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Analysis of matrix metalloproteinase-1 gene polymorphisms and expression in benign and malignant breast tumors

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Abstract

A guanine insertion polymorphism in matrix metalloproteinase-1 promoter (MMP-1 2G) is linked to early onset and aggressiveness in cancer. We determined the role of MMP-1 2G on the level of MMP-1 expression and breast cancer severity in benign breast disease, atypical hyperplasia, invasive and non invasive (*in situ*) breast cancer. We observed no significant difference in genotype distribution among the different breast disease groups. However, the level of MMP-1 expression was significantly higher in atypical ductal hyperplasia compared to benign breast disease; and in invasive breast cancer compared to *in situ* breast cancer. MMP-1 2G insertion polymorphism in the invasive group also correlated significantly with the expression of MMP-1 and breast cancer prognostic markers HER2 and P53.

Introduction

Matrix Metalloproteinases (MMPs) are a large family of calcium-dependent zinc-containing proteinases. They actively participate in the remodeling of extracellular matrix (ECM) by degrading certain ECM components and promoting cell proliferation, migration, differentiation, apoptosis and angiogenesis (1-5). MMPs are not only involved in physical process such as growth, wound healing and fibrosis, but also play an important role in tumor invasion and progression (6-16). Among all the identified MMPs, MMP-1 is the most ubiquitously expressed interstitial collagenase. It is also one of the few MMPs that can cleave collagen I, and III, the major components of ECM, which must be degraded for tumor invasion and metastasis to occur (17). In the literature, MMP-1 has been implicated in both early detection and prognosis of breast cancer and increased expression of MMP-1 has been reported to be significantly associated with precancerous status (18). Over-expression of MMP-1 mRNA has been correlated with high frequency of recurrence and fatal outcome (19).

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Declaration of Interests

The authors report no declarations of interest.

A number of mechanisms can impact the level and the activity of MMPs, among which transcriptional regulation plays one of the most important roles. It has been reported that a single guanine insertion at -1607 bp in the promoter region of MMP-1 creates a binding site (5'-GGAT-3') for transcription factor ETS which can cooperate with the adjacent AP-1 binding site to induce high level expression of MMP-1 through enhancing the activity of the basal transcriptional machinery, but the 1G allele (5'-GAT-3') does not affect the transcriptional activity of MMP-1 (20, 21). MMP-1 2G polymorphism has been associated with lymph node metastasis in breast cancer (22, 23) and has also been linked to the pathogenesis of other cancers, for example, to the early-onset of lung cancer (24), increased risk of nasopharyngeal carcinoma, oral squamous cell carcinoma and colorectal cancer (25-27), aggressiveness of head and neck cancer (28, 29), development and progression of endometrial carcinoma (30) and negative prognosis in patients with ovarian cancer (31).

In this study, we sequenced the genotype of 132 patients categorized as invasive, *in situ* breast cancer; atypical hyperplasia or benign breast disease and analyzed the tissue expression of MMP-1 in these diseases to determine the impact of MMP-1 2G insertion polymorphism and MMP-1 expression on the occurrence and severity of breast cancer. We further explored the possible relationship of MMP-1 expression and MMP-1 polymorphism with other known clinical markers of breast cancer such as HER2 and P53.

Materials and Methods

Subject selection

All participants were fully consented patients recruited under the Institutional Review Board approved protocols of the Clinical Breast Care Project (CBCP). They were women, 18 years of age and above, who were attending the breast clinics of the CBCP at the Walter Reed Army Medical Center (WRAMC) in Washington DC, USA, and the Joyce Murtha Breast Care Center (JMBCC)/Windber Medical Center (WMC) in Windber, PA, USA. A total of 180 patients were recruited out of which we obtained sequencing data for 132 patients consisting of 52 invasive breast cancers [IBC], 50 ductal/lobular carcinoma *in situ* [DCIS/LCIS], 13 atypical ductal hyperplasia [ADH] and 17 benign breast disease. The benign patients were mainly those exhibiting fibrocystic changes (stromal fibrosis, cysts, apocrine metaplasia, sclerosing adenosis, intraductal hyperplasia). Other benign diagnoses included microcalcifications, adenomatous change, columnar cell change, and intraductal papillomas. The median ages for IBC, DCIS/LCIS, ADH and benign groups were 62 years (range: 36 - 86), 55 years (range: 25 - 84), 49 years (19 - 74) and 45 years (range: 26 - 71) respectively. Immunohistochemistry data for MMP-1 was determined for 165 patients. Characteristics of the patients with breast cancer are summarized in Table 1.

