ORIGINAL ARTICLE

Importance of MACC1 expression in breast cancer and its relationship with pathological prognostic markers

Nevroz A. Söyleyici, Figen Aslan[1], Ayla S. Avcıkurt[2], Gizem A. Akgün[3]

Department of Pathology, Institute of Health Science, [1]Departments of Pathology and [2]Medical Biology, School of Medicine, Balıkesir University, Cagıs Yerleskesi, Bigadic Yolu 17. Km Altıeylul, Balıkesir, [3]Department of Pathology, School of Medicine, Dumlupınar University, İstiklal, Okmeydanı Cd., Merkez/Kütahya, Turkey

Address for correspondence:
Dr. Figen Aslan, Department of Pathology, Balıkesir University School of Medicine, Cagıs Yerleskesi, Bigadic Yolu 17. Km Altıeylul, Balıkesir - 10145, Turkey. E-mail: fgenaslan@gmail.com

ABSTRACT

Background: Metastasis associated colon cancer gene 1 (MACC1) is a gene that was first described as a c-Met transcription regulator causing the progression of colon cancer. In this study, protein and messenger RNA (mRNA) expression of MACC1 in breast cancer and its relationship with clinicopathological prognostic parameters were investigated. **Methods:** Sixty-six cases with tumors underwent radical mastectomy for invasive ductal carcinoma and 25 control cases operated for mammoplasty were included in the study. In paraffin blocks of tumor and control tissues, MACC1 expression was investigated by the immunohistochemical method and Real-time polymerase chain reaction (Real-Time PCR). In addition, vascular endothelial growth factor (VEGF) expression was examined immunohistochemically in tumor tissues. The relationship between MACC1 expression in tumor tissues, clinicopathological prognostic parameters, and VEGF was investigated. **Results:** In this study, protein and mRNA expressions of MACC1 were found to be higher in tumor tissues compared with normal breast tissues. MACC1 protein expression was also associated with significant poor prognostic markers, such as high histologic grade, ER negativity, and HER2 positivity. However, there was no correlation between MACC1 expression and VEGF. **Conclusion:** According to these results, MACC1 expression may be a marker of breast carcinoma as well as an independent predictor of poor prognosis. In addition, MACC1 may not affect angiogenesis in breast cancer or even if it has an effect, it may not be associated with VEGF. However, it would be appropriate to support these results in a larger series by investigating *in vivo* and *in vitro* studies.

KEY WORDS: Breast cancer, expression, Metastasis Associated Colon Cancer Gene 1, prognostic marker

Access this article online

Website: www.ijpmonline.org

DOI: 10.4103/IJPM.IJPM_658_19

Quick Response Code:

INTRODUCTION

Metastasis Associated Colon Cancer Gene 1 (MACC1) was first described as a c-Met transcription regulator causing the progression of colon cancer and the main regulator of tumor progression and metastasis mediated by hepatocyte growth factor (HGF)/c-mesenchymal epithelial transition (MET) factor gene signaling in colon cancer.[1,2] The HGF/c - Met pathway regulates various biological activities, including proliferation, motility, and invasion in cancer.[3]

MACC1 shows higher expression in primary and metastatic colon cancer than normal colon mucosa and is associated with poor clinical course in colon cancer.[1] High expression of MACC1 after colon cancer has been associated with poor clinical course in many cancers.[3-12] However, the potential relationship between MACC1 expression and prognosis in breast cancer has not yet been fully explored.

In this study, MACC1 expression in invasive ductal carcinoma cases and normal breast tissues were examined by immunohistochemical and molecular methods, and the relationship between tumor expression and clinicopathological prognostic parameters was investigated. In addition, the relationship between MACC1 and vascular endothelial growth factor (VEGF) was evaluated to investigate the effect of MACC1 expression on angiogenesis.

How to cite this article: Söyleyici NA, Aslan F, Avcıkurt AS, Akgün GA. Importance of MACC1 expression in breast cancer and its relationship with pathological prognostic markers. Indian J Pathol Microbiol 2020;63:19-24.

