Clin Oncol Cancer Res (2011) 8: 114–119
DOI 10.1007/s11805-011-0568-6

MTDH Expression in Invasive Micropapillary Carcinoma of the Breast

Jin-yan HAO
Yi-ling YANG
Fang-fang LIU
Shuai LI
Wei-dong LI
Xiao-long QIAN
Estifanos Paulos
Li FU

Department of Breast Cancer Pathology and Research Laboratory of Tianjin Medical University Cancer Institution and Hospital, Key Laboratory of Breast Cancer Research Prevention and Therapy, Tianjin Medical University, Ministry of Education; Key Laboratory of Cancer Prevention and Therapy, Tianjin 300060, China.

Correspondence to: Li FU
Tel: 86-22-2334 0123 ext. 5221
E-mail: fulijyb@hotmail.com

Received April 21, 2011; accepted May 19, 2011

E-mail: editor@cocronline.org
Tel (Fax): 86-22-2352 2919

OBJECTIVE To clarify the expression of MTDH in invasive micropapillary carcinoma of the breast (IMPC) and analyze the relationship between MTDH expression and clinicalpathologic parameters of the IMPC patietns.

METHODS The expression of MTDH protein was analyzed using immunohistochemical staining in 86 cases with IMPC and another 86 cases with invasive ductal carcinoma not otherwise specified (IDC-NOS). The association between MTDH expression and clinicalpathologic parameters of the IMPC patients was analyzed.

RESULTS Immunohistochemical analysis revealed the high expression of MTDH in 64 of the 86 (64/86, 74.4%) IMPC patients and in 45 of the 86 (45/86, 52.3%) IDC-NOS patients. Statistical analysis showed a statistically significant difference in MTDH expression between IMPC and IDC-NOS ($P < 0.05$). In IMPC, the expression of MTDH was correlated with lymph nodes metastasis ($P < 0.05$). The expression of MTDH was negative in normal breast tissue of IMPC and IDC-NOS patients.

CONCLUSION High expression of MTDH is one of the molecular mechanisms, which facilitates lymph node metastasis of IMPC, therefore, the expression level of IMPC plays an important role in lymph node metastasis of IMPC.

KEY WORDS: breast carcinoma, invasive micropapillary carcinoma, metadherin, lymph node metastases.

Introduction

Metadherin (MTDH), also known as astrocyte elevated gene-1 (AEG-1), is a new oncogene found in recent years. Published studies have suggested that MTDH expression is up-regulated in several kinds of tumors, such as breast cancer, prostate cancer, non-small cell lung cancer, squamous cell carcinoma and renal cell carcinoma et al.[1–5] and the level of the MTDH expression is associated with tumor invasion and metastasis. Importantly, MTDH enhances the invasiveness of breast cancer cells by inducing epithelial to mesenchymal transition[6].

Invasive micropapillary carcinoma (IMPC) is a rare and histological special type of breast cancer with high prevalence of lymphovascular invasion, high incidence of lymph node metastases and poor prognosis[7–10]. In the resent study, Li et al.[11] found that the increased proportion of $CD44^+/CD24^{-/low}$ tumor cells and the epithelial-mesenchymal transition may play an important role in tumor invasion and metastasis of breast IMPC.

To our knowledge, evaluation of the expression of MTDH in breast IMPC has never been reported before. For this reason, in this study, the expression of MTDH protein was examined in 86 cases diagnosed as IMPC and in a control group of another 86 cases diagnosed as invasive ductal carcinoma not otherwise specified type (IDC-NOS) using immunohistochemistry, and the association between MTDH expression and lymph node metastasis of IMPC was discussed in this paper. Furthermore, we attempted to further investigate the potential role of MTDH in lymph node metastasis of breast IMPC.