Genotyping

Blood clot was obtained after processing peripheral blood into serum. Blood DNA was extracted from blood clots using the QIAamp blood DNA commercial kit (Qiagen). The DNA was then utilized for polymerase chain reaction to amplify MMP-1 gene as earlier described (21). Briefly, PCR reaction was carried out in a total volume of 50 µl with 100ng genomic DNA, 0.2mM dNTP, 2mM MgSO₄, 50nM each of the two primers and 1 unit of platinum Taq DNA high fidelity polymerase (Invitrogen). The primer sequences for amplifying MMP-1 were: forward primer 5'TTGCCAGATGGGACAGTGTATGAG-3'; reverse primer 5'-ACATTAAATTGTCTTGGGTACTGGT-3'. The PCR reaction started with 30 seconds incubation at 94°C followed by 10 cycles of 30 seconds incubation at 94°C, 30 seconds incubation at 52°C, and 2 minutes incubation at 68°C; 30 cycles of 30 seconds incubation at 94°C, 30 seconds incubation at 55°C, and 1 minute 30 seconds at 68°C with 4 minutes incubation at 68°C. The amplification was verified on 1.2% agarose gel.

PCR products were cleaned up with Exonuclease I & Shrimp Alkaline Phosphatase (Affymetrix) to remove primers. Cycle sequencing reactions were performed with a nested primer (5'-AGTGTCTTTGGTCTCTGC-3') and purified by ethanol precipitation (including 7.5M ammonium acetate). DNA pellets were then dried, resuspended in MegaBACE Loading Solution and sequenced using the MegaBACE 1000 (GE Healthcare). Data was analyzed with Sequence Analysis v4.0 software (GE Healthcare).

Immunohistochemistry (IHC)

Formalin-fixed paraffin embedded sections of the selected cases were retrieved from the tissue repository to determine expression level of MMP-1 by IHC. The pathological diagnosis of each case was confirmed by the CBCP pathologist using a freshly prepared Hematoxylin and Eosin slide. For all confirmed cases, seven slides of 4 microns sections were cut and subjected to IHC analysis for MMP-1. Antibody optimization was performed to establish the required optimal conditions. For the determination of MMP-1 expression, we utilized a polyclonal rabbit antibody at 1:80 dilutions (LabVision, Catalog # RB-1536-P). The antibody was derived against a synthetic peptide from the middle region of the human MMP-1 and can recognize both the 52 kDa (unglycosylated) /57 kDa (glycosylated) species of the pro-form and 42 kDa (unglycosylated)/47 kDa (glycosylated) active form of MMP-1(32, 33). Detailed information on antibody specificity are as earlier described (32, 33). All IHC was performed at MDR Global Inc., Windber, PA, using standardized procedures and protocols. Briefly, each section was placed on positively charged slides which were then dried at 59°C, before placing in SubX solution prior to deparaffinization and antigen recovery using Dako PT Module. The non-enzymatic antigen recovery step was performed in a patented microwave oven and the slides were washed thoroughly in distilled water before placing in TBS buffer. Immunostaining was performed on the Dako Autostainer with the application of primary antibody followed by washing the slides again with TBS buffer. The appropriate detection reagent was then dispensed onto the slides followed by another wash in TBS buffer and Diaminobenzidine "DAB"/hydrogen peroxide for color visualization. All the slides were counterstained with hematoxylin. Pre-analytical processes such as antigen retrieval and dilution determination were performed on a known positive control, in this case, placenta (syncytiotrophoblast layer). Placenta provided internal negative control as well (blood vessels and lymphatics) and these elements were evaluated in each run. The immunohistochemistry result for MMP-1 was assessed according to MMP-1 staining intensity in tumor cells as follows: 0, no staining; 1+, mild staining; 2+, moderate staining and 3+, intense staining (Figure1). Available IHC data for ER, PR, HER2, and P53 was queried from the clinical database of the CBCP. Evaluation of the expression of ER, PR, P53 was based on the percentage of nuclear staining of the tumor cells. For ER/PR: 0-5% negative, 5% positive; for P53: 0-50% negative, 50% positive. HER2 expression was assessed according to ASCO/CAP guideline and was based on the intensity and percentage of the membrane staining of tumor cells; 0/1+ negative, 2+ reflex to FISH, and 3+ positive (34). Detailed information on the antibodies used to detect the protein markers is provided in Supplementary Tables 1.

