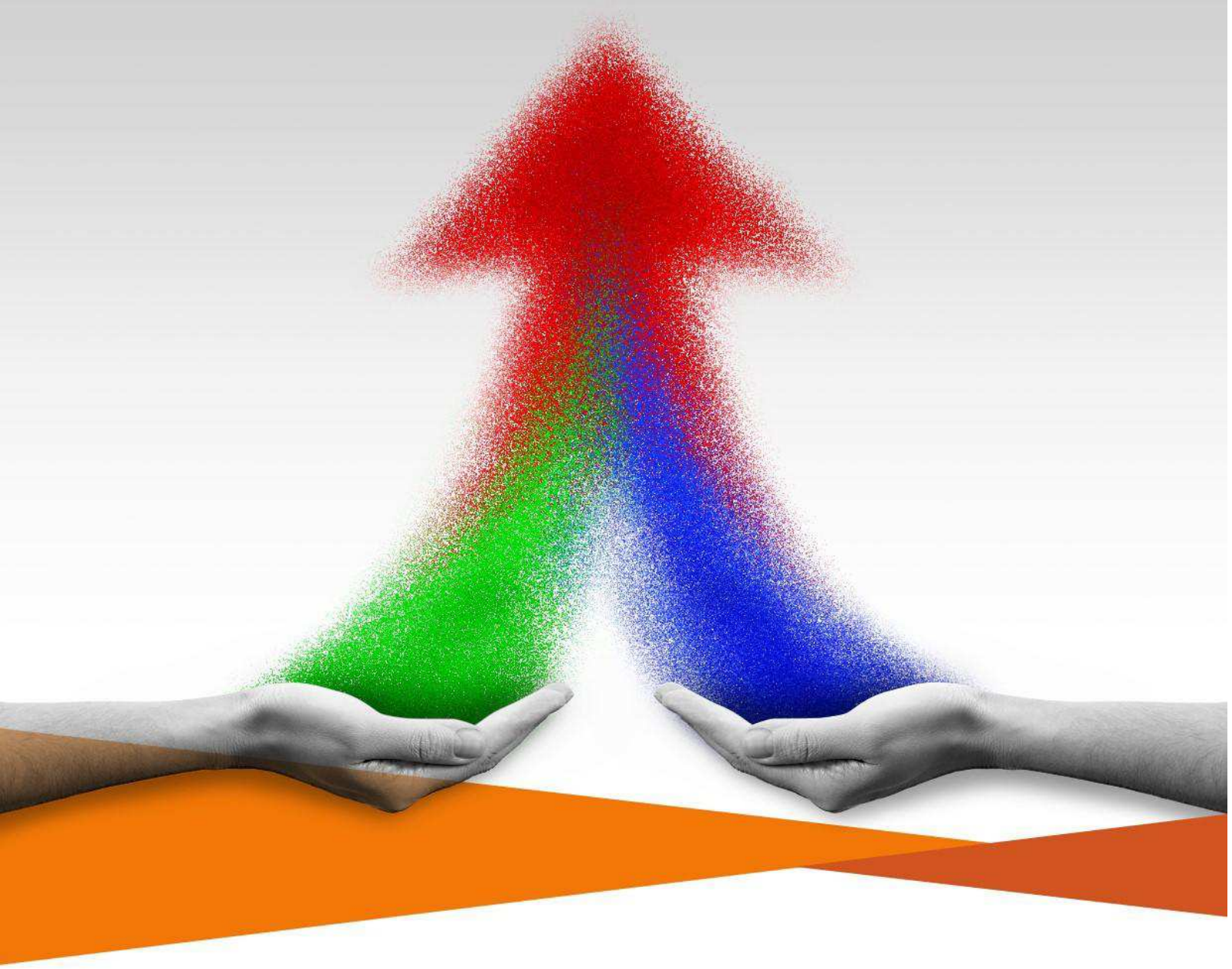

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HYPOMETHYLATION OF PERICENTROMERIC DNA IN BREAST ADENOCARCINOMAS

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Drug-induced DNA demethylation in normal human cells and inherited localized hypomethylation in mitogen-stimulated lymphocytes from patients with a rare recessive disease (ICF: immunodeficiency, centromeric region instability, facial anomalies) are associated with karyotypic instability. This chromosomal recombination is targeted to heterochromatin in the vicinity of the centromere (pericentromeric region) of human chromosome 1. Pericentromeric rearrangements in this chromosome as well as overall genomic hypomethylation are frequently observed in many kinds of cancer, including breast adenocarcinoma. We found that almost half of 25 examined breast adenocarcinomas exhibited hypomethylation in satellite 2 DNA, which is located in the long region of heterochromatin adjacent to the centromere of chromosome 1 and is normally highly methylated. One of the 19 examined non-malignant breast tissues displaying fibrocystic changes was similarly hypomethylated in this satellite DNA. We also looked at an opposing type of methylation alteration in these cancers, namely, hypermethylation in a tumor-suppressor gene region that is frequently hypermethylated in breast cancers. We found that increased methylation in the E-cadherin promoter region and decreased methylation in satellite 2 DNA were often present in the same breast cancers. While hypermethylation in certain tumor-suppressor gene regions may favor tumorigenesis by repressing transcription, demethylation of other DNA sequences may predispose to cancer-promoting chromosomal re-arrangements. *Int. J Cancer* 77:833–838, 1998.
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We have found that low doses of the DNA methylation inhibitors 5-azacytidine and 5-azadeoxycytidine specifically induce rearrangements in the heterochromatin in and around the centromere (pericentromeric heterochromatin) of chromosome 1 (Chr1) in cultured cells of normal human origin (a pro-B cell line, FLEB14; Hernandez *et al*., 1997). These structural aberrations are targeted with extraordinary efficiency. About 70% of all cytogenetically detected rearrangements in FLEB14 cells treated with these DNA methylation inhibitors were in this chromosomal region, which contains satellite DNA that is normally highly methylated. The relative frequency of pericentromeric rearrangements in chromosomes of these azacytidine-treated cells was Chr1 >> Chr16 > Chr9, with no pericentromeric rearrangements seen in any other chromosome (Hernandez *et al*., 1997; Ji *et al*., 1997). Chr1 contains the longest region of heterochromatin adjacent to the centromere (juxtacentromeric heterochromatin) of any human chromosome. The juxtacentromeric heterochromatin region of Chr16, which is much shorter than that of Chr1, consists mostly of satellite 2 (Sat2) DNA, as does the corresponding heterochromatin region in Chr1. Chr9 also contains an unusually long region of juxtacentromeric heterochromatin. However, the major juxtacentromeric satellite DNA of Chr9 is Sat3, rather than Sat2.

