The Prompting Effects of MT1-MMP on EMT in Breast Cancer

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Abstract

Objective

Epithelial to mesenchymal transition (EMT) was the initial process of invasion and metastasis leading to a relapse of breast cancer following resection and chemo-radiotherapy. Membrane type-1 Matrix Metalloproteinase (MT1-MMP) was confirmed to play an important role in EMT in various cancers. However, the MT1-MMP effects on EMT in breast cancer had not yet been studied. Here, we investigated the MT1-MMP effects on breast cancer EMT onset, invasion, and migration abilities in MCF-7 cells.

Methods

Expressions of MT1-MMP and EMT-associated proteins including E-cadherin, N-cadherin, and Vimentin were detected by immunohistochemistry in 71 breast cancer resection samples. The relationships of MT1-MMP with clinic-pathological parameters were statistically analyzed, as well as EMT-associated proteins. Western blots were performed to test MT1-MMP and EMT-associated proteins expression levels in MCF-7 cells transfected by MT1-MMP plasmid. Wound-healing and transwell experiments were used to estimate MT1-MMP-induced invasion and migration.

Results

Overexpression of MT1-MMP was verified in 71 breast cancer patients. MT1-MMP levels were observed to be correlated with the breast cancer clinical TNM stage, lymph node metastasis, and tumor size. The EMT-associated proteins including N-cadherin and Vimentin expression levels were higher both in MT1-MMP strong positive breast cancer resection samples and MCF-7 cells transfected with MT1-MMP plasmid. Furthermore, MCF-7 cells also acquired more ability to migrate and invade according to the results of the wound-healing assays and transwell experiments.

Conclusion

MT1-MMP was overexpressed in breast cancer tissue, and MT1-MMP promoted breast cancer EMT incidence, which was closely associated with breast cancer invasion and migration.

Introduction

Breast cancer (BC), a common life-threatening malignancy, has become the leading cause of cancer-related deaths in women worldwide. Globally, breast cancer accounted for approximately 1.67 million new cases and 0.5 million deaths in 2012 [1]. Despite the early detection and treatment of breast cancer, it remains the second leading cause of cancer-related death in America[2].

Cancer relapse and metastasis are primary reasons for poor survival and prognosis among patients receiving successful resection or chemo-radiotherapy. Epithelial to mesenchymal transition (EMT) is the initial process of cancer progression, during which tumor cells dissociate from the primary tumor to
invade into the neighboring tissue, traveling through the blood vessels, and finally forming colonies at a secondary site[3].

EMT has been defined as a critical step in metastasis progression. Cancer cells can use EMT to initiate invasion and metastasis[4]. Tumor cells, when combined with immunofluorescence, have identified EMT as a very early event in a pancreatic ductal adenocarcinoma mouse model[5]. EMT activation in human breast cancer cells could also enhance metastatic dissemination[6].

EMT is characterized by the decrease of E-cadherin expression (which is an epithelial protein marker) and the increase of N-cadherin (the cadherin switch, which is one of the hallmarks of EMT)[7], as well as vimentin (the mesenchymal proteins in the main extracellular matrix)[8].

Membrane type 1 Matrix metalloproteinase (MT1-MMP) is the first discovered membrane-associated Matrix metalloproteinase, which has been confirmed to be extensively associated with many kinds of tumor growths and negative prognosis[9]. Its indirect effect on the EMT and tumorigenesis process was also displayed in cultured endocardial cushion cells and transgenic animals by activation of MMP-2,-3,-8,-13[10].

Whereas, further research of MT1-MMP on EMT of breast cancer samples was rarely reported, thus MT1-MMP-affected breast cancer progression is still unknown.

In this study, the expressions of MT1-MMP in breast cancer tissues verified that MT1-MMP was associated with some breast cancer clinicopathological parameters. Furthermore, based on the above analysis, MT1-MMP plasmid-transfected breast cancer cells were used to verify that MT1-MMP was involved with the EMT procedure, the progression of breast cancer cell migration, and invasion.

Materials And Methods

Immunohistochemistry: 71 breast cancer cases were observed in the first affiliated hospital of the Shihezi University, school of medicine, Xinjiang, China. The median ages were 43. All cases were diagnosed as Ductal Breast Cancer by the pathologist. 13 cases were grouped into stage I, 41 into stage II, and 17 into the advanced stage. All cases were grouped according to the UICC 2009 TNM clinical staging standard. Paired normal breast tissue samples were taken from the sites at more than 3 centimeters (cm) away from the cancerous lesions, and were used as controls. Informed consent was obtained from all patients in this study, which was authorized by the institutional ethics committee of the First Affiliated Hospital of Shihezi University, School of Medicine.