MATERIAL AND METHODS

Patients and tissue samples

As a result of the retrospective analysis of the archives of our center between 2011 and 2018, 66 patients with invasive ductal carcinoma who underwent radical mastectomy and axillary dissection were included in this study. The patients included in the study had not previously received neoadjuvant therapy was taken into consideration. Twenty-five cases were selected for our control group among patients who had no breast disease problems and had only operated for mammoplasty. Hematoxylin and eosin (H and E) stained slides and pathology reports were retrospectively reviewed. Clinicopathological prognostic parameters, including age, tumor diameter, lymph node involvement, the histologic grade of the tumor, ER, PR, HER2 expression status, and Ki67 proliferative index, were recorded for each tumor. The modified Bloom–Richardson grading system was used for the histological grade of the tumor. To control, paraffin blocks, which best represented normal and tumor tissue, were obtained from the pathology archive.

Immunohistochemistry

MACC1 and VEGF protein expressions in 66 tumors and 25 control tissues were studied by immunohistochemical method. First, approximately three 5-4μ thick sections were taken from the tumor paraffin blocks on a positively charged slide. Sections were kept in a 65°C incubator for 30-45 min and made ready for deparaffinization. Sections whose paraffin was partially removed were studied on Ventana BenchMark XT immunohistochemical staining device.

The immunohistochemical staining protocol was performed through the following steps:

- Sections taken on positively charged slides were placed in the device (Ventana BenchMark XT) and the paraffin melting was performed
- Antigen release was performed with Cell Conditioner 1 (CC1) solution on slides
- Masking of endogenous peroxidase was performed by applying hydrogen peroxide to the slides and incubating for 7 min
- Primary antibodies VEGF (Thermo Scientific RB-9031-R7-1: 100) and MACC1 (Millipore Polyclonal Human-Mouse Anti-MACC1-1: 400) were manually instilled and incubated for 30 min
- Ultra View Universal DAB Detection kit secondary antibody was administered for 10 min
- Background staining was performed with hematoxylin for 8 min and then conveyed with Bluing Reagent solution
- After this stage, slides were removed from the device, rinsing, dehydration with alcohol, and clearing with xylene were performed. Entellan was dropped to the preparations and covered with lamella.

Interpretation of immunohistochemistry

Two pathologists evaluated the MACC1 and VEGF protein expressions, which were studied immunohistochemically, at 400x (40x objective lens, 10x ocular lens) with an ergonomic side-by-side light microscope (Nikon 300038) without any clinical and pathological information about the cases.

The degree of cytoplasmic staining in the epithelium of tumor cells was considered for both antibodies. Stromal areas were not evaluated. As both the antibodies showed expression in all of the tumor tissues but at different concentrations, the intensity of staining was considered while evaluating the expression. MACC1[13] and VEGF[14] expressions were scored according to the staining intensity in the tissues as follows:

- If there is no staining- 0
- If there is poor staining (light yellow)- 1
- f there is moderate staining (yellow-brown)- 2
- If there is strong staining (brown- 3.

Statistical analysis was conducted by grouping the staining score as 0 and 1 as negative and 2 and 3 as positive.

Real-time PCR method

RNA isolation was performed using RNeasy FFPE (QIAGEN) kit from the paraffin blocks of 66 patients and 25 control patients according to user protocol. Complementary DNA isolation from RNA was performed in two steps using the kit (Gene All, Hyperscript first-strand synthesis kit, Cat no.: 601-005, Lot no.: FS015B04002). The Real-time PCR step was performed with the Applied Biosystem 7500 Fast Real-Time PCR instrument (GeneAllSybr Green Master Mix, Cat no.: 801-520, Lot no.: QP116G25001). Samples were studied in three replicates. Actin beta (ACTB) was used as control. Gene expression levels were measured using 7500 Fast Real-Time Sequence detection system Software (Applied Biosystems, Foster City, CA, USA). Gene expression was normalized to cycle threshold (CT) values by the 2– ΔΔCT method and ACTB, the internal control gene.[11,15]

The primers used in the study were as follows:

- ACTB forward: 5' CCTGACTGACTACCTCATGAAGATCCTC 3'
- Reverse: 5' CGTAGCACAGCTTCTCCTTAATGTCAC 3' (103 bp)
- MACC1 forward: 5' GATGAACTTGATGTGCATCA GTTACTTAG 3'
- Reverse: 5' GTCGTGTAGTAGGATCTGGTCAGAGTTATG 3' (150 bp).

