Altered expression and loss of heterozygosity of the migration and invasion inhibitory protein (*MIIP*) gene in breast cancer

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Abstract. Previous studies have characterized the migration and invasion inhibitory protein (MIIP) as a novel putative tumor-suppressor gene that regulates cell migration and invasion as well as the mitotic checkpoint. The *MIIP* gene is located on chromosome 1p36.22, a common site for deletion in many solid tumors including breast cancer. In the present study, we evaluated *MIIP* expression and allelic deletion to gain insight into the role of the *MIIP* gene in breast cancer. *MIIP* gene mRNA and protein expression was assessed in 86 matched breast cancer and adjacent normal tissues. Loss of heterogeneity (LOH) of the *MIIP* gene was determined using single-nucleotide polymorphism (SNP) and microsatellite (MS) markers in 149 breast carcinomas and the corresponding normal lymphocytes. The analysis revealed that the expression levels of *MIIP* mRNA and protein were downregulated in tumor specimens compared to those in corresponding adjacent tissues. Advanced clinical stage and tumor size >2 cm were associated with a decreased MIIP expression level. Twenty-six percent (37/142) of tumors were shown to have LOH at the *MIIP* locus by MS and SNP markers. Breast cancer patients with LOH at the SNP marker rs2295283 experienced shorter survival time. The attenuated expression and LOH of the *MIIP* gene may contribute to the poor prognosis of breast cancer, supporting a tumor-suppressing role of *MIIP* gene in the pathogenesis of this disease.

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Abbreviations: TSG, tumor-suppressor genes; MIIP, migration and invasion inhibitory protein; IIp45, invasion inhibitory protein 45; IGFBP2, insulin-like growth factor binding protein 2; SNP, single-nucleotide polymorphism; LOH, loss of heterogeneity; ER, estrogen receptor; PR, progesterone receptor; MS, microsatellite; HDAC6, histone deacetylase 6

Key words: breast cancer, gene inactivation, LOH, MIIP, mitosis

Introduction

Breast cancer is the most common type of cancer among women worldwide, and its incidence is on the increase (1). Clearly identified genetic susceptibilities to early onset familial breast cancer are associated with mutations in tumor-suppressor genes (TSGs) *BRCA1* and *BRCA2*, which, however, are not important in sporadic breast cancer (2). Evidence suggests that allelic loss at chromosome 1p36 is a common genetic event in a various types of human cancer including breast cancer, suggesting the presence of at least one TSG located in this region (3). One of these genes is *p73* (1p36.33), a p53 homologue (4). However, the allelic status of other genes mapped to this chromosomal region has yet to be sufficiently elucidated.

The migration and invasion inhibitory protein (MIIP; or invasion inhibitory protein 45, IIp45) gene, located on chromosome 1p36.22, has emerged as a key gene that plays a critical role in tumorigenesis (5). MIIP was initially identified in a yeast two-hybrid screen for proteins that interact and inhibit insulin-like growth factor binding protein 2 (IGFBP2) (6). Advances in characterizing this protein have demonstrated that MIIP regulates cell migration and mitosis (7), two essential microtubule and cytoskeleton-associated processes for homeostasis of tissues and fetal development. Additionally, MIIP is underexpressed in human glioma, and an *in vivo* study using transgenic mouse models showed that exogenously introduced *MIIP* reduced the incidence and grade of glioma (8). For breast cancer, a case-control epidemiological study conducted by our team identified a functional genetic single-nucleotide polymorphism (SNP) of *MIIP* that is associated with the risk of breast cancer development and prognosis (9).

However, unlike the classical TSGs such as *TP53*, *PTEN* and *Rb*, in which frequent mutations are identified in cancers, previous mutation screening among glioma and pancreatic cancer tissues only detected rare mutations in the *MIIP*

gene (6,10), similar to the low mutation frequency found in the *p73* gene in human cancers. Haploinsufficiency or loss of one allele in another group of TSGs has been described as an alternative important mechanism leading to gene inactivation and cancer development. Genes in this group include the *CHD5* gene on 1p36 and the *PTEN* gene on 10q23 (11,12). In the present study, we evaluated loss of heterogeneity (LOH) of the *MIIP* locus in breast cancers and investigated the expression of MIIP in breast cancer tissues by reverse-transcription-quantitative PCR (RT-PCR) and western blotting.