The induction of pericentromeric rearrangements in FLEB14 cells was specific to DNA methylation inhibitors (Ji *et al*., 1997). A variety of non-demethylating genotoxins that induced chromosomal rearrangements in the FLEB14 cells gave <0.2% of these rearrangements in the pericentromeric (centromeric or juxtacentromeric) region of Chr1, Chr16 or Chr9. DNA hypomethylation is further implicated in these 5-azacytidine- and 5-azadeoxycytidine-induced pericentromeric Chr1 rearrangements because the diagnostic feature of a rare human genetic disease, ICF (immunodeficiency, centromeric region instability, and facial anomalies), is a very high frequency of chromosomal rearrangements in these regions in mitogen-stimulated lymphocytes and ICF is always associated with abnormal hypomethylation of Sat2 DNA sequences (Jeanpierre *et al*., 1993). The importance of juxtacentromeric heterochromatin in these pericentromeric rearrangements is suggested by these rearrangements being seen almost only in Chr1, Chr9 and Chr16, with their considerable lengths of juxtacentromeric heterochromatin, and mostly in Chr1, just as for DNA methylation inhibitor-induced pericentromeric rearrangements in normal cultured cells (Hernandez *et al*., 1997; Smeets *et al*., 1994).

In a wide variety of cancers, pericentromeric rearrangements in Chr1 are over-represented with respect to the percentage of the human genome present in the pericentromeric heterochromatin (Mertens *et al*., 1997). For example, the most frequent karyotypic abnormality in breast cancer is chromosomal rearrangement in the pericentromeric region of Chr1, usually involving isochromosomal formation, whole-arm deletions of Chr1, or unbalanced translocations of 1q to 16p with the fusion of these chromosomal arms in the juxtacentromeric or the centromeric heterochromatin (Heim and Mitelman, 1995). These Chr1 pericentromeric rearrangements are sometimes the sole karyotypic abnormality in primary breast adenocarcinoma or its metastasis (Pandis *et al*., 1995), suggesting a role in carcinogenesis by causing the numerical arm imbalances that result from these rearrangements. Abnormal dosages (Heim and Mitelman, 1995) for proto-oncogenes and tumor-suppressor genes inferred to be located on the arms of Chr1 may contribute to tumor formation in cancers with these pericentromeric rearrangements. In this study, we demonstrate that hypomethylation of Sat2 DNA in the pericentromeric regions of Chr1 and Chr16 is often found in breast adenocarcinomas.

MATERIAL AND METHODS

Tissue samples and DNA isolation

Twenty-five primary mammary adenocarcinoma samples were obtained in the course of mastectomy or lumpectomy from women aged 33 to 87 years (mean 52.5 years) who were treated for breast cancer. The neoplastic samples selected for DNA isolation (Gama-Sosa *et al*., 1983) were those with a high percentage of cancer cells as determined by examination of frozen sections by light microscopy. Almost all of the tumors were infiltrating ductal carcinomas of the moderately differentiated type. None of the patients received hormonal therapy or radiation therapy prior to surgery. Also, breast

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tissue with benign pathology was collected from 18 female patients and 1 male patient. Ten of these specimens showed mild fibrocystic changes; 2 had moderate fibrocystic changes; 5 were fibroadenomas; 1 was a benign phylloides tumor; and 1 was male-derived tissue displaying fibrocystic changes. Five of the samples displaying fibrocystic changes also showed mild epithelial cell hyperplasia. Also, in 5 patients with mild fibrocystic changes, there was a personal history of breast cancer but no evidence of carcinoma in or near these tissue samples. DNA was also obtained from the following non-neoplastic tissues: histologically normal breast tissue from a part of a mastectomy sample distant from the adenocarcinoma; various tissues derived from autopsy samples of trauma victims; pooled normal sperm samples; and term placenta. Also, for analysis of the hypermethylation of an E-cadherin gene region, a cellular fraction enriched in breast epithelial cells was isolated from non-malignant (reductive mammoplasty) breast tissue (Graff *et al*., 1997).

