Clinical significance of Mena and Her-2 expression in breast cancer

J.W. Du, K.Y. Xu, L.Y. Fang, X.L. Qi

School of Medicine, Anhui University of Science & Technology, Huainan, Anhui Province (P.R. China)

Summary

Objective: The aim of this study was to determine the expression patterns of Mena and Her-2 in breast cancer tissues and to explore their clinical significance and correlation with clinicopathological parameters. *Methods:* The expression of Mena and Her-2 was detected in 40 breast cancer tissues and 14 normal breast tissues by immunohistochemistry, and the relationship of Mena and Her-2 expression with clinicopathological parameters was analyzed. *Results:* Both Mena (70%) and Her-2 (40%) were more commonly expressed in breast cancer than in normal breast tissue (7.1%, 0%, respectively; $p < 0.05$); further, Mena and Her-2 expression in breast cancer were positively correlated ($r = 0.530$, $p < 0.05$). In comparing expression with clinicopathological parameters of tumor samples, Mena and Her-2 were both associated with axillary lymph node metastasis and TNM stage ($p < 0.05$), but not with patient age or pathological type. *Conclusions:* Mena and Her-2 are related to the malignancy degree and metastasis of breast cancer, and thus may play a coordinating role in the occurrence and progression of breast cancer.

Key words: Breast cancer; Mena; Her-2; Immunohistochemistry; Clinicopathological parameter.

Introduction

In many countries, the incidence of breast cancer has continued to increase in recent years, and it is commonly ranked as the most frequent malignancy in women [1-3]. Indeed, although mortality rates have declined through improved screening and treatment, this cancer still claims many lives each year [2]. Breast cancer prognosis and mortality are heavily influenced by tumor invasion and metastasis [4, 5]. Thus, the development of novel clinical prognostic indicators is crucial to improving survival rate and reducing mortality of patients with breast cancer [6]. Both diagnosis and therapy can be improved by understanding the molecular mechanisms behind tumor initiation and metastasis and by identifying factors that can estimate metastasis potential and/or alter this ability of tumors to invade other sites.

One factor of great interest as a promoter of metastasis in breast cancer is Mena. This protein is a member of the Ena/VASP family of actin regulatory proteins and is rarely expressed in normal breast tissue. However, Mena expression correlates with tumor development and increases with malignancy grade. Further, overexpression of Mena can promote infiltration and metastasis of breast cancer cells [7, 8]. The association of Mena with tumor development, progression, and metastasis has made it an important molecule for clinical investigation, with potential wide applications as a diagnostic, prognostic, and therapeutic marker for breast cancer [9].

Another molecule that promotes metastasis of breast tumors [10, 11], human epidermal growth factor receptor 2 (Her-2), also known as c-erbB2, is commonly used as a marker in clinical research and practice. However, although both Mena and Her-2 are known to promote metastasis, no report has demonstrated whether they share the same mechanism or have synergistic effects on metastasis. Further research is needed to know whether both molecules may be targeted at once to block tumor cell invasion and metastasis more effectively, lengthening patient survival and improving cure rates.

To attempt to understand whether Mena and Her-2 share similar mechanisms and/or synergistic effects for promoting breast cancer metastasis, we used immunohistochemistry to detect Mena and Her-2 protein expression in 40 cases of breast cancer and 14 cases of normal breast tissue. The relationship between Mena and Her-2 expressions and their relationship with clinicopathological parameters were determined.

