J. Cell. Mol. Med. Vol 14, No 6B, 2010 pp. 1468-1475

Familial and racial determinants of tumour suppressor genes promoter hypermethylation in breast tissues from healthy women

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Received: June 17, 2009; Accepted: August 11, 2009

Abstract

To determine the hypermethylation status of the promoter regions of tumour suppressor genes in breast tissues from healthy women and identify the determinants of these epigenetic changes. Questionnaires and breast tissues were collected from healthy women without a history of cancer and undergoing reduction mammoplasty ($N = 141$). Methylation for $p16^{INK4}$, *BRCA1*, $ER\alpha$ and $RAR\text{-}\beta$ promoter regions from breast tissues were determined by methylation specific PCR. Associations were examined with chi-square and Fisher's exact test as well as logistic regression. All statistical tests were two-sided. $p16^{INK4}$, *BRCA1*, $ER\alpha$ and $RAR\text{-}\beta$ hypermethylation were identified in 31%, 17%, 9% and 0% of the women, respectively. Women with *BRCA1* hypermethylation had an eight-fold increase in the risk of $ER\alpha$ hypermethylation ($P = 0.007$). $p16^{INK4}$ hypermethylation was present in 28% of African-Americans, but 65% in European-Americans ($P = 0.02$). There was an increased likelihood of $p16^{INK4}$ or *BRCA1* hypermethylation for women with family history of cancer (OR 2.3; 95%CI: 1.05–4.85 and OR 5.0; 95%CI: 1.55–15.81, respectively). $ER\alpha$ hypermethylation was associated with family history of breast cancer (OR 6.6; 95%CI: 1.58–27.71). After stratification by race, $p16^{INK4}$ in European-Americans and *BRCA1* hypermethylation in African-Americans were associated with family history of cancer (OR 3.8; 95%CI: 1.21–12.03 and OR 6.5; 95%CI: 1.33–31.32, respectively). Gene promoter hypermethylation was commonly found in healthy breast tissues from women without cancer, indicating that these events are frequent and early lesions. Race and family history of cancer increase the likelihood of these early events.

Keywords: $p16^{INK4}$ • *BRCA1* • $ER\alpha$ CpG islands hypermethylation • breast biology • family history of cancer

Introduction

There is evidence that breast carcinogenesis may begin early in life, perhaps even *in utero* [1–3]. However, very little is known about the biological events in morphologically normal breasts. Early genetic and epigenetic lesions presumably occur, and the study of these may allow for a better understanding of breast carcinogenesis *in vivo,* and subsequent cancer risk.

DNA CpG promoter hypermethylation of tumour suppressor genes is commonly found in breast tumours [4], and these epigenetic changes are involved in early tumourigenesis [5–7]. Specifically, it is known that promoter hypermethylation of the tumour suppressor gene $p16^{INK4}$ occurs in breast tumours [8], and that it has been associated with gene silencing in both pre-invasive breast lesions and breast tissues from apparently healthy women [9]. *BRCA1* promoter hypermethylation, which occurs in 13% of sporadic breast cancers [10], has also been observed in periareolar cytologic samples (5–22%) from women at high risk of developing breast cancer [11]. Oestrogen receptor α ($ER\alpha$), with a crucial role in normal mammary gland growth and differentiation, as well as in the development and progression of breast cancer [12], also is found silenced by hypermethylation in breast cancer cell lines and tumours [13, 14]. $ER\alpha$

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doi:10.1111/j.1582-4934.2009.00924.x

hypermethylation frequency increases from ductal carcinoma *in situ* to metastatic lesions [15]. Another gene of interest is *RARβ*, a putative tumour suppressor gene that regulates differentiation and cellular growth mediated by retinoids also is found silenced by promoter hypermethylation in breast cancer cell lines [16, 17] and breast carcinoma [18–20]. Methylation of this gene was observed in 32% of benign breast samples from cancer patients, but only in 9% of similar samples from unaffected women [21]. *RARβ* hypermethylation has also been detected in ductal lavage fluid from healthy women who are *BRCA1* gene mutation carriers, providing evidence that this epigenetic change may be an early event in breast tumourigenesis [22]. However, for all of the above genes, little is known about how early these changes occur in the carcinogenic process and what other factors may be related to their occurrence.

In a cross-sectional study of women undergoing elective reduction mammoplasty, with no history of any cancer, we examined the frequency of gene promoter hypermethylation and the association of other exogenous and endogenous factors with the hypermethylation phenotypes. We focused on *p16^{INK4}, BRCA1, ERα* and *RAR-β* genes since these genes are each part of several critical pathways important for breast carcinogenesis, and because they are commonly hypermethylated in breast tumours [4, 8, 15, 23–26].

