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MUC1 oncogene amplification correlates with protein overexpression in invasive breast carcinoma cells

E. Lacunza[a], M. Baudis[b], A.G. Colussi[a], A. Segal-Eiras[a], M.V. Croce[a], M.C. Abba[a,*]

[a]*Centro de Investigaciones Inmunológicas Básicas y Aplicadas (CINIBA), Facultad de Ciencias Médicas, Universidad Nacional de La Plata, Calle 60 y 120, La Plata, CP:1900, Argentina*

[b]*Institute of Molecular Life Sciences, University of Zurich, Winterhurerstrasse 190, CH-8057, Zurich, Switzerland*

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Abstract The *MUC1* gene is aberrantly overexpressed in approximately 90% of human breast cancers. Several studies have shown that *MUC1* overexpression is due to transcriptional regulatory events. However, the importance of gene amplification as a mechanism leading to the increase of *MUC1* expression in breast cancer has been poorly characterized. The aim of this study was to evaluate the role of *MUC1* gene amplification and protein expression in human breast cancer development. By means of real-time quantitative polymerase chain reaction and immunohistochemical methods, 83 breast tissue samples were analyzed for *MUC1* gene amplification and protein expression. This analysis showed *MUC1* genomic amplification and a positive association with the histopathological group in 12% (1 out of 8) of benign lesions and 38% (23 out of 60) of primary invasive breast carcinoma samples ($P = 0.004$). Array–comparative genomic hybridization meta-analysis of 886 primary invasive breast carcinomas obtained from 22 studies showed MUC1 genomic gain in 43.7% (387 out of 886) of the samples. Moreover, we identified a highly statistical significant association between *MUC1* gene amplification and MUC1 protein expression assessed by immunohistochemistry and Western blot test ($P < 0.0001$). In conclusion, this study demonstrated that *MUC1* copy number increases from normal breast tissue to primary invasive breast carcinomas in correlation with MUC1 protein expression.

1. Introduction

DNA amplification is a common mechanism used by cancer cells to upregulate the activity of critical genes associated with tumor development and progression [1,2].

In breast cancer, a number of discrete amplicons have been identified, involving *ERBB2* (17q12), *MYC* (8q24), *CCND1* (11q13), *AIB1* (20q13), and *FGFR* (10q26) genes [3–7]. The most common regions of increased DNA copy number in breast cancer as determined by comparative genomic hybridization (CGH) include the chromosome arm 1q, where an unambiguous target gene has not been identified yet [8,9]. Although regional genomic copy number amplifications frequently increase the expression of relevant oncogenes, they may also serve as an indicator of genomic instability [10]. As such, presence of one or several gene amplifications may have prognostic significance.

MUC1 oncoprotein is aberrantly overexpressed in approximately 90% of human breast cancers [11–13], which has been associated with cell adhesion inhibition as well as increased metastatic and invasive potential of tumor cells [14]. This overexpression allows MUC1 to interact with members of the ERBB family of receptor tyrosine kinases [15]. In addition, the MUC1 cytoplasmic domain, which comprises the last 72 amino acids, also interacts with diverse effectors that have been linked to transformation, such as c-Src, β-catenin, and IKβ/NF-κB [15–17].

Several studies have shown that *MUC1* overexpression is in part due to transcriptional regulatory events [18–21]. Furthermore, MUC1 regulation has been also associated to epigenetic changes in cancer cells [22]. However, the importance of gene amplification as a mechanism leading to the increase of *MUC1* expression in breast cancer has been poorly characterized. In an early study, Bièche and Lidereau have postulated a concordance between *MUC1* mRNA expression and gene dosage in breast carcinomas [23]. Similar observations were further reported affecting ovary, thyroid, and gastric carcinomas [24–26]. Interestingly,

* Corresponding author. Tel.: +54-221-4236711; fax: +54-221-4258989.

E-mail address: mcabba@conicet.org.ar (M.C. Abba).

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a recent array−comparative genomic hybridization (a-CGH) study coupled to genomewide transcriptome map showed a cluster of genes, located at 1q21.3−1q22, exhibiting the strongest association between copy number and gene expression in primary breast carcinomas [27]. In addition, *in silico* analysis of the transcriptomic profile, based on our previously published Serial Analysis of Gene Expression (SAGE) data sets [28,29], showed that *MUC1* is the most frequently upregulated gene at 1q21.3−1q22 chromosomal region in primary invasive breast carcinomas. Thus, the possible association between MUC1 expression and gene amplification could reveal novel information about *MUC1* as a predictive breast cancer gene. The aim of this study was to evaluate the role of *MUC1* gene amplification and protein expression in human breast cancer progression.

