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Original Article

ADAM12 expression predicts clinical outcome in estrogen receptor-positive breast cancer

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Abstract: Objectives: Our study was aimed to make sure whether *ADAM12* could serve as a prognostic biomarker of estrogen receptor (ER) -positive breast cancer. Methods: 127 patients with ER-positive breast cancer were included in the present study. The level of *ADAM12* was assayed through real-time quantitative PCR (RT-qPCR). Levels of *ADAM12* in tumor tissues and adjacent normal tissues were compared with paired t-test. The association of *ADAM12* expression with clinical characteristics was analyzed via χ^2 test. Kaplan-Meier survival curve was used to evaluate the role of *ADAM12* expression in overall survival (OS) of patients. Cox-regression analysis was performed to judge if *ADAM12* could serve as a prognostic marker in breast cancer. Results: The level of *ADAM12* was upregulated in tumor tissues of breast cancer compared to that of adjacent normal tissues ($P < 0.05$). The expression of *ADAM12* was closely related to the Ki-67 and HER2 status ($P < 0.05$ for both). The results of Kaplan-Meier survival curve showed that patients with higher level of *ADAM12* exhibited shorter survival time compared to that of low level of *ADAM12* ($P < 0.001$). Cox regression analysis showed that *ADAM12* might be a biomarker in predicting prognosis of patients with ER-positive breast cancer (HR = 7.116, 95% CI = 3.329-15.212). Conclusion: *ADAM12* appears to be a prognostic marker in ER-positive breast cancer.

Keywords: ADAM12, clinical outcome, estrogen receptor, breast cancer

Introduction

Breast cancer is a leading cause of cancer-related death in women [1], which is characterized by diverse clinical behavior and outcomes [2]. The newly diagnosed patients are commonly presented with early-stage breast cancer, however 20% of them will suffer recurrence in 10 years after diagnosis [3]. In recent years, despite the advances in early diagnosis and treatment therapy, there are still many patients died from cancer recurrence and metastasis. Until now, there has been many studies that proposed biomarkers for tumor behavior and clinical outcome in breast cancer [4-8], however, the identification of new markers is still necessary [9]. Among the factors related with cancer progression, abnormal genes expression has attracted a lot of attention, some of which have been demonstrated as independent biomarkers for breast cancer.

ADAMs, a group of membrane-associated metalloproteinases, plays an important role in regulating integrin-mediated cell adhesion, cell signal transduction and proteolytic release [10-12]. The family contains 20 members, 9 out of which are produced beyond the reproductive system: ADAM8, -9, -10, -12, -15, -17, -19, -28 and -33. The expression of *ADAM12* is upregulated in various cancers [13-15]. Moreover, *ADAM12* has been demonstrated as a susceptibility gene of breast cancer [16]. Fröhlich et al. reported that overexpression of *ADAM12* accelerated the progression of breast cancer [17]. The related study also suggested that the level of *ADAM12* was associated with disease status and pathologic stage in breast cancer [14, 17]. However, there are no studies investigating the effects of *ADAM12* expression on the survival of patients with breast cancer.

Our study was aimed to ensure if *ADAM12* expression served as a prognostic biomarker in

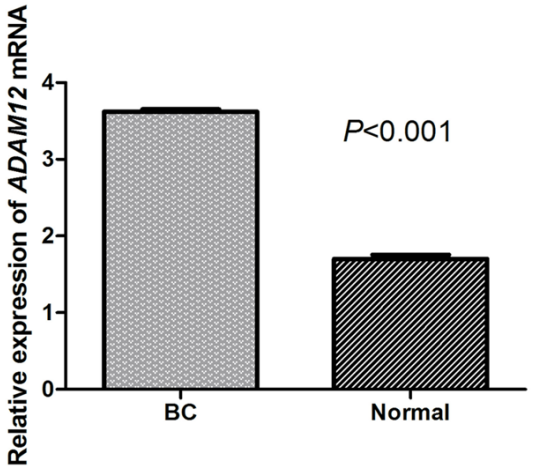

Figure 1. The expression level of *ADAM12* in tumor tissues and adjacent normal tissues of ER-positive breast cancer. The level of *ADAM12* in tumor tissues was significantly higher than that of normal tissues ($P < 0.001$). BC indicates breast cancer.

breast cancer. The expression level of *ADAM12* in serum and tissues (tumor tissues and adjacent normal tissues) were tested with real-time quantitative PCR (RT-qPCR). Kaplan-Meier and Cox analyses were conducted to the role of *ADAM12* in breast cancer.