Methods

Subjects

One hundred and forty-one healthy women undergoing reduction mammoplasty at Georgetown University Medical Center, the University of Maryland and the Washington Hospital Center were recruited for this study between 2000 and 2007. All participating women were at least 16 years of age and had no prior history of any cancer and underwent reduction mammoplasty surgery mostly for cosmetic reasons, but never for a past history of breast cancer. Within 24 hrs prior to surgery, a questionnaire that included information regarding demographic characteristics, recent lifestyle, medications, last menstrual period and other exposures was administered in order to evaluate recent exposures. A more extensive questionnaire was administrated either at that time or shortly after surgery; the second questionnaire addressed personal medical history, family medical history, occupation, diet and alcohol, smoking history and reproductive history. Race was determined by self-report. Smokers were defined as women who had smoked more than 100 cigarettes during their lifetime. Current drinking, defined as the intake of alcohol in the last 24 hrs as well as ever drinking, defined as consumption of 12 or more alcoholic beverages over the course of the lifetime were assessed based on self-report. Post-menopausal women (over age 45) were those who had not had a menstrual period in the last 12 months and had had a surgical induced menopause. Family history of any cancer was defined as history of any cancer for any first or second-degree family relatives. Family history of breast cancer was defined as the occurrence of breast cancer in any first- or second-degree family relatives. None of the participants had more than one family member with breast cancer.

All participants provided informed consent and the Institutional Review Boards at all the participating institutions approved the study.

Biospecimen collection

Surgically removed breast tissue that was not medically needed was inspected and determined to be free from gross pathologic abnormalities. Pathological examinations of tissues, although not from the specific flash frozen tissue used in this study, revealed them as normal. Epithelial tissues were dissected and snap frozen in liquid nitrogen within 1 hr of removal. Breast tissue samples were stored at −80°C.

Hypermethylation analysis

DNA was extracted from the dissected epithelial tissues that were flash frozen in liquid nitrogen by standard methods using a Puregene DNA purification kit (Gentra Systems, Minneapolis, MN, USA). The CpGenome™ Universal DNA Modification Kit from Chemicon (Millipore Co., Billerica, MA, USA) was used for bisulfite modification of DNA. CpG island methylation patterns of *p16^{INK4}*, *BRCA1*, *ERα* and *RARβ* were determined by methylation-specific PCR (MSP). Briefly, for the *p16^{INK4}* gene, a nested, two-stage PCR was used to detect one methylated allele in >50,000 unmethylated alleles, as previously reported [27]. Primer sequences and PCR conditions used to detect *BRCA1, ERα* and *RARβ* methylation status have been described previously [14, 19, 28]. All MSP products were analysed on 2% agarose gels in 1XTBE.

Statistical analysis

The associations between promoter hypermethylation of *p16^{INK4}*, *BRCA1* and *ERα* genes and subject characteristics were examined with chi-square tests; except for contingency tables containing a cell count lower than 5 when Fisher's exact test was used. For binary characterization of methylation, odds ratios and 95% confidence intervals were estimated using unconditional logistic regression (SAS Software, Cary, North Carolina, USA). All *P*-values are based on two-tailed tests.

Results

The subject characteristics are shown in Table 1. The mean age of the 141 study participants was 35 years (SD = 11) with a range of 16–65. Study subjects were 51% European-Americans (EA) and 49% African-Americans (AA). Among these women, 65% never smoked, 18% were current smokers and 7% were former smokers. Also, 66% were current drinkers and 34% never drank. Fourteen per cent of participants were post-menopausal, 54% had had children, 47% had a family history of cancer and among those 23% had a family history of breast cancer.

p16^{INK4} promoter hypermethylation was assayed in 141 subjects, while for the other three genes promoter hypermethylation was assayed in 100 subjects. We detected *p16^{INK4}* promoter

