DNA Methylation of K_v1.3 Potassium Channel Gene Promoter is Associated with Poorly Differentiated Breast Adenocarcinoma

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Key Words

Breast • Cancer • Human • Potassium channels • DNA methylation

Abstract

Background: DNA methylation is an important mechanism for gene silencing and has already been described for several genes in breast cancer. A previous immunohistochemistry study demonstrated a decrease of K_v1.3 potassium channel expression in breast adenocarcinoma compared to normal breast tissue. **Methods:** Methyl-specific PCR (MSP), immunohistochemistry and RNA extraction were performed on breast adenocarcinoma. MSP and DNA extraction were also performed on one breast carcinoma cell line and on primary culture normal cells. **Results:** DNA methylation of K_v1.3 gene promoter was observed in 42.3% of samples (22/52). The methylated status was associated with poorly differentiated tumors ($p=0.04$) and younger patients ($p=0.048$). Decreased K_v1.3 expression was observed in grade III tumors, at both the mRNA and protein levels, while methylation increased in grade III tumors. Finally, K_v1.3 gene promoter was methylated in the MCF-7 breast carcinoma cell line while promoter methylation was absent in

primary culture of normal breast cells (HMEpC). **Conclusion:** We report, for the first time, the methylation of the K_v1.3 gene promoter in breast adenocarcinoma. Our data suggest that DNA methylation is responsible for a decrease of K_v1.3 gene expression in breast adenocarcinoma and is associated with poorly differentiated tumors and younger patients.

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Introduction

Breast cancer is the leading cause of cancer-related mortality in women worldwide. Like other tumor types, breast carcinoma is the result of activation of oncogenes and inactivation of tumor suppressor genes. Recent studies indicate that deregulation of potassium (K⁺) channels could play a role in carcinogenesis [1-4]. Among K⁺ channels, the voltage-gated K⁺ channels family represents a structurally and functionally diverse group of membrane proteins and the K_v1.3 type has been implicated in the regulation of many cellular functions including resting membrane potential, cell-volume, cell-adhesion, apoptosis and proliferation [4]. K⁺ channels may also regulate tumor cell apoptosis by various mechanisms

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[5], such as cell volume reduction or implication of intracellular pro-apoptotic mediators [6]. Overexpression of bcl2, an anti-apoptotic oncoprotein, inhibits apoptosis in pulmonary artery smooth muscle cells by decreasing the activity of voltage-gated K⁺ channels [7].

Decreased gene expression could be due to various mechanisms such as mutation or deletion of the gene or post-transcriptional deregulation. The large number of genes found to undergo hypermethylation in various malignancies [8] suggests a role for epigenetic changes such as methylation in the initiation and/or progression of cancer [9]. Aberrant methylation of promoter region has been described for various genes involved in carcinogenesis of breast tissue such as p16, p14, BRCA1, Cyclin D2 or estrogen receptor (ER α) [10-12]. In a recent study [2], we showed decreased expression of the K_v1.3 channel protein in 30 breast adenocarcinomas compared to 30 normal tissues [2]. Because aberrant methylation of the K_v1.3 gene promoter can repress gene transcription, thereby affecting protein expression, we hypothesized that the K_v1.3 gene promoter may be methylated in breast cancer. A review of the literature revealed only one article concerning DNA methylation of a K⁺ channel promoter (KCNH5) in non-small-cell lung cancer [13]. Methylation of K⁺ channels in breast cancer or breast cancer cell lines has never been described and only a limited number of studies have compared methylation of the promoter region and protein expression [14, 15].

In this study, we report for the first time an aberrant methylation of the K_v1.3 K⁺ channel gene promoter in breast cancer. This methylated status is associated with decreased K_v1.3 expression and poorly differentiated tumor. Finally, K_v1.3 K⁺ channel gene promoter was methylated in the MCF-7 breast carcinoma cell line but remained unmethylated in primary culture of normal breast epithelial cells.