Materials and methods

Participants

In this research, 205 women from Affiliated Central Hospital of Huzhou Teachers College with breast cancer were selected. Among them, 76 individuals were diagnosed as estrogen receptor (ER) negative and 129 were ER positive. 129 women with ER positive breast cancer were included. They were aged 25-75 years old at the time of diagnosis. The patients who had metastatic disease, tumor history and received neoadjuvant chemotherapy were excluded. After the selection, 127 patients were selected.

Pathological information was collected: primary tumor size, Ki-67 status and HER2 status. Before surgery, the blood samples of each individual were collected. Meanwhile, 127 tumor samples and adjacent normal samples were also collected for further analysis. The follow-ups were performed after the treatments. In the survey, survival time, date of death and date of last follow-up were recorded. The overall survival time was defined from the time as treatment began to death time or date of last follow-up.

All the participants signed the written consent before the study and the study was approved by The Ethical Committee of the hospital.

RT-qPCR assay

Total RNA was extracted from serum and tissue samples by Qiagen DNeasy Blood & Tissue Kit, followed by cDNA synthesis with First Strand cDNA Synthesis Kit (Thermo Scientific). *GAPDH* was used as internal control. The expression of *ADAM12* was analyzed with Applied Biosystems 7500 real-time PCR system (Applied Biosystems, Foster City, CA, USA). The adopted calculation method was $2^{-\Delta\Delta CT}$. All the samples were tested in triplicate.

Statistics

All the analysis was completed in SPSS 12.0. The differences in the level of *ADAM12* between tumor tissues and adjacent normal tissues were compared with paired t-test. The relationship of expression of *ADAM12* with clinical features was analyzed via χ^2 test. Kaplan-Meier analysis was adopted to evaluate the effects of *ADAM12* expression on the overall survival (OS) of patients with breast cancer. Whether *ADAM12* could serve as a prognostic marker in breast cancer was estimated by Cox-regression analysis. The figures were finished in Graphpad prism 5. All the tests were two-tailed, and $P < 0.05$ indicated that there was significant difference.

Results

The expression level of ADAM12 in serum and tissues

The level of *ADAM12* in serum and tissues were tested with RT-qPCR technology. The relative content of *ADAM12* in serum was 3.74. It was obvious that *ADAM12* expression level was higher in tumor tissues compared to that of adjacent normal tissues ($P < 0.05$) (**Figure 1**).

Basic characteristic of patients with ER-positive breast cancer

The clinical characteristics of patients with ER-positive breast cancer were listed in **Table 1**. The patients were divided into two groups

Table 1. Relationship between *ADAM12* expression and clinical features of patients

Characteristics	Case NO.	Expression		χ^2	*P* value
		High	Low		
Age				0.961	0.327
≤ 49	60	41	19		
> 49	67	51	16		
Family history				1.298	0.255
Yes	72	55	17		
No	55	37	18		
Tumor size				0.339	0.561
≤ 3.5	82	58	24		
> 3.5	45	34	11		
Ki-67				4.958	0.026
Positive	71	57	14		
Negative	56	35	21		
HER2				4.013	0.045
High	76	60	16		
Low	51	32	19		