Statistical Analysis

Statistical analyses were carried out using SAS 9.1 for Windows (SAS institute, Cary, NC). The association between MMP-1 expression/polymorphism and disease status was determined using Chi-square or Fisher's exact test when the numbers of the cases were small. Jonckheere-Terpstra test for trend was used to assess the correlation between MMP-1 genotype and expression level. Jonckheere-Terpstra test is a nonparametric statistical test, which examines the ordered differences of MMP-1 expression level among polymorphism classes. Odds Ratios (ORs) and 95% Confidence Intervals (CIs) were calculated by logistic regression. In all cases, $P < 0.05$ was required for significance.

Results

MMP-1 Expression in subjects

MMP-1 was primarily detected in the cytoplasm of normal and neoplastic cells (Figure 1). In the ADH group, 91% (10 out of 11) of the patients expressed MMP-1 at high levels (2+/3+), while in the benign group, only 48% (12 out of 25) had high levels of expression of MMP-1. The expression of MMP-1 was significantly associated with the disease status ($P = 0.016$; Table 2). Compared to subjects expressing low levels (0/1+) of MMP-1, the odds for subjects expressing high levels (2+/3+) of MMP-1 to develop ADH increased 10.83 times (OR = 10.83; 95% CI, 1.2-97.8; Table 2). When the expression of MMP-1 in IBC was compared with *in situ* breast cancer (DCIS/LCIS), we found that 58% of the IBC patients had high MMP-1 expression, which was significantly higher than that of the DCIS/LCIS group ($\chi^2 = 4.79$, Df = 1, $P = 0.03$; Table 2). Patients expressing high levels of MMP-1 had a 2.20 fold increased risk of developing invasive breast cancer (OR = 2.20; 95% CI, 1.09-4.44; Table 2).

The distribution of MMP-1 1G/2G genotype in subjects

The distribution of 1G/2G genotypes in the 132 selected patients was according to the Hardy-Weinberg equilibrium [20]. There was no significant difference in the distribution of the MMP-1 1G/2G genotypes between benign and ADH groups or between IBC and DCIS/LCIS groups ($P > 0.05$; Table 3). Allele frequencies between these groups were also not statistically significant ($P > 0.05$; Table 3).

Correlation of MMP-1 expression and MMP-1 genotypes

We observed a significant association between the expression of MMP-1 and MMP-1 genotypes in the IBC group (Table 4; $P = 0.032$). In this group, 57% of the patients expressing low MMP-1 had the 1G/1G homozygote genotype while 80% of those expressing high MMP-1 (3+) were either 1G/2G carriers (40%) or homozygous for 2G (40%). This data is displayed graphically in Figure 2(a). The 2G allele frequency for IBC patients expressing low MMP-1 was 0.29 (for 1+) and 0.44 (for 2+), while IBC patients expressing high MMP-1 (3+) had a 2G allele frequency of 0.6 (Figure 2(b)). Thus in the IBC patients, the expression of MMP-1 was positively correlated with the 2G allele. This association between the MMP-1 2G insertion polymorphism and MMP-1 expression was not observed in the DCIS/LCIS group ($P = 0.23$; Table 4).