2. Materials and methods

2.1. Tissue samples and cell lines

A total of 83 formalin-fixed, paraffin-embedded breast tissue specimens were obtained from different hospitals associated to the National University of La Plata, Argentina. They included 15 normal tissues from individuals who underwent cosmetic surgery, 8 benign lesions, and 60 primary invasive ductal carcinomas (IDC; ICD-O 3 8500/3). Because the hypothetical multistep model for breast carcinogenesis suggests that invasive carcinoma arises from a series of intermediate hyperplasic lesions to *in situ* and invasive carcinoma, benign lesions included in this study were all usual epithelial hyperplasia. Stage at time of diagnosis was based on the tumor, node, metastasis system classification. The breast cancer cell lines MCF7, T47D, and ZR75-1, and the MRC5 normal lung fibroblast cells were also included. The study was performed with respect to the ethical standards of the 1975 Declaration of Helsinki, as revised in 1983.

2.2. Cell culture

The estrogen-dependent breast cancer cell lines MCF7, T47D, and ZR75-1 were grown in RPMI-1640 medium (Gibco, Gaithersburg, MD) supplemented with 10% (v/v) fetal bovine serum (Bioser, Buenos Aires, Argentina), 10 IU/mL penicillin G, and 10 μg/mL streptomycin. The human diploid fibroblast cell strain MRC5 was grown in minimal essential medium (Gibco), supplemented with nonessential amino acids and 0.1% sodium pyruvate (Gibco). Cell cultures were collected by rinsing the monolayer with cold lysis buffer; the cell suspension was centrifuged and the pellet recovered before tissue digestion (see below).

2.3. DNA extraction and purification

Paraffin-embedded samples from breast tissues were processed by routine techniques for histological analysis. Briefly, four 10-μm paraffin embedded tissue from each biopsy sample were used for DNA extraction. Tissue digestion was performed by incubating in extraction buffer (1 mmol/L Tris−HCl, pH 8; 1 mmol/L EDTA; 1% Triton X-100; 0.5% Tween 20) with proteinase K at 56°C overnight. Finally, DNA from tissue samples and cell lines was purified by the phenol−chloroform−isoamyl alcohol method (25:24:1) followed by ethanol precipitation, as previously described [30]. The DNA was resuspended in distilled water. DNA concentration was measured on the Nanodrop spectrophotometer and adjusted to 30 ng/μL.

2.4. Real-time quantitative polymerase chain reaction analysis

DNA quantitative polymerase chain reaction analysis (Q-PCR) was performed by the PerfeCta SYBR Green SuperMix (Quanta Biosciences, Gaithersburg, MD) and the Stratagene MX30005P Real-Time PCR System. The efficiency-corrected comparative $\triangle\triangle C_t$ method was applied, and the $\triangle C_t$ of a human normal diploid lung fibroblast cell strain (MRC5 cells, not immortal), run in parallel with each assay, was used for calibration to get the relative quantification for each tumor or cell line sample versus a normal chr1 gene copy number control. In this experiment, *MUC1* and *PUM1* (*Pumilio homolog 1*) were used as the target and normalizing reference genes, respectively. *MUC1* gene maps in the chromosome region 1q21.3−q22, whereas *PUM1* is located at the chromosome region 1p35.2. The MUC1 amplicon (86 bp) (Fig. 1A) does not include the tandem repeat region present within the *MUC1* gene, and the *PUM1* gene has been well established as a reference control in breast cancer samples [31].

The PCR reactions were performed with 12.5 μL of the SuperMix (Quanta Biosciences), 5 μL of DNA templates, and 1 μL of *MUC1* (forward 5′-gctgctcctcacagtgctt-3′ and reverse 5′-ccacagaaagaccacgaaga-3′) or *PUM1* (forward 5′-tccatcttcatcctaccgc-3′ and reverse 5′-aagggacaatctgctcgt-tag-3′) primers at 10 pmol/μL. The reactions were cycled as follows: 1 cycle of 45°C for 5 minutes, 1 cycle of 95°C for 3 minutes followed by 40 cycles of 95°C for 30 seconds, 57°C for 30 seconds, and 72°C for 20 seconds. PCR amplification was followed by melting curve analysis to assure the presence of a single PCR product (Fig. 1B). Samples were considered to be affected by genomic amplification for *MUC1* gene when the relative quantitative values were greater than +3 standard deviations (99% confidence interval; $P < 0.01$) with respect to the values observed for the normal breast samples. All samples were analyzed in triplicate to confirm the obtained values.