Materials and Methods

Breast cells

Two breast cell lines and a primary culture of normal cells were used in this study. The monoclonal breast carcinoma cell line MCF-7 was purchased from the American Type Culture Collection (LGC Promochem, Molsheim, France). Cells were grown in Eagle's Minimum Essential Medium (EMEM) supplemented with 5% fetal calf serum (FCS), 2 mM L-glutamine, 0.06% HEPES buffer, and a mixture of penicillin (50 IU/ml)/streptomycin (50 μ g/ml). The EMEM solution was renewed every 2 days. Cells were grown in an atmosphere saturated with humidity at 37°C and 5% CO₂. The monoclonal normal breast epithelial cell line MCF 10A was grown in DMEM/F12 supplemented with

10% FCS, 20 ng/ml EGF, 0.01 ng/ml of insulin and 500 ng/ml of hydrocortisone. Finally, normal human breast epithelial cells (HMEpC) obtained from primary culture were grown in Mammary Epithelial Cell Growth Medium KIT C-21110 (Promocell). 10⁶ cells were used for DNA or RNA extraction.

Tissues specimens and patient characteristics

Sixty-one primary breast adenocarcinomas were obtained from surgical specimens from women undergoing surgery at Amiens University Hospital, France between 2006 and 2008. The mean age of these patients was 56.1 years. All tissue specimens were derived from mastectomy or tumorectomy specimens. At diagnosis, 30 tumors were associated with lymph node metastasis, without distant metastasis (clinical examination, bone scintigraphy and liver scan).

Tumor grade was determined according to the Scarff, Bloom and Richardson grade. In this series of 61 human breast cancer specimens, 19 were Grade I (well differentiated), 26 were Grade II (moderately differentiated) and 16 were Grade III (poorly differentiated). *In situ* carcinoma was not observed in any samples included in this series.

Informed consent to use a portion of the tissue for research purposes (form approved by the local ethics committee) was obtained from each patient before surgery.

Immunohistochemistry

Immunohistochemistry was performed on 61 tumor samples as described in a previous report [2]. Briefly, three-micrometer-thick sections of formalin-fixed and paraffin-embedded tissue samples were performed. Immunohistochemical staining was performed on a Ventana XT immunostainer, using antibodies directed against the K_v1.3 potassium channel (anti-K_v1.3 (Kcna3) (1/50 dilution), rabbit polyclonal, Alomone Labs, Ltd, Jerusalem, Israel), anti-estrogen receptor (ER) (DAKO, clone 6F11, 1/20 dilution, Dako, Trappes, France), anti-progesterone receptor (PR) (DAKO, clone PgR636, 1/50 dilution, Dako, Trappes, France), anti-Ki67 (proliferation index) (DAKO, clone KiS5, 1/20 dilution, Dako, Trappes, France), anti-ErbB2 (Her2/neu receptor) (DAKO, A485, 1/1500 dilution, Dako, Trappes, France). The avidin-biotin-peroxidase complex technique was then used. Reactions were developed using a chromogenic reaction in DAB (diamino-3,3'-benzidine tetrahydrochloride) substrate solution (DAB, Sigma Fast). Counterstaining was performed with hematoxylin solution. The channel antibody was certified for immunohistochemistry by Alomone Labs. For K_v1.3 immunohistochemistry, a negative control was performed according to the same technique without primary antibody.

Immunostaining levels for K_v1.3 channels were determined by subjective visual scoring of the brown stain. Two operators independently evaluated antigen expression. Four intensities were determined (-, +, ++, +++) according to the intensity and distribution of staining (no staining (-); < 30% with low intensity (+); 30-60% with low intensity (++); > 60% with low intensity or > 30% with high intensity (+++); high intensity < 30% was never observed). Two groups were combined for quantitative analysis: high/medium (+++/++) and low/no stain (+/-).

ER, PR and Ki67 staining was evaluated according to the percentage of positive nuclear staining. Expression was con-

Table 1. MSP and semiquantitative primer PCR conditions (M: primer for methylated PCR; U: primer for unmethylated PCR).

Gene name	Primers	T _m (°C)	PCR cycles	Size (bp)
K _v 1.3	F-R certified from Qiagen	55	40	103
CK19	F 5'-GATTGCCACCTACCGC -3' R 5'-CCATCCCTCTACCCAG -3'	58	35	136
β-actin	F 5'-CAGAGCAAGAGAGGGCATCCT -3' R 5'-ACGTACATGGCTGGGGTG -3'	58	20	210
K _v 1.3 - M	F 5'-TTTTTCGCGGGGCGTCGAGGT -3' R 5'-ATTCCCGCCCACCGCGCGA -3'	63	40	131
K _v 1.3 - U	F 5'-TTTTTGTGGGGTGTGAGGT -3' R 5'-ATTCCACCCACACACAA -3'	63	40	131

sidered positive when > 0% epithelial cells presented strong staining for ER and PR and when ≥ 10% epithelial cells presented strong staining for Ki67. ErbB2 staining was evaluated according to the DAKO kit (0, 1+, 2+, 3+). Expression was considered positive when score was 2+ or 3+.