5-micrometer (μm) thick sections of formalin-fixed, paraffin-embedded tissue were used to perform the immunohistochemical staining. Mouse anti-human monoclonal primary antibody (Epitomics, America) at a dilution of 1:300 was used to detect MT1-MMP. Mouse anti-human E-cadherin (1:500; Abcam America), rabbit anti-human monoclonal antibody vimentin (1:400; Abcam, America), and anti-N-cadherin (1:800;
Abcam, America) primary antibodies were incubated with slides overnight at 4°C. All positives (in the kit) and negative-controls (PBS instead of the first antibody) in the experiments were performed to verify the specificity of the immunostaining reaction. The second antibody EnVision kit (Dako, America) (Cat:201503, Lot:10051369) was used for immunohistochemical staining, which was assessed by a semi-quantitative scoring system [11] as follows:

In terms of the staining intensity, no staining was recorded as 0 (negative), and positivity required that at least 10% of the tumor cells would be stained. Pale yellow was recorded as 1 (weakly positive), brownish-yellow was recorded as 2 (positive), and brown was recorded as 3 (intensive positive). No significant age difference was found between the elementary stage group (Ⅰ+Ⅲ) and the advanced stage group (Ⅰ+Ⅲ).

**Cell culture:** Human Breast cancer cell line MCF-7 was purchased from the Shanghai Institute of Biochemistry and Cell Biology (Shanghai, China). Cells were cultured in Dulbecco’s Modified Eagle Medium (DMEM), supplemented with 100 U/mL penicillin, 0.1 g/L streptomycins, and 10% fetal bovine serum (10% FBS, (GIBCO), and let to grow in a humidified atmosphere containing 5% CO₂ at 37 °C.

**Plasmids and cell transfection:** MT1-MMP plasmid was linked to a green fluorescent protein (GFP) at the N-terminal which was gifted from Prof. Amanda L at the Willis Weiss Lab University of Michigan, United States. MCF-7 Cells (1×10⁶/well) cultured in a 6-well cell-culture plate and were transiently transfected with 3.0 μL of MT1-MMP plasmid or empty pcDNA3.1 (Invitrogen) (negative control) using 7 μL Lipofectamine® 2000 (Invitrogen), according to the manufacturer's protocol. Transfection efficiency was assessed by monitoring GFP expression, using the Laser scanning confocal microscope technique (confocal laser scanning microscopy (CLSM), ZEISS LSM 510). MCF-7 cell line transfected with the MT1-MMP construct. Empty vector plasmid was cultured for 48 hours, seeded onto coverslips, and fixed with 4% paraformaldehyde. After cells were 75% congregated, they were observed on the coverslips. After being blocked by the goat serum (Epitomics, US) for 20 minutes, the coverslips were stained with MT1-MMP mouse anti-human monoclonal antibody (Epitomics, US) and incubated overnight at 4 degrees Celsius with phosphate-buffered saline (PBS) as the negative control. IgG-phodamine goat anti-mouse secondary antibody (Golden Bridge in Zhongshan, Beijing China) was added in the samples on the coverslips and incubated under room temperature avoiding light for 30 minutes. Then washed and soaked by PBS for 30 minutes under dark conditions. The glycerol-sealed coverslips were then examined under CLSM.

**Wound-healing assay:** MCF-7 cells transfected with control plasmid and MT1-MMP plasmid were seeded at a density of 1×10⁶/well in 6-well tissue culture plates overnight. Once confluence was reached, 3 parallel lines were drawn on the bottom of the 6-well plates before seeding the cells. Using a sterilizing 100 uL pipette tip, an area vertical to the lines, approximately 5 mm in width, was cleared to create a scratch. Then the cells were maintained in DMEM without fetal bovine serum (FBS) medium for 24 and 48 hours, in which repopulation and closure were subsequently monitored daily under phase contrast microscope 4×objective lens. The closure was quantified using Image J 2.0.0 software by measuring the width of the scratch each day and normalizing it to the initial size of the scratch.
**Transwell assay in vitro**: MT1-MMP and pcDNA 3.1 control vector were transiently transfected into MCF-7 cells \( (5\times10^4) \), which were then allowed to migrate for 24h through Transwell\textsuperscript{®} cell culture inserts (8-μm pore size, 6.5-mm diameter; Costar, Cambridge, MA). Transwell\textsuperscript{®} inserts were coated with Matrigel\textsuperscript{®}, and the lower compartment was filled with 600 μL of DMEM supplemented with 20% fetal bovine serum. After incubation at 37 °C in an incubator for 24 h, filters were rinsed with phosphate-buffered saline, fixed with 4% paraformaldehyde (15min at -4 °C), and stained with crystal violet (0.1%; Thermo Fisher Scientific) for 15min. Cells on the upper surface of the filters were removed with a cotton swab. Cells that migrated through the filters were counted under the microscope at a magnification of 400×. Each clone was tested in triplicate in at least three independent assays. Data was expressed as mean ± standard error.

