Int J Clin Exp Pathol 2016;9(11):11826-11831
www.ijcep.com /ISSN:1936-2625/IJCEP0020149

Original Article

High expression of *STC1* is associated with poor prognosis of breast cancer patients

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Received November 19, 2015; Accepted January 23, 2016; Epub November 1, 2016; Published November 15, 2016

Abstract: Background: Breast cancer, the most common female malignant tumor which incidence increased year by year, has become severe threat to women's health. Stanniocalcin 1 (STC1) was firstly identified as hypocalcemic hormone in bony fish. Then studies suggested *STC1* can function as an oncogene in human. In this study, we investigated the relationship between *STC1* expression and its prognostic value in breast cancer patients. Methods: Quantitative real-time RT-PCR (qRT-PCR) was used to detect the mRNA expression level of *STC1* in 157 breast cancer tissue samples and corresponding noncancerous breast tissue specimens. The Kaplan-Meier and Cox regression analysis were performed to evaluate the prognostic value of the *STC1* in breast cancer patients. Results: The results showed that the *STC1* mRNA expressed significantly higher in breast cancer tissues than that in paired noncancerous tissues ($P < 0.05$). The expression of *STC1* was significantly correlated with tumor size, TNM stage and lymph node metastasis (all $P < 0.05$). In addition, the Kaplan-Meier survival curve indicated that high *STC1* expression implied poor overall survival of breast cancer patients. Moreover, Cox analysis showed that the *STC1* expression was an independent prognostic factor for breast cancer patients ($P = 0.049$, HR = 1.825 and 95% CI: 1.003-3.321). Conclusion: All the results suggested that up-regulation of *STC1* expression may act as a novel and specific biomarker of the aggressive poor prognosis for breast cancer patients.

Keywords: *STC1*, prognosis, breast cancer

Introduction

Breast cancer is the most common cancer in women worldwide which incidence and mortality are increasing year by year, and it already becomes a serious threat to the health of women [1, 2]. In 2008, there were more than one million new breast cancer cases which, accounting for 23% of the total new cancer cases and 14% (458,400) of the total cancer deaths in the global scope [3, 4]. An early diagnosis of breast cancer is a crucial step to reduce the mortality [5]. However, in many cases, most breast cancer patients have been advance stage, and approximately 20% to 30% of early breast cancer cases will eventually become metastatic [6, 7]. Although medical technology advances, and the traditional therapeutic strategies constantly improved, the prognosis of breast cancer is still poor. At present, it is important to look for a novel and more reliable biomarker to help estimating clinicopathologic characteristics and prognosis of breast cancer.

Stanniocalcin 1 (STC1) is a glycoprotein hormone originally discovered in bony fish, which involved in calcium and phosphate homeostasis to prevent high blood calcium [8, 9], and in 1995, Chang et al. found *STC1* existed in human and mammals [10]. Human *STC1* gene located on chromosome 8p11.2-p21, which contains four exons, encodes a polypeptide containing 247 amino acid residues. Mature *STC1* consists of 230 amino acid residues contained 11 cysteine residues [11]. *STC1* is investigated to have multiple functions in activities of physiological and pathological, including the growth of muscles and bones [12], angiogenesis [13], organogenesis [14-16], wound healing [17], apoptosis [18] and so on. Recently, studies found that STC1 can be a carcinogen closely related with

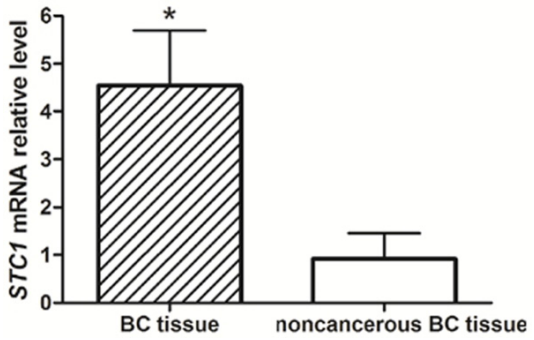

Figure 1. It shows *STC1* mRNA expression level between breast cancer tissues and matched noncancerous breast tissues. The expression level of *STC1* mRNA was higher in breast cancer evaluated by qRT-PCR.

various tumors, such as colorectal cancer [19], gastric cancer [20], and ovarian cancer [21], but little is known about the association between *STC1* expression and prognostic value in breast cancer.

In our study, we aimed to explore the relationship between clinicopathologic characteristics and *STC1* expression and to assess the potential prognostic value of *STC1* in breast cancer patients.