Table 1 Characteristics of participants in the reduction mammoplasty study

Demographic characteristics of the reduction mammoplasty subjects ($n = 141$)	
Age (mean ± SD)	35 ± 11 (range: 16–65)
BMI	32 ± 7 (range: 18–58)
Race (%)	
European-Americans (EA)	71 (50.7%)
African-Americans (AA)	69 (49.3%)
Ever smoking (%)	
Yes	47 (34.8%)
Never	88 (65.2%)
Current smoking (%)	24 (17.9%)
Ever alcohol (%)	
Yes	87 (66.4%)
Never	44 (33.6%)
Current drinking (%)	43 (34.4%)
Pre-menopausal (%)	119 (86.2%)
Have children (%)	67 (53.6%)
Family history of any cancer (%)	63 (47.4%)
Family history of breast cancer (%)	31 (22.6%)

hypermethylation in 31% ($n = 43$) of the study participants, *BRCA1* hypermethylation in 17% ($n = 17$) and *ERα* hypermethylation in 9% ($n = 9$). *RARβ* promoter hypermethylation was not found in any participant's breast tissue samples. Thus, there are no results to report for *RARβ* in this manuscript.

No subject had all four of the examined genes hypermethylated. There was a significant association for having both *BRCA1* and *ERα* genes hypermethylated; 7% of women had both. Women with *BRCA1* hypermethylation had an eight-fold increase in the likelihood of *ERα* hypermethylation (OR = 8.2, 95%CI: 1.9–35.02). Hypermethylation of $p16^{INK4}$ was not associated with hypermethylation of the other two genes.

In Table 2, associations between demographic variables, breast cancer risk factors and gene promoter hypermethylation are shown. Given the age effects on hypermethylation and breast cancer risk, we examined the association of the variable in Table 2 and gene hypermethylation with adjustment for age; however, the age-adjusted analysis did not change the associations found. AA women were less likely to have the $p16^{INK4}$ gene hypermethylation than EA women (OR = 0.4, 95%CI: 0.2–0.9). There was a tendency for women who were ever consumers of alcohol to have greater prevalence of $p16^{INK4}$ hypermethylation although the confidence interval included the null (OR = 2.3, 95%CI: 0.97–5.31).

Family history of cancer was associated with promoter hypermethylation. There was an increased likelihood of both $p16^{INK4}$ and *BRCA1* for women with family history of any cancer compared to those without such a history (OR = 2.3, 95%CI: 1.05–4.85 and OR = 5.0, 95%CI: 1.55–15.81, respectively). While the OR for *ERα* was similarly elevated, it was not statistically significant (OR = 3.1, 95%CI: 0.7–13.92). There was also an increased likelihood of *ERα* hypermethylation for women with a family history of breast cancer (OR = 6.6, 95%CI: 1.58–27.71) and a trend toward elevated risk for *BRCA1* hypermethylation (OR = 2.8, 95%CI: 0.88–8.95).

The likelihood of promoter hypermethylation within strata of breast cancer risk factors (race, family history of any cancer and breast cancer) was assessed, although the number of subjects in some strata was small. The associations of breast cancer risk factors with likelihood of $p16^{INK4}$, *BRCA1* and *ERα* hypermethylation after stratification by race are presented in Table 3. The associations of $p16^{INK4}$ methylation with family history of any cancer and with alcohol consumption appeared to be limited to EA women. For EA women ($n = 71$), the likelihood of having $p16^{INK4}$ hypermethylation was significantly higher among those with a family history of any cancer (OR = 3.8, 95%CI: 1.21–12.03). Similar to analyses of both racial groups combined, there was a non-significant trend for increased hypermethylation among EA drinkers, although the point estimate was higher (OR = 2.3, 95%CI: 0.97–5.31). Among AA women ($n = 69$), family history of any cancer and alcohol consumption was not associated with hypermethylation of $p16^{INK4}$. In AA women, *BRCA1* hypermethylation was associated with family history of any cancer (OR = 6.5, 95%CI: 1.33–31.32). An association between *BRCA1* hypermethylation and family history of breast cancer was also observed (OR = 4.4, 95%CI: 0.91–21.29).

Although the sample size was small and confidence intervals wide, there was an indication that age was significantly associated with *ERα* hypermethylation for AA women ($n = 49$; OR = 14.6, 95%CI: 1.53–138.51; $P = 0.01$). Additionally, age at first birth was associated with methylation of this gene for the AA participants, with increased methylation for those with a later age at first birth (OR = 20.0, 95%CI: 1.74–229.5; $P = 0.01$). In both groups, EA ($n = 51$) and AA women with a family history of breast cancer were more likely to have *ERα* hypermethylation (OR = 11.1, 95%CI: 0.89–140.12 and OR = 5.00, 95%CI: 0.83–30.08; respectively).

