Int J Clin Exp Pathol 2016;9(9):8817-8823
www.ijcep.com /ISSN:1936-2625/IJCEP0030723

Review Article

Correlation analysis of metadherin expression and breast cancer progression

Xin Wang*, Zhongzhao Wang*, Wenyan Wang, Jie Wang, Jiaqi Liu, Zeyu Xing, Xiang Wang, Jidong Gao

*Department of Breast Surgery, National Cancer Center, Cancer Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing, China. *Equal contributors.*

Received April 17, 2016; accepted July 19, 2016; Epub September 1, 2016; Published September 15, 2016

Abstract: Metadherin (MTDH) plays an important role in various malignant tumors' occurrence and development. This study investigated the relationship between MTDH expression and primary breast cancer clinicopathological characteristics, aiming to clarify the role of MTDH in breast cancer prognosis. A total of 156 cases of primary breast cancer patients in our hospital were selected. MTDH expression in cancer tissue was detected by qRT-PCR and immunohistochemistry. The correlation relationship between MTDH expression and patients' clinicopathological characteristics were analyzed. The relationship between patient survival rate and MTDH expression was measured by Kaplan-Meier method. MTDH presented highly consistency at mRNA level and protein level. MTDH highly expression was associated with breast cancer metastasis, ER expression, PR expression, and recurrence. It was also related to patient survival rate. MTDH expression was associated with breast cancer metastasis, ER expression, PR expression, recurrence, and survival rate. It could be treated as a molecular marker for breast cancer.

Keywords: MTDH, breast cancer, tumor progression, prognosis

Introduction

In recent decades, breast cancer showed gradually increased incidence worldwide. It also accounts for the leading morbidity among female malignant tumors in our country [1]. Following the improvement of breast cancer early screening and treatment, breast cancer treatment has obtained significant progress [2]. Due to the complexity of breast cancer causes, breast cancer patients may present different therapeutic effect to the same treatment strategy. Therefore, finding the effective molecular markers to evaluate prognosis is of great significance to improve the therapeutic effect of breast cancer [3].

Metadherin (MTDH) is widely distributed on the membrane of vertebrate cells [4]. At present, numerous studies found that MTDH was associated with multiple types of cancers' proliferation and metastasis, such as lung cancer, gastric cancer, and hepatic cancer, etc. [5-8]. MTDH mediates cancer cell adhering to the vascular wall, thus promoting cancer cells metastasis [9]. Therefore, MTDH expression in cancer cells is usually higher than that in normal cells [10]. In this study, we intends to explore the relationship between MTDH expression and the clinical pathological features of primary invasive breast cancer, so as to further clarify the role of MTDH in breast cancer diagnosis and prognosis.

Materials and methods

Objects selection

A total of 156 breast cancer patients between Jan 2008 and Mar 2015 in the oncology department of Cancer Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College were enrolled. The mean age was 53.6 ± 8.7 years old. There were 47 cases in stage I, 54 cases in stage II, and 26 cases in stage III according to TNM grading. All the selected patients were primary and did not receive any therapy before surgery.

This study has been pre-approved by the ethical committee of Cancer Hospital, Chinese Academy of Medical Sciences and Peking Union

Table 1. Primer sequence

Gene		Sequence	Tm
β-actin	F	5'- CGTACCACTG GCATCGTGAT -3'	55. 9°C
	R	5'- GTGTTGGCGTACAGGTCTTTG -3'	55.7°C
MTDH	F	5'-TCCGAGAAGCCCAAACCAAAT-3'	54.4°C
	R	5'-CTTCACCCTCAGCCACTTCAA-3'	54.5°C

Medical College. All subjects have signed the consent forms before recruitment in this study.

qRT-PCR

Tissue RNA was extracted using human total RNA extraction kit (QIAGEN) according to the manual. RNA was reverse transcripted to cDNA at 37°C for 2 h. RT-PCR primers for MTDH mRNA were designed based no its sequence (Genebank, NM_178812) (**Table 1**). PCR reaction was performed on Mx3000p real time PCR amplifier (Agilent) using the RT-PCR kit (TianGen). PCR reaction was consisted of 95°C for 5 min, followed by 30 cycles of 95°C for 1 min, 58°C for 30 s, and 72°C for 1 min. The results were calculated by $2^{-\Delta\Delta Ct}$ method [11].