Materials and methods

Patients and specimens

157 breast cancer tissue samples and corresponding noncancerous breast tissue specimens from 2006-2009 that all conformed by pathologists were obtained from The First Affiliated Hospital of HENAN University of Science and Technology. And none of those breast cancer patients received any chemotherapy or radiotherapy before surgery. The written consents were obtained from all patients and their families. This study was approved by the ethics committee of The First Affiliated Hospital of HENAN University of Science and Technology. The tissue specimens were immediately frozen in liquid nitrogen and then stored at -80°C until use. The breast cancer patients were followed up every 3 months in the first 2 years and then every half a year for the subsequent one year, and then annually. The clinicopathological features were collected from the breast cancer patients, including age, tumor size, estrogen receptor (ER) status, progesterone receptor (PR) status, histological type, TNM stage, and lymph node metastasis.

RNA extraction and quantitative real-time RT-PCR (qRT-PCR)

Total RNA of all tissue samples was extracted with the TRIzol reagent (Invitrogen, Carlsbad, CA, USA) following the manufacturer's instructions. The concentration and quality of extracted RNA were measured with a NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies). Then first-strand cDNA was synthesized by AMV reverse transcription system (Promega, USA). In this study, qRT-PCR was performed using SYBR Green PCR master mix (Applied Biosystems, USA) by the 7300 Real-Time PCR System (Applied Biosystems, USA) to evaluate the levels of mRNA expression. *β-actin* was amplified as the endogenous control. The primer sequences for *STC1* and *β-actin* were described previous [22]. All data were evaluated by normalizing with the *β-actin* expression and using the $2^{-\Delta\Delta Ct}$ method.

Statistical analysis

Student's t test was used to analyze the difference of the expression of *STC1* between breast cancer tissues and corresponding noncancerous breast tissues. Chi-square test was applied to analyze the associations between *STC1* expression and clinicopathological characteristics. The survival curves were estimated using the Kaplan-Meier method, and compared by the log-rank test. Cox regression analysis was performed to explore the influence of *STC1* expression and the clinicopathological variables on survival. All the differences with *P* value of 0.05 or less were statistically significant in this study.

Results

High expression of STC1 in breast cancer tissues

QRT-PCR was performed to compare the expression of *STC1* mRNA in breast cancer tissues and matched noncancerous breast tissues. The results showed that the expression level of *STC1* mRNA was higher in breast cancer tissues than that in the paired noncancerous tissues, and the difference was statistically significant (**Figure 1**, $P < 0.05$).

The relationship between STC1 expression and clinicopathologic parameters of breast cancer

We researched the correlation between *STC1* expression and clinicopathologic characteris-

Table 1. Association of *STC1* protein expression with clinicopathological features of breast cancer patients

Clinicopathologic features	Number of cases	*STC1* expression		χ^2	*P* value
		Low	High		
Age (years)				0.292	0.589
< 50	77	37	40		
≥ 50	80	35	45		
Tumor size				6.032	0.014
≤ 2.0 cm	58	34	24		
> 2.0 cm	99	38	61		
ER status				0.090	0.764
Positive	83	39	44		
Negative	74	33	41		
PR status				0.510	0.475
Positive	78	38	40		
Negative	79	34	45		
Histological type				0.004	0.950
Ductal	65	30	35		
Lobular	92	42	50		
TNM stage				21.592	0.000
Stage I-II	71	47	24		
Stage III	76	25	61		
Lymph node metastasis				14.418	0.000
Positive	93	31	62		
Negative	63	41	23		

ER: estrogen receptor; PR: progesterone receptor.

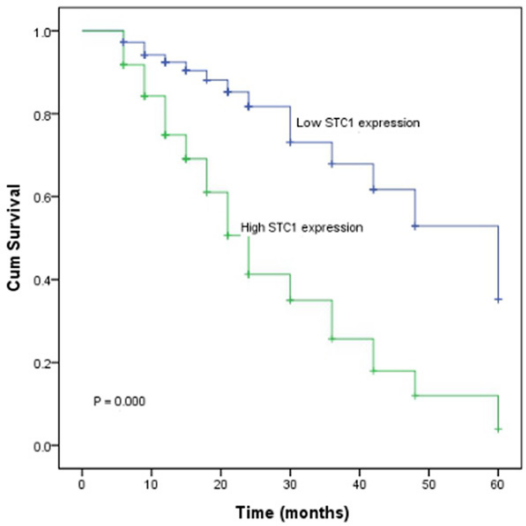

Figure 2. Kaplan-Meier analyzed breast cancer patients based on the expression of *STC1*. The breast cancer patients with high expression of *STC1* means have poor prognosis, (log rank test, *P* = 0.000).

tics in breast cancer patients, and all the data of breast cancer patients were listed in the **Table 1**. The results indicated that the expression of *STC1* was closely related with tumor size (*P* = 0.014), TNM stage (*P* = 0.000) and lymph node metastasis (*P* = 0.000). However, there was no significant association with the age, ER status, PR status and histological type of the breast cancer patients (all *P* > 0.05; **Table 1**).