DNA extraction and bisulfite modification of DNA

Gene methylation status was evaluated using sodium bisulfite modification of DNA and subsequent Methylation-specific PCR (MSP). Genomic DNA was obtained from 52 paraffin-embedded breast tumors, two breast cell lines and from the primary culture cells using a DNA kit extraction (QIAmp DNA Mini kit, Qiagen). Paraffin-embedded tissue was not available in 9 cases. Briefly, without macro- or microdissection of tumor cells, paraffin rolls were dissolved in xylene twice then dehydrated with absolute ethanol twice. Pellets were suspended in ATL buffer with proteinase K at 56°C overnight for enzyme digestion. 10⁶ breast cells were used for DNA extraction.

Sulfonation was performed on 1 µg of genomic DNA according to the Epitect bisulfite kit instructions (Epitect bisulfite kit, Qiagen). DNA was eluted to 20 µL and was almost always used immediately for PCR.

Methylation-specific PCR

Primer sequences used for MSP are listed in Table 1, with annealing temperature TM, PCR product size and number of PCR cycles. The K_v1.3 gene promoter was obtained using the promoter database (accession number 3180). Primers were designed to contain a minimum of 2 CpG sites. For the reaction, 1 µl of bisulfite-converted DNA was added for a total volume of 25 µl containing 2.5 µl of 10x buffer, optimal Mg²⁺ concentration (0.5 µl for unmethylated PCR, 2 µl for methylated PCR), 1 µl of each primer (20 pmol) and 0.1 µl of Taq polymerase (HotStarTaq DNA polymerase, Qiagen). PCR products were analyzed by electrophoresis on 2% agarose gels. Primers for unmethylated DNA were used on all samples to confirm the presence of DNA in the absence of a methylation-specific band. A positive control for methylated PCR was performed using SSSI methylase (Ozyme). After methylation, positive control DNA was converted by bisulfite modification in the same way as for the other DNA samples. A negative control was performed using H₂O.

Sequence analysis

PCR products were purified by ExoSAP-IT® (Amersham), with 2 µl of enzyme for 5 µl of PCR product. Exonuclease activity was performed at 37°C for 15 min followed by enzyme denaturation at 80°C for 15 min. Direct sequencing, with each sense and antisense primer (same primers as those used for MSP), was performed on these purified PCR products, using the DTCS Quick Start Kit (Beckman Coulter) with a modification of the manufacturer's instructions. Briefly, 3 µl of purified DNA was sequenced with 2 µl of DTCS Quick Start Master Mix, 1 µl of 1x CEQTM buffer, 0.4 pmol of one primer in a 10 µl final volume. The sequencing program was 30 cycles of 96°C for 20 sec, 52°C for 20 sec and 60°C for 4 sec. Sequencing products were purified by ethanol precipitation before electrophoresis on an CEQTM8000 Genetic Analysis System Beckman CoulterTM. Electropherograms were analyzed in both sense and anti-sense directions, using SequencherTM software in combination with the K_v1.3 reference promoter sequence.

Semiquantitative PCR

RNA extraction of the 14 frozen tumor tissues available was performed with the RNeasy Micro Kit (Qiagen). Frozen tissue was not available for other tumors. Methylene blue-stained sections were reviewed for each sample to identify areas of tumor. Macrodissection was performed to ensure greater than 80% tumor volume for each case. Pieces of tissue (50 mg of tumors) were placed in a lysis buffer and homogenized using a Polytron homogenizer (PRO-200, Fisher Bioblock Scientific). Total RNA was isolated according to the manufacturer's standard protocols and used (1 µg) for first-strand cDNA synthesis with oligodT primers and MultiScribeTM Reverse Transcriptase (Applied Biosystems).

For breast cells, total RNA was extracted from approximately 1 x 10⁶ cultured cells using the TRIzol method (Invitrogen, Carlsbad, CA) according to the manufacturer's instructions. RNA samples were treated with 1 U of DNase I (Promega, France) at 37°C for 30 min. A phenol/chloroform (v/v) extraction was performed and RNA was precipitated with ethanol and dissolved in 20 µl of sterile distilled water. The RNA level was measured by spectrophotometry (optical density at 260 nm) and 1 µg was reverse-transcribed into cDNA using an SSII kit (Invitrogen) according to the manufacturer's instructions.