Immunohistochemistry

Immunohistochemistry was applied to detect MTDH expression in breast cancer tissue. The tissue was fixed by paraffin embedding and sliced. Next, the paraffin section was dewaxed, hydrated, and antigen repair. Then the section was incubated in rabbit anti human MTDH monoclonal primary antibody (1:100) and biotin tagged secondary antibody. After HRP-tagged streptavidin incubation, the section was washed and developed. The section was sealed after hematoxylin redyeing and observed under microscope.

The cytoplasmic staining degree under high power field of vision was scored. No obvious staining was considered as 0, shallow staining was read as 1, middle staining was considered as 2, and deep staining was treated as 3. The cell with score ≥ 2 was defined as MTDH highly expressed cell. Tissue sample with positive cell number greater than 10% of the total cells were treated as MTDH highly expressed sample.

ER, PR, and HER-2 detection

Estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER-2) expressions in metastatic breast cancer cells were detected using immunohistochemistry. Mouse anti human ER monoclonal antibody, mouse anti human PR monoclonal antibody, and mouse anti human HER-2 monoclonal antibody (DAKO) at 1:500 were applied for detection.

Result interpretation: for ER and PR detection, brown particle appeared in cell nucleus was defined as ER or PR positive. For HER-2 detection, brown cell surface was treated as HER-2 positive. The ratio of positive cell number and total cell number was considered as positive rate. According to positive rate, positive rate at 0% was considered as 0, at 1-30% was treated as 1, at 31-70% was read as 2, and at 71-100% was treated as 3. According to staining degree, no obvious staining was considered as 0, shallow staining was read as 1, middle staining was considered as 2, and deep staining was treated as 3. Total score was the sum of two terms. Score > 0 was considered as positive for ER and PR, while score ≥ 5 was treated as positive for HER-2.

Statistical analysis

Statistical analysis was performed on JMP10.0 software. Correlation relationship between MTDH expression and clinicopathological features were tested by chi-square test and Fisher exact test. Patient survival rate was drawn through Kaplan-Meier method. $P < 0.05$ was considered as statistical significance.

Results

Immunohistochemistry detection of MTDH expression in breast cancer

MTDH expression in breast cancer was detected by immunohistochemistry. After staining, the tissue was divided into four groups according to staining degree (**Figure 1**). Only the score ≥ 2 was considered as MTDH highly expressed cells, and the samples with positive cell ratio > 10% were treated as positive sample. Forty-two patients showed positive among all 156 patients (26.9%).

qRT-PCR detection of MTDH expression in breast cancer

MTDH mRNA expression level in breast cancer tissue was detected by qRT-PCR. The results in positive samples and negative samples judged by immunohistochemistry were compared

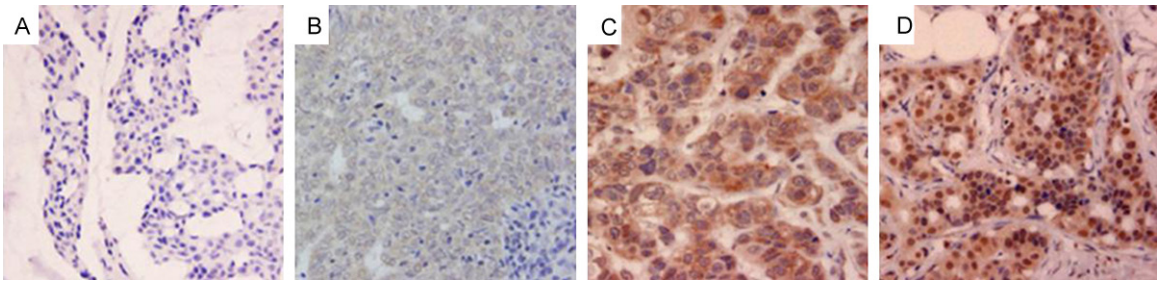

Figure 1. MTDH expression in breast cancer tissue (×400). A. No cytoplasmic staining (score 0); B. Shallow cytoplasmic staining (score 1); C. Middle cytoplasmic staining (score 2); D. Deep cytoplasmic staining (score 3).