Ethics

In this study, the investigation protocol was in accordance with the Helsinki committee requirement and was approved by the Institutional Ethical Committee of The Balıkesir University School of Medicine (Decision no: 2017/41).

Statistics

Statistical analysis was performed using IBM Statistical Package for the Social Sciences (SPSS) software, v. 22.0 for Windows. In addition to descriptive statistics (mean, standard deviation [SD]), the Fischer Exact test was used for the immunohistochemical data

and Pearson Chi-square test was used for the qualitative data. In the molecular data, student's *t*-test was used for comparison of independent groups. The results were evaluated at $P < 0.05$ significance levels.

RESULTS

In our study, there were 91 patients, including 66 tumor cases and 25 control cases. All the patients were females who had been operated for invasive ductal carcinoma. The mean age of the tumor patients was 57.4 ± 3 years while the mean age of the control patients was 39.7 ± 4 years. MACC1 protein expression and mRNA expression were evaluated in tumor and control cases.

Immunohistochemical findings

MACC1 expression was positive in 45 (68.1%) whereas it was negative in 21 (31.8%) of the tumor cases [Figure 1].

MACC1 expression score and number-% distribution in tumor cases are shown in Table 1.

MACC1 expression was significantly higher in tumor cases (68.1%) than normal breast cases (40%) ($P = 0.014$). The MACC1 expression status of control and tumor tissues are presented in Table 2 and Figure 2.

VEGF protein expression was positive in 53 tumor cases (80.3%) while it was negative in 13 cases (19.6%) [Figure 3]. There was no significant relationship between MACC1 expression and VEGF expression in tumor cases ($P = 0.31$) [Table 3].

Table 1: MACC1 expression score and number and percentage immunohistochemical distribution in tumor cases

MACC1 Score	*Number*	*%*
Score 0	4	6
Score 1	17	25.7
Score 2	36	54.5
Score 3	9	13.6

MACC1: Metastasis associated colon cancer gene 1

Table 2: MACC1 expression status of control and tumor cases

MACC1 Expression	*Control*		*Tumor*	
	Number	*%*	*Number*	*%*
(−) (Score 0-1)	15	60	21	31.8
(+) (Score 2-3)	10	40	45	68.1

MACC1: Metastasis associated colon cancer gene 1

Table 3: The relation between MACC1 expression and VEGF expression in tumor cases

VEGF Expression	*MACC1 (−)*		*MACC1 (+)*		*P*
	Number	*%*	*Number*	*%*	
VEGF (+) (*n*=53)	15	28.3	38	71.6	0.31**
VEGF (-) (*n*=13)	6	46.1	7	53.8	

**Fischer Exact, MACC1: Metastasis associated colon cancer gene 1, VEGF: Vascular endothelial growth factor

COMPARISON OF MACC1 PROTEIN EXPRESSION WITH CLINICOPATHOLOGICAL PARAMETERS

MACC1 protein expression was compared with clinicopathological parameters in tumor cases; age (≤50, >50), tumor diameter (≤2 cm, >2 cm), lymph node metastasis (+,–), ER (+,–), PR (+,–),

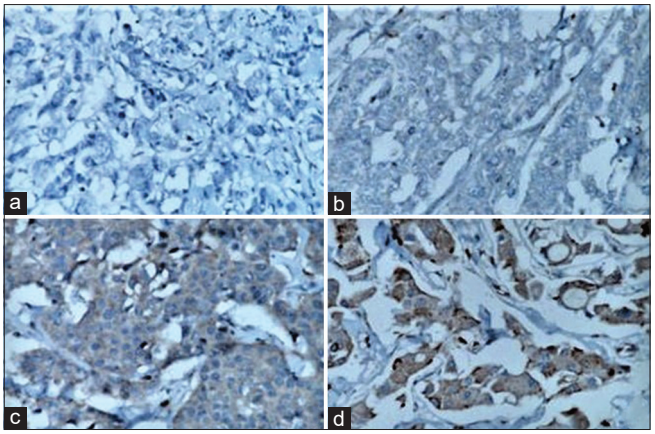

Figure 1: MACC1 protein expression in the tumor cases (MACC1, x400). (a) Score-0 (b) score-1 (c) score-2 (d) score-3