Materials and Methods

General information

Specimens were collected from 40 breast cancer patients who had been pathologically confirmed and received surgical resection in the Affiliated Hospital of Anhui University of Science and Technology (Huainan City, Anhui Province, China) from June 2010 to October 2011. For each case detailed clinical and pathological data were available; none had received any preoperative chemotherapy or radiotherapy. All patients were female. Patients ranged in age from 24 to 72 years (mean age 54.1 ± 9.4 years); 21 patients were < 55 years, and 19 patients were ≥ 55 years. Pathological diagnoses revealed that 36 patients had invasive ductal carcinoma, three patients had invasive lobular carcinoma, and one case had medullary carcinoma; 32 patients had axillary lymph node metastasis, and eight had no axillary lymph node metastasis. TNM stages were classified according to standards established in 2002 by Union Internationale Contre Le Cancer (UICC): 25 cases were in Stage I+II, and 15 cases were in Stage III+IV. In addition, 14 specimens were also collected from normal breast tissues (> 5 cm away from edge of cancer and confirmed by pathological diagnosis) as control.

Revised manuscript accepted for publication March 1, 2012

Immunohistochemistry

Tissues were fixed in neutral formalin, dehydrated, and embedded in paraffin by conventional methods. Samples were sectioned (4 μm) and collected onto glass slides. Slices were then dewaxed with dimethylbenzene, rehydrated, soaked, and heated for antigen retrieval. Hydrogen peroxide solution (3%) was used to block endogenous peroxidase activity, then sections were covered with non-specific serum, placed in a wet box, and incubated at room temperature. Primary antibodies (Mena, goat anti-mouse monoclonal; Her-2/neu, mouse anti-human monoclonal; Santa Cruz Biotechnology) were added to the wet box, and the box was incubated at 4ºC overnight. Slides were washed three times with PBS before addition of biotinylated secondary antibodies and incubation at room temperature. Slides were again washed three times in PBS before adding streptococcus avidin-peroxidase (Zhongshang-Golden Bridge Biotechnology, Ltd., Beijing) and incubating at 37ºC for 30 minutes. Staining was developed with DAB chromogen (Zhongshang-Golden Bridge Biotechnology). Sections were counterstained with hematoxylin, dehydrated with ethanol gradient, and sealed. Known positive tissue slices were used as positive controls, and PBS was used as a negative control instead of primary antibodies.

Staining patterns indicating expression were yellowish-brown granules in cancer cell cytoplasm for Mena, and for Her-2 brownish-yellow in the membranes of cancer cells. To assess staining, ten high-power fields were selected per sample. Staining intensity was classified into four grades as follows: no staining visible was scored as 0; faint yellow was scored as 1; yellow as 2; and brownish-yellow as 3. Staining frequency was assessed by the proportion of positive cells per total tumor cells, as follows: ≤ 5% positive cells was assigned a 0; 6-25% positive, 1; 26-50% positive, 2; 51-75% positive, 3; and ≥ 76% positive was assigned a 4. Total scores for each case reflect the sums of scores of staining intensity and frequency, with total scores of 0 indicated as (-), total scores of 1-2 as (+), total scores of 3-5 as (++), and total scores of 6-7 as (+++).

Statistical methods

SPSS17.0 statistical software was used for statistical analysis; χ^2 test was used to compare expression of Mena and Her-2 protein among groups, and Spearman rank correlation was used to analyze correlations between Mena and Her-2 protein expression. Analyses were performed with two-sided tests; $p < 0.05$ was considered statistically significant.

Results

Expression of Mena and Her-2 protein in breast cancer and normal breast tissue

Breast tumor samples more commonly exhibited both Mena and Her-2 expression than did normal breast tissues. Specifically, for 40 breast cancer tumors, 70% (Table 1) and 40% (Table 2) expressed Mena and Her-2, respectively. In contrast, as few as 7.1% of normal breast samples expressed Mena, while 0% exhibited staining for Her-2. The positive rates of Mena and Her-2 expression in breast cancer were significantly higher compared to normal breast tissue ($p < 0.05$). Further, Mena and Her-2 expression in breast cancer were positively correlated with one another ($r = 0.530$, $p < 0.05$; Table 3).

Table 1. — *Expression of Mena in breast cancer and normal breast tissue* [n (%)] *detected by*.