Correlation between STC1 expression and prognosis of breast cancer patients

The association between *STC1* expression level and survival time of the breast cancer patients was evaluated by Kaplan-Meier survival analysis. Apparently, patients with lower *STC1* expression had worse overall survival compared with those patients who had high expression of *STC1* (log-rank test *P* < 0.05, **Figure 2**). It suggested an increasing risk of dying of breast cancer patients with high *STC1* expression.

To estimate the prognostic value of STC1 for breast cancer patients, we used Cox regression analysis. From **Table 2**, we could see that *STC1* expression was an independent prognostic factor for overall survival in patients with breast cancer (HR = 1.825, 95% CI: 1.003-3.321, *P* = 0.049). It elucidated that *STC1* could be a novel and reliable biomarker for the prognosis of breast cancer patients.

Discussion

Breast cancer is one of the most prevalent neoplasm, accounting for more than one million new cases each year in the worldwide [23-25]. Although breast cancer is the fifth of the main cause of cancer-related deaths, but in the developing countries and developed regions, it is still the most common cause of cancer-related deaths in women [3]. Recent years in our country, the breast cancer patients respond well to treatment, standard guideline has improved. However, breast cancer is still the second most common cause of cancer-related deaths, and every year approximately 1.2 million people died of breast cancer in China [26, 27]. Thus, new technologies to predict the prognosis are important for timely and appropriate treatment for breast cancer patients, and fur-

Table 2. Univariate Cox regression analyses for *STC1* in 157 breast cancer patients

Variables	*P*-value	HR	95% CI
STC1	0.049	1.825	1.003-3.321
TNM Stage	0.002	3.401	1.539-7.516
Lymph node metastasis	0.016	2.416	1.178-4.955

ther studies are needed to find novel and specific biomarker of prognosis for breast cancer.

STC was first found in bony fish as a glycoprotein hormone and later found in human and mammals [28]. In human, *STC1* is ortholog of fish STC. *STC1* mainly exist in kidney, duodenum, brain, ovary and other organizations under normal conditions to function by paracrine and autocrine way [29]. Originally, *STC1* was cloned for searching cancer-related genes, and growing evidences indicated that *STC1* was associated with carcinogenesis. Studies have confirmed that *STC1* can promote the proliferation of tumors perhaps because of the role of a stable factor to promote the formation of new blood vessels [13]. *STC1* also can improve the concentration of Pi in the cytoplasm and increase the level of metabolic enzymes of the glucose metabolism glycolytic pathway in cancer cells [30]. *STC1* was found plays an oncogenic role in breast cancer [31], but little studies was reported about the relationship between *STC1* expression level and the prognostic value of breast cancer. In this study, we examined the role of *STC1* in breast cancer patients. In the present study, we found that the mRNA expression level of *STC1* were higher in breast cancer tissues than that in the matched noncancerous breast tissues by qRT-PCR. Our results were consistent with the previous studies [31].

In addition, in our research we focused on the relationship between the *STC1* expression level and the clinicopathological features of breast cancer patients. The results showed that the mRNA expression of *STC1* was significantly correlated with tumor size, TNM stage and lymph node metastasis. However there were no relationship with other clinical characteristics, such as age, ER status, PR status and histological type. Because metastasis was crucial factor affecting the prognosis of patients, *STC1* might be a potential prognostic marker for patients with breast cancer. Previous researches were also found that up-regulated *STC1* expression was correlated with a poor prognosis in many cancers. Zheng et al. reported that up-regulation of *STC1* acted an important role in gastric cancer development [32]. Gerritsen et al. and Tamura et al. indicated higher *STC1* expression in colorectal cancer [19, 33]. Based on the conjecture and the results above, more researches were performed to further study the correlation of *STC1* expression and the prognosis of breast cancer patients. Kaplan-Meier survival analysis showed that breast cancer patients with high *STC1* expression correlated with poor breast cancer patients' survival. Moreover we also studied the prognosis value of *STC1* expression in breast cancer. Cox regression analysis further demonstrated that *STC1* expression level was an independent prognostic factor for the survival of breast cancer patients.

In conclusion, our data provide a novel, specific, and clinically useful biomarker for the prognosis of breast cancer patients. However, further investigation should be implemented about the exact mechanism of high expression and functions of *STC1* in breast cancer.

Disclosure of conflict of interest

None.

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