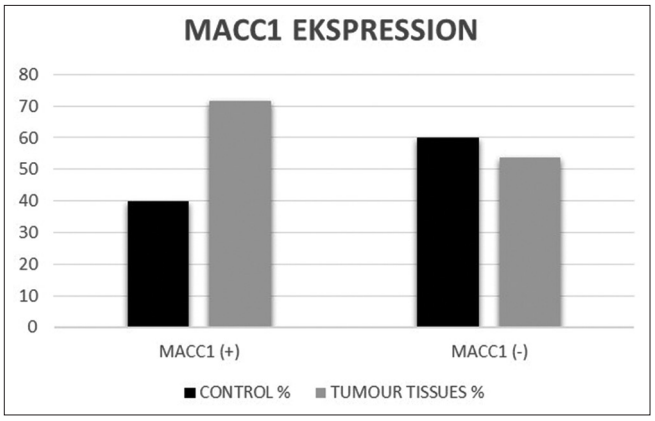

Figure 2: Relationship between MACC1 protein expression and tumor and control cases

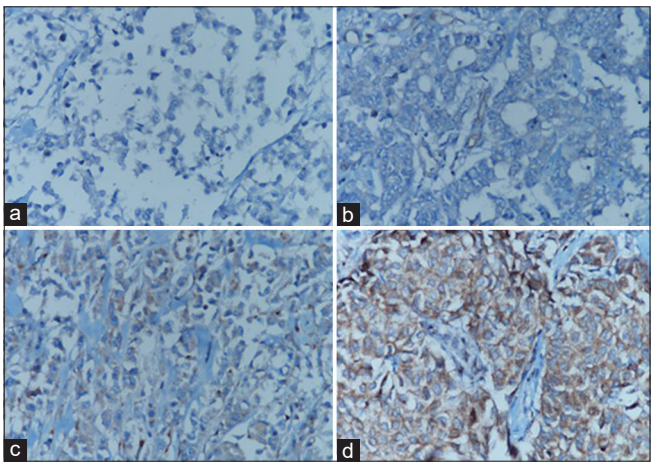

Figure 3: VEGF protein expression in the tumor cases (VEGF1, x400). Score-0 (a), score-1 (b), score-2 (c), and score-3 (d)

HER2 (+,–), Ki-67 (<15%, ≥15%), and histological grade of the tumor (Grade I–II, Grade III).

The relationship between MACC1 protein expression and clinicopathological parameters in tumor cases is shown in Table 4.

MACC1 protein expression was correlated with ER and HER2 status and grade, one of the prognostic parameters of the tumor. MACC1 expression was significantly higher in ER (–) cases compared with ER (+) cases ($P = 0.02$). Moreover, MACC1 protein expression was significantly higher in Grade III cases than in Grade I–II cases ($P = 0.02$). No correlation was found between MACC1 protein expression and other prognostic parameters such as age, tumor diameter, lymph node involvement, PR status, and Ki-67 proliferative index.

RT-PCR findings

mRNA expression of MACC1 measured by real-time PCR (qRT-PCR) was compared between tumor tissues and control tissues, between tumor groups with negative and positive MACC1 protein expression, and between tumor groups with VEGF (+) and VEGF (–) immunohistochemically [Figure 4].

The mRNA expression of MACC1 was significantly higher in tumor tissues than in control tissues ($P = 0.00$). MACC1 mRNA expression was higher in the tumor group with positive MACC1 protein expression ($P = 0.043$). In VEGF (+) and VEGF (–) cases, MACC1 mRNA expression was very close to each other and there was no statistically significant difference ($P = 0.779$).

No statistically significant difference was found between the subjects in terms of mRNA expression depending on the status of prognostic parameters (patient age (≤50,>50), tumor diameter (≤2,>2), lymph node involvement (+,–), ER (+,–), PR (+, -), HER2 (+, –), Ki-67 proliferative index (≥ 15, <15), and Grade (Grade I–II and Grade III)), respectively ($P = 0.888$, $P = 0.475$, $P = 0.340$, $P = 0.874$, $P = 0.550$, $P = 0.525$, $P = 0.792$, $P = 0.413$) [Figure 5].