**Western blot**: Cellular proteins were prepared using cell lysis buffer (50 mM Tris-HCl, pH 8.0, 1% Nonidet P-40, 2mM ethylenediaminetetraacetic acid, 10mM NaCl, 2mg/mL aprotinin, 5mg/mL leupeptin, 2mg/ml pepstatin, 1mM dithiothreitol, 0.1% sodium dodecyl sulfate, and 1mM phenylmethylsulfonyl fluoride). Equal amounts of protein samples (8μg) were separated by polypropylene gel electrophoresis with 5% concentrated gel and 12% separating gel under a stable voltage of 80 for 60 minutes, and then transferred to PVDF membrane with the conditions of stable 110 voltage lasting 58 minutes for MT1-MMP, 83 minutes for N-cadherin, 90 minutes for E-cadherin, and 52 minutes for Vimentin, separately. To block the non-specific protein, PVDF membranes were all incubated by the solution prepared by skimmed milk powder and dissolved in Tris Buffered Saline Tween (TBST buffer) for 2 hours. Then the different, diluted, first antibody was added and incubated overnight at 4 degrees Celsius in MT1-MMP mouse anti-human monoclonal antibody (Abcam company, US) dilution degree 1:1000, Vimentin Rabbit anti-Human Monoclonal Antibody 1:100(Abcam company, US), E-Cadherin mouse anti-Human Monoclonal Antibody 1:500 (Abcam company, US), and N-Cadherin Rabbit anti-Human Monoclonal Antibody 1:1000 (Abcam company, US), respectively. This procedure was followed by incubation with sheep anti-mouse (1:20,000) or anti-rabbit (1:20,000) horseradish peroxidase-labeled secondary antibodies for 2h at room temperature. β -actin immunoblots served as loading controls. The membrane was photographic, developed and fixed, and an ECL reagent kit (Thermo Systems) was used to detect the bands on the membrane. Finally, the data and images were collected by the Gel Imaging System (Bio-Rad, America) and X-ray exposure.

**Results**

2.1 Immunohistochemical analysis of the association of MT1-MMP protein expressions and clinicopathological factors in Breast Cancer cases.

Immunohistochemical analysis for MT1-MMP expression was almost positive in 71 breast cancer samples (weak positive or strong positive), and negative expressions were observed in the paired-noncancerous tissues of adjacent normal breast samples. MT1-MMP immunohistochemical staining was orientated on the plasma membrane and partly in the cytoplasm of the tumor cells in Breast cancer specimens. Strong expression of MT1-MMP was also observed in the nest of infiltrating stroma. (Fig 1 A and B).
The enhanced expression level of MT1-MMP (positive and strong positive expressions) in the lymph node metastasis group was significantly higher than that in the non-lymph node metastasis group (p<0.05, Table 1). It could be seen that the bigger size of tumors were more positively expressed in Breast Cancer. Compared with the clinical stages I and II, the significantly higher expressions of MT1-MMP could be seen in the advanced stages of Breast cancer III and IV (p<0.05, Table 1).

Other clinic-pathological factors such as age, estrogen receptor, and progestogen receptor showed no significant correlation with the expressions of MT1-MMP in Breast cancer tissues.

2.2 The correlation analysis of the immunohistochemical expressions of MT1-MMP and EMT-related proteins - E-cadherin, N-cadherin, and Vimentin in breast cancer specimens. (See Table 2).

E-cadherin immunohistochemical staining was mainly seen in the membrane of cancer cells, and partly in the breast cancer tissues cytoplasm. The positive rates between the normal and cancer group were statistically significant (28.2% in the cancer group, while 45.8% in paracancerous tissue, p<0.05). The E-cadherin expression levels in the MT1-MMP weak positive breast cancer group (± or +) were significantly stronger than that in the MT1-MMP strong positive breast cancer group (++ or +++). (χ²=12.236, p =0.000)

N-cadherin immunohistochemical staining was observed almost in the membrane of the breast cancer cell. The expressions of N-cadherin were significantly higher in cancer cells compared with that in the paracancerous samples (61.9% in the cancer group, while 40.2% in paracancerous tissue, p<0.05). The level of N-cadherin expression varied directly with that of MT1-MMP expression (χ²=4.410, p =0.036).

The membrane expression of Vimentin in breast cancer tissue was more than that in the cytoplasm. A higher positive rate of the Vimentin expression was observed in cancer with a significant difference (60.6% in the cancer group, while 38.2% in paracancerous tissue, p<0.05). The level of Vimentin immunohistochemical expressions was strongly related to that of MT1-MMP (χ²=6.459, p =0.011).