Southern blot analysis for satellite DNA hypomethylation

DNA samples (1 to 2 μg) were digested with 20 U of the CpG methylation-sensitive enzyme BstB I (New England Biolabs, Beverly, MA) in a total volume of 60 μl for 18 hr under standard conditions with an internal control for complete digestion. Blot hybridization under stringent conditions [0.12 M sodium phosphate (pH 7.2), 0.25 M NaCl, 7% SDS, 50% formamide, 43°C] was done with the following probes: cloned Chr1-specific and Chr16-specific Sat2 inserts excised from recombinant plasmids, pUC1.77 (Almeida *et al*., 1993) and D16Z3 (Moyzis *et al*., 1987); a Chr1-specific Satα DNA, D1Z5 (Waye *et al*., 1987); and a Sat2 consensus oligonucleotide, 5′-TCGAGTCCATTCGATGAT-3′ (Tagarro *et al*., 1994).

Methylation-specific PCR (MSP) for analyzing E-cadherin gene methylation in the 5′ CpG island

Bisulfite modification-based MSP analysis was done on 0.5 to 1 ng of DNA modified with sodium bisulfite and amplified with primers for CpG island 3 of E-cadherin (Graff *et al*., 1997). DNA from an E-cadherin-expressing breast cancer cell line (MCF-7) was included in every set of reactions and found to be negative for methylation at island 3.

RESULTS

Methylation of juxtacentromeric Sat2 DNA sequences in normal tissues and in breast tissues displaying fibrocystic changes

Because juxtacentromeric heterochromatin has been implicated in chromosomal rearrangements, we compared methylation of its main component, Sat2 DNA, in various normal tissues, breast cancers and fibrocystic or fibroadenoma breast tissue. We first examined methylation of this sequence in normal tissues. DNA samples were digested with a CpG methylation-sensitive restriction endonuclease, BstB I, recognizing a site present twice, 522 bp apart, in the cloned 1,352-bp repeat of the Sat2 sequence (HSSAT2). These digests were blot-hybridized with a cloned DNA probe specific for the Sat2 sequences of Chr1, another for the Sat2 DNA sequences of Chr16 and a consensus sequence oligonucleotide probe for Sat2 DNA. The oligonucleotide probe should hybridize to both Chr1 and Chr16 Sat2 DNA sequences but will mostly anneal to the much more abundant Chr1 sequence (Tagarro *et al*., 1994).

A high degree of methylation was observed in a variety of normal postnatal somatic tissues, including histologically normal breast tissue, in this juxtacentromeric satellite DNA with the Chr1-specific probe and the consensus sequence probe (Figs. 1, 2; data not shown). In contrast to the postnatal somatic tissues, sperm DNA was extensively cleaved by BstB I in Chr1 Sat2. Given the difference in m.w. between the sperm DNA fragments and these somatic tissue DNA fragments, this result indicates that sperm DNA is highly hypomethylated in this sequence; however, the very high copy number of the satellite repeat and its attendant intragenomic sequence heterogeneity interfere with quantitation of its

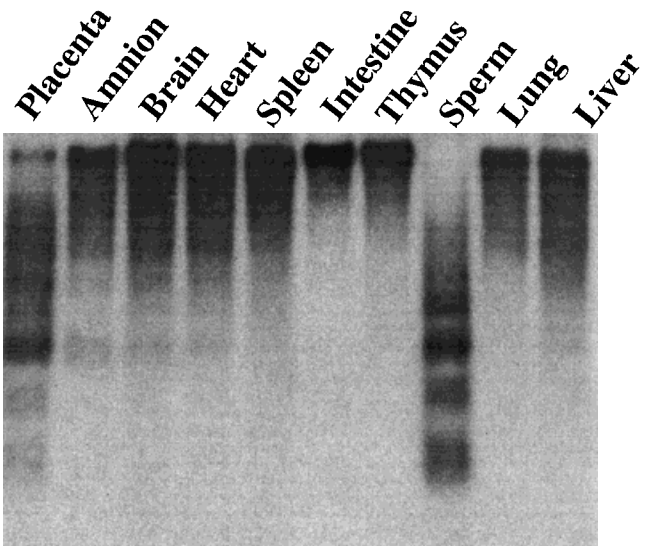

FIGURE 1 – Hypermethylation of the juxtacentromeric Sat2 DNA from chromosome 1 in normal postnatal somatic tissues. DNA from normal postnatal somatic tissues, sperm or term placenta was digested with BstB I and subjected to blot hybridization with a ^{32}P-labeled Chr1 Sat2 DNA probe. The 4 low-m.w. bands seen in the sperm DNA digest contain 0.8-, 1.3-, 1.8- and 2.2-kb fragments.