Semiquantitative PCR was performed in duplicate using $K_v1.3$ and cytokeratin 19 (CK19) primers. PCR conditions and primers are summarized in table 1. Cytokeratin 19 was used as reference for breast adenocarcinomas because of its expression in breast adenocarcinoma [16]. Actin was used as reference for human breast cells. The intensity of the PCR band was analyzed by Quantity One software. The ratio between $K_v1.3$ and cytokeratin 19 or $K_v1.3$ and actin mRNA level was determined. Mean of duplicate tests was used for statistical analysis.

Statistics

Fisher tests (two-tailed) were used in GraphPad software to estimate the correlation between $K_v1.3$ channel methylation and grade subgroups and between immunohistochemical results and grade subgroups. Anova test was used in Origin 7 software to estimate the correlation between mRNA level and grade subgroups, and between mRNA level and immunohistochemical results. A correlation was considered significant when $p < 0.05$.

Results

Methylation and expression of $K_v1.3$ channel in human breast cells

Methylation-specific PCR was performed on the MCF-7 breast cancer cell line, a normal breast epithelial cell line and primary culture of normal cells (HMEpC) as described in Material and Methods. HMEpC cells presented an unmethylated $K_v1.3$ K^+ channel promoter while the $K_v1.3$ K^+ channel promoter was methylated in the breast cancer cell line MCF-7 and in the normal monoclonal cell line MCF 10A. Representative MSP results are shown in Figure 1a.

Semiquantitative PCR was performed on breast cells. Expression of the $K_v1.3$ K^+ channel was decreased in MCF-7 cells compared to primary culture of normal cells ($p=0.01$, Figure 1b). No difference was observed for the MCF 10A cell line.

Methylation of $K_v1.3$ channel in adenocarcinoma

Methylation-specific PCR was performed on 52 samples as described in Material and Methods. The $K_v1.3$ K^+ channel promoter was methylated in 22 (42.3%) of the 52 tumor samples (Table 2). Unmethylated DNA was observed in all but one of the patients in whom only methylated DNA was observed. Representative MSP results are shown in Figure 2a.

Methylated and unmethylated sequences were confirmed by sequencing. Sequencing of bisulfite-modified DNA from tumors showing methylated bands revealed methylated sequences with intact CG sites whereas PCR

Fig. 1. A: Methylated status of $K_v1.3$ promoter. Only unmethylated DNA was observed for primary culture of normal cells (HMEpC) while only methylated DNA was observed for the cell lines. Positive and negative controls for Unmethylated (U) and Methylated (M) PCR products are shown on the left-hand of the gel. B: $K_v1.3$ K^+ channel expression by semiquantitative PCR analysis in breast cells. mRNA level decreased in MCF-7 cells compared to HMEpC cells ($p=0.01$).

products from unmethylated tumors obtained with unmethylated-specific primers showed unmethylated sequences with TG at CG sites (Figure 2b).

Correlation between methylated status and clinicopathological data

A correlation between methylation status and clinicopathological data was then investigated. Analysis of clinical data showed that the 22 patients with methylated $K_v1.3$ tumors (22/52) were younger than the 30 patients with unmethylated $K_v1.3$ tumors (30/52) (54.4 versus 60.7 years old respectively, $p=0.048$, Table 2). No correlation was observed between methylated $K_v1.3$ tumors and mean tumor size (26.7 versus 19.4 mm respectively, $p=0.19$, Table 2).

Table 2. Correlation between K_v1.3 promoter methylation and clinicopathological data for 52 breast adenocarcinomas (SBR: Scarff-Bloom-Richardson; ER: estrogen receptor; PR: progesterone receptor). Methylated status is associated with younger age (p=0.048) and poorly differentiated tumors (p=0.040 between Grade I and Grade III tumors).

SBR Grade	
Methylated status	Grade III>Grade I p=0.040
mRNA level	Grade I > Grade III p=0.009
Protein expression	Grade I > Grade III p=0.047

Table 3. Results of statistical analysis of methylated status, mRNA level and K_v1.3 protein expression according to tumor grade. These results show a correlation between methylation status which was increased in grade III tumors and gene expression which was decreased in grade III tumors. (SBR: Scarff-Bloom-Richardson).