Materials and methods

Patient characteristics. All 149 patients enrolled in the present study were newly diagnosed and histologically confirmed breast cancer cases from the Breast Cancer Research Center in Tianjin Medical University Cancer Institute and Hospital (TMUCIH) from January 1, 2006 to June 1, 2008. The patients recruited in the present study had not received any chemo- or radiotherapy prior to obtaining blood samples. The blood samples were obtained prior to surgery. All the patients met the following inclusion criteria: Tianjin residents aged 18-65 years with no previous diagnosis of cancer. The Ethics Committee of TMUCIH approved the study protocol. Written informed consent was obtained from all the patients participating in the present study.

The participants were personally interviewed using a structured questionnaire regarding their socio-demographic characteristics, lifestyle, and family history of cancer. Clinical information on tumor features and disease severity, including morphology, tumor size, lymph node and organ metastasis, tumor stage, and estrogen and progesterone receptor (ER and PR) status was also collected. Each subject donated 20 ml of blood, which was collected in heparinized tubes. Matched tumor and adjacent normal tissues from 86 cases were obtained from the Tissue Bank Facility of TMUCIH, which collects all solid tumor tissues from surgical patients when possible.

RNA extraction and RT-qPCR. Total RNA were extracted and purified from the 86 pairs of snap-frozen breast cancer and adjacent normal tissues according to the protocol of TRIzol reagent (Invitrogen Corporation, Grand Island, NY, USA). The reverse-transcribed products, cDNA, were synthesized from 5.0 ng total RNA in a 20 μl reaction mixture using M-MLV reverse transcriptase (Invitrogen Life Technologies, Carlsbad, CA, USA). RT-qPCR was performed on ABI PRISM 7900 Fast Real-Time system (Applied Biosystems, Foster City, CA, USA) using TaqMan gene expression assay for human *MIIP* according to the manufacturer's instructions [Takara Biotechnology (Dalian) Co., Ltd., China]. The human *GAPDH* housekeeping gene was measured as an endogenous control. The probe used was purchased from Applied Biosystems (Hs00976263_m1 for *MIIP*). Each PCR was performed in triplicate in a 5-μl reaction volume containing 100 ng cDNA as template to detect *MIIP* gene expression. Amplification was performed under the following conditions: 50˚C for 2 min, then 95˚C for 10 min, followed by 40 cycles of denaturation at 95˚C for 15 sec and annealing/extension at 60˚C for 1 min. The 7900 Sequence Detection System 2.3 software (Applied Biosystems) was used to calculate the relative fold-change in *MIIP* expression by the $2^{-\Delta\Delta Ct}$ method with 95% confidence (13).

Protein extraction and western blotting. Approximately 100-150 mg frozen tissues were homogenized with pestle and mortar containing liquid nitrogen, and then dissolved in 500-1,000 μl lysis buffer (500 μl protein lysate, 5 μl DTT and 5 μl protease inhibitor). Following incubation on ice for 15 min, the resulting lysates were centrifuged at 140,000 x g for 15 min at 4˚C in an ultracentrifuge. Supernatant fractions were collected on ice and the total protein concentration was measured according to the protocol of the BCA Protein Quantitative Detection kit (Sangon Biological Engineering Technology and Services Corporation, Shanghai, China). Twenty microliters supernatant (~40-50 ng protein) was mixed with 5X SDS loading buffer containing 62.5 mM Tris-HCl, pH 6.8, 4% SDS, 10% glycerol, 5% β-mercaptoethanol and 0.1% bromophenol blue, and then boiled at 95˚C for 5 min. The denatured proteins of each group were loaded into lanes with 30% SDS-polyacrylamide gel electrophoresis, and then transferred to nitrocellulose membrane. Blots were blocked with 5% skim milk at room temperature for 1 h and incubated overnight at 4˚C with monoclonal rabbit anti-IIP45 antibody (1:800; Sigma-Aldrich, St. Louis, MO, USA) and then with HRP-conjugated goat anti-rabbit IgG (1:2,000; Santa Cruz Biotechnology, Inc., Dallas, TX, USA) as secondary antibody at room temperature for 1 h. β-actin was used as an internal reference and the antibodies (mouse anti-β-actin antibody and the secondary HRP-linked goat anti-mouse IgG antibody) were purchased from Santa Cruz Biotechnology. Immunoreactive bands were visualized using the Immobilon™ Western Chemiluminescent HRP Substrate (Millipore Corporation, Billerica, MA, USA), and exposed to film. The bands were subjected to densitometric quantification by Image J 2X [National Institutes of Health (NIH), Bethesda, MD, USA].