Correlation of MMP-1 expression with clinicopathological parameters

Correlation of MMP-1 expression with clinicopathological parameters in patients with breast cancer is summarized in Table 5. We did not observe significant correlation of MMP-1 expression with age, ethnicity, menopausal status, tumor stage, tumor size, lymph node metastasis or ER, PR, and P53 status. However, we observed significant correlation of MMP-1 expression with HER2 status ($P = 0.004$). In the HER2- group, 71% (27 out of 38) patients had high MMP-1 expression, while in HER2+ group, only 35% (9 out of 26) of the patients expressed high levels of MMP-1 (Table 5). We also found marginal correlation of MMP-1 expression with tumor histological grade ($P = 0.0496$) (Table 5).

Correlation of MMP-1 genotype with clinicopathological parameters

We analyzed the data to determine if the MMP-1 2G insertion polymorphism had any association with other clinicopathological parameters in patients with breast cancer. No significant correlation between genotype distribution of MMP-1 with age, ethnicity, menopausal status, lymph node metastasis, tumor stage, histograde, tumor size and ER/PR status was observed ($P > 0.05$; Table 6). However, we observed a significant difference in the

genotype distribution of the MMP-1 between the HER2+ and HER2- group in IBC patients (Table 6). Notably, the percentage of 1G/1G homozygote was similar in the two groups, but the percentage of 1G/2G heterozygotes was significantly decreased and the percentage of the 2G/2G homozygotes was significantly increased in HER2+ group compared to the HER2- group ($P = 0.0401$; Table 6). Patients carrying the 2G/2G genotype had a 7.5-fold (95%CI, 1.48-37.9; $P = 0.032$; Table 6) increased odds of expressing HER2 compared to the 1G/2G carriers. However, the odds for 1G/1G carriers expressing HER2 was only increased 2.78 fold, which was not statistically significant ($P = 0.29$).

We also observed significant association between MMP-1 genotype distribution and the expression of P53 in IBC patients ($P = 0.0399$; Table 6). Compared with 1G/2G carriers, the odds for the 2G/2G carriers to express P53 increased 5.88 fold (95%CI, 5.26-33.3; $P = 0.049$), while the odds for the 1G/1G carriers to express P53 increased 5.26 fold (95%CI, 1.18-25; $P = 0.038$).

Discussion

Expression of MMP-1 on breast diseases development

We determined the expression of MMP-1 in patients with benign breast disease, ADH (a precancerous condition which poses an increased risk of breast cancer development), DCIS/LCIS (*in situ*) and IBC. We observed significantly increased expression of MMP-1 in ADH patients compared to benign breast disease ($P = 0.016$) and a dramatic increase in the expression of MMP-1 in IBC compared to DCIS/LCIS ($P = 0.014$). Interestingly, we also found that the expression of MMP-1 in the *in situ* foci of IBC tumors are significantly higher than that of pure DCIS specimens ($P = 0.035$, data not shown), but not significantly different from that of the invasive foci of IBC specimens ($P = 0.595$, data not shown). These observations indicate an association of MMP-1 expression with breast disease status/severity and are consistent with earlier studies that support (i) the role of MMP-1 as a candidate precancerous marker for early detection of breast cancer (18) and (ii) the notion that over-expression of MMP-1 is important for the invasion and progression of breast cancer (19-21).

MMP-1 has been reported as a useful marker for the identification of ADH patients without a history of cancer who would likely develop breast cancer in the future (17). Our limited data on follow up history of these ADH patients prevents us from further discussion on the role of MMP-1 in identifying the ADH patients with potential to develop breast cancer.