2. MCF-7 breast cancer cell lines transfected with MT1-MMP plasmid exhibited promoting BC cancer cell migration and invasion in vitro.(Fig. 2).

2.1 The MT1-MMP plasmid was transfected to MCF-7 cells to examine the role of MT1-MMP on the metastasis of breast cancer cell lines. MT1-MMP plasmid was transfected into the MCF-7 cell line. The positive signal green fluorescence of GFP protein could be observed in MCF-7 cells after 24 hours of transfection (panel 1). Furthermore, MT1-MMP was observed on the plasma membrane and a 70% transfection efficiency was confirmed by the immunofluorescence assay under the confocal microscope (panel 2).

Compared with the controls, MCF-7 cells transfected with the MT1-MMP plasmid were cultured for 1 week, and the morphology of the cells was observed under a microscope at the magnification of 400×.
The results showed that the morphology of the cells changed distinctly with controls, presenting elongated, fibroblast-like cells with less cell-to-cell contact (panel 3).

2.2 Next, MT1-MMP protein expression was estimated by Western blot analyses, which revealed that the protein of MT1-MMP band (66-kDa) was seen in transient transfection MCF-7 cell lines, which was not observed in a control cell line with pcDNA3.1 vector-transfected (see Fig. 3). However, the β-actin band (42-kDa) was detected in both groups of cells. The results showed that MCF-7 with overexpression of MT1-MMP was established successfully, furthermore, the association of EMT and MT1-MMP could be testified effectively in vitro.

3. MCF-7 cells, with MT1-MMP overexpression, underwent EMT. After MT1-MMP plasmid was transfected into MCF-7 cells for 48 hours, a Western blot analysis was performed to detect protein expression differences of MT1-MMP and EMT key markers, for example E-cadherin, N-cadherin and vimentin in MCF cells transfected by MT1-MMP plasmid and pcDNA3.1 vector (Fig. 3). Cells transfected with MT1-MMP exhibited a decreased expression level of the epithelial marker E-cadherin but increased expression levels of mesenchymal markers N-cadherin and vimentin.

4. MCF-7 cells transfected with MT1-MMP plasmid exhibited increased cell migration in vitro. Next, a Wound Healing Assay was used to examine whether the MT1-MMP was involved in the MCF-7 breast cancer cells migration. (see Fig. 4). MCF-7 cells transfected transiently with MT1-MMP plasmid or empty vector were cultured for 24 and 48 hours, respectively, to observe which cells of the two groups migrated into the wounded area faster. A significantly higher closure percentage of the wounded gap was seen in the MT1-MMP over-expressed MCF-7 cells in comparison with the control MCF-7 cells \( (P<0.05, \text{Fig. 4a}) \). The remaining wounded gap of the MCF-7 cells transfected with empty pc DNA3.1 vector varied slightly in 24- and 48-hour culture (Fig. 4b).

5. MCF-7 cells transfected with MT1-MMP plasmid accelerated the invasion ability in vitro. (see Fig. 5) To examine the effect of MT1-MMP expressions on the invasion of MCF-7 cells, a Transwell experiment was used to investigate the ability of MCF-7 cells transfected with MT1-MMP plasmid or empty vector pcDNA3.1 to migrate through the Matrigel-coated filter for 24 hours. The number of MT1-MMP-transfected MCF-7 cells on the reversed filters of transwell was observed to be significantly more than that of empty vector-transfected MCF-7 cells \((329.33\pm10.99 \text{ VS } 229.89\pm8.40, P<0.05, \text{Fig.5})\). The result suggested that MCF-7 cells on which MT1-MMP over-expressed was more invasive than the control cells which had low MT1-MMP expression.

**Discussion**

To find effective diagnostic strategies and targeted therapies, it was essential to understand well the MT1-MMP biology and molecular expressions in breast cancer and to identify the relationship between MT1-MMP and Breast cancer EMT. In this study, we first examined the MT1-MMP protein expression level through immunohistochemistry in 71 surgically resected breast cancer cases and their paired adjacent normal breast tissue samples, respectively. The results showed that MT1-MMP was strongly expressed in
breast cancer tissues compared with their paracancerous tissues, which suggested that the overexpression of MT1-MMP in breast cancer tissues was positively related to the incidences of breast cancer disease. Similar results were found in a microarray analysis in nasopharyngeal carcinoma[12], and a high expression of MT1-MMP was also observed in human lung cancer[13], prostate cancer[14], and glioblastomas[15]. Gigantic strides in animal experiments were also identified and related to abnormal MT1-MMP over-expression induced mammary gland abnormalities and adenocarcinoma in transgenic mice[10].