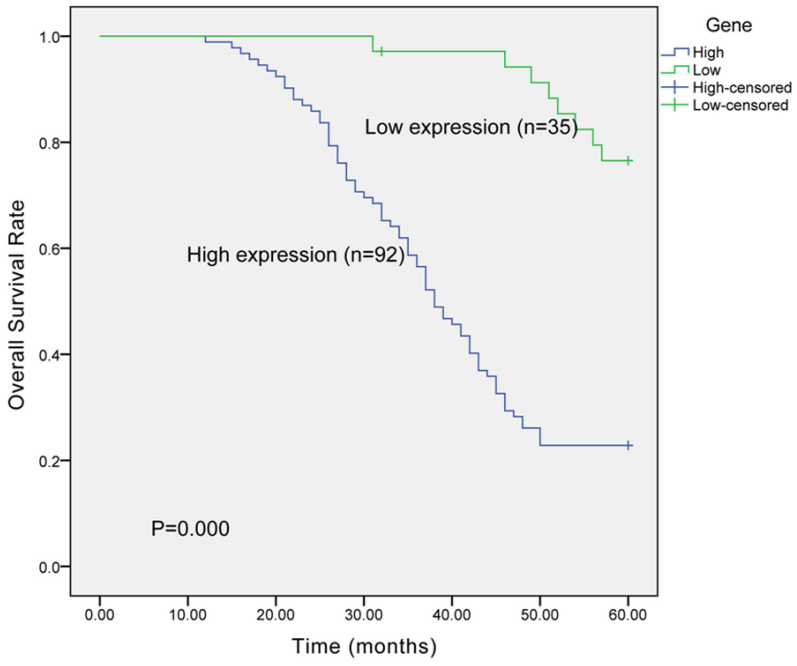

Figure 2. Kaplan-Meier survival curve. Increased expression of ADAM12 predicted worse clinical outcome of patients with ER-positive breast cancer.

according to the level of *ADAM12*. There were no obvious differences in age, family history and tumor size between two groups ($P > 0.05$ for all). While, it turned out that *ADAM12* level was significantly related with Ki-67 and HER2 status ($P < 0.05$ for both), which indicates that *ADAM12* may be correlated with prognosis in breast cancer.

Kaplan-Meier survival curve and Cox regression analysis

Kaplan-Meier survival curve was constructed to determine the differences in survival situation of two patients. The result illustrated that high level of *ADAM12* predicted worse prognosis of patients with ER-positive breast cancer ($P < 0.001$) (**Figure 2**). Further analysis of Cox regression provided evidence that *ADAM12* was a promising prognostic biomarker in ER-positive breast cancer (HR = 7.116, 95% CI = 3.329-15.212) (**Table 2**).

Discussion

ADAMs have specific extracellular domains, such as a prodomain, metalloproteinases domain, epidermal growth factor (EGF)-like repeat domain, disintegrin domain, cysteine-rich domain, transmembrane domain and cytoplasmic domain. ADAMs show many important biological activities through these domains. First, ADAMs can shed growth factors of TGF-α and HB-EGF, which results in promoted cell proliferation. Second, ADAMs serve as adhesion molecules by binding to inregrins with cysteine-rich and disintegrin domains. Third, ADAMs incline to regulate cell proliferation signals via integrins. Fourth, the proteinase activity of ADAMs to membrane-anchored molecules of cytokines, chemokines or its receptors laid basis for its role in cancer progression. The fact

Table 2. Multivariate analysis of *ADAM12* expression in ER-positive breast cancer

Characteristics	*P* value	HR	95% CI
Age	0.161	0.725	0.462-1.137
Tumor size	0.145	0.691	0.420-1.136
Ki-67	0.347	1.275	0.768-2.116
HER2	0.615	1.132	0.698-1.835
ADAM12 expression	0.000	7.116	3.329-15.212

is that ADAMs contribute to the occurrence of many cancers [18].

ADAM12 is a member of ADAM family. It has certain crucial biological functions in tumors. Like other ADAMs, *ADAM12* also could regulate proteolytic ectodomain and result in shedding of growth factors [11, 19, 20]. Besides, it is involved in nonproteolytic protein-protein interactions. The expression level of *ADAM12* is always low. Nevertheless, its increased level was observed in liver cancer, bladder cancer, lung cancer and breast cancer [21-24]. *ADAM12* is commonly expressed as two forms: transmembrane form (ADAM12-L) and secreted form (ADAM12-S). The research of Roy et al. found that overexpression of ADAM12-L and ADAM-12-S in breast cancer cells could promote the estrogen-independent proliferation [25].

In terms of the abnormality behavior of *ADAM12* in cancers or diseases, there has been many studies investigating the specific role in single cancer or disease. Yang et al. concluded that *ADAM12* was a promising diagnostic marker for complete spontaneous abortion and ectopic pregnancy in symptomatic women [26]. A study on oral squamous cell carcinoma indicated that high level of *ADAM12* could accelerate cell proliferation [27]. The positive activation loop between *ADAM12* and *HER2* may contribute to tumor cell migration and invasion in human head and neck cancer [28]. It also appears to be a prognostic marker in resected pethological stage I lung adenocarcinoma [23].