The role of MMP-1 2G polymorphism on MMP-1 expression and breast cancer severity

The reported MMP-1 genotype frequency in healthy Caucasian women are 0.28, 0.54 and 0.18 for 1G/1G, 1G/2G and 2G/2G respectively (35), and this is not significantly different from the genotype frequency we observed in IBC, DCIS/LCIS, ADH or benign breast disease group ($P > 0.05$ respectively). The distribution of the MMP-1 genotype in our studied groups was also not significantly different ($P > 0.05$), suggesting that MMP-1 polymorphism may not contribute to the development of breast cancer. This is consistent with the result from a recent meta-analysis study which failed to show any significant association between MMP-1 2G polymorphism and breast cancer susceptibility (36).

However, we observed an association between the 2G genotype and MMP-1 expression in the IBC patient group, ($P = 0.032$; Table 4) but not in the DCIS/LCIS group ($P > 0.05$; Table 4). This correlation was also not observed in ADH or benign group. These observations indicates that MMP-1 2G polymorphism may contribute significantly to the increased expression of MMP-1 in invasive breast cancer but not in the *in situ* breast cancer or the less severe breast disease conditions, and may thus correlated with breast disease status or breast cancer severity. However, our limited sample size prevents us from making any strong

conclusions. A larger sample size study is required to establish the full potential of MMP-1 polymorphism in breast cancer progression.

It is worth noting that while the 2G polymorphism may contribute to increased expression of MMP-1 in IBC, other factors may contribute more significantly to increased expression of MMP-1 in ADH group. It has been reported that MMP-1 expression can be increased by many stimuli including growth factors, inflammatory interleukins, heat and UV-light, which can stimulate mitogen-activated protein kinases (MAPK) and activate MMP-1 transcription (37-40).

Correlation of MMP-1 2G polymorphism with other clinical markers of breast cancer

Higher 2G allele frequencies have been reported in breast cancer patients with lymph node-metastasis compared with patients without metastasis, indicating the role of MMP-1 2G polymorphism as a potential prognostic marker for breast cancer (22, 23, 41). In our study, although we observed increased 2G/2G genotype frequency (35%) in the lymph node positive group compared with lymph node negative group (19%), the difference did not reach statistical significance ($P = 0.098$). The contradiction may be explained by the smaller sample size in our study or other factors such as ethnic differences of subjects in the different studies.

However, we did find a correlation between MMP-1 genotype with other breast cancer markers, such as HER2 and P53. There was a significant association between the MMP-1 2G polymorphism and HER2 expression in IBC patients ($P = 0.0401$; Table 6). Forty four percent (10 out of 23) of patients expressing HER2, had the homozygous 2G/2G genotype compared to 15% of the patients (4 out of 26) not expressing HER2 (Table 6). The distribution curve of the genotype shifted to a higher frequency of 2G homozygotes in HER2+ group. In breast cancer, the amplification of the Her2/neu oncogene is a well established marker which is associated with relapse and short survival (42, 43). HER2 over-expression has also been correlated with poor outcome (44-46). The association of the MMP-1 2G insertion polymorphism with this clinical biomarker again indicates the potential role of this polymorphism in breast cancer severity.

Studies have shown that both MMP-1 2G polymorphism and HER2 over-expression can up-regulate MMP-1 expression via different molecular mechanisms (20, 21, 47). The 2G single nucleotide polymorphism of MMP-1 affects the basal transcription of MMP-1 through regulating ETS-1 and AP-1(20), while HER2 can further increase MMP-1 expression through activating ER81, another member of ETS family (47). In the current study we did not observe a positive correlation between HER2 and MMP-1 expression in the patients with breast cancer. On the contrary, the MMP-1 expression was higher in HER2 negative group compared with HER2 positive group (Table 5). This could be due to unknown mechanisms, for example, a negative feedback effect from increased MMP-1 expression on HER2 transcription or a result of the overall effect of different regulators on MMP-1 expression. We did, however, observe a correlation between the 2G insertion polymorphism and MMP-1 expression in the IBC patients only ($P = 0.032$) and not in the *in situ* group, which supports the notion that MMP-1 2G polymorphism is associated with disease severity in breast cancer.