Furthermore, in the analysis of clinical statistical data, it was found that the MT1-MMP expression level was positively correlated with the lymph node metastasis and the breast cancer clinical stages, while no significant expression differences were found on other clinical parameters such as age, PR, and ER in breast cancer cases, which indicated that MT1-MMP played a promotional role in breast cancer clinical metastasis and progression.

EMT has been considered as a favored explanation of how tumor cells leave the primary tumor site, disseminate through the body, and eventually form distant metastases. In the multiple-step metastasis, the function of EMT was attributed to the initial events[16]. Similarly, clinical statistics data confirmed that EMT had a strong association with distant metastasis in patients younger than 40 years old and in lymphovascular invasion patients[17].

Based on the significant changes of MT1-MMP on clinical-pathological parameters, E-cadherin, N-cadherin, and vimentin were tested in breast cancer and their adjacent noncancerous tissues to further investigate the association of MT1-MMP expression level and breast cancer EMT by the experiments of immunohistochemistry. Statistical analysis showed that E-cadherin expression was negatively correlated with that of MT1-MMP in breast cancer cases, while N-cadherin and vimentin expressions had significantly positive relationships with MT1-MMP expression, which afforded the evidence of the noteworthy positive association of MT1-MMP expression with the EMT incidence in Breast cancer cases. Moreover, our study in Western blot experiments identified the molecular mechanisms of MT1-MMP’s effects on breast cancer EMT. The results showed that MT1-MMP prompted the expression of N-cadherin and vimentin proteins and inhibited the E-cadherin expressions in MCF-7 breast cancer cells transfected with MT1-MMP plasmid, which indicated that MT1-MMP arose the breast cancer EMT by repressing E-cadherin products and subsequently inducing N-cadherin and vimentin proteins.

Previous studies also revealed that over-expression of MT1-MMP in carcinoma tissues up-regulated expression of EMT-associated genes including N-cadherin, vimentin, snail, and slug in nasopharyngeal carcinoma[12], gastric cancer[18], and esophageal squamous cell carcinoma[19].

Many studies signified that EMT was popularly accepted as an initial process of cancer procession, i.e. gaining the migratory and invasive properties and allowing cancer cells to leave the primary tumor, invading into blood vessels[20][21]. The process was characterized by epithelial cells losing their apicobasal polarity with delocalization of tight and adherens junction proteins (decreasing epithelial
proteins of E-cadherin[22]) but acquiring a spindle-shaped mesenchymal-like morphology with an up-regulated expression of mesenchymal markers, N-cadherin [23] and Vimentin[24].

To identify that MT1-MMP promotes the invasion and metastasis of breast cancer, we did further experiments in vitro to explore the role of MT1-MMP in breast cancer cell migration and invasion. Compared with the empty vector-transfected group (fig.3, panel3), MCF-7 breast cancer cells transfected with MT1-MMP–GFP pcDNA3.1 plasmid were found to obtain morphological mesenchymal changes characterized by the spindle-shaped mesenchymal-like structure. Wound healing assay and transwell experiment results (fig.4 and fig.5) also showed that constitutive activation of MT1-MMP enhanced the breast cancer cell migration and invasion potential in vitro. It seemed that the MT1-MMP expressed in breast cancer cell membrane had positive effects on cancer EMT and invasion ability in this experimental data.

Interestingly, another experiment result revealed that MT1-MMP in the cytoplasmic domain was indispensable for migration but necessary to mediate viability of the MCF-7 breast cancer cell line[25],

Further research of MT1-MMP's different domain's effects on the cell line should be made to fully understand the complex mechanism of MT1-MMP prompting breast cancer migration and invasion.

Collectively, our experimental data demonstrated that MT1-MMP was over-expressed in breast cancer tissue, and MT1-MMP played a critically positive role in breast cancer EMT through the mechanism of repressing E-cadherin and enhancing N-cadherin and Vimentin expressions, whilst MT1-MMP also prompted breast cancer metastasis and migration. Therefore, MT1-MMP was promised to become a potential candidate as the diagnostic or therapeutic measure in breast cancer disease.

**Declarations**

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**Conflict of interest/Competing interests**

All authors declare no conflict of interests, including employment, consultancies, stock ownership, honoraria, paid expert testimony, patent applications/registrations, and grants or other funding except the funding declared.

**Availability of data and material (data transparency)**

All authors declare that the data transparency were confirmed.

**Code availability** (software application or custom code)
software application were available.

Authors' contributions

All authors contributed to the study conception and design, more contributions from Cao weiwei. Material preparation, data collection and analysis were performed by He Jianwei, Zhang Hongmei and Zhang Licui. The first draft of the manuscript was written by Cao weiwei and Inayat Azeem. All authors commented on previous versions of the manuscript and all authors read and approved the final manuscript.