Discussion

DNA promoter hypermethylation of tumour suppressor genes has been shown to be one of the most common abnormalities in cancer [4, 29, 30], although there is little information about when these abnormalities occur and why. To our knowledge, this is the first report regarding the frequency of hypermethylation of this group of important genes, namely *BRCA1* and *ERα* in a large number

Table 2 Association of breast cancer risk factors with likelihood of *p16 INK4*, *BRCA1* and *ERα* hypermethylation in healthy women

	p16 INK4 hypermethylation				*BRCA1* hypermethylation				*ERα* hypermethylation			
	Negative ($n = 97$)	Positive ($n = 44$)	*P*-value	OR 95%CI	Negative ($n = 83$)	Positive ($n = 17$)	*P*-value	OR 95%CI	Negative ($n = 91$)	Positive ($n = 9$)	*P*-value	OR 95%CI
Age												
<34.7	51	19		1.00	49	10		1.00	55	4		1.00
≥34.7	45	25	0.27	1.5 (0.73,3.06)	34	7	0.99	1.00 (0.35,2.91)	36	5	0.48	1.9 (0.48,7.59)
BMI												
<31.0	41	23		1.00	36	7		1.00	41	2		1.00
≥31.0	49	16	0.16	0.6 (0.29,1.36)	39	9	0.76	1.2 (0.39,3.48)	43	5	0.44	2.4 (0.43,12.92)
Age first birth												
<21.8	19	11		1.00	24	2		1.00	25	1		1.00
≥21.8	22	9	0.53	0.7 (0.21,2.09)	17	3	0.43	1.7 (0.24,11.48)	16	4	0.15	5.5 (0.53,56.91)
Race												
EA	43	28		1.00	43	8		1.00	48	3		1.00
AA	54	15	0.02	0.4 (0.2,0.9)	40	9	0.72	1.2 (0.43,3.44)	43	6	0.311	2.2 (0.53,9.48)
Current smoking												
No	74	36		1.00	64	14		1.00	71	7		1.00
Yes	16	8	0.95	1.0 (0.40,2.62)	14	2	0.731	0.7 (0.07,3.40)	14	2	0.651	1.5 (0.27,7.72)
Current drinking												
No	53	29		1.00	37	11		1.00	42	6		1.00
Yes	31	12	0.40	0.7 (0.32,1.58)	30	6	0.48	0.7 (0.13,3.20)	33	3	0.731	0.6 (0.15,2.74)
Ever drinking												
No	35	9		1.00	30	7		1.00	34	3		1.00
Yes	55	32	0.06	2.3 (0.97,5.31)	45	9	0.78	0.9 (0.29,2.55)	50	4	1.001	0.9 (0.19,4.30)
Family history of cancer												
No	55	15		1.00	54	5		1.00	56	3		1.00
Yes	39	24	0.04	2.3 (1.05,4.85)	24	11	0.004	5.0 (1.55,15.81)	30	5	0.141	3.1 (0.7,13.92)
Family history of breast cancer												
No	72	34		1.00	67	11		1.00	74	4		1.00
Yes	23	8	0.51	0.7 (0.30,1.82)	13	6	0.07	2.8 (0.88,8.95)	14	5	0.011	6.6 (1.58,27.71)

[1]Fisher's exact test.
Odds ratios and 95% confidence intervals estimated by logistic regression.

Table 3 Association of breast cancer risk factors with likelihood of *p16 INK4*, *BRCA1* and *ERα* hypermethylation in EA and AA women

***p16 INK4* hypermethylation**								
	European-American women (*n* = 71)				**African-American women (*n* = 69)**			
Variables	**Negative (*n* = 43)**	**Positive (*n* = 28)**	***P*-value**	**OR (95%CI)**	**Negative (*n* = 54)**	**Positive (*n* = 15)**	***P*-value**	**OR (95%CI)**
Current smoking								
No	32	26		1.00	42	9		1.00
Yes	7	2	0.29[1]	0.4 (0.07,1.84)	9	6	0.07	3.1 (0.88,10.96)
Ever drinking								
No	13	3		1.00	22	6		1.00
Yes	28	24	0.08[1]	3.7 (0.95,14.60)	27	8	0.89	1.1 (0.33,3.60)
Family history of cancer								
No	21	5		1.00	34	10		1.00
Yes	22	20	0.02	3.8 (1.21,12.03)	17	4	1.001	0.8 (0.22,2.93)
BRCA1 hypermethylation								
	European-American women (*n* = 51)				African-American women (*n* = 49)			
Variables	Negative (*n* = 43)	Positive (*n* = 8)	*P*-value	OR (95%CI)	Negative (*n* = 40)	Positive (*n* = 9)	*P*-value	OR (95%CI)
Family history of cancer								
No	23	1		1.00	31	4		1.00
Yes	18	6	0.10[1]	7.7 (0.85,69.54)	6	5	0.021	6.5 (1.33,31.32)
Family history of breast cancer								
No	34	6		1.00	33	5		1.00
Yes	7	2	0.63[1]	1.62 (0.27,9.75)	6	4	0.081	4.4 (0.91,21.29)
ERα hypermethylation								
	European-American women (n = 51)				African-American women (n = 49)			
Variables	Negative (*n* = 48)	Positive (*n* = 3)	*P*-value	OR (95%CI)	Negative (*n* = 43)	Positive (*n* = 6)	*P*-value	OR (95%CI)
Family history of cancer								
No	23	1		1.00	33	2		1.00
Yes	22	2	1.00[1]	2.1 (0.18,24.73)	8	3	0.081	6.2 (0.88,43.44)
Family history of breast cancer								
No	39	1		1.00	35	3		1.00
Yes	7	2	0.08[1]	11.1 (0.89,140.12)	7	3	0.101	5.0 (0.83,30.08)