Materials and Methods

Specimens

The paraffin-embedded tumor samples taken from 86 cases with breast IMPC (pure IMPC 23 cases, concurrent IMPC and IDC-NOS 63 cases) and from another 86 cases with IDC-NOS were obtained from the Department of Breast Cancer Pathology and Research Laboratory, Tianjin Medical University Cancer Hospital (Tianjin, China). The patients in both groups were diagnosed during 2008. All the patients were women and their age ranged from 23 to 80 years, with a mean age of 52. None of them had received preoperative radiotherapy or chemotherapy. All the specimens were fixed in 10% formaldehyde and embedded in paraffin. The histopathology was reviewed and then the diagnosis of each case was made and confirmed independently by 3 pathologists[12] based on the 2003 World Health Organization histologic classification of tumors of the breast[13]. The IMPC specimens were divided into 4 groups according to the amount of IMPC presenting in each tumor: IMPC accounting for less than 25% of the whole tumor, for 25%–49%, for 50%–75%, and for more than 75%[11].

Reagents

Rabbit anti-MTDH, mouse anti-ER and PR, kit for immunohistochemical indirect method and DAB substrate kit were all purchased from Beijing Zhongshan Golden Bridge Biotechnology Co. Mouse anti-HER2 (1:800) was purchased from Newmarkers.

Staining methods

Immunohistochemical (IHC) staining was performed using the labeled streptavidin-biotin method (LSAB). The MTDH primary antibody was diluted at 1:500, and the ER, PR, HER2 primary antibody was diluted at 1:150, 1:150 and 1:800, respectively. All antigens were restored with high-pressure cooking in citrate buffer (pH 6.0) for 2.5 min before they were blocked with the serum. The 3.3′-diaminobenzidine (DAB) was used to show the reaction intensity, and hematoxylin was used for counterstain of the sections. Phosphatebuffered saline (PBS) was used instead of primary antibody in the incubation step as a negative control, and the positive controls were used according to the manufacture's recommendation.

Standardization assessments of the results

The degree of immunostaining of formalin-fixed, paraffin-embedded sections was reviewed and scored independently by 2 observers, based on both the proportion of positively stained tumor cells and the intensity of the stain[14,15]. MTDH[2] immunohistochemistry labelled the cytoplasm of tumor cells, the proportion of tumor cells was scored as follows: 0 (no positive tumor cells), 1 (positive tumor cells $< 10\%$), 2 (positive tumor cells between 10%–50%), and 3 (positive tumor cells $> 50\%$). The stain intensity was graded according to the following criteria: 0 (no stain), 1 (weak stain = light yellow), 2 (moderate stain = yellow brown), and 3 (strong stain = brown). The staining of MTDH was calculated as staining intensity score $\times$ proportion of positive tumor cells, and the scores were in the range of 0, 1, 2, 3, 4, 6, and 9. The staining index score of $\geqslant 4$ demonstrated that there was a high expression of MTDH in the tumor and that of ≤ 3 indicated a low expression of MTDH in the tumor, and that of 0 showed none or negative. Cases were scored positive for ER and PR if nuclear immunoreactivity was present in $> 10\%$ of tumor cells[16]. The scoring of HER2 was as follows[17]: Strong, circumferential membrane staining demonstrated in more than 30% of the invasive carcinoma cells was scored as 3+; moderate, circumferential membrane staining demonstrated in 10% or more of the invasive tumor cells or 3+ staining in 30% or less of the invasive tumor cells was scored as 2+ staining; weak and incomplete membrane staining presented in the invasive tumor cells was scored as 1+; and no staining shown in the tumor invasive cells was scored as 0. In the IMPC group, only the staining of the IMPC component was evaluated.

Statistical analysis

Data were analyzed using SPSS 15.0 software package. The methods of statistical analysis were mainly Mann-Whitney U-test, χ^2 test and Spearman's rank correlation test. $P < 0.05$ was considered statistically significant in all the statistical analyses.

Results

Clinical information

Among the 86 cases with IMPC (pure IMPC 23 cases, concurrent IMPC and IDC-NOS 63 cases), there were 32 cases with the IMPC component accounting for less than 25% of the primary tumor, 8 cases with the IMPC component for 25%–49%, 18 cases for 50%–75% and 28 cases for more than75%.

Table 1. The various pathologic parameters between IMPC and IDC-NOS.