Ethics approval

The retrospective protocol of this study was approved by the Ethical Committee of the first affiliated hospital of Shihezi University, School of medicine in China. The procedures were conducted in accordance with the Helsinki Declaration of 1964, as revised in 2000.

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References

1. Ahmedin Jemal, D.F.B.M.M.C.J.F., M; Elizabeth Ward; David Forman, Global cancer statistics. CA CANCER J CLIN 2011. 61(2): p. 22.

2. Rebecca L. Siegel, M.K.D.M., MPH; Ahmedin Jemal, DVM, Cancer Statistics, 2016. CA CANCER J CLIN 2016. 66(1): p. 24.

3. Thomas R. Geiger, D.S.P., Metastasis mechanisms. Biochimica et Biophysica Acta 2009. 1796: p. 15.

4. A. Weinberg, C.L.C.B.P.S.J.E.L.R., EMT, cell plasticity and metastasis. Cancer Metastasis Rev 2016. 35: p. 10.

5. Andrew D. Rhim, E.T.M., Nicole M. Aiello, Anirban Maitra, Jennifer M. Bailey, Florencia McAllister, Maximilian Reichert, Gregory L. Beatty, Anil K. Rustgi, Robert W. Vonderheide, Steven D. Leach and a.B.Z. Stanger, EMT and Dissemination Precede Pancreatic Tumor Formation. Cell, 2012. 148: p. 13.

6. Hung D. Tran1, K.L., Michael Kim1, Kun Zhang, Gregory D. Longmore, and David D. Tran, Transient SNAIL1 expression is necessary for metastatic competence in vreast cancer. Cancer Res, 2014. 74(21): p. 11.

7. Christofori, U.C.a.G., CELL ADHESION AND SIGNALLING BY CADHERINS AND IG-CAMS IN CANCER. NATURE REVIEWS | CANCER, 2004. 4(2): p. 15.
8. Raghu Kalluri, R.A.W., *The basics of epithelial-mesenchymal transition*. J Clin Invest, 2009. **119**(6): p. 9.

9. Radisky, L.S.OÆ.D.C., *Matrix metalloproteinases stimulate epithelial-mesenchymal transition during tumor development*. Clin Exp Metastasis, 2008. **25**(2): p. 8.

10. Hye-Yeong Ha, H.-B.M., Myoung-Soo Nam, Jeong-Woong Lee, Zae-Yoong Ryoo, Tae-Hoon Lee, Kyung-Kwang Lee, Byung-Jan So, Hiroshi Sato, Motoharu Seiki, and Dae- Yeul Yu, *Overexpression of Membrane-type Matrix Metalloproteinase-1 Gene Induces Mammary Gland Abnormalities and Adenocarcinoma in Transgenic Mice*. CANCER RESEARCH 2001. **61**: p. 7.

11. Aditya Bardia, I.A.M., Jennifer R. Diamond, Rebecca L. Moroose, Steven J. Isakoff, Alexander N. Starodub, *Efficacy and Safety of Anti-Trop-2 Antibody Drug Conjugate Sacituzumab Govitecan (IMMU-132) in Heavily Pretreated Patients With Metastatic Triple-Negative Breast Cancer*. J Clin Onco, 2017. **35**(19): p. 8.

12. Tinghua Yan, G.Q., Zhonghao Lin, Jianfeng Mao, Jinhua Jiang, Jinan Xiao, Suiwan Lu1, Miaoan Chen, Huaxing Que, Xiangsheng He, Qingwei Zheng, *MMP14 regulates cell migration and invasion through epithelial-mesenchymal transition in nasopharyngeal carcinoma*. Am J Transl Res 2015. **7**(5): p. 9.

13. Yang, Y.-z.K.-p.A.-b.Z.-c.Y.J.-m.L.Y.-l.M.X.B.Z.-x., *MMP-14 overexpression correlates with poor prognosis in non-small cell lung cancer*. Tumor Biol, 2014. **35**(7): p. 7.

14. Thirupandiyur S. Udayakumar, M.L.C., Elisabeth L. Bair, Dorothea C. von Bredow, Anne E. Cress, Raymond B. Nagle, and G. Timothy Bowden, *Membrane Type-1-Matrix Metalloproteinase Expressed by Prostate Carcinoma Cells Cleaves Human Laminin-5 β3 Chain and Induces Cell Migration*. CANCER RESEARCH, 2003. **63**(5): p. 8.

15. Carine MUNAUT, A.s.N., Olivier HOUGRAND, Jean-Michel FOIDART, Jacques BONIVER, and Manuel DEPREZ, *VASCULAR ENDOTHELIAL GROWTH FACTOR EXPRESSION CORRELATES WITH MATRIX METALLOPROTEINASES MT1-MMP, MMP-2 AND MMP-9 IN HUMAN GliOBlastOMAS*. Int. J. Cancer, 2003. **106**(4): p. 8.