DNA extraction and LOH analysis. LOH was determined on specific PCR products of tumors and corresponding normal peripheral leukocytes. Genomic DNA was extracted from paired snap-frozen tumor tissues and normal controls using the Qiagen DNA Mini kit according to the manufacturer's protocol (Axygen Biosciences, Union City, CA, USA). LOH analysis of the *MIIP* locus was performed using three microsatellite (MS) markers and one SNP marker on 149 human breast cancer tumors. Following a search in the Human Genome Annotation Database (UCSC) and Human Sequence Tag Map (NCBI), no MS marker intragenic to the *MIIP* gene was identified. The three nearest MS loci flanking the 5' and 3' side of the *MIIP* gene (within 0.2 Mb of the gene): *D1S2740*, *D1S489* and *D1S434* were selected. PCR was performed with synthesized labeled primers (Sangon Biological Engineering Technology and Services Corporation; Table I) specific to the MS markers obtained from the NCBI UNISTAT database. The size of amplification products was determined by capillary electrophoresis on a 3730XL Genetic Analyzer (Applied Biosystems). Data were collected and analyzed by Gene-Scan3.1g software (Applied Biosystems).

An SNP with a high heterozygosity status present in the *MIIP* gene was selected from the Ensembl database (Table I).

Table I. Details of the SNP and microsatellite markers used for detecting the LOH status of *MIIP*.

Marker	Primer sequences (5'-3')	Tm (°C)	Product size (bp)
rs2295283	Forward: CTCAGCCTAAATGCTCGCTA		
	Reverse: TGGCACGGTCAGAGAAACAA	58	460
D1S2740	Forward: AGCCTTTTGATACTCTTACTGTGG		
	Reverse: AAAGATTGGGAAGTGGCTCA	55	201
D1S489	Forward: AGCCAGACCAAGTCTCAACA		
	Reverse: ACAAAATGATGGGGTTATGG	55	141-153
D1S434	Forward: AGCTAATTTACATTACCCAAAAAGA		
	Reverse: ACAATACAGCAGCAGGTGGC	55	260

MIIP, migration and invasion inhibitory protein; SNP, single-nucleotide polymorphism; LOH, loss of heterogeneity; Tm, temperature.

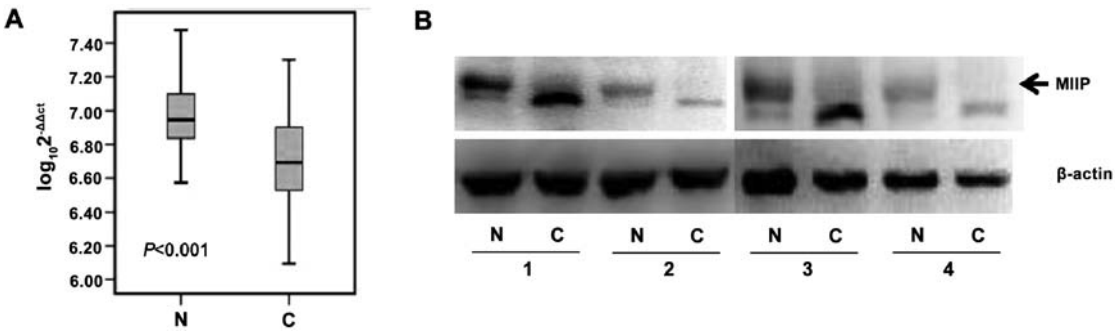

Figure 1. Decreased MIIP expression in breast cancer tissues. (A) *MIIP* mRNA expression in adjacent normal tissues (N) and (C) breast cancer tissues. (B) MIIP protein expression in representative adjacent normal tissues (N) and (C) breast cancer tissues. MIIP, migration and invasion inhibitory protein.