Sample	*n*	–	+	++	+++
Breast cancer	40	12 (30.0)	13 (32.5)	7 (17.5)	8 (20.0)
Normal breast tissue	14	13 (92.9)	1 (7.1)	0	0
Total	54	25 (46.3)	12 (25.9)	7 (13.0)	8 (14.8)

$\chi^2 = 16.672$, $p = 0.001$.

Table 2. — *Expression of Her-2 in breast cancer and normal breast tissue* [n (%)] *detected by*.

Sample	*n*	–	+	++	+++
Breast cancer	40	24 (60.0)	7 (17.5)	5 (12.5)	4 (10.0)
Normal breast tissue	14	14 (10.0)	0	0	0
Total	54	38 (70.4)	7 (13.0)	5 (9.3)	4 (7.4)

$\chi^2 = 7.958$, $p = 0.047$.

Table 3. — *Correlation between Mena and Her-2 expression in breast cancer tissue*.

Mena	*n*	Her-2 [*n*(%)]			
		–	+	++	+++
–	12	11 (91.7)	0	1 (8.3)	0
+	13	9 (69.2)	2 (15.4)	1 (7.7)	1 (7.7)
++	7	2 (28.6)	3 (42.9)	1 (14.3)	1 (14.3)
+++	8	2 (25.0)	2 (25.0)	2 (25.0)	2 (25.0)
Total	40	24 (60.0)	7 (17.5)	5 (12.5)	4 (10.0)

$r = 0.530$, $p = 0.001$

Relationship between expression of Mena and Her-2 protein and clinical/pathological parameters

To determine whether the increased expression of both Mena and Her-2 correlated with the severity of breast tumors, we assessed expression patterns in comparison with clinical and pathological features of the samples. Both Mena and Her-2 protein expression in breast cancer were correlated with axillary lymph node metastasis and TNM stage ($p < 0.05$), but not with age or pathological type.

Discussion

Breast cancer is one of the most common malignancies among women, often metastasizing to the lungs, bone, brain, and other organs. With rapid advances in molecular biology and cytobiology many genes have been found to play important roles in the occurrence, development, and metastasis of breast tumors. At least a subset of these genes may offer potential as molecular markers or therapeutic targets to aid in diagnosis and treatment of the disease.

To invade and metastasize, cancer cells must first evade normal defense systems, then disseminate and infiltrate into the surrounding tissues, blood vessels, and lymphatic vessels. Mena, as a member of the Ena/VASP family of actin regulatory proteins, can enhance motor activity of cancer cells, enabling them to infiltrate lymph nodes and distant organs. Indeed, this protein is associated with the occurrence, development, invasion, and metastasis of various tumors and is overexpressed in lung, colon, and other cancers. Studies performed by DiModugno *et al*.

Table 4. — *Correlation between expression of Mena protein and clinicopathological parameters in breast cancer* [n (%)].

Clinicopathological parameters	*n*	–	+	++	+++	χ^2	*p*
Age							
< 55	21	7 (33.3)	9 (42.9)	3 (14.3)	2 (9.5)	4.310	0.230
≥ 55	19	5 (26.3)	4 (21.1)	4 (21.1)	6 (31.6)		
Pathological type							
Invasive ductal carcinoma	36	11 (30.6)	10 (27.8)	7 (19.4)	8 (22.2)	4.708	0.582
Invasive lobular carcinoma	3	1 (33.3)	2 (66.7)	0	0		
Medullary carcinoma	1	0	1 (100.0)	0	0		
Axillary lymph node metastasis							
No	32	11 (34.4)	13 (40.6)	5 (15.6)	3 (9.4)	13.624	0.003
Yes	8	1 (12.5)	0	2 (25.0)	5 (62.5)		
TNM Stages							
I+II	25	10 (40.0)	10 (40.0)	3 (12.0)	2 (8.0)	9.328	0.025
III+IV	15	2 (13.3)	3 (20.0)	4 (26.7)	6 (40.0)		

Table 5. — *Correlation between expression of Her-2 protein and clinicopathological parameters in breast cancer* [n (%)].