16. Hanqiu Zheng, M.S., Yin-Lian Zha, Wenyang Li, Yong Wei, Mario Andres Blanco, Guangwen Ren, Tianhua Zhou, Peter Storz, Hui-Yun Wang, and Yibin Kang, *PKD1 Phosphorylation-Dependent Degradation of SNAIL by SCF-FBXO11 Regulates Epithelial-Mesenchymal Transition and Metastasis*. Cancer Cell 2014. **26**(9): p. 6.

17. Ritu Lakhtakia, A.A., Muhammad Furrukh, Shyam S. Ganguly, *Epithelial Mesenchymal Transition (EMT) in Metastatic Breast Cancer in OmaniWomen*. Cancer Microenvironment, 2017(10): p. 13.

18. Xiao, W.L.L.D.Y.L.L.C.L., *Decreased MT1-MMP in gastric cancer suppressed cell migration and invasion via regulating MMPs and EMT*. 2015. **36**(9): p. 9.

19. Lijuan Pang, Q.L., Shugang Li, Jianwei He, Weiwei Cao, Jiaojiao Lan, Bin Sun, Hong Zou, Chengyan Wang, Ruixue Liu, Cuilei Wei, Yutao Wei6, Yan Qi, Jianming Hu, Weihua Liang, Wen Jie Zhang, Mei Wan7 & Feng Li, *Membrane type 1-matrix metalloproteinase induces epithelial-to-mesenchymal
transition in esophageal squamous cell carcinoma: Observations from clinical and in vitro analyses. SCIENTIFIC REPORTS 2016. 6: p. 11.

20. Andrew D. Rhim, E.T.M., Nicole M. Aiello, Anirban Maitra, Jennifer M. Bailey, Florencia McAllister, Maximilian Reichert, Gregory L. Beatty, Anil K. Rustgi, Robert H. Vonderheide, Steven D. Leach and Ben Z. Stanger, EMT and Dissemination Precede Pancreatic Tumor Formation. Cell, 2012. 148(1): p. 12.

21. Felicity M. Davis1, T.A.S., Erik W. Thompson and Gregory R. Monteith, Targeting EMT in cancer: opportunities for pharmacological intervention. Trends in Pharmacological Sciences, 2014. 35(9).

22. Anna Fantozzi1, D.C.G., Laura Pisarsky, Chantal Heck, Akiko Kunita, Mahmut Yilmaz, Nathalie Meyer-Schaller, Karen Cornille, Ulrike Hopfer, Mohamed Bentires-Alj, and Gerhard Christofori, VEGF-Mediated Angiogenesis Links EMT-Induced Cancer Stemness to Tumor Initiation. Cancer Res, 2014. 74(5): p. 10.

23. Neha Tiwaria, A.G., Marianthi Tatarib, Gerhard Christoforia, EMT as the ultimate survival mechanism of cancer cells. Seminars in Cancer Biology 2012. 22(2): p. 14.

24. Galatea Kallergi, M.A.P., Eleni Politaki, Dimitris Mavroudis, Vassilis Georgoulis and Sophia Agelaki, Epithelial to mesenchymal transition markers expressed in circulating tumour cells of early and metastatic breast cancer patients. Breast Cancer Research 13(3): p. 11.

25. Mario A. Cepedaa, J.J.P., Caitlin L. Evereda, Hon S. Leongb, Sashko Damjanovskia, The cytoplasmic domain of MT1-MMP is dispensable for migration augmentation but necessary to mediate viability of MCF-7 breast cancer cells. Experimental Cell Research, 2017. 350(11): p. 14.

Tables

Table 1 The relationship between MT1-MMP protein expression with the clinicopathological features in breast cancer.
|                     | MT1-MMP expression |
|---------------------|---------------------|
|                     | ++ (%)              | Negative (%) | χ² | P value |
| n=71                |                     |              |    |         |
| Median age          |                     |              |    |         |
| <40                 | 11 (15.6%)          | 25 (35.2%)   |    |         |
| ≥40                 | 11 (15.6%)          | 27 (38.0%)   | 0.199 | 0.655 |
| Tumour size         |                     |              |    |         |
| <2cm                | 3 (1.0%)            | 18 (25.3%)   |    |         |
| 2-5cm               | 27 (38.0%)          | 13 (18.3%)   |    |         |
| ≥5cm                | 9 (12.7%)           | 1 (1.4%)     | 23.563 | 0.000 |
| Estrogen receptor   |                     |              |    |         |
| positive            | 22 (31.0%)          | 21 (29.6%)   |    |         |
| negative            | 16 (22.5%)          | 12 (16.9%)   | 0.244 | 0.621 |
| Progestogen receptor|                     |              |    |         |
| positive            | 23 (32.4%)          | 20 (28.2%)   |    |         |
| negative            | 15 (21.1%)          | 13 (18.3%)   | 0.000 | 0.995 |
| LN metastases       |                     |              |    |         |
| positive            | 21 (29.6%)          | 28 (39.4%)   |    |         |
| negative            | 1 (1.4%)            | 21 (29.6%)   | 10.421 | 0.000 |
| TNM                 |                     |              |    |         |
| T+                  | 50 (70.4%)          | 4 (5.6%)     |    |         |
| T+                  | 16 (22.5%)          | 1 (1.4%)     | 8.2  | 0.017  |