2.5. Immunohistochemical analysis

Immunohistochemistry (IHC) was performed according to standard procedures as previously reported [32]. Briefly, sections 4 μm thick were cut from block paraffin tissue

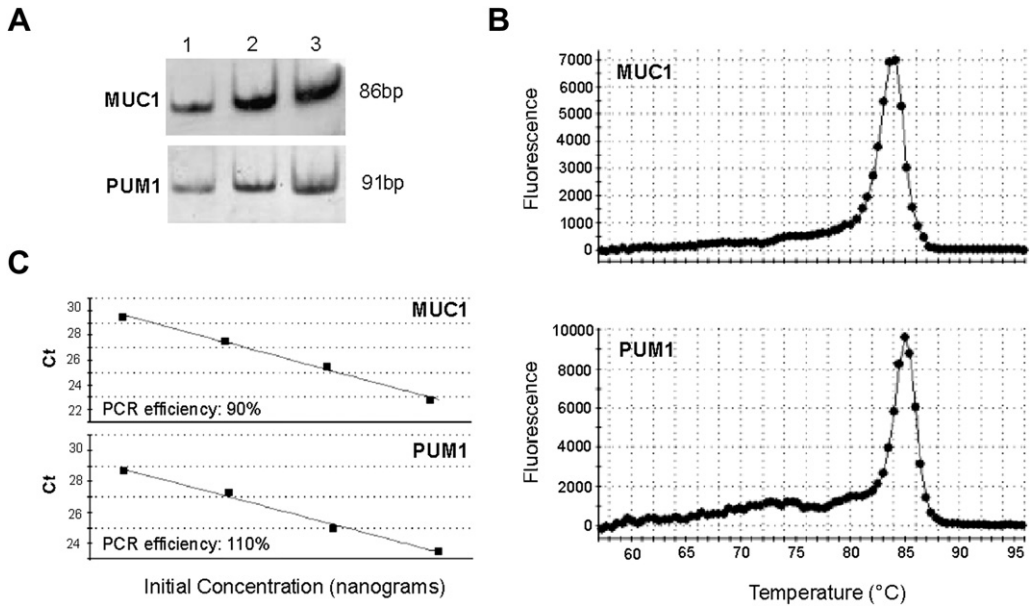

Fig. 1. (A) Polymerase chain reaction (PCR) assay showing the *MUC1* and *PUM1* gene products in MRC5 cell line optimized at different concentrations of DNA template. (B) Dissociation (melting curve) analysis plot showing a single homogeneous peak for *MUC1* and *PUM1* PCR products in MRC5 cell line, which confirms specific amplification. (C) Standard curve plot of *PUM1* and *MUC1* obtained with serial dilutions of MRC5 DNA showing the initial concentration against the C_t generated for each dilution as well as the efficiency of amplification.

samples. The samples were dewaxed and rehydrated with xylene and ethanol, respectively. Endogenous peroxidase activity was blocked by treatment with 0.3% H_2O_2 (Merck, Darmstadt, Germany) in methanol. Slides were then incubated in 10% horse normal serum in 1% bovine serum albumin (Sigma, St. Louis, MO)–phosphate-buffered saline (PBS) to eliminate background reaction, and antigenic recuperation was performed by heating at 100°C in 10 mmol/L sodium citrate buffer, pH 6.0. Mouse antihuman polyclonal antibody against MUC1 (CT33) was used at a dilution of 1:500. This antibody is directed against the last 72 amino acids of the MUC1 cytoplasmic domain. Samples were incubated overnight with the primary antibody at 4°C, whereas negative controls were incubated with PBS under the same conditions. After incubation with secondary peroxidase-labeled anti–Armenian hamster immunoglobulin (1:150; Dako, Glostrup, Denmark), reaction was developed with 3-30-diaminobencidine and 0.03% H_2O_2 in PBS. Finally, sections were counterstained with hematoxylin (Sigma), dehydrated, and covered with mounting media. Samples were evaluated under light microscope. A reaction was considered positive when more than 5% of the cells were stained. The patterns of reaction were as follows: L = linear membrane, C = cytoplasmic, and M = mixed linear and cytoplasmic. Nuclear staining was also recorded. Staining intensity was scored in a semiquantitative manner and was graded as negative (−), low (+), moderate (++), and strong (+++).