Clinicopathological parameters	*n*	–	+	++	+++	χ^2	*p*
Age							
< 55	21	14 (66.7)	4 (19.0)	1 (4.8)	2 (9.5)	2.516	0.472
≥ 55	19	10 (52.6)	3 (15.8)	4 (21.1)	2 (10.5)		
Pathological type							
Invasive ductal carcinoma	36	22 (61.1)	6 (16.7)	4 (11.1)	4 (11.1)	8.249	0.220
Invasive lobular carcinoma	3	2 (66.7)	1 (33.3)	0	0		
Medullary carcinoma	1	0	0	1 (100.0)	0		
Axillary lymph node metastasis							
No	32	22 (68.8)	7 (21.9)	2 (6.3)	1 (3.1)	16.354	0.001
Yes	8	2 (25.0)	0	3 (37.5)	3 (37.5)		
TNM Stages							
I+II	25	21 (84.0)	2 (8.0)	1 (4.0)	1 (4.0)	16.091	0.001
III+IV	15	3 (20.0)	5 (33.3)	4 (26.7)	3 (20.0)		

[9] showed that Mena is rarely expressed in normal breast tissues, but its expression gradually increases with tumor development. Additionally, studies by Philippar *et al*. [12] found that Mena can promote *in vivo* and *in vitro* movement and infiltration of breast cancer cells, enhance effects of epidermal growth factor (EGF) on their invasion and metastasis, and improve their motility. Our findings indicate that Mena is expressed at a higher intensity and in more cells in breast cancer tissue than in normal breast tissue. Additionally, Mena expression correlates with disease severity: increased expression is associated with axillary lymph node metastasis and TNM stages. These findings suggest that changes in Mena expression reflect the invasion and metastasis potential of tumor cells. Thus, Mena may be a useful indicator for evaluating biological behaviors of tumors, and may provide new avenues for tumor diagnosis and treatment.

The association of Her-2, a member of the epidermal growth factor receptor family, with breast cancer has been studied intensively. Typically expressed during human embryonic development, Her-2 participates in growth and development of tissues and organs; however, its expression is low in normal tissues during adulthood. In malignant transformation, the *Her-2* gene becomes activated and abnormally upregulated in many epithelial-origin tumors, such as gastric cancer, breast cancer, ovarian cancer, and colon cancer. Breast cancer patients with Her-2 overexpression usually have a low survival rate, rapid disease progression, recurrence, metastasis, and poor therapeutic efficacy. Many studies have confirmed that Her-2 can enhance the invasion and metastatic potential of breast cancer cells [13-15], resulting in earlier lymph node metastasis and more advanced pathological stages. Thus, as an independent breast cancer prognostic factor, Her-2 can provide reliable indicators for clinical estimation of disease condition and prognosis evaluation and is commonly used in the clinic.

Here, we confirmed that Her-2 is more commonly expressed in breast cancer tissues compared to normal breast tissue; further, its expression correlates with axillary lymph node metastasis and TNM stage, consistent with results reported by Kroger *et al*. [16]. This underscores the role that Her-2 plays in breast cancer occurrence, progression, and metastasis. However, we extended these findings by examining the correlation of Her-2 expression with that of Mena. Mena expression and Her-2 expression were positively correlated with each other, suggesting that Mena and Her-2 may collectively participate in proliferation of breast cancer cells and have synergistic effects on invasion and metastasis of tumor cells.

In summary, expression of both Mena and Her-2 protein in breast cancer were significantly increased and positively correlated. Further, expression was associated with increased disease severity. Thus, both Mena and Her-2 may be used as reference indicators for evaluating biological behavior and prognosis of breast cancer, and Mena may offer a new therapeutic target.

References

[1] Botha J.L., Bray F., Sankila R., Parkin D.M.: "Breast cancer incidence and mortality trends in 16 European countries". *Eur. J. Cancer*, 2003, *39*, 1718.

[2] DeSantis C., Siegel R., Bandi P., Jemal A.: "Breast cancer statistics, 2011". *CA Cancer J. Clin.,* 2011, *61,* 409.