P<0.05 are indicated in bold.

Table 2 The correlation of MT1-MMP expressions with E-cadherin, N-cadherin and vimentin expressions in Breast cancer cases
|             | MMP14 ± or + (%) | MMP14 ++ or +++ (%) | χ²  | P    |
|-------------|------------------|---------------------|-----|------|
| E-Cad       |                  |                     |     |      |
| 弱阳        | 33 (46.5)        | 18 (25.3)           |     |      |
| 强阳        | 12 (16.9)        | 8 (11.3)            | 12.236 | 0.000 |
| N-Cad       |                  |                     |     |      |
| 阴性        | 10 (14.1)        | 17 (23.9)           | 4.410 | 0.036 |
| 阳性        | 20 (28.2)        | 24 (33.8)           |     |      |
| Vimentin    |                  |                     |     |      |
| 阴性        | 7 (23.9)         | 11 (15.5)           | 6.459 | 0.011 |
| 阳性        | 13 (18.3)        | 30 (42.3)           |     |      |

*P<0.05 are indicated in bold.*

**Figures**
Expression of the MT1-MMP and markers of Epithelial-to-Mesenchymal Transition in Breast cancer tissue and adjacent non-cancerous tissues. (A) The MT1-MMP IHC immunoreactivity was in the breast cancer cell membrane and infiltration stroma, and increased expressions in tumor buds (400×). (B) No detectable MT1-MMP expression in the non-cancerous adjacent tissues. (C and D) the representative images of IHC staining for E-cadherin in breast cancer tissue (E) and adjacent tissues (F). The representative images of
IHC staining for N-cadherin (strong positive in breast cancer tissues (E) and weakly positive in non-cancerous tissues (F).

Panel 1

Panel 2

Panel 3

Figure 2

(Panel 1-3). Immunochemical and immunofluorescent staining of MT1-MMP protein expression in MCF-7 cells transfected with MT1-MMP plasmid. Panel 1, A immunofluorescent image showed green fluorescence of the MT1-MMP expression in MCF-7 cells and the transfection efficiency being nearly 70%.
Panel 2, the immunofluorescent staining of MT1-MMP was mainly located on the plasma membrane of MCF-7 cells transfected with the MT1-MMP plasmid. Panel 3, the MCF cells transfected with MT1-MMP plasmids were observed to change the morphology of the cells after 1-week culture, presenting elongated, fibroblast-like cells with less cell-to-cell contact.

**Figure 3**

MT1-MMP, N-cadherin, Vimentin, E-cadherin Western blot tests in MCF-7 cell lines transfected with MT1-MMP plasmid or pcDNA3.1 Vector. β-actin proteins were expressed in both groups, MT1-MMP proteins were detected only in the MCF-7 cells transfected with MT1-MMP plasmid. Compared with the control group, in the MCF-7 transfected with MT1-MMP plasmid group, less expression of E-cadherin but increased expression levels of N-cadherin and vimentin was found. T group: the MCF-7 cells transfected with MT1-MMP plasmid. Con group: the MCF-7 cells transfected with pcDNA3.1 Vector.
Figure 4

(A and B): Migration ability of MCF-7 detected by the wound-healing assay. Panel A: wound-healing assay images in 40× vision field (40×). Panel B: the comparison of migration ability of MCF-7 transfected by pc DNA3.1 victor and MT1-MMP plasmid, respectively. *: P<0.05. LP, low power lens.
Figure 5

Invision of breast cancer cells line MCF-7 transfected by MT1-MMP plasmid detected by Transwell assay (100×). Panel A: Transwell assay image of MCF-7 transfected by empty pc DNA 3.1 plasmid. B: Transwell assay image of MCF-7 transfected by MT1-MMP pc DNA3.1 plasmid showed more MCF-7 cells (methyl violet gentian violet dye) migration. C: the number comparison of MCF-7 cells that have migrated through
the filters in Transwell assay experiments. Numbers were counted from triplicate independent assays bars: standard error of the mean (SEM)**P<0.05.