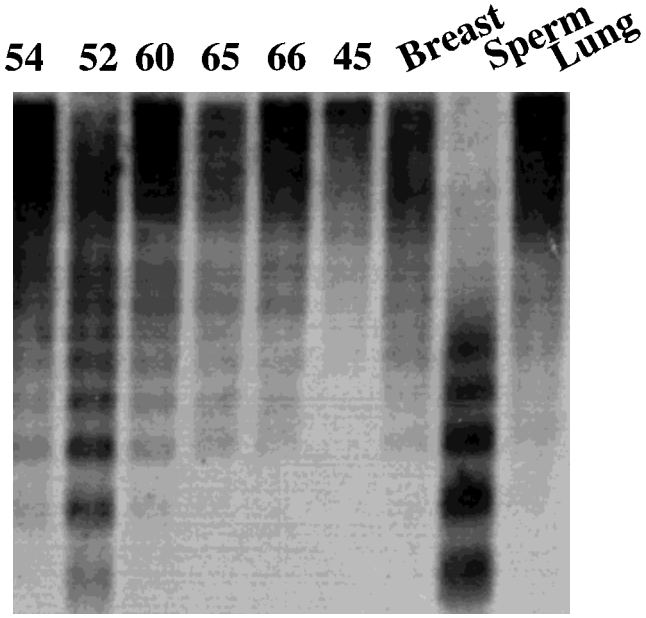

FIGURE 2 – Analysis of methylation of Chr1 Sat2 DNA sequences in benign breast tissue samples. Methylation of Chr1 Sat2 DNA was examined in non-malignant breast tissues, as in Figure 1, and compared to that of sperm as a hypomethylated standard and lung as typical postnatal somatic DNA. DNA digests were from benign breast tissue samples 54, 52 and 45, which showed mild fibrocystic changes; 60, gynecomastia; 65 and 66, fibroadenomas; Breast, histologically normal tissue from a part of a mastectomy sample distant from the adenocarcinoma; and normal sperm and lung. The high-mobility bands seen in the sperm DNA digest and in sample 52 contain fragments of 0.8, 1.3, 1.8 and 2.2 kb and of 2.7 kb, respectively.

percentage of hypomethylated repeats. In term placenta, Chr1 Sat2 also was cleaved to relatively low-m.w. fragments, though not as much as in sperm. In the sperm DNA digests, 5 high-mobility

hybridizing bands, of approximately 0.8-, 1.3-, 1.8-, 2.2- and 2.7-kb DNA fragments, were seen (Figs. 1, 2). In digests of normal DNA that were of neither germ cell (sperm) nor trophoblast (placental) origin, the only 1 of the 3 highest-mobility bands that was occasionally observed was that corresponding to the 1.8-kb fragment, and it was present in only trace amounts relative to the smeared signal at the top of the gel (Fig. 1). Like Chr1 Sat2, Chr16 Sat2 was hypermethylated in all studied normal postnatal somatic tissues, but not in sperm or placenta, and gave patterns of hybridizing bands distinctly different from Chr1 Sat2 in the latter DNA (data not shown).

We analyzed methylation of Sat2 DNA from Chr1 in 19 breast tissue samples displaying benign alterations, tissue with mild or moderate fibrocystic changes, fibroadenoma, gynecomastia or benign phylloides tumor. Only one, sample 52, had an appreciable amount of hybridizing DNA in the 3 highest-mobility bands seen in the analogous sperm DNA digests (Fig. 2). In addition, this sample showed a decrease in the amount of hybridizing DNA near the top of the gel compared to that in high-mobility bands. Duplicate digests for 3 of the fibrocystic samples, including sample 52, and 2 of the fibroadenomas were blot-hybridized to the consensus sequence Sat2 probe with the same results as obtained using the Chr1-specific Sat2 probe. To confirm the non-malignant nature of sample 52, it was re-examined histologically in 4-μ sections cut at 50-μ intervals. Also, DNA was isolated from aliquots of this sample twice and each DNA preparation was subjected to Southern blotting as above. These analyses confirmed that this sample gave no evidence of malignant changes but that the Chr1 Sat2 sequence was hypomethylated (Figs. 2, 3; data not shown). This tissue was from a patient with no evidence of prior or current breast cancer, who continued to show no evidence of disease up to 1 year after excision of this breast tissue.

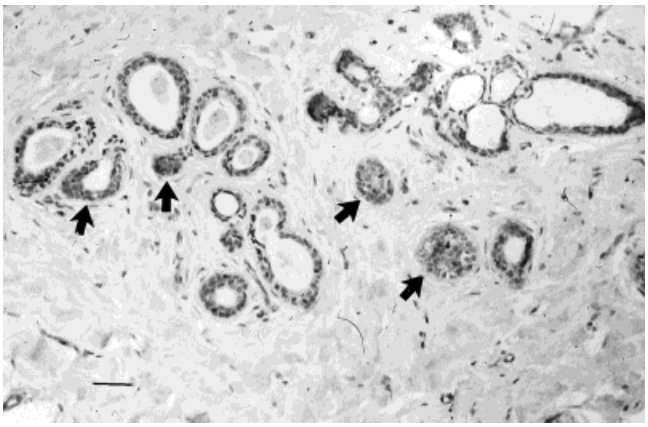

FIGURE 3 – Histological analysis of the benign breast tissue sample displaying Sat2 hypomethylation. H&E section corresponding to sample 52 (see Fig. 2). Benign mammary gland proliferation and fibrosis with epithelial hyperplasia are seen. Some of the areas of hyperplasia are indicated by arrows. Sample 45, which also displayed epithelial hyperplasia as well as fibrocystic changes, did not show evidence of Sat2 hypomethylation (Fig. 2). Scale bar: 62 μM.