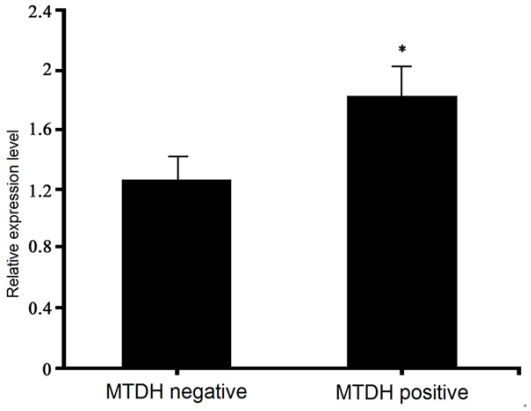

Figure 2. MTDH mRNA relative expression in breast cancer tissue. *P<0.05, compared with MTDH negative group.

(**Figure 2**). It was showed that MTDH mRNA relative expression in negative samples judged by immunohistochemistry was 1.27 ± 0.15, which was significantly lower than that in positive samples as 1.84 ± 0.21 ($P < 0.05$), suggesting that MTDH level was in consistent between protein and mRNA level.

ER, PR, and HER-2 expressions detection

ER, PR, and HER-2 expressions in breast cancer tissue were tested by immunohistochemistry. Brown particle appeared in cell nucleus was defined as ER or PR positive. Brown cell surface was treated as HER-2 positive (**Figure 3**).

Correlation relationship between MTDH expression and clinicopathological features

The relationship between MTDH expression and clinicopathological features were analyzed (**Table 2**). It was found that MTDH high expression was correlated with multiple clinical factors, including age, lymph node metastasis, ER positive expression, PR positive expression, recurrence, and distant metastasis ($P < 0.05$). On the contrary, MTDH high expression showed no correlation with tumor diameter, TNM grading, and HER-2 expression ($P > 0.05$).

Correlation between MTDH expression and prognosis

The patients received postoperative follow-up for average 4.6 (0.6-8.3) years. Kaplan-Meier method was applied to draw the survival curve (**Figure 4**). It was revealed that the disease free survival rate, distant metastasis free survival rate, and total survival rate in MTDH positive patients were obviously lower than that in MTDH negative patients ($P < 0.05$), indicating that MTDH high expression was correlated with breast cancer poor prognosis.

Discussion

Tumor malignant procession refers to the process that multiple gene mutations caused tumor cell proliferation and metastasis [11]. The expression pattern of genes that are closely related to tumor malignant progression became the hotspot in recent years. This article tested MTDH expression in breast cancer patients, analyzed its relationship with multiple clinical pathologic features, and calculated its correlation with disease free survival rate, distant metastasis free survival rate, and total survival rate. The results concluded that MTDH expression in breast cancer patients was associated with tumor malignant progression and poor prognosis.

MTDH is a human cell membrane protein gene discovered and cloned in recent years. Following tumor molecular biology development, more and more research data revealed that MTDH was closely related to various human malignant tumors' behaviors, such as

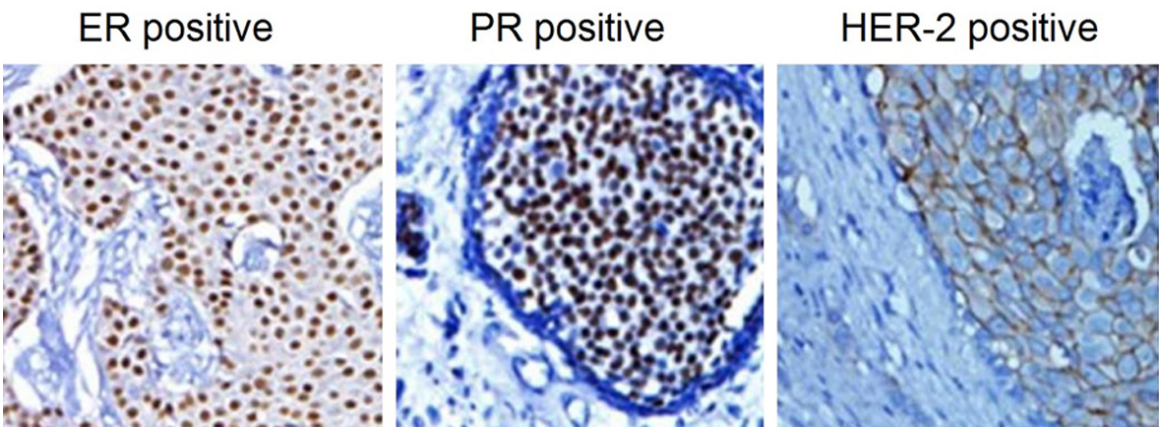

Figure 3. ER, PR, and HER-2 expressions detected by immunohistochemistry.