This functional SNP (rs2295283) has been found to be associated with risk and prognosis of breast cancer in a previous large-scale epidemiological study (9). Genotypic and allelic frequency of this SNP was conducted by using the MGB TaqMan probe assay (Applied Biosystems) as described previously (9).

For LOH analysis, only those sample pairs in which the normal lymphocyte DNA exhibited a heterozygous pattern, i.e., two alleles for each MS or SNP marker, were considered informative (14,15). Among the informative sample pairs for MS loci, LOH was evaluated by comparing the areas of the two alleles in tumor tissue with corresponding peripheral leukocytes (15). An absence or a reduction by >33% for one allele in the tumor DNA as compared to the normal DNA was interpreted as LOH (16). Concerning the SNP site, LOH in informative samples was defined as a total loss of one allele in tumor DNA compared to its normal tissue counterpart (14). For scoring changes, LOH frequency for each MS or SNP marker was calculated separately as a percentage of all the informative samples in that particular locus (14).

Statistical analysis. Statistical analysis was performed with the SPSS version 17.0. Statistical tests were two-sided, and a difference was considered statistically significant at P<0.05. The relative expression levels of *MIIP* mRNA were quantified using the $2^{-\Delta\Delta Ct}$ method, and the data were $\log_{10}$-transformed for analysis. Comparison of MIIP mRNA or protein expression level between tumors and adjacent normal tissues was made by the Wilcoxon rank-sum (Mann-Whitney) test. The χ^2 test was employed to compare the differences in the frequency distribution of age, family history and clinical variables including morphology, tumor size, lymph node metastasis, tumor stage, and ER/PR status between cases with different LOH status in *MIIP* gene. MIIP mRNA (further divided into high- and low-expression groups by the median of $\log_{10}2^{-\Delta\Delta Ct}$ value) and protein expression were correlated with clinical characteristics and *MIIP* gene LOH status by using the χ^2 and Wilcoxon tests. The correlation between survival (overall survival) and LOH status of the *MIIP* gene was analyzed using the Kaplan-Meier method and log-rank test.

Results

MIIP expression in breast cancer. We analyzed *MIIP* mRNA and protein expression in 86 cases with breast cancer and matched adjacent normal breast tissues. *MIIP* mRNA and protein expression was detected in all the adjacent normal tissues and in the majority of the breast carcinomas. The comparison between paired normal adjacent tissue and tumors revealed that *MIIP* transcript expression was significantly decreased in carcinomas (Fig. 1A). On the basis of $\log_{10}2^{-\Delta\Delta ct}$ value, tumors were divided into the high- or low-expression groups by the median expression level. We found that *MIIP* gene exhibited a descending expression pattern with an

Table II. Correlation between clinicopathological characteristics and MIIP expression in 86 breast cancer cases.

	mRNA expression		Protein expression	
Variables	High expression/total (%)	P-value	Median (min-max)	P-value
Age (years)				
≤50	19/41 (46.34)	0.860	14.85 (0.95-54.24)	0.465
>50	20/45 (44.44)		20.05 (0.96-63.45)	
ER				
-	17/32 (53.12)	0.265	21.19 (1.68-63.45)	0.105
+	22/54 (40.74)		14.85 (0.95-63.04)	
PR				
-	20/36 (55.56)	0.107	19.99 (3.51-63.45)	0.956
+	19/50 (38.00)		16.61 (0.95-63.04)	
Benign breast disease				
Never	30/68 (44.11)	0.656	16.61 (0.96-63.45)	0.949
Ever	9/18 (50.00)		18.09 (0.95-40.06)	
Family history of cancer				
No	27/58 (46.55)	0.747	18.09 (0.95-63.45)	0.214
Yes	12/28 (42.86)		14.98 (0.96-63.04)	
Morphology				
Non-invasive	29/57 (50.88)	0.149	16.61 (0.96-63.45)	0.747
Invasive	10/29 (34.48)		17.17 (0.95-63.04)	
Clinical stage				
0	11/13 (84.62)	0.012	15.85 (3.51-63.04)	0.249
I	13/34 (41.18)		19.45 (1.01-63.45)	
II	14/36 (38.89)		16.61 (0.96-56.67)	
III	1/3 (33.33)		12.46 (0.95-23.98)	
Tumor size (cm)				
≤2	14/19 (73.68)	0.005	15.85 (3.51-63.04)	0.289
>2	25/67 (37.31)		17.17 (0.95-63.45)	
Lymph node metastasis				
No	22/43 (51.16)	0.279	18.78 (1.01-63.45)	0.843
Yes	17/43 (39.53)		15.89 (0.95-56.67)	

ER, estrogen receptor; PR, progesterone receptor; MIIP, migration and invasion inhibitory protein.

advanced TNM stage of the tumors (P=0.012) and with an increased tumor size (P=0.005) (Table II).