Hypomethylation of juxtacentromeric Sat2 and centromeric Satα DNA sequences in breast adenocarcinomas

Southern blot analysis revealed that many of the breast cancers were hypomethylated in the juxtacentromeric Sat2 DNA of Chr1 (Fig. 4). Of 25 cancer samples, 11 (indicated in Table I by ↓) showed a considerable fraction of the hybridizing DNA fragments in the 3 highest-mobility bands of 0.8, 1.3 and 1.8 kb and 4 more showed a lesser degree of hypomethylation (indicated by ↓). Furthermore, for most of these samples with prominent low-m.w. bands, the ratio of hybridizing fragments of ≤4 kb to those of >4 kb was 0.5 to 0.8, while the analogous ratio was ≤0.3 for digests of normal tissue DNA. About half of the breast cancer DNA samples were separately digested and probed with the consensus sequence probe for Sat2, with the same results as for the Chr1 Sat2 probe.

For 3 of the breast cancer samples, we knew the overall level of genomic methylation by a precise quantitative HPLC analysis of DNA digested to deoxynucleosides (Ehrlich *et al*., 1982). One of these, O1, had hypomethylated Chr1 Sat2 DNA sequences (Fig. 4)

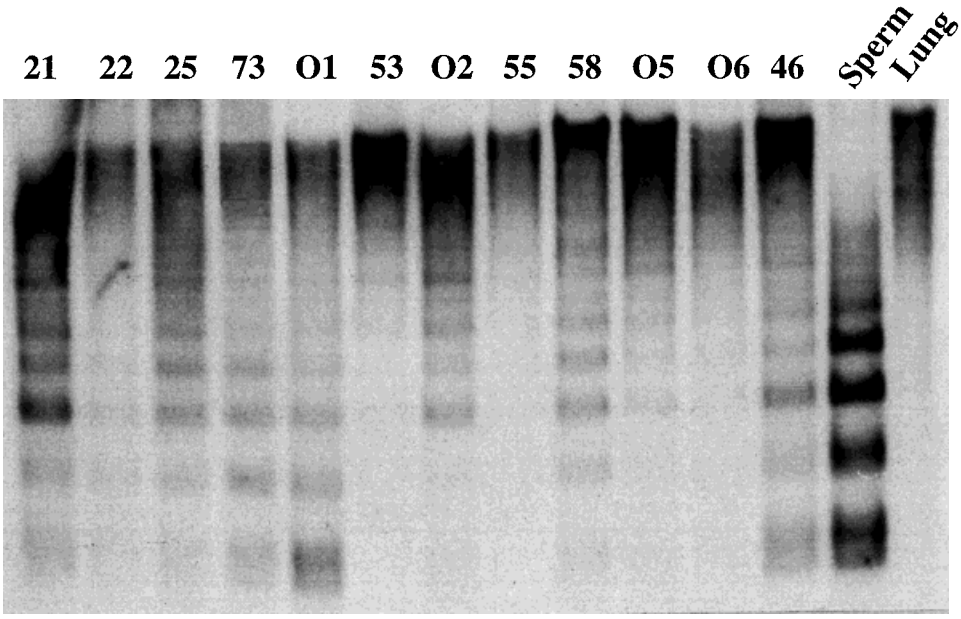

FIGURE 4 – Analysis of methylation of Chr1 Sat2 DNA sequences in malignant breast tissue samples. Breast adenocarcinoma DNA samples, in the first 12 lanes, and sperm and lung DNA samples, in the last 2 lanes, were analyzed for Chr1 Sat2 DNA hypomethylation. The 5 lowest bands in the sperm DNA samples, which were also seen in the breast cancer samples showing considerable hypomethylation (21, 25, 73, O1, 46), had DNA fragments of about 0.8, 1.3, 1.8, 2.2 and 2.7 kb. In addition, a hybridizing fragment migrating at the position for 4.0-kb DNA is seen above the 2.7-kb fragment in samples 21, 25, O2 and 46.

TABLE I – HYPOMETHYLATION IN SATELLITE DNA AND HYPERMETHYLATION IN THE E-CADHERIN GENE 5′ CpG ISLAND IN HUMAN BREAST ADENOCARCINOMAS[1]

Tumor sample number	Changes in DNA methylation in				Tumor sample number	Changes in DNA methylation in			
	Chr1 Sat2[1]	Chr16 Sat2	Chr1 Satα	E-cadherin promoter[2]		Chr1 Sat2	Chr16 Sat2	Chr1 Satα	E-cadherin promoter
21	↓	↓	–	↑	128	–	–	–	↑
22	(↓)	↓	ND[3]	ND	131	–	–	ND	(↑)
25	↓	↓	↓	↑	132	–	–	–	–
31	↓	–	–	ND	134	–	–	–	–
46	↓	↓	ND	(↑)	146	↓	↓	(↓)	↑
53	–	–	–	ND	B016	↓	↓	↓	ND
55	–	–	↓	–	B014	(↓)	–	–	ND
56	–	–	–	(↑)	H007	↓	–	–	ND
58	(↓)	–	↓	ND	O1	↓	↓	–	↑
73	↓	↓	–	(↑)	O2	(↓)	–	–	–
112	↓	↓	ND	–	O5	–	–	–	ND
113	↓	–	–	(↑)	O6	–	–	–	ND
122	–	–	–	↑					