2.6. Western blot analysis

Homogenates from breast tissue samples were filtered through a mesh and centrifuged at 1,500 × *g* at 4°C for 15 minutes; the resulting supernatants were subjected to sodium dodecyl sulfate–polyacrylamide gel electrophoresis and Western blot analysis. Electrophoretic separation of proteins was performed with discontinuous 4% stacking–10% resolving polyacrylamide gels under reductive conditions with a miniVE Vertical Electrophoresis System (Amersham Biosciences, Piscataway, NJ). Gels were transferred to nitrocellulose membranes, which were blocked with 3% dry milk in PBS/Tween 20 (0.05%) at 4–8°C for 3 hours and then washed in PBS/Tween 20 (0.05%). Membranes were incubated with CT33 antibody (1:1,000 in 1% milk/PBS Tween 20) at 4°C overnight. After washing, membranes were incubated with horseradish peroxidase–conjugated anti–Armenian hamster immunoglobulin (Dako) (1:1,000) at room temperature for 1 hour. Finally, reaction was developed with diaminobenzidine (Sigma). As an internal control, β-actin Western blot analysis was performed by incubating membranes with AC15 AMo (1:5,000 in 1% milk/PBS/Tween 20). Western blot band intensity analysis was performed by Scion Image software (Frederik, MD).

2.7. Bioinformatics and statistical analysis

To perform the comparative analysis of the transcriptomic profiles in a set of normal and breast cancer samples, we combined six normal breast SAGE libraries available in public databases (http://cgap.nci.nih.gov/cgap.html) with 23 invasive breast cancer SAGE libraries previously reported by us [29]. To compare the 29 SAGE libraries, we used a modified *t*-test as previously described [28]. This analysis allowed us to identify SAGE tags with

significantly different expression levels between normal and IDCs ($P < 0.01$).

To enable visualization and illustration of the 1q21–q23 chromosome region in MCF7, T47D, and ZR75-1 breast cancer cell lines from the array-CGH data of Pollack et al. and of Chin et al. [9,27], we used the VAMP software (Visualization and Analysis of Array-CGH, Transcriptome and Other Molecular Profiles) (http://bioinfo-out.curie.fr/actudb) [33]. The association between *MUC1* gene amplification, protein overexpression, and histological status of the samples was assessed by Fisher's exact test; the two-way contingency tables were the result of defining the variables amplification status, which was codified as 0 to nonamplified samples and 1 to amplified samples, and protein expression that was coded according to the grade of staining intensity obtained by IHC as 0 (−), 1 (+), 2 (++), and 3 (+++). To test the independence of variables, the basic significance level was fixed at $P < 0.05$. In addition, ordinal-by-ordinal associations were assessed by Kendall's tau-b test. Data analysis was performed by SPSS 17.0 software (SPSS, Chicago, IL).

As reference for overall frequency of DNA imbalances affecting the region 1q21.3–q22, CGH data of 886 primary IDC cases (ICD-O 3 8500/3) from the Progenetix database (http://www.progenetix.net) [34] was used.

3. Results

We analyzed the global gene expression profile in a set of normal and breast cancer SAGE libraries previously published by us [28,29]. Interestingly, we identified the *MUC1* gene among the most highly upregulated group of transcripts in the primary invasive breast cancer SAGE libraries at transcriptomic level (see Supplementary Fig. 1). Specifically, according to the *in silico* analysis of our SAGE database, *MUC1* was detected as being the most frequently upregulated gene located at 1q21.3–1q22 chromosomal region of invasive breast carcinomas.

Array-CGH evidence is available reporting the relative DNA copy number profile of the chromosome 1 in breast cancer cell lines [9,27]. The VAMP resource [33] was used for array-CGH data analysis of chromosome region 1q21.3–q22 from MCF7, T47D, and ZR75-1 breast cancer cell lines. *In silico* analysis of data of Pollack et al. and of Chin et al. [9,27] identified a gain of chromosome regions 1q21.3–q22, including the *MUC1* gene region in T47D cell line (Fig. 2A). Next, we used a Q-PCR method for the interrogation of the presence or absence of *MUC1* genomic amplification in these breast cancer cell lines. The specificity of the MUC1/PUM1 gene products was verified through melting curve analysis after Q-PCR cycles, as well as gel electrophoresis (Fig. 1). Amplicons of the expected size for *MUC1* (86 bp) and *PUM1* (91 bp) were confirmed in the normal diploid MRC5 cell strain (Fig. 1A). In addition, melting curve analysis confirmed the specificity of primers: we did not observe any deviation from the average melting curve profile in all DNA samples analyzed (Fig. 1B). Q-PCR analysis showed *MUC1* gene amplification in T47D cell line, while no amplification was detected for the MCF7 and ZR75-1 cancer cell lines, in agreement with the array-CGH data (Fig. 2B).