Over expression of P53 is also associated with breast cancer progression. Both P53 mutation and accumulation have been associated with poor prognosis (48-51). Thor et al found significant correlation of P53 accumulation with short metastasis-free and overall survival in both sporadic and familial breast cancers (51). Wild type P53 can down regulate MMP-1 expression by disrupting the communication between AP-1 and basal transcriptional complex in human foreskin fibroblast cells (52, 53). However, it has been reported that the

repression effect of P53 on MMP-1 expression is lost in osteogenic sarcoma cells over expressing mutant P53 (52). More detailed study of the effect of accumulated or mutation of P53 on MMP-1 expression in breast cancer will be required to gain a better understanding of the interrelationship between P53 and MMP-1. So far, we did not observe an association between MMP-1 and P53 expression in our study (Table 4), but we observed a significant difference in the distribution of MMP-1 genotypes between P53+ and P53- patients ($P = 0.0399$, Table 6). There was a significant decrease in the number of heterozygotes (1G/2G) and increased number of homozygotes (1G/1G and 2G/2G) in the P53+ patients compared to the P53- patients ($P = 0.010$). The overall effect of this difference on MMP-1 expression in P53+/- patients may need further investigation. Although the observed pattern of MMP-1 genotype distribution in P53 expressing tumors varied from that observed in the HER2 expressing tumors, these observations both point to some level/degree of connectivity between the MMP-1 polymorphism and breast markers associated with disease severity.

In this study, we provide evidence that over-expression of MMP-1 is associated with both increased risk and local invasion in breast cancer. While the MMP-1 2G insertion polymorphism may not contribute to either event, other regulators must be involved in up-regulating MMP-1 levels in the different steps leading to breast cancer onset and invasion. However, the MMP-1 2G polymorphism is associated with breast cancer severity which is supported by its correlation with MMP-1 expression and the expression status of HER2 and P53 in IBC patients. Over-expression of MMP-1 induced by this polymorphism together with other regulators may predict an adverse outcome of breast cancer. More comprehensive studies are required to fully understand the mechanism of the interaction of this polymorphism with other molecular markers, and to explore the impact of this polymorphism alone or with other clinical markers on disease free or overall survival in breast cancer. Such studies will elucidate the impact of MMP-1 2G polymorphism on breast cancer development and progression.

Conclusion

To study the impact of MMP-1 1G/2G polymorphism on breast cancer development, we genotyped 132 patients with benign breast disease, ADH, DCIS/LCIS and IBC and analyzed our results with expression data of the following breast cancer markers, MMP-1, ER, PR, HER2 and P53. We found that over-expression of MMP-1 is associated with breast cancer onset and invasion. We report here, for the first time, that MMP-1 2G insertion polymorphism is significantly associated with HER2 and P53 expression. HER2 over-expression, P53 accumulation, or the coexistence of these two, are known molecular markers in breast cancer progression (42, 44, 46, 51, 54). The association of MMP-1 2G insertion polymorphism with these two biomarkers in invasive breast cancer indicates a role of MMP-1 2G polymorphism in predicting breast cancer severity and may provide new molecular tools for sub classification of invasive breast cancer.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

Immunostaining of MMP-1 expression in benign and malignant breast tumor. MMP-1 expression in breast tissues was visualized by immunohistochemical staining with a specific rabbit anti human polyclonal antibody. MMP-1 was predominantly detected in the cytoplasm of the normal and neoplastic cells. Examples of MMP-1 staining in (A-B) usual type hyperplasia; (C-D) atypical ductal hyperplasia; (E-H) ductal carcinoma *in situ* (DCIS); and (I-L) invasive ductal carcinoma (IDC) Magnification: X200 for all pictures.

Figure 2.