Table 2. Correlation relationship between MTDH expression and clinicopathological features

	MTDH negative expression (n=114)	MTDH positive expression (n=42)	*P* value
Age (year)			
≥52	70 (61.4%)	33 (78.6%)	<0.01
<52	44 (38.6%)	9 (21.4%)	
Gender			
Male	0 (0%)	0 (0%)	>0.05
Female	114 (100%)	42 (100%)	
TNM grading			
T I	29 (25.4%)	6 (14.3%)	>0.05
T II	51 (44.7%)	23 (54.8%)	
T III	34 (29.9%)	13 (31.0%)	
Tumor diameter (cm)			
≤2	34 (29.8%)	9 (21.4%)	>0.05
2.1-5.0	67 (58.8%)	25 (60.0%)	
≥5.1	13 (11.4%)	8 (18.6%)	
Lymph node metastasis			
With	43 (37.7%)	22 (52.4%)	<0.05
Without	71 (62.3%)	20 (47.6%)	
ER			
Positive	89 (78.1%)	18 (42.9%)	<0.01
Negative	25 (21.9%)	24 (57.1%)	
PR			
Positive	68 (59.6%)	17 (40.5%)	<0.05
Negative	46 (40.4%)	25 (59.5%)	
HER-2			
Positive	24 (21.1%)	12 (28.6%)	>0.05
Negative	90 (78.9%)	30 (71.4%)	
Recurrence			
Yes	19 (16.7%)	16 (38.1%)	<0.05
No	95 (83.3%)	26 (61.9%)	
Distant metastasis			
Yes	15 (13.2%)	12 (28.6%)	<0.05
No	99 (86.8%)	30 (71.4%)	

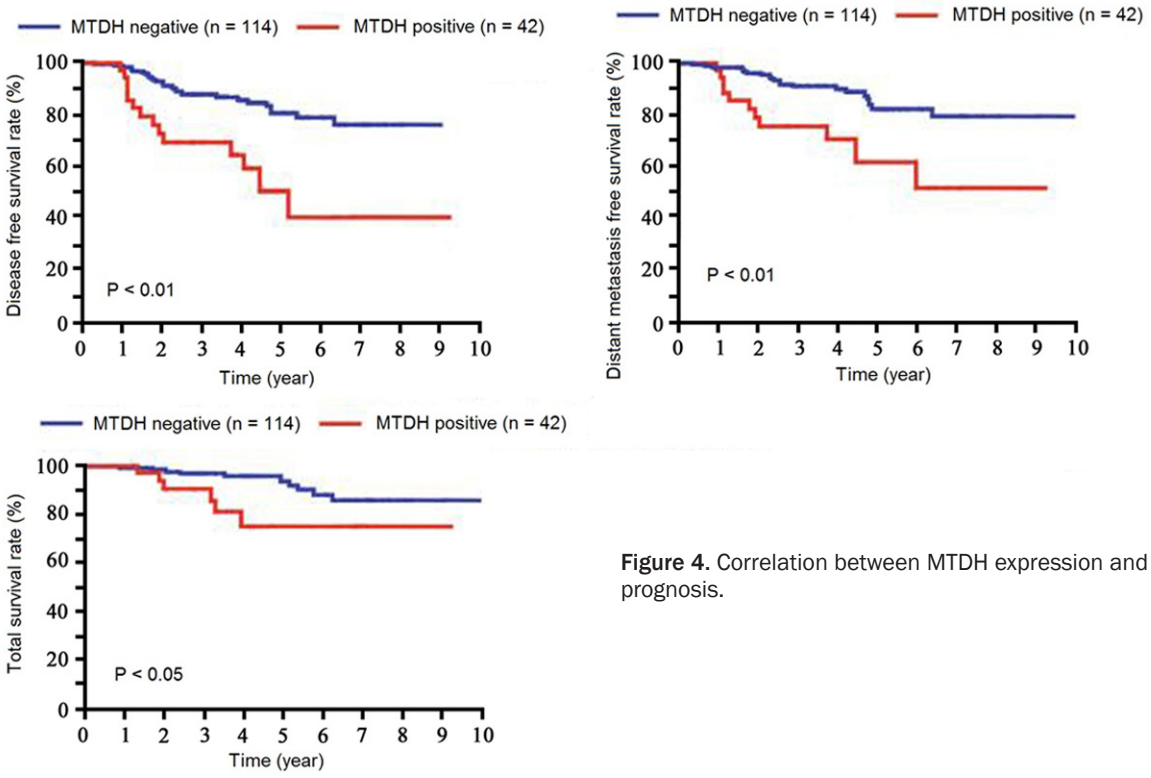

Figure 4. Correlation between MTDH expression and prognosis.