To investigate whether MUC1 protein overexpression correlates with gene amplification in breast cancer development, we analyzed DNA obtained from 83 breast tissue samples. Samples were considered to be affected by *MUC1* genomic amplification when the relative DNA copy number was greater than +3 standard deviations (99% confidence interval; $P < 0.01$) with respect to the values observed in normal specimens. According to these, *MUC1* genomic amplification was detected in 12% (1 out of 8) of benign lesions and 38% (23 out of 60) of primary invasive breast carcinoma samples (Fig. 2C). The statistical analysis revealed a significant positive association between the histopathological group and *MUC1* DNA copy number changes ($P = 0.004$) (Fig. 2). Moreover, to explore the frequency of DNA copy number gains/losses affecting the chromosome region 1q21.3–q22 in independent set of samples, we analyzed chromosomal and array-CGH data of 886 individual IDC cases as collected in the Progenetix online database. In this data set, which did not include cell lines, a gain or genomic amplification involving the *MUC1* locus on 1q22 (UCSC HG18 chr1:153,424,924–153,429,324) could be found in 387 out of 886 cases (43.7%) (Fig. 3).

To further investigate the relevance of *MUC1* gene amplification over protein expression in breast cancer progression, we studied 74 specimens by IHC from the 83 samples previously analyzed by Q-PCR (Table 1). Because MUC1 protein is highly glycosylated and this could block or hamper the binding of antibodies, we used the well-characterized polyclonal antibody CT33, developed against the MUC1 unglycosylated cytoplasmic domain [32]. MUC1 protein expression was detected in all normal breast samples analyzed. As it was expected, the reaction was restricted to the apical membranes of epithelial cells, which displayed a weak or moderate MUC1 immunostaining. However, in benign lesions, a moderate to strong MUC1 expression was observed in approximately 75% of the analyzed cases (Table 1). Furthermore, the pattern of reaction varied from linear to mixed (linear/cytoplasmic). Among IDCs, MUC1 protein was detected in 93% of the analyzed cases with a strong intensity of expression in approximately 72% of the samples (Table 1). Frequently, cytoplasmic staining was observed together with a membrane reaction in a mixed pattern at equal intensity, although some samples showed a linear pattern. It is also important to note that MUC1 reaction comprised the entire cell in many carcinoma samples. More importantly, MUC1 protein expression analysis demonstrated a highly statistical significant association between *MUC1* gene amplification and MUC1 overexpression ($P < 0.0001$) (Fig. 4). In this sense, 23 out of 26 amplified samples displayed a high