Table 4: The relationship between MACC1 protein expression and clinicopathological parameters

Clinicopathological parameters	*MACC1 (–)*		*MACC1 (+)*		*P*
	Number	*%*	*Number*	*%*	
Age ≤50 (*n*=16)	5	31.25	11	68.7	0.60*
Ager >50 (*n*=50)	16	32	34	68	
Tumor Size ≤2 cm (*n*=29)	11	37.9	18	62	0.42*
Tumor Size >2 cm (*n*=37)	10	27	27	72.9	
Lymph Nodes (+) (*n*=40)	15	37.5	25	62.5	0.28*
Lymph Nodes (-) (*n*=26)	6	23	20	76.9	
ER (+) (*n*=52)	20	38.4	32	61.5	0.02**
ER (-) (*n*=14)	1	7.1	13	92.8	
PR (+) (*n*=43)	14	32.5	29	67.4	0.54*
PR (-) (*n*=23)	7	30.4	16	69.5	
HER2 (+) (*n*=42)	9	21.4	33	78.5	0.02*
HER2 (-) (*n*=24)	12	50	12	50	
Ki-67 <%15 (*n*=16)	6	37.5	10	62.5	0.75*
Ki-67 ≥%15 (*n*=50)	15	30	35	70	
Grade I-II (*n*=45)	18	40	27	60	0.02*
Grade III (*n*=21)	3	14.2	18	85.7	

*Pearson Chi-square **Fischer Exact MACC1: Metastasis associated colon cancer gene 1

DISCUSSION

RT PCR first identified MACC1 as a gene associated with poor prognosis. It acts as the main regulator of tumor growth and metastasis through the HGF/c-Met signal pathway in primary and metastatic tumors of the colon.[1,2] High-MACC1 expression is associated with poor prognosis or survival in many cancers, such as lung cancer,[4,5] gastric cancer,[12] hepatocellular carcinoma,[6] ovarian cancer,[7] cervical cancer,[8] endometrial cancer,[9] renal cell carcinoma,[10] and osteosarcoma.[11] In this study, parallel with the study of Huang *et al*.[13] on breast cancer, we found that MACC1 mRNA and protein expressions were correlated with each other and these two expressions were higher compared with normal breast tissue. In another study, investigating triple-negative breast cancer cases, a more aggressive form of breast cancer, similar results were obtained. In that study, immunohistochemical expression of MACC1 in tumor tissues was higher than in control tissues.[16] In a similar study conducted by measuring the serum MACC1 levels via enzyme-linked immunosorbent assay (ELISA) test, the serum MACC1 level was

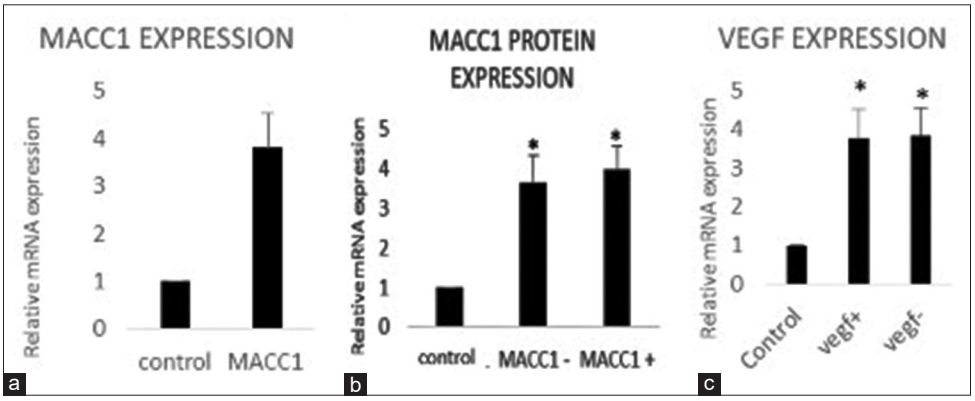

Figure 4: Expression levels of MACC1 mRNA in control and tumor cases (a) The relationship between MACC1 mRNA expression and MACC1 protein expression (b) The relationship between MACC1 mRNA expression and VEGF protein expression (c)