At the protein level, 86 cases were analyzed for MIIP expression by western blotting, which varied in the same manner to the transcriptional level with a lower MIIP expression in cancer tissues than the normal tissues (Fig. 1B). No significant difference was identified in the expression pattern of MIIP protein based on patient clinical characteristics, with the exception of TNM staging. Tumor tissues in the advanced stage (stage III) expressed significantly decreased MIIP levels than those in the early stages (stage 0, I and II): 16.99 (0.96-63.45) vs. 12.46 (0.95-23.98), P=0.043.

LOH analysis of the MIIP locus. To determine the possible mechanism of *MIIP* gene underlying breast cancer onset and development, a LOH analysis with MS/SNP markers for *MIIP* gene was performed in 149 cases. The four MS and SNP markers used were all informative, being clearly biallelic in the normal lymphocyte samples and showing partial or total loss of one allele in the tumor samples (candidate LOH samples) or remained biallelic. Representative LOH profiles of all the markers used in the selected samples are shown in Fig. 2. As shown in Table III, of the 149 cancer tumors analyzed with the intragenic SNP marker rs2295283, 84 samples were informative (heterozygous), 9 of which (10.71%) showed LOH. For the flanking MS loci, 138 of 149 (92.62%) cancer samples were informative. LOH frequency affecting ≥1 MS locus observed was 23.91% (33/138). The most frequently deleted loci were *D1S489* (20/97, 20.62%) and *D1S2740* (14/70, 20.00%), with LOH for *D1S434* marker being similar, at 16.49% (16/97) in all the samples studied.

A comparison of the rates of LOH for the four MS/SNP markers in breast cancer patients with different subtypes of tumor characteristics demonstrated no correlations among the