	Methylated promoter region n (%)	Unmethylated promoter region n (%)	
Total n=52	22 (42.3)	30 (57.7)	
Age mean (yrs)	54.4 SD 10.8	60.7 SD 11.4	p=0.048
Mean tumor size (mm)	26.7 SD 27	19.4 SD 12.7	p=0.19
SBR Histological grade			
Grade I (16)	2 (12.5)	14 (87.5)	
Grade II (23)	12 (52.1)	11 (47.9)	
Grade III (13)	7 (53.9)	6 (46.1)	p=0.040
Nodal involvement			
Positive (24)	12 (50)	12 (50)	
Negative (28)	10 (35.7)	18 (64.3)	p=0.4
ER status			
Negative (10)	3 (30)	7 (70)	
Positive (42)	19 (45.2)	23 (54.8)	p=0.48
PR status			
Negative (18)	6 (33.3)	12 (66.7)	
Positive (34)	16 (47)	18 (53)	p=0.38
ErbB2 status			
Negative (30)	13 (43.3)	17 (56.7)	
Positive (22)	9 (40.9)	13 (59.1)	p=1
Ki67 status			
≤ 10% (10)	3 (30)	7 (70)	
≥ 10% (42)	19 (45.2)	23 (54.8)	p=0.48

Analysis of pathological data showed that methylation of the K_v1.3 gene promoter was associated with poor differentiation with 53.9% of methylation in grade III tumors and only 12.5% of methylation in grade I tumors (p=0.040, Table 2). In grade II tumors, methylation of the K_v1.3 promoter was observed in 52.1 % of the cases (12/23).

Finally, no difference was observed between methylated and unmethylated tumors in terms of lymph node status, estrogen and progesterone receptor expression, ErbB2 expression and expression of the Ki67 proliferation index (Table 2).

K_v1.3 channel expression in breast adenocarcinoma

As gene methylation may repress expression of the gene, K_v1.3 K⁺ channel expression was also investigated. K_v1.3 mRNA was evaluated by semiquantitative PCR in all 14 cases for which frozen tissue was available and

immunohistochemistry was performed on 61 samples. K_v1.3 mRNA level was lower in grade III tumors than in grade I tumors (p=0.009, Figure 3a). There was no difference in the mRNA level between grade I and grade II tumors (p=0.34) but in grade II tumors, K_v1.3 mRNA level was higher than grade III tumors (p=0.05). These results were confirmed by immunohistochemistry which revealed a high expression of the K_v1.3 channel protein in 36.8% of Grade I tumors, in 19.2 % of Grade II tumors and in 6.3 % of grade III tumors. A statistical difference is observed between grade I and grade III subgroups (p=0.047, Figure 3b).

Correlation between methylation and K_v1.3 channel expression

The results of statistical analysis of methylated status, mRNA level and K_v1.3 K⁺ channel protein expression according to tumor grade are summarized in Table 3. A correlation was observed between methylated sta-

Fig. 2. MSP $K_v1.3$ gene promoter in breast adenocarcinomas
A: Panel viewed from left to right shows a 50-bp ladder as a molecular weight marker, a positive control (+) and a water control (-) for unmethylated PCR (U), then a positive control (+) and a water control (-) for methylated PCR (M). Methylated and unmethylated DNA were detected in tumor samples from patients 2, 5 and 6, while only unmethylated DNA was detected in tumor samples from patients 1, 3 and 4. B: Sequencing analysis: forward sequences of methylated (FM) and unmethylated (FU) PCR products. CG sites are intact in methylated sequences although no Cytosine is visible in unmethylated sequences.

tus and gene expression. Methylation of the $K_v1.3$ gene promoter was increased in grade III tumors whereas $K_v1.3$ K^+ channel expression was decreased in grade III tumors in terms of both mRNA and protein.

Discussion

This study reports for the first time methylation of the $K_v1.3$ gene promoter in breast adenocarcinoma. This DNA methylation is associated with poorly differentiated tumors and decreased $K_v1.3$ K^+ channel expression.

Overall, aberrant methylation of the $K_v1.3$ K^+ channel promoter was observed for the first time in 42.3% of breast ductal adenocarcinomas. A higher frequency of young patients was also observed among methylated tumors. Sharma et al [10] have already described a higher proportion of cell-cycle gene methylation in younger breast cancer patients, which could be one of the factors accounting for tumor aggressiveness in younger breast cancer patients.