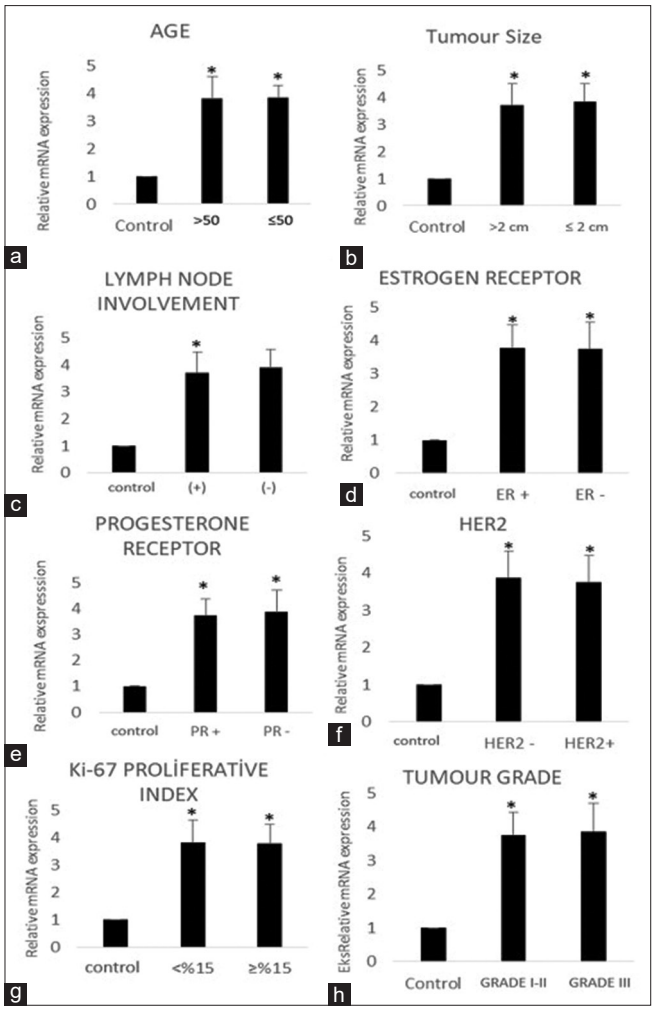

Figure 5: MACC1 mRNA expression was compared with clinicopathological parameters in tumor cases (a-h)

found to be higher in breast cancer patients than in benign breast disease or healthy subjects. In addition, this study indicated the association of high-MACC1 levels in serum with short disease-free survival and a significant prognostic value in breast cancer.[17] However, MACC1 in serum might be high in other cancers, such as lung and colorectal cancers, and might not be specific to tissue expression for breast cancer.

ER positivity shows better clinical outcomes than ER negative patients for the ER receptor, an important prognostic marker of breast cancer.[18] In our study, it was found that the MACC1 protein expression was significantly higher in ER (–) cases compared with ER (+) cases. Prguda-Mujic *et al.* showed that MACC1 protein expression was correlated with ER, as well as shorter disease-free survival, in patients who did not receive adjuvant chemotherapy and had ER (+).[19] Furthermore, MACC1 expression was found to be positively associated with TNM staging of the tumor but not associated with ER, PR and HER2 status in the first study investigating the relationship between MACC1 and prognosis in breast cancer.[13] Huang *et al.* reported the prognostic value of MACC1 expression in both ER (+) and ER (–) breast cancer subgroups. Thus, it may be possible to benefit from new biomarkers such as MACC1 in determining the need for chemotherapy after complete resection for patients with ER (–) tumor.[13]

HER2, a member of the receptor tyrosine kinase (RTK) family, is hyperactive in breast cancer and plays an important role in the onset and progression of the disease.[20] In this study, it was found that MACC1 protein expression was also associated with HER2 positivity and high tumor Grade, which are important poor prognostic markers as well as ER negativity. Muendlein *et al.* showed the association of MACC1 polymorphism with clinical outcomes in HER2 (+) breast cancer patients.[21] Lymph node involvement is one of the most important prognostic factors in disease-free and overall survival in breast cancer patients. According to the study conducted by Huang *et al.*, MACC1 protein expression is associated with lymph node positivity.[13] Moreover, according to Han *et al.*, there is a relationship between MACC1 expression and lymph node involvement in triple negative breast cancer.[16] In this study, we could not find this relationship. In another study conducted by Pruguda-Mujic *et al.* with 105 primary breast cancer patients, a negative correlation was found between high MACC1 expression and disease-free survival in patients with negative lymph node involvement.[19] Tumor Grade, another important prognostic parameter in our study, was positively correlated with MACC1 expression. However, Pruguda-Mujic *et al.* showed no relation between MACC1 and tumor Grade.[19] In the study of Han *et al.*, MACC1 expression in triple-negative breast cancer was associated with lymph node involvement and TNM stages, but not with patient age, tumor diameter, location, type, or grades.[16] Therefore, although the number of studies to determine the prognostic role of MACC1 expression on breast cancer is few, there are different and conflicting results between MACC1 expression and prognostic markers (age, tumor diameter, lymph node involvement, ER, PR, HER2, histologic Grade, and TNM).[13,17,19] These differences in results may be because the tumor groups in the studies contain molecular and histological diversity and the studies have not yet been performed in a large series. This suggests that MACC1 should be investigated as an independent prognostic marker, apart from other prognostic markers.