[1]Summary of Southern blot results at the given satellite DNA sequence of the breast cancer sample: ↓, considerable hypomethylation; (↓), a small amount of hypomethylation; –, no hypomethylation.–[2]MSP analysis of the 5′ CpG island of the E-cadherin gene: ↑, hypermethylation; (↑), a small amount of hypermethylation; –, no hypermethylation.–[3]ND, not determined.

and a very low level of DNA methylation, namely, 0.79% of all the bases as m^5C (mol% m^5C). This is to be compared to the m^5C contents of a wide variety of normal post-natal somatic tissues, which range from 0.87 to 1.00 mol%, depending on the tissue (Ehrlich *et al*., 1982). The other 2 breast cancer samples with known genomic m^5C contents were O5 and O6, which differed greatly in their level of m^5C, 0.83 and 0.99 mol%, respectively. Neither of these cancers displayed hypomethylation in Chr1 Sat2 (Fig. 4). Therefore, a moderate extent of overall genomic hypomethylation in sample O5 was not accompanied by Sat2 DNA hypomethylation.

Most of the breast cancer DNA samples that were subjected to Southern blot analysis of Chr1 Sat2 methylation were analyzed for methylation at BstB I sites with the Chr16-specific Sat2 DNA probe. Of the 11 cancer DNAs that showed considerable hypomethylation in Chr1 Sat2, 8 were similarly hypomethylated in Chr16 Sat2 (Table I). No samples that appeared hypomethylated in Chr16 Sat2 showed the high level of methylation in Chr1 Sat2 seen in normal tissues.

As for the centromere-adjacent Chr1 Sat2, the centromeric Satα of Chr1 is highly methylated in normal post-natal somatic tissues but hypomethylated in sperm DNA (Fig. 5; data not shown). Hypomethylation of Satα sequences of Chr1 was seen in 4 of the 21 breast cancer DNA samples examined for methylation at BstB I sites in this satellite DNA (Fig. 5; Table I).

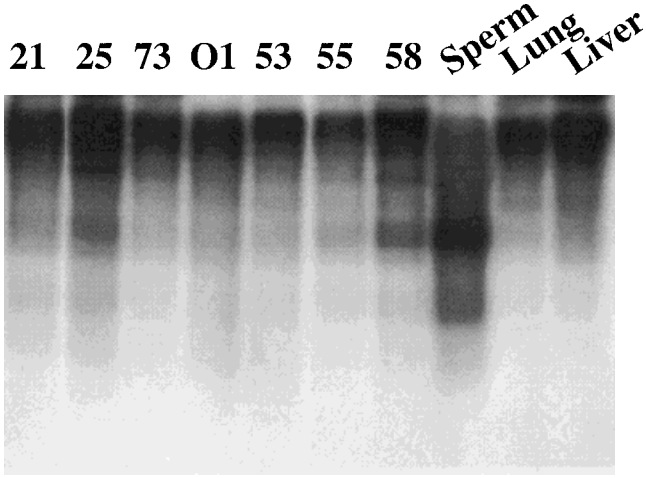

FIGURE 5 – Analysis of methylation of the centromeric Satα DNA repeat of Chr1 in malignant breast tissue samples. The indicated normal tissue and adenocarcinoma DNA samples were digested with BstB I, and a Chr1 Satα probe was used for hybridization. The major band in the sperm DNA digest, which is also seen as a prominent high-mobility band in samples 58, 55 and 25, contains a 1.7-kb fragment. The bands above and below it in digests of DNA from sperm and sample 58 contain 3.1-kb and 0.8-kb fragments, respectively.

Hypermethylation of the E-cadherin gene in many of the breast cancer samples showing hypomethylation of satellite DNA sequences

We next analyzed 16 of the breast cancer samples for the opposite aberration in DNA methylation, increased methylation, specifically, hypermethylation in the CpG island that overlaps the transcription start site of the E-cadherin gene. DNA from MCF-7 and MDA-231 breast cancer cell lines served as negative and positive standards, respectively, for methylation in this region (Graff *et al*., 1997). Cancer-associated methylation in the 5′ region of this gene in breast cancers has been demonstrated by Southern blot analysis and MSP (Graff *et al*., 1995, 1997). Relative to histologically normal breast tissue or an enriched epithelial cell fraction from normal breast, 11 of the breast cancers, including 7 that were hypomethylated in Chr1 Sat2 DNA sequences, showed hypermethylation in a fraction of the examined E-cadherin alleles (Fig. 6*a,c;* Table I). The percentage of copies of this CpG island that were hypermethylated in these samples was low, as indicated by the need for a high number of amplification cycles to visualize the product from the methylation-specific primers (Fig. 6*c*). We also examined 11 benign breast tissue samples (Fig. 6*b;* data not shown). In these fibroadenomas or tissues with fibrocystic changes, such hypermethylation was detected only in the fibrocystic sample 52, which was negative for hypermethylation in 3 of 5 standard 35-cycle MSP assays (Fig. 6*b*) but weakly positive in 2 (data not shown). This indicates an extremely low number of methylated copies of the studied E-cadherin sequence in sample 52. This was the only fibrocystic sample which was hypomethylated in Sat2 DNA (Fig. 2). In quadruplicate MSP assays, benign breast tissue samples 1, 4 and 8 were negative for hypermethylation, while breast cancer sample O1 was positive.