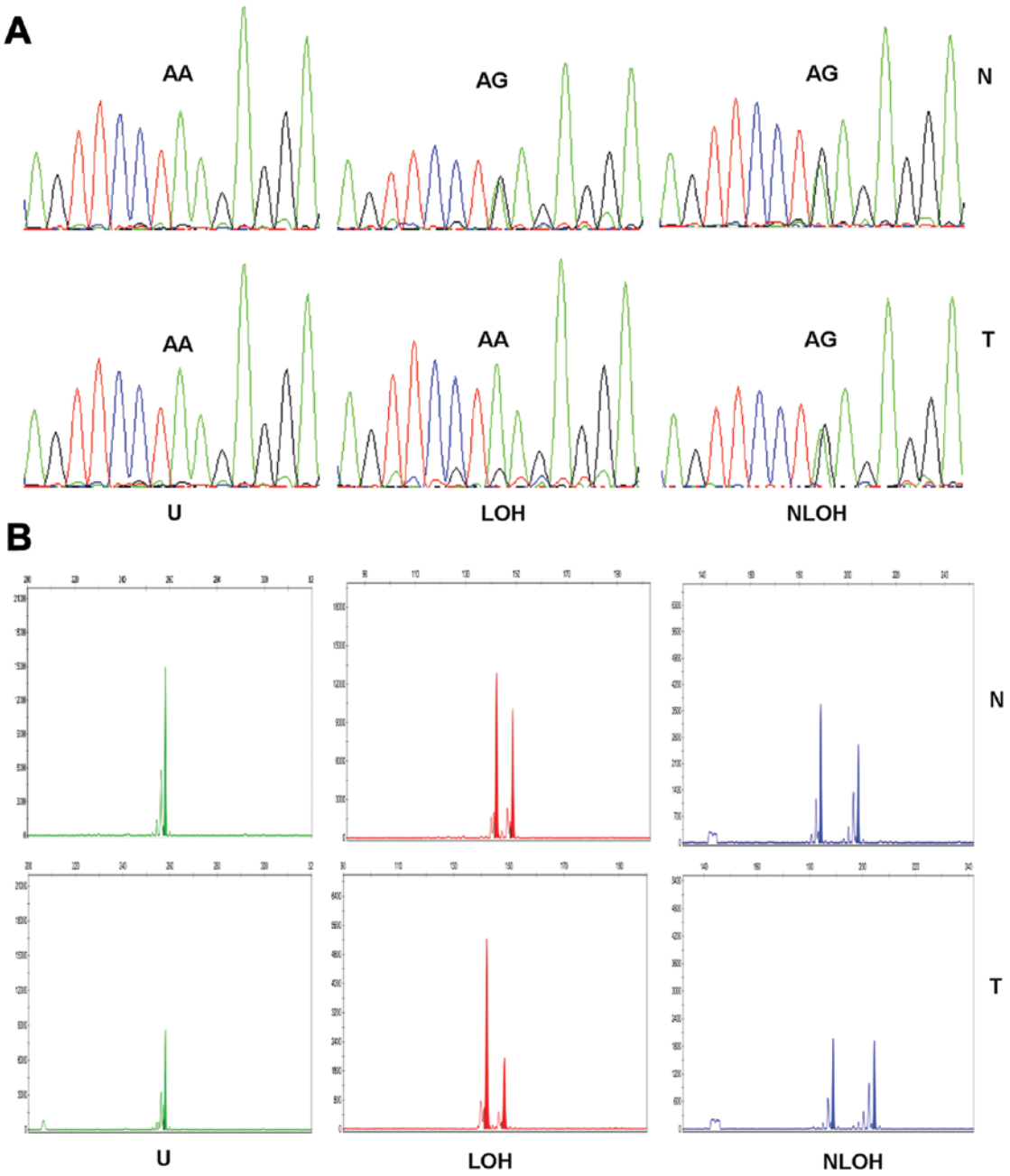

Figure 2. Representative capillary electrophoretic photographs for (A) SNP and (B) MS markers indicating LOH, non-LOH (NLOH) and uninformative (U) results in breast cancer. N, normal leucocyte DNA. T, tumor DNA. SNP, single-nucleotide polymorphism; MS, microsatellite; LOH, loss of heterogeneity.

four markers (Table IV). However, survival analysis revealed that breast cancer patients with LOH in the SNP marker rs2295283 had a poorer prognosis (P=0.035) (Fig. 3; Table V). A similar trend was observed for MS loci, with an association with overall survival reaching significance (P=0.054) (Table V).

Additionally, we analyzed the correlations among the four MS/SNP markers and MIIP expression, and found MIIP expression was not affected by LOH status in the *MIIP* gene (Table VI).

Discussion

Cell motility and cell cycle regulation are essential processes for normal tissue development and remodeling. Disruption

Table III. Summary of LOH analysis of *MIIP* gene locus in 149 breast cancer cases using SNP and MS markers.

Loci	Informative samples/ total (%)	LOH per informative sample (%)
rs2295283	84/149 (56.38)	9/84 (10.71)
D1S2740	70/149 (46.98)	14/70 (20.00)
D1S489	97/149 (65.10)	20/97 (20.62)
D1S434	97/149 (65.10)	16/97 (16.49)
Overall MS loci	138/149 (92.62)	33/138 (23.91)

LOH, loss of heterogeneity; MS, microsatellite; SNP, single-nucleotide polymorphism; MIIP, migration and invasion inhibitory protein.

Table IV. Association between the *MIIP* gene LOH and clinicopathological characteristics in breast cancer patients.

