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Over-Expression of POU Class 1 Homeobox 1 Transcription Factor (*Pit-1*) Predicts Poor Prognosis for Breast Cancer Patients

Authors' Contribution:
Study Design A
Data Collection B
Statistical Analysis C
Data Interpretation D
Manuscript Preparation E
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Background: The POU class 1 homeobox 1 transcription factor (*POU1F1*, also known as *Pit-1*) was reported to be associated with tumor progression and metastasis. The purpose of this study was to evaluate the prognostic value of *Pit-1* in breast cancer patients.

Material/Methods: The relative expression levels of *Pit-1* in breast cancer patients were detected by quantitative real-time PCR (qRT-PCR). Chi-square analysis was used to analyze the association between *Pit-1* expression and clinical features. The Kaplan-Meier method was used to estimate the overall survival of the patients and Cox regression analysis was used to analyze the prognostic value of *Pit-1*.

Results: Increased expression of *Pit-1* was detected in the tumor tissues compared with the normal tissues (1.086 *vs.* 0.541) and the abnormal expression was associated with tumor size, clinical stage, tumor grade, and lymph node metastasis ($P<0.05$). High expression level of *Pit-1* was significantly associated with poor overall survival of the patients ($P=0.001$) and Cox regression analysis indicated that *Pit-1* might be a prognostic factor for breast cancer prognosis (HR=1.955, 95% CI=1.295–3.035, $P=0.003$).

Conclusions: *Pit-1* may be a potential prognostic biomarker for breast cancer patients and it is associated with tumor progression.

MeSH Keywords: Inflammatory Breast Neoplasms • Prognosis • Transcription Factor Pit-1

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Background

Breast cancer is one of the most common cancers among women, and is the leading cause of cancer death in this group [1,2]. Although advances have recently been made in tumor diagnostic and therapeutic strategies, the clinical outcomes of the patients are still poor due to the metastasis and heterogeneity of the cancer [3,4]. Therefore, there is a need to develop biomarkers that can be used as valuable tools in identifying high-risk patients and to predict disease prognosis [5]. The genes that are expressed abnormally during tumor progression and metastasis may be used as markers to provide prognostic information beyond standard clinical assessment [6,7].

The POU class 1 homeobox 1 transcription factor (*POU1F1*, also known as *Pit-1*) was originally detected in the pituitary gland; it can regulate cell differentiation and is an activator for pituitary gene transcription during organogenesis [8,9]. In *Pit-1*-deficient mouse models, there were no somatotropes, lactotropes, or thyrotropes generated, leading to anterior pituitary hypoplasia and dwarfism [10]. *Pit-1* was also detected in other cell lines and tissues, such as human placenta [11], human breast adenocarcinoma cell line [12,13], and hemopoietic and lymphoid tissues [14]. Moreover, abnormal expression of *Pit-1* in these tissues was reported to be associated with tumor progression [15–17]. In breast cancer cells, *Pit-1* was reported to be associated with tumor growth and metastasis [18], but the prognostic value of *Pit-1* in breast cancer had not been reported yet.

In this study we aimed to evaluate the prognostic significance of *Pit-1* in breast cancer.

Material and Methods

Patients and specimens

We enrolled 106 female patients with invasive breast cancer confirmed by clinical and pathological diagnoses in Linyi People's Hospital from October 2008 to January 2015. Tumor tissues and adjacent normal tissues of the patients were immediately stored in liquid nitrogen, then kept at –80°C until use. None of the patients had received any chemotherapy or radiotherapy before the surgery.

The study was approved by the Institutional Research Ethics Committee and complies with the precepts of the Helsinki Declaration. All examinations were performed after obtaining written informed consent from the participants. All the patients were enrolled in a 5-year investigation and the clinical characteristics and survival status of the patients during the follow-up were collected for analyzing the clinical significance of *Pit-1*.