Clinico-pathological variables in breast cancer patients and abnormalities in DNA methylation

Neither satellite DNA hypomethylation nor E-cadherin CpG island hypermethylation correlated with any of the following clinico-pathological variables in the breast cancer patients: the expression status of estrogen/progesterone receptors, the pre- or

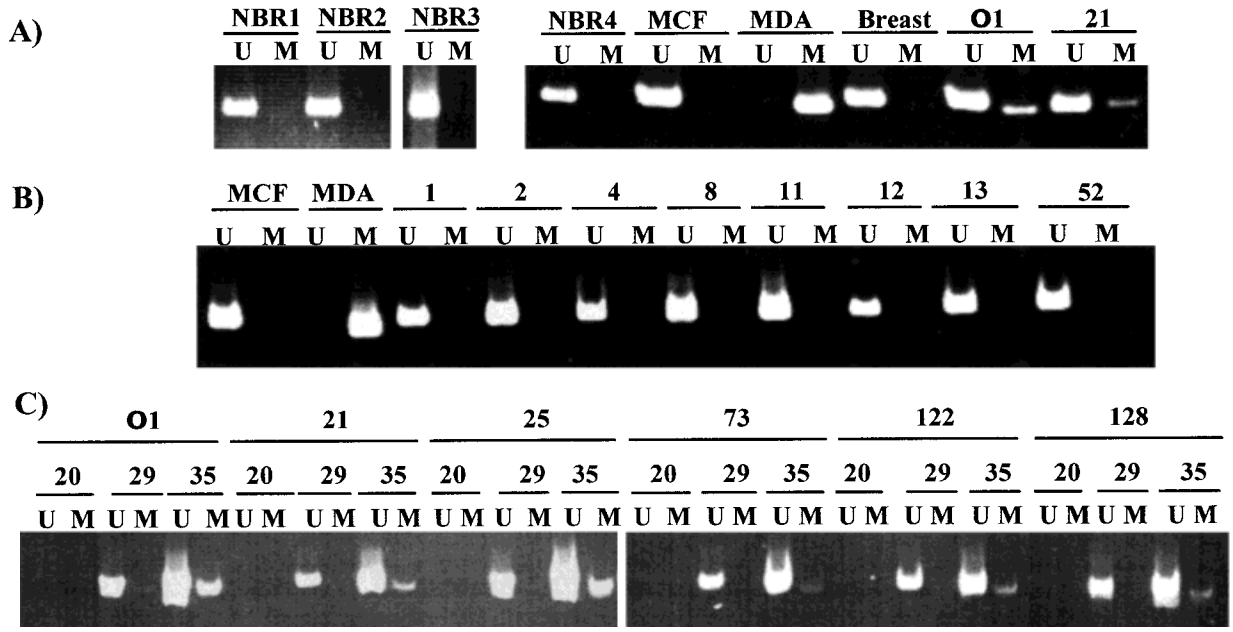

FIGURE 6 – Analysis of hypermethylation in malignant and benign breast tissue samples by MSP. After treatment with bisulfite and then alkali, which selectively deaminates unmethylated C residues, PCR assays were done with either CpG methylation-specific (M) or unmethylated CpG-specific (U) primers for the CpG island overlapping the transcription start site of the E-cadherin gene. *(a)* NBR1, NBR2, NBR3 and NBR4, epithelial cell fractions from normal breast tissues; MCF-7 and MDA-231, breast cancer cell lines that are hypomethylated or hypermethylated, respectively, in the examined region; Breast, unfractionated breast tissue with no abnormal pathology from a mastectomy sample; O1 and 21, breast adenocarcinomas. *(b)* MCF-7 and MDA-231 cell lines: 1, fibroadenoma; 2, 4, 8, 11, 12, 13 and 52, breast tissues displaying mild fibrocystic changes. *(c)* Breast adenocarcinomas. PCR involved 35 cycles of amplification in all analyses for *(a)* and *(b)*. In *(c)*, 20, 29 or 35 cycles of amplification were used, as indicated. The sizes of the PCR products from the methylated and unmethylated sequences are 204 and 211 bp, respectively.

post-natal menopausal status or age ($\leq$55 or $>$55), the clinical stage of the tumor or follow-up data on patients. Almost all of the examined tumors were stage II. The 2 patients with stage I cancers did not display hypomethylation of the studied satellite DNA samples or hypermethylation of E-cadherin; however, as described above, Chr1 Sat2 hypomethylation was seen in one of the non-malignant samples with fibrocystic changes (Fig. 2).