Based on the previous studies, our study tested the expression level of *ADAM12* in serum and tissues. The results showed that the level of *ADAM12* was upregulated in tumors serum and tissues. Moreover, its expression was associated with Ki-67 and HER2 status. The Kaplan-Meier survival curve illustrated that the patients with high level *ADAM12* were more likely to experience short survival time. Cox regression analysis indicated that *ADAM12* was an independent marker for predicting clinical outcome in breast cancer.

Taken together, *ADAM12* may serve as a promising biomarker for predicting prognosis of patients with breast cancer. The present study only considered the effects of single gene on the survival situation, future researches should take more factors into account to get better understand on the pathological mechanism of breast cancer. ADAMs play its roles by many ways, so further studies to make sure the function mechanism of *ADAM12* in breast cancer are necessary for promoting the treatments of patients.

Disclosure of conflict of interest

None.

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References

[1] Malvezzi M, Bertuccio P, Levi F, La Vecchia C and Negri E. European cancer mortality predictions for the year 2014. Ann Oncol 2014; 25: 1650-1656.

[2] Cadoo KA, Fornier MN and Morris PG. Biological subtypes of breast cancer: current concepts and implications for recurrence patterns. Q J Nucl Med Mol Imaging 2013; 57: 312-321.

[3] Early Breast Cancer Trialists' Collaborative Group (EBCTCG), Effects of chemotherapy and hormonal therapy for early breast cancer on recurrence and 15-year survival: an overview of the randomised trials. Lancet 2005; 365: 1687-1717.

[4] Sorensen KP, Thomassen M, Tan Q, Bak M, Cold S, Burton M, Larsen MJ and Kruse TA. Long non-coding RNA expression profiles predict metastasis in lymph node-negative breast cancer independently of traditional prognostic markers. Breast Cancer Res 2015; 17: 55.

[5] Tanaka M, Ichikawa-Tomikawa N, Shishito N, Nishiura K, Miura T, Hozumi A, Chiba H, Yoshida S, Ohtake T and Sugino T. Co-expression of S100A14 and S100A16 correlates with a poor prognosis in human breast