	rs2295283			D1S2740			D1S489			D1S434			Overall MS loci		
Variables	-	+	P-value	-	+	P-value	-	+	P-value	-	+	P-value	-	+	P-value
Age (years)															
≤50	39	3	0.483	24	5	0.627	40	8	0.341	35	7	0.968	47	15	0.944
>50	36	6		32	9		37	12		46	9		58	18	
ER															
-	28	2	0.480	27	7	0.905	33	5	0.145	32	7	0.752	46	12	0.450
+	47	7		29	7		44	15		49	9		59	21	
PR															
-	28	4	0.726	26	7	0.811	30	7	0.745	31	6	0.954	45	14	0.965
+	47	5		30	7		47	13		50	10		60	19	
Benign breast disease															
Never	57	8	0.677	38	10	1.000	59	18	0.550	62	13	1.000	80	25	0.959
Ever	18	1		18	4		17	3		19	6		25	8	
Family history of cancer															
No	44	6	0.733	39	8	0.525	51	11	0.351	52	8	0.258	71	17	0.080
Yes	31	3		17	6		26	9		28	8		33	16	
Morphology															
Non-invasive	56	7	1.000	39	11	0.742	59	5	1.000	62	11	0.533	26	10	0.527
Invasive	19	2		17	3		18	15		19	5		79	23	
Clinical stage															
0+I	39	2	0.091	32	10	0.329	39	11	0.729	43	12	0.106	56	22	0.178
II+III	36	7		24	4		38	9		38	4		49	11	
Tumor size (cm)															
≤2	15	0	0.352	10	4	0.457	15	2	0.511	14	6	0.091	17	8	0.295
>2	60	9		46	10		62	18		67	10		88	25	
Lymph node metastasis															
No	36	2	0.174	30	10	0.227	34	12	0.206	43	11	0.249	55	21	0.257
Yes	39	7		26	4		43	8		38	5		50	12	

-, non-LOH (NLOH); +, LOH; MIIP, migration and invasion inhibitory protein; ER, estrogen receptor; PR, progesterone receptor; LOH, loss of heterogeneity.

of this homeostasis is involved in tumorigenesis and tumor progression through aberrant increase in cell migration and invasion as well as the loss of control for the cell cycle checkpoint. leading to genome instability (17). MIIP, as a novel candidate tumor suppressor, inhibits tumor cell migration and invasion and regulates mitosis through the IGFBP2 and histone deacetylase 6 (HDAC6) downstream target proteins (7). Song *et al* (6) first reported a significantly reduced MIIP expression in highly-invasive glioblastoma multiformes compared with normal brain and anaplastic glioma tissues. Subsequently, Wu *et al* (18) found a decrease in expression of MIIP in high-grade compared to lower-grade gliomas. Similarly, in the present study, we detected a decreased MIIP expression at the mRNA and protein level. This downregulation is more evident in patients with advanced tumor stage and larger tumor size. Therefore, MIIP may be important in the tumorigenic process and in the tumor development of breast cancer.

Although not fully elucidated, the mechanism by which MIIP is downregulated in tumors was examined in the present study. Considering its localization on chromosome 1p36 (refined in 1p36.22) where frequent deletions are observed in various cancer types (5), we hypothesized that attenuation of the *MIIP* gene is a genetic event contributing to carcinogenicity. However, this hypothesis was not further supported by the mutation screening of 59 glioma samples and pancreatic cancer tissues and cell lines, among which only rare frequent mutations were observed in the *MIIP* gene (6,10). This observation suggests that MIIP may not be a classic TSG such as p53. In the present study, we provide evidence that *MIIP*

Table V. Univariate analysis for overall survival of breast cancer patients.

Loci	Survival times (months)		P-value
	Means	95% CI	
rs2295283			
-	50.73	48.92-52.54	0.035
+	42.15	34.37-49.93	
D1S2740			
-	46.30	42.25-50.34	0.286
+	36.16	25.90-46.42	
D1S489			
-	49.75	46.49-53.02	0.436
+	48.64	43.33-53.95	
D1S434			
-	44.87	40.87-48.87	0.782
+	45.48	38.44-52.53	
Overall MS loci			
-	50.09	48.21-51.96	0.054
+	46.02	40.47-51.59	

-, non-LOH (NLOH); +, LOH; MS, microsatellite; LOH, loss of heterogeneity.