Pathologic parameters	IMPC	IDC-NOS	Z	P
Tumor size (cm)			−4.351	0.000
< 2	17	42		
2–5	54	40		
> 5	15	4		
Lymph node metastasis			−5.893	0.000
-	13	50		
+	73	36		

Compared with the IDC-NOS patients in the control group, IMPC patients had larger tumor size (Z=−4.351, P=0.000, Table 1) and a higher rate of lymph node metastasis ($Z=-5.893$, $P=0.000$, Table 1). Among IMPC patients, 73 (73/86, 84.9%) cases exhibited lymph node metastasis. However, only 36 IDC-NOS patients (36/86, 41.9%) had nodal metastasis. Noticeably, of the 73 cases with breast IMPC exhibiting nodal metastasis, 46 cases with their metastatic carcinoma presenting in the lymph nodes was diagnosed as IMPC, 21 cases diagnosed as concurrent IMPC and IDC-NOS and 6 cases diagnosed as IDC-NOS. The rate of lymph node metastasis of the amount of IMPC component in primary tumour varied from < 25%, 25%–49%, 50%–75% and > 75% was 93.8% (30/32), 87.5% (7/8), 72.2% (13/18), 82.1% (23/28), respectively. No correlation was found between the amount of IMPC components in the primary tumor and the rate of lymph node metastasis of the primary tumor ($r_s=-0.157$, $P=0.149$).

Expression of MTDH in IMPC and IDC-NOS

In the 86 cases with IMPC, high expression of MTDH was demonstrated in 64 cases (64/86, 74.4%), low and none expression of MTDH presented in 22 cases (22/86, 25.6%), and the negative expression of MTDH shown in normal breast tissue of the IMPC patients (Fig.1A–D). No significant association was observed between the amount of IMPC components in the primary tumor and the expression level of MTDH ($r_s=-0.078$, $P=0.476$). In contrast, in the control group, only 45 IDC-NOS cases (45/86, 52.3%) presented high expression of MTDH, 41 cases (41/86, 47.7%) showed low or none expression of MTDH. MTDH expression was negative in the normal breast tissue of the IDC-NOS patients (Fig.1E–H). Compared with IDC-NOS, IMPC showed significantly higher expression of MTDH ($\chi^2=9.042$, $P=0.003$, Table 2). Of the 86 cases with IMPC, the rate of lymph node metastasis in the patients exhibiting high expression of MTDH was 90.6% (58/64), and that in the patients exhibiting low or none expression of MTDH was 68.2% (15/22). MTDH expression was positively correlated with lymph node metastasis in the IMPC patients ($P=0.011$). No correlation was found between the expression of MTDH and each of tumor size, ER, PR and HER2 ($P > 0.05$, Table 3).

Table 2. Expression of MTDH in IMPC and IDC-NOS.

Pathologytype	n	MTDH expression		χ^2	P
		Low and none	High		
IMPC	86	22	64	9.042	0.003
IDC-NOS	86	41	45		

Discussion

MTDH, a Ha-ras regulated gene, located at chromosome 8q22, was originally identified by Su et al. as a protein induced in primary human fetal astrocytes infected with HIV-1 or treated with HIV gp120 or tumor necrosis factor-α[18–20]. It contained 12 exons and 11 introns with a full-length of 86,082 bp and pI 9.3[21]. Additional studies have shown that MTDH could promote tumor progression, including transformation, initiation of apoptosis, invasion, metastasis, chemoresistance and angiogenesis through many signaling pathways, such as the phosphatidylinositol-3-kinase/Akt pathway, the Rac/Rho pathway, the Rac/c-Jun-NH2-kinase, and Rac/p38 pathways, et al.[22,23] Our present study demonstrated that the expression level of MTDH was related to lymph node metastasis of IMPC, suggesting that MTDH might promote the invasion and metastasis of tumor cells in IMPC by some signaling pathways.

It has been found that the expression level of MTDH is elevated in many types of human malignancy, including breast cancer, prostate cancer, non-small cell lung cancer, squamous cell carcinoma and renal cell carcinoma et al.[1–5], and its high expression is related to tumor invasion and metastasis. Importantly, some reports on breast cancer have demonstrated that MTDH enhances the invasiveness of breast cancer cells by inducing epithelial to mesenchymal transition (EMT)[6]. Some studies have suggested that EMT could generate breast cancer stem cells[24,25]. Breast cancer cells with a $CD^{44+}/CD^{24-/low}$ phenotype has been associated with stem cell-like characteristics, such as enhanced invasive properties[26,27]. Recently, our study has revealed that $CD^{44+}/CD^{24-/low}$ tumor cells in IMPC

Table 3. The relationship between the expression of MTDH and the various pathologic parameters in IMPC.