[3] Long N., Moore M.A., Chen W., Gao C.M., Lai M.S., Mizoue T. *et al*.: "Cancer epidemiology and control in north-East Asia - past, present and future". *Asian Pac J. Cancer Prev.,* 2010, *11* (suppl. 2), 107.

[4] Mazurek M.S.: "Sedation and analgesia for procedures outside the operating room". *Semin. Pediatr. Surg.,* 2004, *13,* 166.

[5] Mao J., Hai J., Shu H.P., Wu Y.H., Ou H.Y., Luo S.Y. *et al*.: "Expressions of survivin and nm23 and the relationship among the expressions and the axillary lymph node metastasis in breast cancer". *Zhong Nan Da Xue Xue Bao Yi Xue Ban,* 2005, *30,* 312.

[6] Liu B.B., Wei J., Fu L.: "Updates on genes related to breast cancer metastasis". *Zhonghua Bing Li Xue Za Zhi*, 2008, *37,* 266.

[7] Kwiatkowski A.V., Gertler F.B., Loureiro J.J.: "Function and regulation of Ena/VASP proteins". *Trends Cell. Biol*., 2003, *13,* 386.

[8] Hoffman L.M., Jensen C., Kloeker S., Wang C.L., Yoshigi M., Beckerle M.C.: "Genetic ablation of zyxin causes Mena/VASP mislocalization, increased motility, and deficits in actin remodeling". *J. Cell Bio.,* 2006, *172*, 771.

[9] Di Modugno F., Mottolese M., Di Benedetto A., Conidi A., Novelli F., Perracchio L. *et al*.: "The cytoskeleton regulatory protein hMena ENAH is overexpressed in human benign breast lesions with high risk of transformation and human epiderma growth factor receptor 2 positive hormonal receptor negative tumors". *Clin*. *Cancer Res*., 2006, *12*, 1470.

[10] Milier J.K., Shattuck D.L., Ingalla E.Q., Yen L., Bowowsky A.D., Young L.J. *et al*.: "Suppression of the negative regulator contributes to ErbB2 overexpression in breast cancer". *Cancer Res*., 2008, *68*, 8286.

[11] Browne B.C., O'Brien N., Dufly M.J., Crown J., O Donovan N.: "HER-2 signaling and inhibition in breast cancer". *Curr*. *Drug*. *Targets*, 2009, *9,* 419.

[12] Philippar U., Roussos E.T., Oser M., Yamaguchi H., Kim H.D., Giampieri S. *et al*.: "A Mena invasion isoform potentiates EGF-induced carcinoma cell invasion and metastasis". *Dev*. *Cell*., 2008, *15,* 813.

[13] Xu Z.H., Ford B.D.: "Upregulation of erbB receptors in rat brain after middle cerebral arteria occlusion". *Neurosei Lett*., 2005, *375,* 181.

[14] Wang Y.J., Wang N., Wang B., Qin W.X., Xue C.Y.: "Comparison of clinicopathologic characteristics and prognosis of triple-negative with Her-2-overexpressing breast cancer". *Zhonghua Zhong Liu Za Zhi,* 2009, *31,* 346.

[15] Spector N.L., Blackwell K.L.: "Understanding the mechanism behind trastuzumab therapy for human epidermal growth factor receptor2 positive breast cancer". *J*. *Clin*. *Oncol*., 2009, *27,* 5838.

[16] Kröger N., Milde-Langosch K., Riethdorf S., Schmoor C., Schumacher M., Zander A.R. *et al*.: "Prognostic and predictive effects of immunohistochemical factors in high-risk primary breast cancer patients". *Clin*. *Cancer Res*., 2006, *12*, 159.

Address reprint requests to:
J.W. DU, M.D.
School of Medicine
Anhui University of Science & Technology
Dongshanzhong Road 25
Huainan City 232001, Anhui (Province China)
e-mail: djwdxswy@126.com