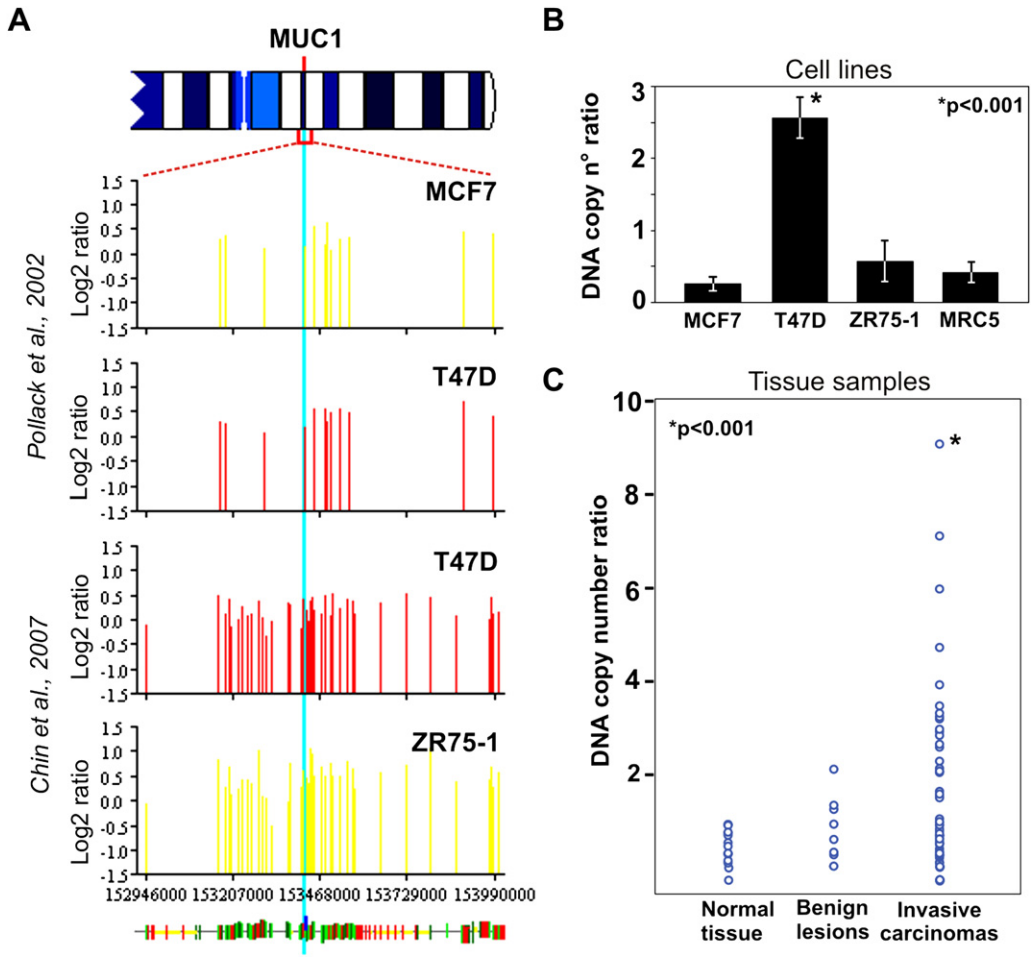

Fig. 2. *MUC1* gene amplification analyses in breast samples. (A) Visualization of 1q21.3–q22 microarray-based comparative genomic hybridization profile of MCF7, T47D, and ZR75-1 breast cancer cell lines obtained from the studies of Pollack et al. and Chin et al. [9,27]. Red lines indicate chromosomal regions of gain. These data suggest a region of genomic gain located at 1q21.3–q22 involving the *MUC1* gene. (B) Quantitative polymerase chain reaction assay showed *MUC1* gene amplification in T47D breast cancer cell line in agreement with data from Pollack et al. and Chin et al. (C) *MUC1* DNA copy number ratios of breast tissue samples. Differences between normal ($n = 15$), benign ($n = 8$), and invasive carcinomas ($n = 60$) were detected ($P < 0.001$).

expression of MUC1 protein, whereas the remainder samples (3 out of 26) showed moderate intensity of MUC1 protein expression (Table 1). Nonstatistically significant associations were detected between MUC1 protein expression/gene amplification and Estrogen Receptor alpha status, histologic tumor grade, and tumor stage ($P = 0.05$).

4. Discussion

Breast cancer progresses as a result of the accumulation of genomic changes, which is thought to underlie the clonal evolution of cancer disease [35]. Moreover, in a significant subset of human breast cancers, as in other solid tumors, gene amplification is an important mechanism for oncogene activation during tumor progression [9].

The *MUC1* oncogene, located at the 1q21.3–q22 chromosomal region, is markedly overexpressed in breast cancer and has been associated with aggressive tumor behavior [14]. In this study, in silico analysis of previously published SAGE databases [28,29] led us to identify the *MUC1* gene as the most upregulated transcript from the 1q21.3–q22 chromosomal region in invasive breast carcinomas. Indeed, upregulation of MUC1 seems to be specifically associated with adenocarcinomas from various sources, which has been correlated with increased metastatic potential and poor prognosis [14,25]. Many studies have revealed that the increase of MUC1 expression is related with transcriptional regulatory events or epigenetic changes [18–20,22]. However, in an early study, Bièche and Lidereau [23] found a concordance between the *MUC1* DNA relative copy numbers and *MUC1* mRNA expression levels in primary breast carcinomas, suggesting a gene dosage effect. Interestingly, in a wide variety of carcinoma types, including ovary, papillary thyroid, and gastric cancers, *MUC1* amplification and overexpression was also found [24–26]. These observations support a functional role of MUC1 as part of the frequent DNA copy number gains observed in a variety of epithelial neoplasias and possibly other malignancies [36].