Pathologic parameters	n	MTDH expression		r_s	P
		Low and none	High		
Tumor size (cm)				0.023	0.833
<2	17	6 (35.3)	11(64.7)		
2–5	54	11 (20.4)	43(79.6)		
>5	15	5 (33.3)	10(66.7)		
Lymph node metastasis				0.273	0.011
-	13	7(53.8)	6(46.2)		
+	73	15(20.5)	58(79.5)		
ER				0.023	0.835
−	22	6(27.3)	16(72.7)		
+	64	16(25.0)	48(75.0)		
PR				0.140	0.198
−	33	11(33.3)	22(66.7)		
+	53	11(20.8)	42(79.2)		
HER2				0.023	0.833
−/+	65	17(26.2)	48(73.8)		
++/+++	21	5(23.8)	16(76.2)		

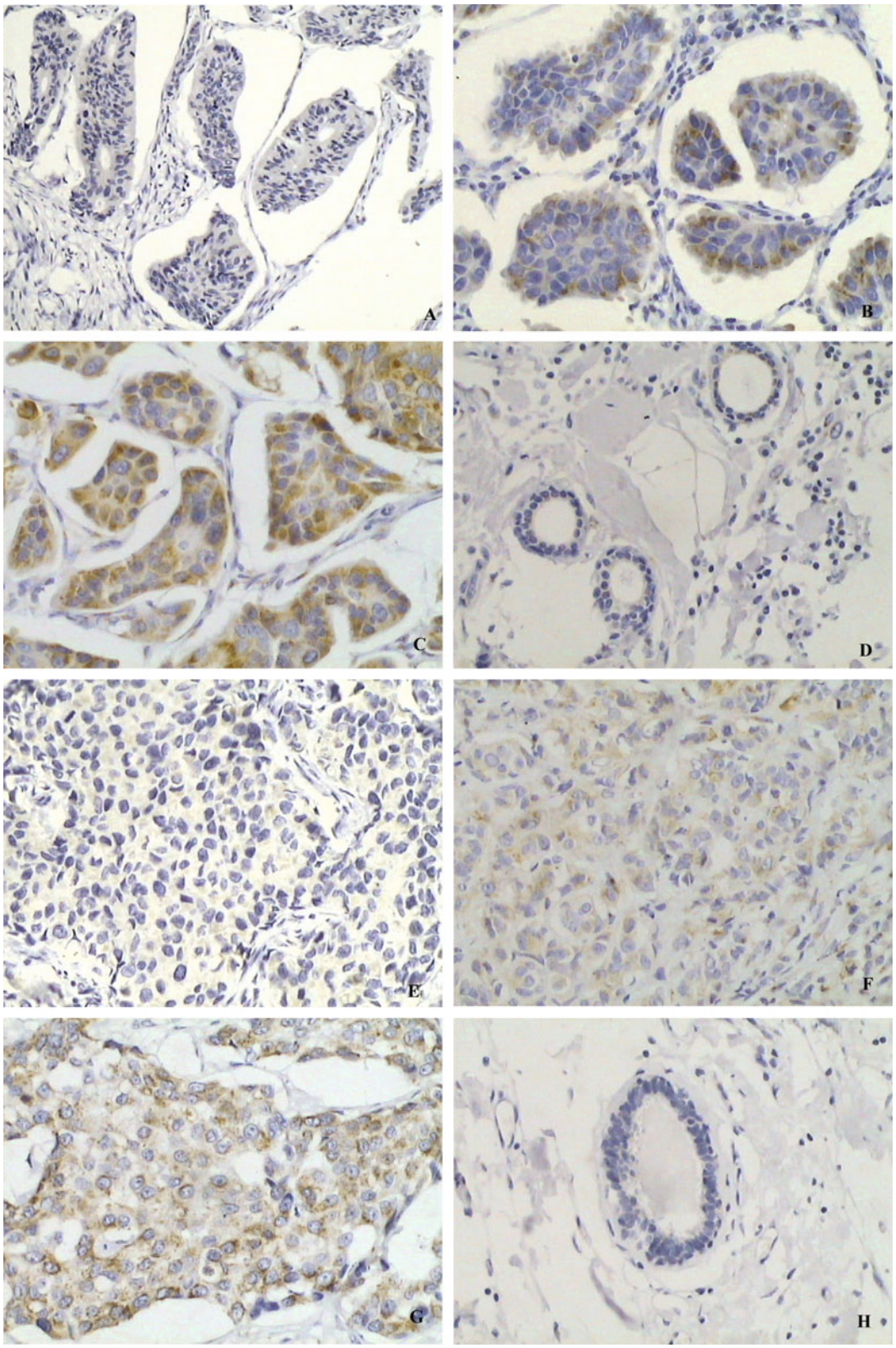

Fig.1. A, Negative expression of the MTDH protein in IMPC (IHC LSAB, ×200); B, Low expression of the MTDH protein in IMPC (IHC LSAB, ×200); C, High expression of the MTDH protein in IMPC (IHC LSAB, ×200); D, Negative expression of the MTDH protein in normal breast tissue of IMPC (IHC LSAB, ×200); E, Negative expression of the MTDH protein in IDC-NOS (IHC LSAB, ×200); F, Low expression of the MTDH protein in IDC-NOS (IHC LSAB, ×200); G, High expression of the MTDH protein in IDC-NOS (IHC LSAB, ×200); H, Negative expression of the MTDH protein in normal breast tissue of IDC-NOS (IHC LSAB, ×200).

may arise through an EMT transdifferentiation process[11]. In addition, the growing pattern of the micropapillary group might be the initial stage of the EMT[28]. It seems reasonable, therefore, to hypothesize that MTDH could promote the invasion and lymph node metastasis of IMPC through EMT. In addition, the previous studies have demonstrated that there were many microvessel in IMPC[29] and the VEGF-C high expression was associated with lymph node metastasis of IMPC[30]. The significance of MTDH expression was in correlation with VEGF in triple-negative breast cancer[31]. MTDH is indeed a direct regulator of angiogenesis by up-regulating key components, such as vascular endothelial growth factor (VEGF), which plays an important role in the process of blood vessel