(a). MMP-1 2G insertion polymorphism is correlated with MMP-1 expression in IBC. Subjects with IBC were grouped according to the expression level of MMP-1(1+, 2+ and 3+). The percentage of homozygotes (1G/1G and 2G/2G) and heterozygotes (1G/2G) were then plotted against MMP-1 expression level based on the Immunohistochemical score of 1+, 2+ and 3+. As MMP-1 expression increased, the percentage of 1G/1G homozygotes decreased, while the percentage of 2G carriers (1G/2G heterozygotes) and homozygotes (2G/2G) increased. Results show a relationship between 2G insertion and MMP-1 expression in IBC patients.

(b). The MMP-1 2G allele frequency correlates with MMP-1 expression level. Allele frequency of subjects in the IBC group were plotted against the expression level of MMP-1(1+, 2+ and 3+) as obtained from immunohistochemistry readings. The 2G allele frequency increased with increasing expression of MMP-1.

Table 1

Clinicopathological characteristics of patients with breast cancer

Parameters		n=136 (%)
<i>Age(years)</i>	<59	58(43)
	59	61(45)
<i>Ethnicity</i>	AA	29(21)
	White	77(57)
	Other	13(10)
<i>Menopausal status</i>	Premenopausal	36(26)
	Postmenopausal	82(60)
<i>Tumor stage</i>	0	64(47)
	I	22(16)
	II	26(19)
	III	9(7)
	IV	8(6)
<i>Histological grade</i>	G1	14(10)
	G2	34(25)
	G3	29(21)
<i>Tumor size</i>	T1	29(21)
	T2	32(24)
	T3	6(4)
<i>Nodal status</i>	Negative	97(71)
	Positive	32(24)
<i>ER</i>	Negative	24(18)
	Positive	88(65)
<i>PR</i>	Negative	48(35)
	Positive	64(47)
<i>HER2</i>	Negative	40(29)
	Positive	30(22)
<i>P53</i>	Negative	29(21)
	Positive	28(21)

Table 2

The expression of MMP-1 in subjects with breast diseases.

	MMP1 expression		OR	95%CI	P
	Low (0/1+) n (%)	High (2+/3+) n (%)			
Benign (n=25)	13 (52)	12 (48)	1		
ADH (n=11)	1 (9)	10 (91)	10.83	1.20-97.80	0.016*
In situ (n=64)	39 (61)	25 (39)	1		
Invasive (n=65)	27 (42)	38 (58)	2.2	1.09-4.44	0.014**

*The expression of MMP1 in ADH is significantly higher than in benign breast disease ($p < 0.05$, Fisher's exact test).

**The expression of MMP1 in invasive breast cancer is significantly higher than in *in situ* breast cancer ($p < 0.05$, χ^2 test)

ADH-atypical ductal hyperplasia

Table 3
MMP-1 genotype distribution and allele frequencies in patients with breast diseases

Genotype	Benign (n=17)		ADH (n=13)		<i>In situ</i> (n=50)		Invasive (n=52)	
	n	f	n	f	n	f	n	f
1G/1G	3	0.18	5	0.39	10	0.20	19	0.36
1G/2G	10	0.59	6	0.46	29	0.58	18	0.35
2G/2G	4	0.24	2	0.15	11	0.22	15	0.29
<i>P</i>	0.068 *				0.051 **			
Allele								
1G	16	0.47	16	0.62	49	0.49	56	0.54
2G	18	0.53	10	0.38	51	0.51	48	0.46
<i>P</i>	0.197 *				0.489 **			

* The difference in genotype distribution or allele frequencies between benign breast disease and ADH groups is not statistically significant ($p>0.05$, Fisher's exact test).
** The difference in genotype distribution or allele frequencies between *in situ* and invasive breast cancer groups is not statistically significant ($p>0.05$, χ^2 test).
ADH-atypical ductal hyperplasia

Table 4
Correlation between MMP-1 expression and genotype in patients breast cancer

Genotype	MMP-1 Expression					
	Invasive (n=41)			In Situ (n=33)		
	1+(n=14) n (%)	2+(n=17) n (%)	3+(n=10) n (%)	1+(n=12) n (%)	2+(n=12) n (%)	3+(n=9) n (%)
1G/1G	8 (57)	6 (35)	2 (20)	2 (17)	3 (25)	0(0)
1G/2G	4 (29)	7 (41)	4 (40)	7 (58)	5 (42)	6 (67)
2G/2G	2 (14)	4 (24)	4 (40)	3 (25)	4 (33)	3 (33)
P	0.032			0.230		

Jonckheere-Terpstra test

Table 5

Correlation of MMP-1 expression with clinicopathological parameters in patients with breast cancer.