Moreover, like the p16 or RAR β 2 genes [11, 12], methylation of the $K_v1.3$ gene promoter is associated with poorly differentiated breast adenocarcinoma. In 2006, Abdul et al [4] showed a decrease of $K_v1.3$ protein expression in high-grade prostate tumor and also suggested an association between decreased $K_v1.3$ protein expression and poor prognosis. Promoter methylation is often associated with silencing gene expression. Some studies have shown a direct correlation between methylation of the gene and decreased mRNA level. Recently, Wang [14], studying different prostate cancer cell lines, observed in that the level of endonuclease G was inversely correlated with the level of methylation of EndoG promoter and that inhibition of DNA methylation by decitabine (a

Fig. 3. Correlation between $K_v1.3$ K^+ channel expression and tumor grade. A: Semiquantitative PCR analysis. mRNA level decreased inversely to tumor grade ($p=0.009$ between grade I and III tumors, grade I: $n=3$, grade II: $n=3$, grade III: $n=8$) B: $K_v1.3$ protein expression by immunohistochemistry. As for mRNA level, protein expression decreased inversely to tumor grade ($p=0.047$ between grade I and grade III tumors, grade I: $n=19$, grade II: $n=26$, grade III: $n=16$) (Negative control: immune reaction without primary antibody). (Leitz, Laborlux S, x200. Sony 3CCD color camera).

DNA methylation inhibitor) induced endoG expression. Because promoter methylation is one of the major mechanisms for gene silencing but cannot always be correlated with decreased gene expression, $K_v1.3$ gene expression

was also studied in these breast tissue samples. Our results show a decrease in both $K_v1.3$ mRNA level and $K_v1.3$ protein expression in grade III tumors. These data are correlated with increased methylation in grade III tumors.

Tumor DNA extraction was performed on paraffin-embedded specimens without macro- or microdissection of tumor cells. In one case, only methylated DNA was detected. This could be explained by the absence of normal cells in the samples and complete methylation of all cancer cells. However, Holst et al [17] reported the presence of rare foci of morphologically normal epithelial cells with hypermethylation of the p16 promoter sequence and loss of p16 protein in normal breast tissue. They identified these abnormalities as premalignant lesions before morphological changes. In our previous report [2], complete loss of $K_v1.3$ protein expression was also observed in some normal breast tissue samples. It would therefore be interesting to study whether methylation of the $K_v1.3$ gene promoter constitutes a premalignant lesion.

Various genes involved in apoptotic pathways have been described to be methylated in prostate cancer [18, 19]. Prostate and breast cancers present certain similarities and $K_v1.3$ K^+ channel expression is decreased in both prostate and breast cancers [2, 4]. Due to methylation of the $K_v1.3$ gene promoter in breast cancer and its association with poorly differentiation, the $K_v1.3$ gene could be one more gene involved in the apoptotic process and in prostate and breast carcinogenesis.

Finally, $K_v1.3$ gene promoter was methylated in the breast cancer cell line MCF-7 and in the monoclonal transformed “normal” breast cell line MCF 10A, while this methylation was absent in normal breast cells derived from primary culture. Differences of methylated status

have already been observed between breast cancer cell lines and have sometimes been directly correlated with gene expression [20]. Decreased $K_v1.3$ K^+ channel expression was observed in MCF-7 cells compared to primary culture cells. No decreased $K_v1.3$ K^+ channel expression was observed in MCF 10A cell line, probably because hypermethylation is one of the mechanisms involved in gene silencing but other regulators could also interact in these morphologically “normal” cells. Nevertheless, methylation of the $K_v1.3$ gene promoter in MCF 10A cells suggests that these monoclonal cells are also not entirely “normal” cells. Finally, the fact that only the $K_v1.3$ promoter from benign breast epithelial cells derived from primary culture remained unmethylated suggests that $K_v1.3$ promoter methylation is an early step in breast carcinogenesis.

In conclusion, we report for the first time an aberrant methylation of the $K_v1.3$ gene promoter in breast adenocarcinomas and show that this methylated status is correlated with decreased $K_v1.3$ K^+ channel expression. Methylation of the $K_v1.3$ gene promoter and decreased $K_v1.3$ expression were also both correlated with poorly differentiated tumors and younger age of patients. These data support the idea that loss of $K_v1.3$ K^+ channels may be a marker of poor prognosis of breast tumors.

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