formation[32,33]. Therefore, we assume that MTDH could promote lymph node metastasis of IMPC by up-regulating VEGF. These notions are supported by the findings from this present study, in which MTDH expression was shown much higher in IMPC than in IDC-NOS and the expression level of MTDH was associated with lymph node metastasis of IMPC.

The expressions of MTDH in normal breast tissue, UDH (usual ductal hyperplasia), ADH (atypical ductal hyperplasia), DCIS (ductal carcinoma in situ) and invasive breast cancer are significantly different, suggesting that the overexpression of MTDH may contribute to breast cancer progression[34]. MTDH expression is higher in breast tumors than in normal breast tissue at mRNA and protein level and there is significant association between MTDH expression and each of tumor size, clinical stage and lymph node metastasis[2]. In addition, MTDH expression is higher in renal cell carcinoma than in the paired normal tissue from the same patient and is correlated with tumor grade, clinical stage and TNM classification[5]. Song et al.[35] also found that MTDH expression was markedly correlated with TNM classification and histological differentiation in colorectal carcinoma. The results in our present study also reveals that the expression of MTDH is closely correlated with lymph node metastasis of IMPC. No correlation was found between the expression of MTDH and each of tumor size, ER, PR and HER2 in IMPC. These studies have provided new insights into the potential role of MTDH in the invasion and metastasis of IMPC, and high expression of MTDH is one of the molecular mechanisms, which facilitates lymph node metastasis of IMPC.

It is remarkable that a new report reveals that MTDH DNA vaccine could effectively inhibit the growth and metastasis of breast cancer[36]. This indicates that it has provided new strategies for clinical treatment of breast cancer.