cancer and promotes cancer cell invasion. BMC Cancer 2015; 15: 53.
[6] Akter S, Choi TG, Nguyen MN, Matondo A, Kim JH, Jo YH, Jo A, Shahid M, Jun DY, Yoo JY, Ngo YN, Seo SW, Ali L, Lee JS, Yoon KS, Choe W, Kang I, Ha J, Kim J and Kim SS. Prognostic value of a 92-probe signature in breast cancer. Oncotarget 2015; 6: 15662-80.
[7] Hedau S, Batra M, Singh UR, Bharti AC, Ray A and Das BC. Expression of BRCA1 and BRCA2 proteins and their correlation with clinical staging in breast cancer. J Cancer Res Ther 2015; 11: 158-163.
[8] Cheng CL, Thike AA, Tan SY, Chua PJ, Bay BH and Tan PH. Expression of FGFR1 is an independent prognostic factor in triple-negative breast cancer. Breast Cancer Res Treat 2015; 151: 99-111.
[9] Banin Hirata BK, Oda JM, Losi Guembarovski R, Ariza CB, de Oliveira CE and Watanabe MA. Molecular markers for breast cancer: prediction on tumor behavior. Dis Markers 2014; 2014: 513158.
[10] Blobel CP, Carpenter G and Freeman M. The role of protease activity in ErbB biology. Exp Cell Res 2009; 315: 671-682.
[11] Murphy G. The ADAMs: signalling scissors in the tumour microenvironment. Nat Rev Cancer 2008; 8: 929-941.
[12] Murphy G. Fell-Muir Lecture: Metalloproteinases: from demolition squad to master regulators. Int J Exp Pathol 2010; 91: 303-313.
[13] Iba K, Albrechtsen R, Gilpin BJ, Loechel F and Wewer UM. Cysteine-rich domain of human ADAM 12 (meltrin alpha) supports tumor cell adhesion. Am J Pathol 1999; 154: 1489-1501.
[14] Kveiborg M, Frohlich C, Albrechtsen R, Tischler V, Dietrich N, Holck P, Kronqvist P, Rank F, Mercurio AM and Wewer UM. A role for ADAM12 in breast tumor progression and stromal cell apoptosis. Cancer Res 2005; 65: 4754-4761.
[15] Tian B, Wen J, Zhang M, Li Q and Xie D. [Expression of ADAM12 (Meltrin-alpha) gene in giant cell tumor of bone]. Zhonghua Bing Li Xue Za Zhi 2001; 30: 350-352.
[16] Sjoblom T, Jones S, Wood LD, Parsons DW, Lin J, Barber TD, Mandelker D, Leary RJ, Ptak J, Silliman N, Szabo S, Buckhaults P, Farrell C, Meeh P, Markowitz SD, Willis J, Dawson D, Willson JK, Gazdar AF, Hartigan J, Wu L, Liu C, Parmigiani G, Park BH, Bachman KE, Papadopoulos N, Vogelstein B, Kinzler KW and Velculescu VE. The consensus coding sequences of human breast and colorectal cancers. Science 2006; 314: 268-274.
[17] Frohlich C, Nehammer C, Albrechtsen R, Kronqvist P, Kveiborg M, Sehara-Fujisawa A, Mercurio AM and Wewer UM. ADAM12 produced by tumor cells rather than stromal cells accelerates breast tumor progression. Mol Cancer Res 2011; 9: 1449-1461.
[18] Duffy MJ, Mullooly M, O'Donovan N, Sukor S, Crown J, Pierce A and McGowan PM. The ADAMs family of proteases: new biomarkers and therapeutic targets for cancer? Clin Proteomics 2011; 8: 9.
[19] Kveiborg M, Albrechtsen R, Couchman JR and Wewer UM. Cellular roles of ADAM12 in health and disease. Int J Biochem Cell Biol 2008; 40: 1685-1702.
[20] Jacobsen J and Wewer UM. Targeting ADAM12 in human disease: head, body or tail? Curr Pharm Des 2009; 15: 2300-2310.
[21] Le Pabic H, Bonnier D, Wewer UM, Coutand A, Musso O, Baffet G, Clement B and Theret N. ADAM12 in human liver cancers: TGF-beta-regulated expression in stellate cells is associated with matrix remodeling. Hepatology 2003; 37: 1056-1066.
[22] Frohlich C, Albrechtsen R, Dyrskjot L, Rudkjaer L, Orntoft TF and Wewer UM. Molecular profiling of ADAM12 in human bladder cancer. Clin Cancer Res 2006; 12: 7359-7368.
[23] Mino N, Miyahara R, Nakayama E, Takahashi T, Takahashi A, Iwakiri S, Sonobe M, Okubo K, Hirata T, Sehara A and Date H. A disintegrin and metalloprotease 12 (ADAM12) is a prognostic factor in resected pathological stage I lung adenocarcinoma. J Surg Oncol 2009; 100: 267-272.
[24] Lendeckel U, Kohl J, Arndt M, Carl-McGrath S, Donat H and Rocken C. Increased expression of ADAM family members in human breast cancer and breast cancer cell lines. J Cancer Res Clin Oncol 2005; 131: 41-48.
[25] Roy R and Moses MA. ADAM12 induces estrogen-independence in breast cancer cells. Breast Cancer Res Treat 2012; 131: 731-741.
[26] Yang J, Wu J, Guo F, Wang D, Chen K, Li J, Du L and Yin A. Maternal serum disintegrin and metalloprotease protein-12 in early pregnancy as a potential marker of adverse pregnancy outcomes. PLoS One 2014; 9: e97284.
[27] Uehara E, Shiiba M, Shinozuka K, Saito K, Kouzu Y, Koike H, Kasamatsu A, Sakamoto Y, Ogawara K, Uzawa K and Tanzawa H. Upregulated expression of ADAM12 is associated with progression of oral squamous cell carcinoma. Int J Oncol 2012; 40: 1414-1422.
[28] Rao VH, Kandel A, Lynch D, Pena Z, Marwaha N, Deng C, Watson P and Hansen LA. A positive feedback loop between HER2 and ADAM12 in human head and neck cancer cells increases migration and invasion. Oncogene 2012; 31: 2888-2898.