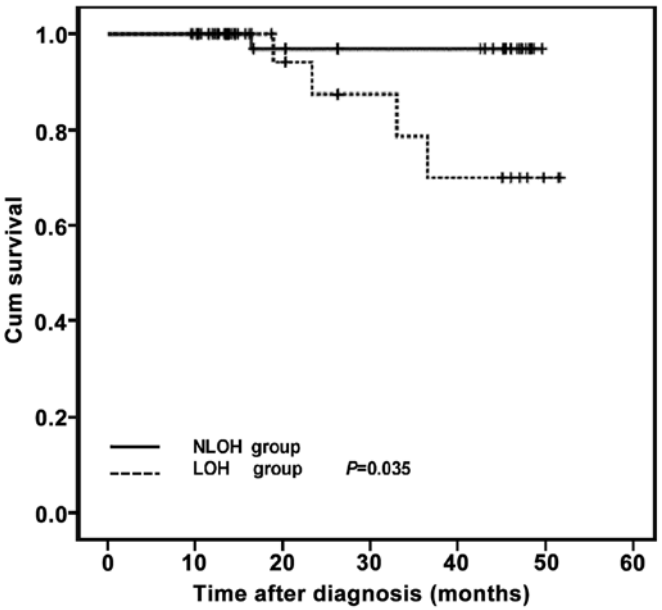

Figure 3. Kaplan-Meier curves of overall survival in breast cancer by the LOH status of *MIIP* SNP rs2295283. P-value is from the log-rank test. MIIP, migration and invasion inhibitory protein; SNP, single-nucleotide polymorphism; MIIP, migration and invasion inhibitory protein; LOH, loss of heterogeneity.

functions as a TSG in another important manner, similar to the *CHD5* and *PTEN* genes whose inactivation occurs, not because of mutations but due to haploinsufficiency or the loss of one allele (11,12).

Frequent LOH of a specific chromosomal region is regarded as an indicator of a loss of a closely linked TSG. The classic method for LOH analysis is using MS markers. For its low coverage and uneven distribution on the chromosome, MS loci selected are usually distant from the relevant genes with poor representativeness (19). However, this method is prone to false-negative (such as non-symmetrical amplification

Table VI. Relationship between the LOH status and mRNA/protein expression of *MIIP* gene in breast cancer.

Loci	mRNA expression		Protein expression	
	High expression/total (%)	P-value	Median (min-max)	P-value
rs2295283				
-	16/40 (0.40)	0.454	20.05 (0.95-63.04)	0.424
+	2/49 (0.22)		14.35 (4.04-56.67)	
D1S2740				
-	14/27 (0.52)	0.564	20.12 (1.68-40.73)	0.414
+	8/13 (0.62)		22.78 (3.51-63.04)	
D1S489				
-	17/42 (0.41)	0.788	16.61 (0.95-54.24)	0.599
+	7/19 (0.37)		16.80 (3.81-63.04)	
D1S484				
-	19/42 (0.45)	0.919	16.61 (0.95-63.45)	0.470
+	7/16 (0.42)		16.99 (3.51-63.04)	
Overall MS loci				
-	16/45 (0.36)	0.541	19.04 (0.95-63.45)	0.877
+	16/38 (0.42)		16.23 (3.51-63.04)	

-, non-LOH (NLOH); +, LOH; MIIP, migration and invasion inhibitory protein; LOH, loss of heterogeneity.

phenomenon) and false-positive (such as sliding) products, affecting the determination of the results (19). SNPs are the most common and widely distributed sequence variations in the human genome. Thus, LOH status of certain SNP may be the specifically localized gene itself (15,19) and has been analyzed by many studies on other specific chromosomal loci (14,20,21).

In the present study, we selected one intragenic SNP (rs2295283) (9) and the three nearest MS loci flanking the *MIIP* gene (*D1S2740*, *D1S489* and *D1S434*) to evaluate the LOH of the *MIIP* gene. In our series of 142 informative samples of paired breast tumors and normal genomic DNA counterparts, we identified 37 tumors (~26%) showing LOH, which clearly showed the allelic loss of one or more polymorphic sites within and close to the *MIIP* sequences. Furthermore, a significant association of allelic loss of MIIP with unfavorable overall survival in breast cancer cases was observed, strengthening the potential role of the locus in the development or progression of the breast tumors. No correlation was identified between MIIP downregulation and LOH, suggesting there may be other to-be-determined mechanisms inactivating MIIP in breast cancer, such as homozygous deletion and methylation.

In conclusion, we present direct evidence that the *MIIP* sequences are susceptible to LOH in breast cancer, and suggested a possible association between this gene and development or progression of this disease. We cannot exclude the possibility of other candidate TSGs on chromosome 1p36 as potential LOH targets in breast cancer, Nevertheless, the occurrence of allelic deletion in the *MIIP* gene and loss of the gene expression in breast tumors presented in this study are useful for further functional and clinical exploration of this gene in cancer.

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