MCF-7 cells. Recent studies showing the increase of DNA hypomethylation with advanced stage of breast,¹⁰ ovarian²⁷ and urothelial³⁵ cancers, support our finding.

The majority of cytosine methylation in mammalian cells resides in transposable elements,^{36,37} which are interspersed repeated sequences that constitute almost 50% of human genome.³⁸ At the same time, more than 50% of HpaII sequences in the human genome are located within transposons.³⁹ Because of this, we may conclude that loss of cytosine methylation in breast cancer cells, as detected by HpaII-cytosine extension assay, predominantly occurred at transposable elements. Decrease in DNA methylation is often associated with broad varieties of chromosomal and genomic instability, and that instability of genome in human breast cells is an important contributor to tumorigenic process.⁴⁰ Based on this evidence, we suggest that more extensive DNA hypomethylation in MDA-MB-231 cells may lead to a greater degree of chromosomal abnormalities during progression of tumors. Indeed, MDA-MB-231 cells are characterized by higher number of DNA lesions and chromosomal aberrations compared to MCF-7 cells.^{19,41} Similar observations regarding the decrease of DNA methylation and increased genomic and chromosomal instability have been made in patients with advanced stages of cancer as well.⁸ In addition to increased genomic

instability, DNA hypomethylation can also lead to gene activation. Based on these observations and our finding of a profound loss of DNA methylation in cancer cells, especially in MDA-MB-231 cells, we hypothesize that MDA-MB-231 cells are characterized by increased expression of the genes that are associated with formation of more aggressive tumor phenotype, especially genes responsible for invasion and metastasis. Indeed, MDA-MB-231 cells are distinguished by high level of expression of urokinase (uPA),³³ interleukin-6 (IL-6),⁴² synuclein γ (SNCG),⁴³ which is also known as breast-cancer-specific gene 1 (BCSG1), and transglutaminase⁴⁴ genes. In contrast, these genes are not expressed in MCF-7 cells. Additionally, these genes are regulated by methylation status of their promoter regions, which are completely unmethylated in MDA-MB231 cells, and densely methylated in normal breast epithelial cells and MCF-7 cancer cells.^{33,42-44}

The tight relationship between DNA methylation and histone modifications²¹⁻²⁶ is also expected to be disrupted in cancer cells, but no profile of overall histone modifications in the transformed cells has been identified.²¹ Moreover, changes that occur during tumor progression have not been addressed yet. In the current study, the breast cancer cells, especially MDA-MB-231 cells, were characterized by increased acetylation of histone H4 and substantial loss of histone H4-Lys20 trimethylation. These findings are in good agreement with previous observations of association between DNA hypomethylation and histone acetylation,^{6,24} and recent study that revealed that hyperacetylation of histone H4 prevents histone H4-Lys20 trimethylation.⁴⁵ To determine the possible mechanism that may be involved in alteration of DNA and histone methylation in breast cancer cells, we studied the expression of proteins responsible for these methylation reactions. Breast cancer cells are characterized by increased expression of DNMT1 and MeCP2 and MBD2 methyl-binding proteins and diminished expression of Suv39h1 and Suv4-20h2 HMTs. Our findings of increased expression of DNMT1 in breast cancer cells support a recent study by Agoston et al.³⁴ that has linked elevated expression of DNMT1 in breast cancer to the increased protein stability. The molecular mechanisms of DNA hypomethylation during tumor progression are undefined. One of the possible mechanisms contributing to loss of DNA methylation in breast cancer cells may be related to the increased expression of MBD2 and its recently identified demethylase activity.⁴⁶ In this study, elevated MBD2 levels in breast cancer cells go in parallel with the genome hypomethylation, indirectly suggesting that its possible role as demethylase has to be further investigated.⁴⁶

The most intriguing findings of our study are substantial loss of histone H4-Lys20 trimethylation, decreased expression of Suv39h1 and Suv4-20h2 HMTs, connection of these changes with DNA hypomethylation, and association with advanced stages of breast cancer. Emerging evidence suggests an important role of Suv39h1 and Suv4-20h2 HMTs and histone H4-Lys20 trimethylation in tumor suppression and in the maintenance of the genomic stability.⁴⁷⁻⁵¹ Genetic inactivation of Suv39h1 and/or Suv4-20h2 HMTs resulted in genomic and chromosomal instabilities in variety of somatic cells and was associated with increased tumor risk.^{49,50} In mice, inactivation of Suv39h1 HMT led to development of aggressive lymphomas in response to oncogenic Ras.⁵⁰ It is known that persistent inhibition of protein function at the gene-product level is essentially similar to mutational inactivation. Therefore, inhibition of expression of Suv39h1 and Suv4-20h2 may lead to the same consequences as mutational inactivation of the gene and result in an abnormal chromatin pattern.⁵¹

Recently, it has been revealed that trimethylated histone H4-Lys20 is present through much of the genome but does not concentrate at any single area, and trimethylation of histone H4-Lys20 does not have any apparent role in the regulation of gene expression, rather plays crucial role in the DNA damage checkpoint control.⁵² In the absence of histone H4-Lys20 methylation, cells are not able to maintain cell cycle arrest. Substantial loss of trimethylation of histone H4-Lys20 has been recently detected in human cancers.²¹ This alteration occurred early and is considered a hallmark of cancer cells.

In summary, the results of present study demonstrate that human breast cancer cells are characterized by prominent changes in cellular epigenetic status. The epigenetic alterations were the most significant in the most malignant cells. Such dysregulations may contribute to and may be indicative of formation of more aggressive tumor phenotype during tumor progression.

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