occurrence, malignant progression, and drug resistance [6, 8, 12]. MTDH can regulate the regulatory factor of the ras proto-oncogene, and can activate NF-κB signaling pathway and PI3K/AKT signaling pathway [13]. Among them, as a nucleus factor, NF-κB can regulate various apoptosis inhibiting gene expressions on the transcriptional level, including XIAP, cIAP1, and Bcl-xL [14-16], thus suppressing cell apoptosis. PI3K/AKT has been confirmed the role in promoting cell division and inhibiting cell apoptosis [17, 18]. Therefore, MTDH gene upregulation in cancer cells has important significance in activating cancer cells proliferation and inhibiting apoptosis.

This study found that breast cancer patients with MTDH high expression presented higher rate of postoperative lymph node metastasis, distant metastasis, and recurrence compared with MTDH negative patients. Thus, we speculate that MTDH may promote breast cancer metastasis. It was demonstrated that MTDH can mediate the adhesion effect between cancer cells and vascular epithelial cells, also can promote the cancer engraftment in target organs, thus affecting the cancer cells malignant progression [19, 20]. However, some scholars found that MTDH expression showed no significant relationship with tumor cell's ability to penetrate vascular epithelial tissue [21]. The specific molecular mechanism of MTDH in the process of malignant tumor metastasis still needs further in-depth investigation.

Breast cancer has the highest incidence among female malignant tumors. Although the treatment of breast cancer has made great progress, some patients still show poor prognosis [1]. Finding effective molecular markers to predict prognosis of breast cancer is of great significance in improving the therapeutic effect and relieving patient's pain. This study analyzed the relationship between MTDH expression and corresponding clinical pathological characteristics, and the correlation relationship of MTDH expression with breast cancer poor prognosis and malignant progression, which provides theoretical basis for breast cancer treatment and prognosis evaluation.

Conclusion

MTDH presented highly consistency at mRNA level and protein level. MTDH highly expression was associated with breast cancer metastasis,

ER expression, PR expression, and recurrence. It was also related to patient survival rate.

Acknowledgements

This project supported by the youth project of the capital health development and scientific research (NO. 2016-4-4026).

Disclosure of conflict of interest

None.

Address correspondence to: Drs. Xiang Wang and Jidong Gao, Department of Breast Surgery, National Cancer Center, Cancer Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College, Panjiayuan South Lane 17, Beijing, China. Tel: +86-010-87787130; Fax: +86-010-87787130; E-mail: xiangw@vip.sina.com (XW); ab168@cicams.ac.cn (JDG)

References

[1] Fan L, Strasser-Weippl K, Li JJ, St Louis J, Finkelstein DM, Yu KD, Chen WQ, Shao ZM and Goss PE. Breast cancer in China. Lancet Oncol 2014; 15: e279-289.

[2] Scott JM, Jones LW, Hornsby WE, Koelwyn GJ, Khouri MG, Joy AA, Douglas PS and Lakoski SG. Cancer therapy-induced autonomic dysfunction in early breast cancer: implications for aerobic exercise training. Int J Cardiol 2014; 171: e50-51.

[3] DeMichele A, Clark AS, Tan KS, Heitjan DF, Gramlich K, Gallagher M, Lal P, Feldman M, Zhang P, Colameco C, Lewis D, Langer M, Goodman N, Domchek S, Gogineni K, Rosen M, Fox K and O'Dwyer P. CDK 4/6 inhibitor palbociclib (PD0332991) in Rb+ advanced breast cancer: phase II activity, safety, and predictive biomarker assessment. Clin Cancer Res 2015; 21: 995-1001.

[4] Yuan R, Wang K, Hu J, Yan C, Li M, Yu X, Liu X, Lei J, Guo W, Wu L, Hong K and Shao J. Ubiquitin-like protein FAT10 promotes the invasion and metastasis of hepatocellular carcinoma by modifying beta-catenin degradation. Cancer Res 2014; 74: 5287-5300.

[5] Yao Y, Gu X, Liu H, Wu G, Yuan D, Yang X and Song Y. Metadherin regulates proliferation and metastasis via actin cytoskeletal remodelling in non-small cell lung cancer. Br J Cancer 2014; 111: 355-364.