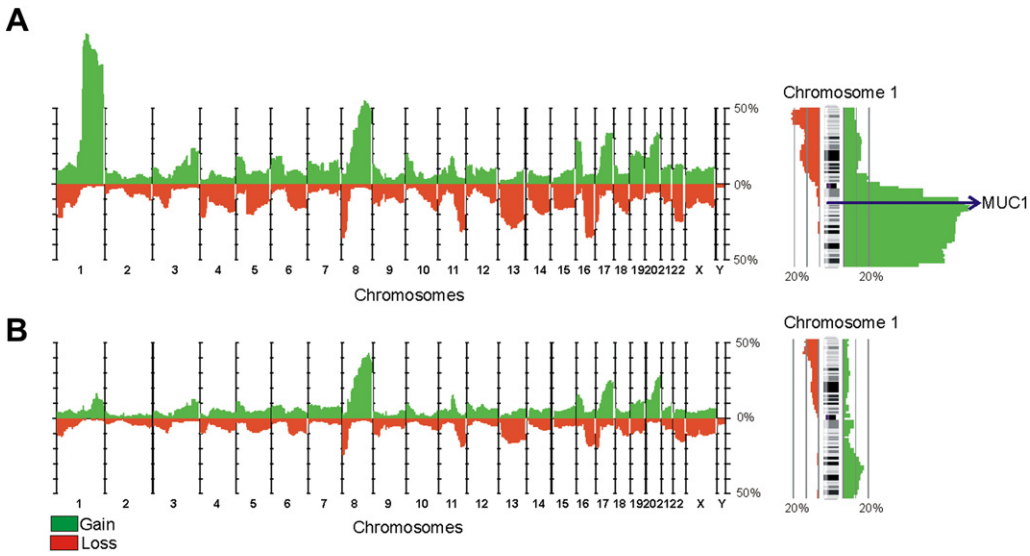

Fig. 3. Frequency of regional genomic imbalances in 886 invasive ductal carcinomas (ICD-O 3 8500/3) as determined by chromosomal or array comparative genomic hybridization. (A) Subset of 387 cases (43.7%) showing genomic gains involving the *MUC1* locus on 1q22 (arrow on chromosome 1 plot). The overview histogram shows the frequency plot for regional genomic gains (green, upward) and losses (1q22 = 100% for gains). (B) Corresponding plot for the 499 cases negative for 1q22 gains. With the whole or near-complete 1q gains removed, an additional distal gain hot spot becomes apparent at band 1q32. Although the distribution pattern of genomic gains and losses is of a similar shape, the overall lower aberration frequency in (B) may be due to the selection bias introduced through exclusion of 1q22 gains.

In this study, we performed a Q-PCR and IHC analyses on malignant, benign, and normal breast epithelial tissue samples and breast cancer cell lines in order to determine whether gene amplification is a mechanism leading to MUC1 overexpression. Q-PCR has proven to be a very powerful tool to analyze gene copy variations in cancer [25,37]. By this method, we detected *MUC1* gene amplification in 12% of benign lesions and 38% of invasive breast carcinomas. We also found gene amplification in the T47D breast cancer cell line as was previously evidenced by array-CGH analysis. The results of our study show that *MUC1* gene copy number increases in invasive breast samples and in the hyperplasic lesions, which means that this gene could have an early causal role in the breast cancer development.

To determine whether *MUC1* gene amplification status is also correlated with protein overexpression in breast tumors, we analyzed MUC1 protein expression by IHC in a set of 74 samples previously characterized for *MUC1* DNA copy number. Previous studies have demonstrated that the MUC1 oncoprotein is overexpressed in breast cancer and other solid tumors [38–40]. The NH_2 terminal mucin domain of MUC1 in normal breast epithelial cells carries numerous branched O-linked carbohydrates, which can block binding of antibodies directed against this region, whereas MUC1 molecules derived from carcinomas usually contain shorter nonbranched glycans, allowing the access of the antibodies to their epitopes. For this reason, to avoid confusing results, we used the CT33 polyclonal antibody [32] developed against the unglycosylated last 72 amino acids of the COOH terminal domain of MUC1. Although the degree of expression and cellular localization varied significantly between tumor and normal tissue and among tumor samples, we observed MUC1 overexpression in 62.5% of benign lesions and 72% of invasive breast carcinomas. In addition, in most carcinoma specimens the pattern of reaction was mixed or cytoplasmic, which confirms the loss of polarity of MUC1 compared with

Table 1
MUC1 protein expression and gene amplification in breast tissue samples

	MUC1 protein expression, *n* (%)				
Characteristic	Absent	Weak	Moderate	Strong	No. of cases
MUC1 gene status					
Nonamplified	5 (10%)	7 (15%)	9 (19%)	27 (56%)	48
Amplified	0 (0%)	0 (0%)	3 (11%)	23 (89%)	26
Histology					
Normal tissue	0 (0%)	2 (33%)	2 (33%)	2 (33%)	6
Benign lesions (usual epithelial hyperplasia)	1 (12%)	1 (12%)	1 (12%)	5 (63%)	8
Invasive carcinomas	4 (7%)	4 (7%)	9 (15%)	43 (72%)	60