[1]Fisher's exact test.
Odds ratios and 95% confidence intervals estimated by logistic regression.

of apparently healthy women with no history of cancer, and the largest study for *p16 INK4*. We found differences in the frequency of hypermethylation by family history of any cancer and family history of breast cancer, as well as some indications that other breast cancer risk factors may be associated with differences in methylation prevalence.

Hypermethylation of promoter regions for *p16 INK4, BRCA1, ERα* and *RAR-β* was present in 31%, 17%, 9% and 0%, respectively, of the breast tissues from healthy women undergoing reduction mammoplasty. Similar to our finding, in a smaller study, Tlsty and coworkers previously found that 29% (4 of 14) of human mammary epithelial cells in histologically normal breast tissues analysed by MSP-ISH showed hypermethylated *p16 INK4A* promoter [31]. Bean and coworkers reported that 34% (29 of 86) of a group of women at high risk for development of breast cancer also had evidence of *p16 INK4* hypermethylation in periareolar fine needle aspiration samples [32]. There is evidence that clones of cultured human mammary epithelial cells, derived from histologically normal breast tissues, can exhibit *de novo* methylation of the *p16 INK4A* CpG islands and can escape M(0) growth arrest [33]. These 'variant' HMECs with *p16 INK4A* epigenetically modified promoters are thought to accumulate chromosomal changes, including aneuploidy and telomeric associations [31], similar to those detected in pre-malignant and malignant breast cancer lesions [34]. Although our results do not provide information on the percentage of cells that are hypermethylated in these tissues, or if the hypermethylation is occurring in epithelial or stroma cells, the finding that some apparently healthy women have cells with hypermethylation may be of importance in our understanding of early stages of breast carcinogenesis, *e.g.* before morphological changes are detected, as has been previously hypothesized [5].

In this study, a family history of breast cancer was associated with *ERα* and *BRCA1* hypermethylation, although only the former was statistically significant. These data are consistent with the hypothesis that susceptibility to development of a hypermethylation phenotype may be a heritable trait for increasing breast cancer risk. Also, having *BRCA1* hypermethylated, was associated with an increased risk for having *ERα* hypermethylated, suggesting that both *BRCA1* and *ERα* may act together in increasing the susceptibility to breast cancer.

Women with a family history of any cancer had increased likelihood of hypermethylation for each of the three genes, although the association was statistically significant only for *p16 INK4* and *BRCA1.* A biological explanation for this finding is that there may be a hypermethylation phenotype that increases risk for all types of cancers, but it is only one of many carcinogenic pathways among a diverse set of cancer types, and so the association of family history of any cancer to breast cancer risk is not easily detected. A recent study showed that double strand breaks occurring in the promoter region of a gene may be an event that initiates the silencing of the promoter, leading to a mechanism by which oxidative or other DNA damage can induce epigenetic silencing, including promoter CpG island DNA hypermethylation of tumour suppressor genes [35].

The four genes studied herein are hypermethylated with different frequencies, and a few women had more than one gene hypermethylated. The presence of a CpG island methylator phenotype (CIMP), characterized by the simultaneous methylation of multiple CpG islands in two or more genes in colon cancers, was first described by Toyota and coworkers in 2000 [36]. The occurrence of the CIMP in more than two genes in these morphologically normal tissues may emerge with the analysis of a larger number of genes.