[6] Haug S, Schnerch D, Halbach S, Mastroianni J, Dumit VI, Follo M, Hasenburg A, Kohler M, Dierbach H, Herzog S, Proske A, Werner M, Dengjel J, Brummer T, Lassmann S, Wasch R and Zeiser R. Metadherin exon 11 skipping variant enhances metastatic spread of ovarian cancer. Int J Cancer 2015; 136: 2328-2340.

[7] Tang Y, Liu X, Su B, Zhang Z, Zeng X, Lei Y, Shan J, Wu Y, Tang H and Su Q. microRNA-22 acts as a metastasis suppressor by targeting metadherin in gastric cancer. Mol Med Rep 2015; 11: 454-460.

[8] Gong Z, Liu W, You N, Wang T, Wang X, Lu P, Zhao G, Yang P, Wang D and Dou K. Prognostic significance of metadherin overexpression in hepatitis B virus-related hepatocellular carcinoma. Oncol Rep 2012; 27: 2073-2079.

[9] Hu G, Wei Y and Kang Y. The multifaceted role of MTDH/AEG-1 in cancer progression. Clin Cancer Res 2009; 15: 5615-5620.

[10] Tang J, Shen L, Yang Q and Zhang C. Overexpression of metadherin mediates metastasis of osteosarcoma by regulating epithelial-mesenchymal transition. Cell Prolif 2014; 47: 427-434.

[11] Semenza GL. Molecular mechanisms mediating metastasis of hypoxic breast cancer cells. Trends Mol Med 2012; 18: 534-543.

[12] Zhang N, Wang X, Huo Q, Li X, Wang H, Schneider P, Hu G and Yang Q. The oncogene metadherin modulates the apoptotic pathway based on the tumor necrosis factor superfamily member TRAIL (Tumor Necrosis Factor-related Apoptosis-inducing Ligand) in breast cancer. J Biol Chem 2013; 288: 9396-9407.

[13] Zhang J, Zhang Y, Liu S, Zhang Q, Wang Y, Tong L, Chen X, Ji Y, Shang Q, Xu B, Chu M and Wei L. Metadherin confers chemoresistance of cervical cancer cells by inducing autophagy and activating ERK/NF-kappaB pathway. Tumour Biol 2013; 34: 2433-2440.

[14] Galban S and Duckett CS. XIAP as a ubiquitin ligase in cellular signaling. Cell Death Differ 2010; 17: 54-60.

[15] Jiang C and Lin X. Regulation of NF-kappaB by the CARD proteins. Immunol Rev 2012; 246: 141-153.

[16] Go HS, Seo JE, Kim KC, Han SM, Kim P, Kang YS, Han SH, Shin CY and Ko KH. Valproic acid inhibits neural progenitor cell death by activation of NF-kappaB signaling pathway and up-regulation of Bcl-XL. J Biomed Sci 2011; 18: 48.

[17] Tan W, Gu Z, Shen B, Jiang J, Meng Y, Da Z, Liu H, Tao T and Cheng C. PTEN/Akt-p27(kip1) Signaling Promote the BM-MSCs Senescence and Apoptosis in SLE Patients. J Cell Biochem 2015; 116: 1583-1594.

[18] Ying J, Xu Q, Liu B, Zhang G, Chen L and Pan H. The expression of the PI3K/AKT/mTOR pathway in gastric cancer and its role in gastric cancer prognosis. Onco Targets Ther 2015; 8: 2427-2433.

[19] Sarkar D and Fisher PB. AEG-1/MTDH/LYRIC: clinical significance. Adv Cancer Res 2013; 120: 39-74.

[20] Tokunaga E, Nakashima Y, Yamashita N, Hisamatsu Y, Okada S, Akiyoshi S, Aishima S, Kitao H, Morita M and Maehara Y. Overexpression of metadherin/MTDH is associated with an aggressive phenotype and a poor prognosis in invasive breast cancer. Breast Cancer 2014; 21: 341-349.

[21] Zhou Z, Deng H, Yan W, Huang H, Deng Y, Li Y and Tian D. Expression of metadherin/AEG-1 gene is positively related to orientation chemotaxis and adhesion of human hepatocellular carcinoma cell lines of different metastatic potentials. J Huazhong Univ Sci Technolog Med Sci 2012; 32: 353-357.