Parameters	MMP-1 Expression		Total n	P
	Low(0/1+) n (%)	High(2+/3+) n (%)		
Age (years)				
59	36(65)	19(35)	55	0.0545
>59	26(47)	29(53)	55	
Ethnicity				
African American	14(58)	10(42)	24	0.7142
White	40(54)	34(46)	74	
Menopausal				
Pre-menopausal	19(59)	13(41)	32	0.6437
Post-menopausal	42(55)	35(45)	77	
Stage				
0	40(65)	22(35)	61	0.057
I	8(38)	13(62)	21	
II	9(39)	14(61)	23	
III/IV	9(64)	5(36)	14	
Histological Grade				
G1	7(54)	6(46)	13	0.0496
G2	8(25)	24(75)	32	
G3	13(54)	11(46)	24	
Tumor Size				
2 cm	10(37)	17(63)	27	0.3177
>2cm	16(50)	16(50)	32	
Lymph Node status				
Negative	49(54)	41(46)	90	0.9355
Positive	15(54)	13(46)	28	
ER Status				
Negative	8(40)	12(60)	20	0.1959
Positive	46(56)	36(44)	82	
PR status				
Negative	23(52)	21(48)	44	0.8328
Positive	31(54)	26(46)	57	
HER2/neu Protein				
Negative	11(29)	27(71)	38	0.0039
Positive	17(65)	9(35)	26	
P53 Status				
Negative	9(39)	14(61)	23	0.7435
Positive	9(35)	17(65)	26	

Table 6
Correlation of MMP-1 genotype and clinicopathological characteristics in patients with breast cancer

Parameters	Genotype			Total n	P
	1G/1G n (%)	1G/2G n (%)	2G/2G n (%)		
Age (years)					
≤55	13(33)	18(45)	10(25)	40	0.8624
>55	15(36)	16(38)	11(26)	42	
Ethnicity					
African American	7(37)	9(47)	3(16)	19	0.6340
White	20(33)	24(39)	17(28)	61	
Menopausal					
Pre-menopausal	9(32)	13(46)	6(21)	28	0.7592
Post-menopausal	18(33)	21(39)	15(28)	54	
Stage					
0	10(23)	25(57)	9(20)	44	0.2323
I	7(39)	6(33)	5(28)	18	
II	6(38)	7(44)	3(19)	16	
III/IV	4(36)	2(18)	5(45)	11	
Histological Grade					
G1	6(60)	3(30)	1(10)	10	0.5673
G2	6(29)	8(38)	7(33)	21	
G3	8(38)	7(33)	6(29)	21	
Tumor Size					
<2 cm	8(36)	8(36)	6(27)	22	0.9759
>2cm	9(38)	8(33)	7(29)	24	
Lymph Node status					
Negative	20(29)	35(51)	13(19)	68	0.0982
Positive	8(40)	5(25)	7(35)	20	
ER Status					
Negative	7(47)	3(20)	5(33)	15	0.1050
Positive	19(31)	30(49)	12(20)	61	

Parameters	Genotype			Total n	P
	1G/1G n (%)	1G/2G n (%)	2G/2G n (%)		
PR status					
Negative	12(38)	11(34)	9(28)	32	0.1217
Positive	14(32)	22(50)	8(18)	44	
HER2/neu Protein					
Negative	10(38)	12(46)	4(15)	26	0.0401
Positive	9(39)	4(17)	10(43)	23	
P53 Status					
Negative	5(24)	13(62)	3(14)	21	0.0399
Positive	10(45)	5(23)	7(32)	22	