VEGFA, also known as VEGF, is one of the most important endothelial growth factors involved in angiogenesis. Signaling mediated by VEGFA leads to angiogenesis by providing endothelial cell proliferation and migration.[22] Previous studies have shown increased VEGF expression in tumor tissue and it was significantly correlated with microvessel density and poor prognosis in many cancers including breast cancer.[14,23] To investigate the effect of MACC1 on angiogenesis, the relationship between MACC1 and VEGF angiogenesis was investigated in various tumors. In studies conducted to date, MACC1 was found to be positively correlated with VEGF in gastric cancer[12] and cholangiocarcinoma,[22] and angiogenesis in cervical cancer.[8] However, as far as we can see, the effect of MACC1 on angiogenesis in breast cancer has not yet been investigated. Therefore, we aimed to investigate the effect

of MACC1 on angiogenesis as a poor prognostic factor in breast cancer. However, we could not see the relationship between mRNA and protein expression of MACC1 and VEGF expression in breast cancer tissues. According to these results, MACC1 may not affect angiogenesis in breast cancer, or if this effect is present, it may not be related to VEGF.

In conclusion, we found that MACC1 mRNA and protein expression was higher in tumor tissues than normal breast tissues, and MACC1 protein expression was associated with significant poor prognostic markers such as high histological Grade, ER negativity, and HER2 positivity. Therefore, we think that MACC1 expression may be a therapeutic target by pointing to poor prognosis in addition to being a marker of breast carcinoma. Furthermore, we could not see the relationship between mRNA and protein expression of MACC1 and VEGF expression in breast cancer tissues. Accordingly, MACC1 may not affect angiogenesis in breast cancer or, even if the effect is present, it may not be associated with VEGF. Nevertheless, it would be appropriate to support these results in a larger series by investigating *in vivo* and *in vitro* studies.

Financial support and sponsorship
Nil.

Conflicts of interest
There are no conflicts of interest.