The expression of MTDH is negative in normal breast tissue, and high in breast cancer cells, so it might be a potential diagnostic marker and therapy target in breast cancer. Further studies are needed to investigate large size of the sample that comprises other histological types of breast cancer in order to validate the findings obtained in this present study and to further explore the place of MTDH for the purpose of predicting prognosis and monitoring response to the therapy of the breast cancer patients.

Acknowledgments

This work was supported by National Natural Science Foundation of China (No. 30930038), Program for Changjiang Scholars and Innovative Research Team in University (No. IRT0743), National High Technology Research and Development Program (''863''Program) of China (No.2006AA02A249), National ''973'' program of China (No. 2009CB521700).

Conflict of interest statement

No potential conflicts of interest were disclosed.

References

1 Kikuno N, Shiina H, Urakami S, et al. Knockdown of astrocyte-elevated gene-1 inhibits prostate cancer progression through upregulation of FOXO3a activity. Oncogene 2007; 26:7647–7655.
2 Li J, Zhang N, Song LB, et al. Astrocyte elevated gene-1 is a novel prognostic marker for breast cancer progression and overall patient survival. Clin Cancer Res 2008; 14: 3319–3326.
3 Song L, Li W, Zhang H, et al. Overexpression of AEG-1 significantly associates with tumor aggressiveness and poor prognosis in human non-small cell lung cancer. J Pathol 2009; 219:317–326.
4 Yu C, Chen K, Zheng H, et al. Overexpression of Astrocyte Elevated Gene-1 (AEG-1) Is Associated with Esophageal Squamous Cell Carcinoma (ESCC) Progression and Pathogenesis. Carcinogenesis 2009; 30: 894–901.
5 Chen W, Ke Z, Shi H, et al. Overexpression of AEG-1 in renal cell carcinoma and its correlation with tumor nuclear grade and progression. Neoplasma 2010; 57: 522–529.
6 Li X, Kong X, Huo Q, et al. Metadherin enhances the invasiveness of breast cancer cells by inducing epithelial to mesenchymal transition. Cancer Sci 2011 Mar 3.
7 Nassar H. Carcinomas with micropapillary morphology: clinical significance and current concepts. Adv Anat Pathol 2004; 11: 297–303.
8 Amendoeira I, Magalhaes J, Damasceno M. Invasive micropapillary carcinoma of the breast: are the pure forms more aggressive than the mixed forms? Breast J 2003; 9:337–338.
9 Chen L, Fan Y, Lang RG, et al. Breast carcinoma with micropapillary features: clinicopathologic study and long-term follow-up of 100 cases. Int J Surg Pathol 2008; 16: 155–163.
10 Li YS, Kaneko M, Sakamoto DG, et al. The reversed apical pattern of MUC1 expression is characteristics of invasive micropapillary carcinoma of the breast. Breast Cancer 2006; 13: 58–63.
11 Li W, Liu F, Lei T, et al. The clinicopathological significance of $CD44^{+}/CD24^{-/low}$ and $CD24^{+}$ tumor cells in invasive micropapillary carcinoma of the breast. Pathol Res Pract 2010; 206: 828–834.
12 Fangfang Liu, Ronggang Lang, Jia Wei, et al. Increased expression of SDF-1/CXCR4 is associated with lymph node metastasis of invasive micropapillary carcinoma of the breast. Histopathology 2009, 54, 741–750.
13 Tavassoli FA, Devilee P. World Health Organization classification of tumors. Pathology and genetics of tumors of the breast and female genital organs. Lyon: IARC Press 2003; pp35–36.