DISCUSSION

About 15–40% of human breast cancers exhibit pericentromeric rearrangements in Chr1, *i.e.,* rearrangements of the heterochromatin in or adjacent (juxtacentromeric) to the centromere of this chromosome (Lu *et al*., 1993; Pandis *et al*., 1995). We found that about 45% of 25 analyzed breast adenocarcinomas were appreciably hypomethylated in the major satellite DNA (Sat2) of the juxtacentromeric heterochromatin of Chr1. All of the normal postnatal somatic tissues examined, including histologically normal breast, were highly methylated in Sat2 DNA of Chr1, while sperm DNA was hypomethylated in this sequence, as is the case for many human DNA sequences (Gama-Sosa *et al*., 1983; Zhang *et al*., 1987). The assessment of methylation changes in breast cancer samples is an under-estimate because such samples may contain up to 50% normal cells, including infiltrating lymphocytes, although histological examination indicated that most of the breast cancer samples consisted of more than 70% neoplastic cells. Furthermore, the Chr1 Sat2 repeat has a very high copy number so that incomplete hypomethylation of this sequence might be due to only a fraction of the repeats in a given Chr1 homologue being hypomethylated.

One of 19 non-malignant breast tissues showed an extent of hypomethylation in this region of the DNA that was similar to that seen in some of the breast cancers. Cancer-associated genetic and epigenetic alterations have been previously reported in benign but abnormal tissues (*e.g.*, Feinberg *et al.,* 1988). Given the multistep nature of oncogenesis, it is not surprising to find such changes in DNA in benign tissues. These include genetic alterations that are known to contribute to oncogenesis, such as cancer-associated *p53* mutations in breast tissue displaying non-proliferative fibrocystic changes (Millikan *et al*., 1995). Most non-proliferative fibrocystic alterations or non-complex fibroadenomas of the breast are not considered to be pre-malignant because they are very common and, when uncoupled to a family history of breast adenocarcinoma, not correlated with an appreciably increased risk of this disease (Bodian *et al*., 1993). Five of the examined fibrocystic samples displayed some epithelial hyperplasia and 2 of these and 3 other samples were from non-malignant regions of the breast in patients who had breast adenocarcinoma. The one fibrocystic sample that was hypomethylated in Chr1 Sat2 was not from a cancer patient, but this sample did show evidence of epithelial hyperplasia (Fig. 3).

Sat2 DNA is the major DNA component of the juxtacentromeric heterochromatin of both Chr1 and Chr16. Most of the breast cancer samples showing hypomethylation of Sat2 DNA in Chr1 were also deficient in methylation of Sat2 of Chr16 (Table I). Hypomethylation of these sequences is also found in leukocytes from patients with the ICF syndrome, which is often fatal in childhood. This hypomethylation is seen prior to mitogen-induced rearrangements, indicating that it is not the consequence of the Chr1 and Chr16 pericentromeric rearrangements diagnostic for this genetic disease (Jeanpierre *et al*., 1993). Hypomethylation of Chr1 Sat2 DNA in ICF cells is associated with frequent undercondensation of this normally heterochromatic region (Smeets *et al*., 1994), which may result from altered interactions of the CpG-rich satellite DNA with chromatin proteins. This local undercondensation, in turn, could lead to Chr1:Chr1 or Chr1:Chr16 somatic associations in the pericentromeric region (Smeets *et al*., 1994), which could predis-

pose to chromosomal rearrangements because of the highly repetitive nature of sequences in this region.

Only a low percentage of breast cancer DNA samples that were hypomethylated in the juxtacentromeric Sat2 DNA of Chr1 were also detectably hypomethylated in the adjacent centromeric Satα region. Therefore, the observed cancer-associated DNA hypomethylation is not necessarily targeted to all of the constitutive heterochromatin in the pericentromeric region of Chr1. However, it is possible that alterations in DNA methylation during carcinogenesis might contribute to oncogenic transformation and then be reversed, *e.g.*, if there is a tendency for hypomethylated pericentromeric satellite DNA sequences to become *de novo* methylated in postnatal somatic cells. This possibility will have to be taken into account in future studies of satellite DNA methylation in breast cancer samples that have been subjected to karyotypic analysis on these solid tumors.

Although many types of cancer have DNA methylation levels much lower than the range of genomic m^5C contents of normal somatic tissue DNA (Gama-Sosa *et al*., 1983; Cheng *et al*., 1997), localized DNA hypermethylation, *e.g.*, at the 5' CpG island of the E-cadherin gene, is frequently observed in a wide variety of cancers (*e.g*., Graff *et al*., 1997). In this study, many of the tumors that were hypomethylated in Sat2 DNA of Chr1 and Chr16 displayed hypermethylation of this E-cadherin CpG island in some of the cells in the tumor sample, though the percentage of the satellite repeat that was hypomethylated appeared to be much greater than that of alleles of the E-cadherin gene 5′ CpG island that was hypermethylated. That opposite alterations in DNA methylation can occur in different regions of the genome in a given breast cancer is consistent with the occurrence of both global demethylation and *de novo* methylation during early embryonic differentiation. During oncogenesis, both of these types of perturbation in DNA methylation may contribute to malignant transformation or tumor progression. For example, the E-cadherin gene hypermethylation may favor metastasis in a fraction of the tumor cells displaying such increased methylation of the transcription regulatory region of the gene (Graff *et al*., 1995). In contrast, DNA demethylation, especially of repetitive DNA sequences and possibly of recombination-associated genes, might predispose cells to rearrangements (unbalanced translocations, deletions, isochromosome formation) that create imbalances in gene copy numbers, which contribute to tumor formation.

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