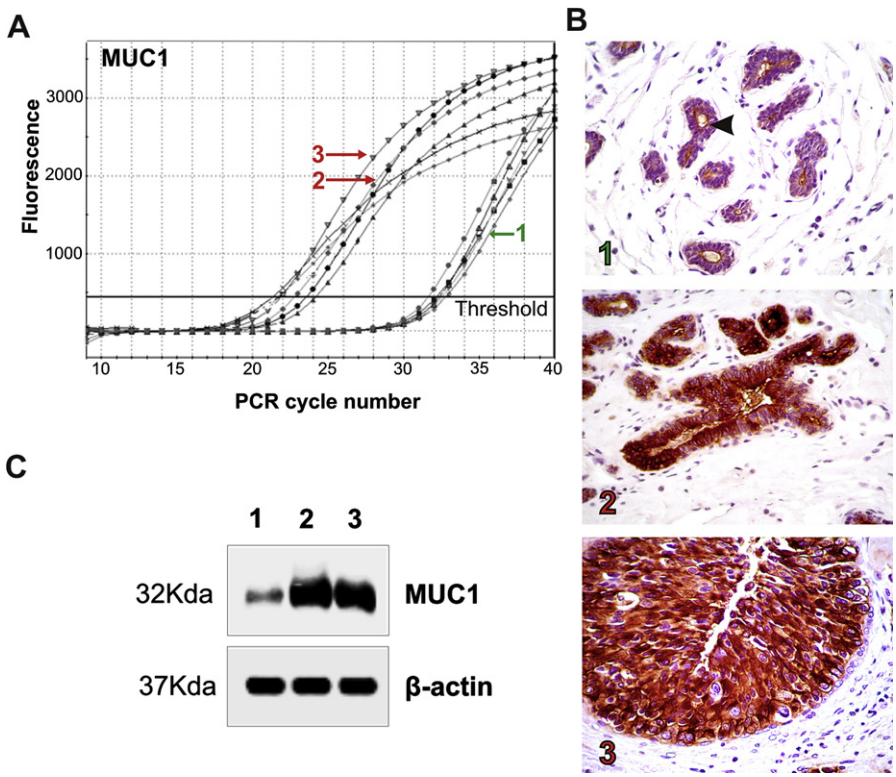

Fig. 4. *MUC1* gene amplification and protein expression analyses in breast samples. (A) Plot of real-time quantitative polymerase chain reaction of 11 breast samples of *MUC1* (5 invasive ductal carcinomas [IDC], 1 benign lesion, and 5 normal breast epithelia). The *MUC1* plot shows all the IDC samples and the benign lesion (arrows 3 and 2, respectively) amplified with respect to the normal samples (arrow 1). (B) Immunohistochemical results obtained with CT33 polyclonal antibody. 1, 2, and 3 are the same samples indicated in the MUC1 amplification plot. (1) Normal breast epithelial tissue (10×). A moderate reaction is observed at the apical membranes (linear pattern) of the epithelial cells, indicated by an arrowhead. (2) Benign lesion (usual epithelial hyperplasia) (10×). A strong reaction with a mixed pattern is clearly observed in the epithelial cells of ducts. (3) Invasive ductal carcinoma (10×). A strong reaction is observed in the entire cell surface with a high percentage of reaction. (C) Western blot analysis of the same samples. Compared with lane 1 (normal breast tissue), a more intense band is observed in lanes 2 and 3, corresponding to benign and carcinoma samples, respectively.

normal tissue samples. More importantly, the results obtained by IHC showed a significant correlation with *MUC1* gene amplification status of the analyzed cases ($P = 0.0001$). Although almost all amplified samples showed a strong MUC1 protein expression (23 out of 26 amplified samples), some breast carcinomas showed overexpression of MUC1 in the absence of gene amplification, suggesting that the upregulation of MUC1 expression in these samples would be mediated by another mechanism. Interestingly, array-CGH/DNA microarray evidence is available reporting amplification and overexpression of the chromosomal region 1q21.3–q22 (the same region to which *MUC1* maps) in breast cancer cell lines and primary breast carcinomas [27]. Moreover, a genomewide transcriptome map revealed clusters of genes located at 1q21 exhibiting nonrandom increased expression in breast cancer cells [41].

In conclusion, this study demonstrates that MUC1 protein overexpression correlates with gene amplification and that this mechanism could be evident in the early stages of breast cancer development. The frequent regional copy number gains involving 1q21.3–q22 in breast carcinomas and a variety of other neoplasias may modulate tumor cell behavior acting through MUC1 as one of the (presumably multiple) oncogenetic target genes in this region. However, gene amplification would not be the only explanation for the MUC1 overexpression at the protein level, suggesting that other molecular mechanisms, such as regulatory transcriptional events and epigenetic control would have effect alone or in concert with copy number alteration in deregulating *MUC1* gene expression in breast cancer cells.

Acknowledgments

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at 10.1016/j.cancergencyto.2010.05.015.

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