14 Song LB, Liao WT, Mai HQ, et al. The clinical significance of twist expression in nasopharyngeal carcinoma. Cancer Lett 2006; 242: 258–265.
15 Fukuoka J, Fuji T, Shih JH, et al. Chromatin remodeling factors and BRM/BRG1 expression as prognostic indicators in non-small cell lung cancer. Clin Cancer Res 2004; 10: 4314–4324.
16 Cui LF, Guo XJ, Wei J, et al. Overexpression of TNF-α and TNFRII in invasive micropapillary carcinoma of the breast: clinicopathological correlations. Histopathology 2008; 53: 381–388.
17 Hanley KZ, Birdsong GG, Cohen C, et al. Immunohistochemical detection of estrogen receptor, progesterone receptor, and human epidermal growth factor receptor 2 expressions in breast carcinomas: comparison on cell block, needle-core, and tissue block preparations. Cancer 2009; 117:279–288.
18 Brown DM, Ruoslahti E. Metadherin, a cell surface protein in breast tumors that mediates lung metastasis. Cancer Cell 2004; 5: 365–374.
19 Su ZZ, Kang DC, Chen Y, et al. Identification and cloning of human astrocyte genes displaying elevated expression after infection with HIV-1 or exposure to HIV-1 envelope glycoprotein by rapid subtraction hybridization, RasH. Oncogene 2002; 21: 3592–3602.
20 Su ZZ, Chen Y, Kang DC, et al. Customized rapid subtraction hybridization (RasH) gene microarrays identify overlapping expression changes in human fetal astrocytes resulting from human immunodeficiency virus-1 infection or tumor necrosis factor-alpha treatment. Gene 2003; 306: 67–78.
21 Kang DC, Su ZZ, Sarkar D, et al. Cloning and characterization of HIV-1-inducible astrocyte elevated gene-1, AEG-1. Gene 2005; 353: 8–15.
22 Rodriguez-Viciana P, Tetsu O, Oda K, et al. Cancer targets in the Ras pathway. Cold Spring Harb Symp Quant Biol 2005; 70: 461–467.
23 Schubbert S, Bollag G, Shannon K. Deregulated Ras signaling in developmental disorders: new tricks for an old dog. Curr Opin Genet Dev 2007; 17: 15–22.
24 Mani SA, Guo W, Liao MJ, et al. The epithelial mesenchymal transition generates cells with properties of stem cells.Cell 2008; 133: 704–715.
25 Morel AP, Lièvre M, Thomas C, et al. Generation of breast cancer stem cells through epithelial-mesenchymal transition. PLoS One 2008; 3: e2888.
26 Ponti D, Costa A, Zaffaroni N, et al. Isolation and in vitro propagation of tumorigenic breast cancer cells with stem/progenitor cell properties. Cancer Res 2005; 65: 5506–5511.
27 Sheridan C, Kishimoto H, Fuchs RK, et al. CD44+/CD24- breast cancer cells exhibit enhanced invasive properties: an early step necessary for metastasis. Breast Cancer Res 2006; 8: R59.
28 Fan Y, Lang RG, Wang Y, et al. Relationship between expression of cell adhesion molecules and metastatic potential in invasive micropapillary carcinoma of breast. Zhonghua Bing Li Xue Za Zhi 2004; 33: 308–311.
29 Cui LF, Guo XJ, Wei J, et al. Significance of interleukin-1beta expression and microvascular density in invasive micropapillary carcinoma of breast. Zhonghua Bing Li Xue Za Zhi 2008; 37: 599–603.
30 Guo X, Chen L, Lang R, et al. Invasive micropapillary carcinoma of the breast association of pathologic features with lymph node metastasis. Am J Clin Pathol 2006; 126: 740–746.
31 Li C, Li R, Song H, et al. Significance of AEG-1 expression in correlation with VEGF, microvessel density and clinicopathological characteristics in triple-negative breast cancer. J Surg Oncol 2011; 103:184–192.
32 Sarkar D, Park ES, Emdad L, et al. Molecular basis of nuclear factor-kappaB activation by astrocyte elevated gene-1. Cancer Res 2008; 68: 1478–1484.
33 Yoo BK, Emdad L, Su ZZ, et al. Astrocyte elevated gene-1 regulates hepatocellular carcinoma development and progression. J Clin Invest 2009; 119: 465–477.
34 Su P, Zhang Q, Yang Q. Immunohistochemical analysis of Metadherin in proliferative and cancerous breast tissue. Diagn Pathol 2010; 5: 38.
35 Song H, Li C, Li R, et al. Prognostic significance of AEG-1 expression in colorectal carcinoma. Int J Colorectal Dis 2010; 25:1201–1209.
36 Qian BJ, Yan F, Li N, et al. MTDH/AEG-1-based DNA vaccine suppresses lung metastasis and enhances chemosensitivity to doxorubicin in breast cancer. Cancer Immunol Immunother 2011 Mar 13.