Breast cancer death rates among AA women are 36% higher than in EA women, despite lower incidence rates [37]. Even taking stage and socio-economic factors into account, there is evidence that AA women have more aggressive tumours [38, 39]. In addition, it has been shown that the frequency of multiple hypermethylated genes is higher in tumours from AA women than in those from EA women, especially those that are oestrogen and progesterone receptor negative [40]. In our study, we found some evidence of higher frequency of hypermethylation of *BRCA1* and *ERα* and an indication of lower frequency in *p16 INK4A* for AA compared to EA women. Additionally, there appeared to be differences by race in the factors related to methylation. Family history of any cancer was associated with hypermethylation of *p16 INK4A* among EA and of *BRCA1* among AA. Further, among AA but not EA women, there was an indication that age was related to the likelihood of *BRCA1* and *ERα* hypermethylation and age at first birth was associated with *ERα* hypermethylation. For all of the race-specific analyses, sample sizes were small and these findings are necessarily preliminary.

One of the strengths of this study is that we were able to collect large amounts of breast tissue from healthy women undergoing reduction mammoplasty as well as detailed questionnaire data regarding breast cancer risk factors. The tissues were collected rapidly and there was pathological confirmation that the tissues were histologically normal. There also are limitations for this study, however. Although this is a large study examining hypermethylation in healthy women, it is still limited in statistical power, particularly for the analyses stratified on race. The study did have sufficient power to identify association for the larger group analyses. A further limitation of this study may be in the extrapolation of our results to the general population of women. The underlying breast biology in the study participants may differ from that of other women because of the large size of their breasts, increased body mass index and other factors leading to self-selection for elective surgery. And so while the frequency of hypermethylation might be different for women more generally, there is no *a priori* reason to believe that comparisons within these women (*e.g.* family history or race) would differ because of the accrual methods. In this study, only four genes were examined. Interestingly, it is known that women with larger breasts, especially those with overall lower BMIs have an increased cancer [41] and thus this group of women might be considered a susceptible population. Another limitation was the narrow number of genes studied herein. While this candidate-gene approach was based on *a priori* hypotheses, there may be other genes that would provide additional insight, as would a genome-wide methylation scan. Another limitation is that we only assayed gene hypermethylation, but not gene expression

and so we do not have data on the biological effect of the observed hypermethylation, *e.g.* decreased $p16^{INK4}$, *BRCA1* and $ER\alpha$ expression in these breast tissues. However, it is reasonable to believe that in those cells with hypermethylation, there is reduced expression of the corresponding protein. Separately, it should be noted that subjects were accrued from three different hospitals, and variability in subject characteristics, tissue collection procedures was introduced, even though all the hospitals followed the same accrual and tissue collection procedures. To address this, we analysed data taking into account collection site and found no significant differences in hypermethylation frequency by site (data not shown). Lastly, we do not know whether the women in our study will go on to develop breast cancer; in fact the reduction mammoplasty procedure involving removal of a considerable portion of their breast tissue may decrease risk by as much as by 28% [42, 43] particularly when a large volume of tissue is removed [44]. Further, given that about 10% of women develop breast cancer in their lifetime [45], the presence of $p16^{INK4A}$ and *BRCA1* hypermethylation in a larger percentage of women might be sensitive as an indicator of susceptibility but would not have a degree of specificity. Therefore, following these patients (most of them young) for a number of years is almost unrealistic. Thus, promoter hypermethylation might be a necessary but not sufficient cause of breast cancer, and that there are subpopulations of cells with hypermethylation that do not evolve into clinical cancer or regress.

The presence of promoter hypermethylation of several tumour suppressor genes, including $p16^{INK4}$, *BRCA1* and $ER\alpha$ genes in breast tissue occurs in morphologically normal tissues at ages well below the typical onset of clinical breast cancer. The association of family history (breast cancer and any cancers) with promoter hypermethylation indicates that hypermethylation of promoter regions may be a genetic trait. The association for hypermethylation with race further highlights the possibility of a genetic trait, although lifestyle and exposure likely plays a role. Further understanding of the process of epigenetic changes in breast tissue may provide important insight into the biology of breast cancer.

Acknowledgements

We would like to thank Drs. Robert Dickson (in memoriam) and Michael Johnson for their support and helpful discussions. We would also like to acknowledge Leonidas Leondaridis and David Goerlitz together with our repository team for providing tissue samples and creating and maintaining the laboratory database. Department of Defense, Alcohol Center of Excellence grant (DOD BC022346) supported this work.

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