REFERENCES

1. Stein U, Walther W, Arlt F, Schwabe H, Smith J, Fichtner I, *et al*. MACC1, a newly identified key regulator of HGF-MET signaling, predicts colon cancer metastasis. Nat Med 2009;15:59-67.
2. Stein U, Smith J, Walther W, Arlt F. MACC1 controls Met: What a difference an Sp1 site makes. Cell Cycle 2009;8:2467-69.
3. Maulik G, Shrikhande A, Kijima T, Ma PC, Morrison PT, Salgia R. Role of the hepatocyte growth factor receptor, c-MET, in oncogenesis and potential for therapeutic inhibition. Cytokine Growth Factor Rev 2002;13:41-59.
4. Wang Z, Cai M, Weng Y, Zhang F, Meng D, Song J, *et al*. Circulating MACC1 as a novel diagnostic and prognostic biomarker for nonsmall cell lung cancer. J Cancer Res Clin Oncol 2015;141:1353-61.
5. Shimokawa H, Uramoto H, Onitsuka T, Chundong G, Hanagiri T, Oyama T, *et al*. Overexpression of MACC1 mRNA in lung adenocarcinoma is associated with postoperative recurrence. J Thorac Cardiovasc Surg 2011;141:895-8.
6. Sun DW, Zhang YY, Qi Y, Liu GQ, Chen YG, Ma J, *et al*. Prognostic and clinicopathological significance of MACC1 expression in hepatocellular carcinoma patients: A meta-analysis. Int J Clin Exp Med 2015;8:4769.
7. Jeong H, Jung J, Oh HE, Choi JW, Lee ES, Kim YS, *et al*. Prognostic characteristics of MACC1 expression in epithelial ovarian cancer. Biomed Res Int 2018;2018:9207153. doi: 10.1155/2018/9207153.
8. Zhou X, Xu CJ, Xu CJ, Wang JX, Dai T, Ye YP, *et al*. Metastasis-associated in colon cancer-1 associates with poor prognosis and promotes cell invasion and angiogenesis in human cervical cancer. Int J Gynecol Cancer 2015;25:1353-63.
9. Chen S, Zong ZH, Wu DD, Sun KX, Liu BL, Zhao Y. The role of metastasis-associated in colon cancer 1 (MACC1) in endometrial carcinoma tumorigenesis and progression. Mol Carcinog 2017;56:1361-71.
10. Jin Z, Xu N, Guo K, Xu P, Li P, Zhang Y, *et al*. Increased expression of metastasis-associated in colon cancer-1 in renal cell carcinoma is associated with poor prognosis. Int J Clin Exp Pathol 2015;8:3857-63.
11. Mohammadi M, Goudarzi PK, Rahmani O, Kaghazian P, Yahaghi E, Taheriazam A, *et al*. Retracted artıcle: Evaluation of gene expression level of CDC5L and MACC1 in poor prognosis and progression of osteosarcoma. Tumor Biol 2016;37:8153-57.
12. Wang L, Zhou R, Zhao Y, Dong S, Zhang J, Luo Y, *et al*. MACC-1 promotes endothelium-dependent angiogenesis in gastric cancer by activating TWIST1/VEGF-A signal pathway. Plos-One 2016;11:137-57.
13. Huang Y, Zhang H, Cai J, Fang L, Wu J, Ye C, *et al*. Overexpression of MACC1 and its significance in human breast cancer progression. Cell Biosci 2013;3:16.
14. Srabovic N, Mujagic Z, Mujanovic-Mustedanagic J, Softic A, Muminovic Z, Rifatbegovic A, *et al*. Vascular endothelial growth factor receptor-1 expression in breast cancer and its correlation to vascular endothelial growth factor a. Int J Breast Cancer 2013;2013:746749.
15. Weaver S, Dube S, Mir A, Qin J, Sun G, Ramakrishnan R, *et al*. Taking qPCR to a higher level: Analysis of CNV reveals the power of high throughput qPCR to enhance quantitative resolution. Methods 2010:50:271-76.
16. Han Z, Gong X, Zhu B, Wu S, Yu L, Song W, *et al*. Expression of ALDH1, MACC1, and KAI1 in the triple-negative breast cancer and their clinical significance. Int J Clin Exp Pathol 2017:10:5655-64.
17. Tan W, Xie X, Li L, Tang H, Ye X, Chen L, *et al*. Diagnostic and prognostic value of serum MACC1 in breast cancer patients. Oncotarget 2016;7:84-8.
18. Utsumi T, Kobayashi N, Hanada H. Recent perspectives of endocrine therapy for breast cancer. Breast Cancer 2007;14:194-99.
19. Prguda-Mujic J, Milde-Langosch K, Mueller V, Suljagic M, Coric J, Ler D. The predictive significance of metastasis-associated in colon cancer-1 [MACC1] in primary breast cancer. Ann Clin Lab Sci 2018;48:191-6.
20. Slamon DJ, Godolphin W, Jones LA, Holt JA, Wong SG, Keith DE, *et al*. Studies of the HER-2/neu proto-oncogene in human breast and ovarian cancer. Science 1989;244:707-12.
21. Muendlein A, Hubalek M, Geller-Rhomberg S, Gasser K, Winder T, Drexel H, *et al*. Significant survival impact of MACC1 polymorphisms in HER2 positive breast cancer patients. Eur J Cancer 2014;50:2134-41.
22. Peng T, Li Z, Li D, Wang S. MACC1 promotes angiogenesis in cholangiocarcinoma by upregulating VEGFA. Oncotargets and Therapy 2019;12:1893.
23. Toi M, Matsumoto T, Bando H. Vascular endothelial growth factor: its prognostic, predictive, and therapeutic implications. Lancet Oncol 2001;2:667-73.