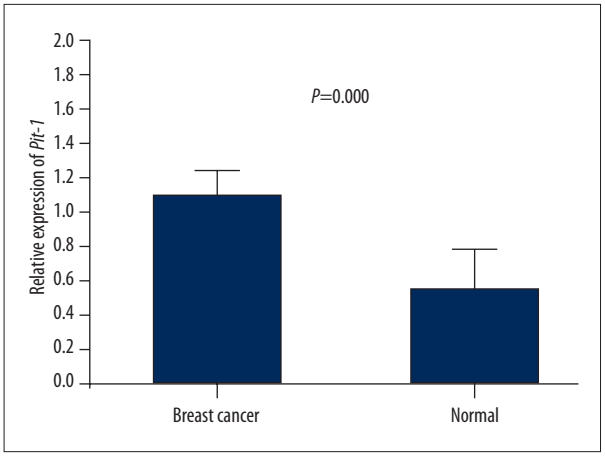

Figure 1. Relative expression of *Pit-1* in tumor tissues and corresponding normal tissues of breast cancer patients.

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

Total RNA were extracted from the tumor tissues and corresponding normal tissues using Trizol reagent (Invitrogen, Carlsbad, CA) according to the manufacturer's instructions. DNase was used to deal with the residuary DNA in RNA samples. The concentration of RNA was detected by UV absorbance (A260/A280) and 1% agarose gel electrophoresis was used for evaluating the quality of RNA.

The Prime Scrip RT reagent kit (Takara, Biotechnology Co., Ltd) was used for synthesizing cDNA and qRT-PCR was performed in triplicate with SYBR Premix Ex Taq™ II (TaKaRa). β-actin was used for internal reference. The data were analyzed by $2^{-\Delta\Delta Ct}$ method.

Statistical analysis

All the statistical analyses were performed using SPSS 18.0 software (SPSS Inc, IL, USA). The difference of *Pit-1* expression in tumor tissues and normal tissues were analyzed by the *t* test and the results are shown as mean ± standard deviation (SD). The relationship between *Pit-1* expression and the clinical features of breast cancer patients was estimated by chi-square analysis. Kaplan-Meier method was used for evaluating the overall survival of the patients at different levels of *Pit-1*. The prognostic value of *Pit-1* was analyzed by Cox regression analysis. $P<0.05$ was considered to be statistically significant.

Results

Increased expression of Pit-1 in breast cancer tissues

Relative expression of *Pit-1* was detected by qRT-PCR. The expression level of *Pit-1* in tumor tissues was 1.086±0.149,

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Table 1. Association between *Pit-1* expression and clinical characteristics of breast cancer patients.

Characteristics	Total number (n)	*Pit-1* expression		χ^2	*P*
		High (n)	Low (n)		
Age				1.691	0.193
≥45	88	39	49		
<45	18	11	7		
Tumor size				8.740	0.03
≥5 cm	50	16	34		
<5 cm	56	34	22		
Clinical stage				3.982	0.046
Early	57	32	25		
Advanced	49	18	31		
Pathology				0.968	0.325
IDC	54	28	26		
ILC	52	22	30		
Tumor grade				6.739	0.009
Early	58	34	24		
Advanced	48	16	32		
Lymph node metastasis				6.121	0.013
Yes	60	22	38		
No	46	28	18		

while the expression in the normal tissues was 0.541±0.238. The difference in *Pit-1* expression between breast cancer tissue and normal tissue was statistically significant (*P*=0.000), as shown in Figure 1.

Correlation between *Pit-1* expression and clinicopathologic characteristics of breast cancer patients

The clinical characteristics of the patients are summarized in Table 1. The patients were divided into 2 groups based on the average expression level of *Pit-1*. The chi-square test was used to analyze the relationship between *Pit-1* expression and the clinical features. The results suggested that *Pit-1* level was associated with tumor size (*P*=0.03), clinical stage (*P*=0.046), tumor grade (*P*=0.009), and lymph node metastasis (*P*=0.013), but was not significantly correlated with other clinical features such as age and pathology (*P*>0.05 for all).

Association of *Pit-1* expression with prognosis in breast cancer patients

The association between *Pit-1* expression and survival of breast cancer patients was investigated by Kaplan-Meier method. The

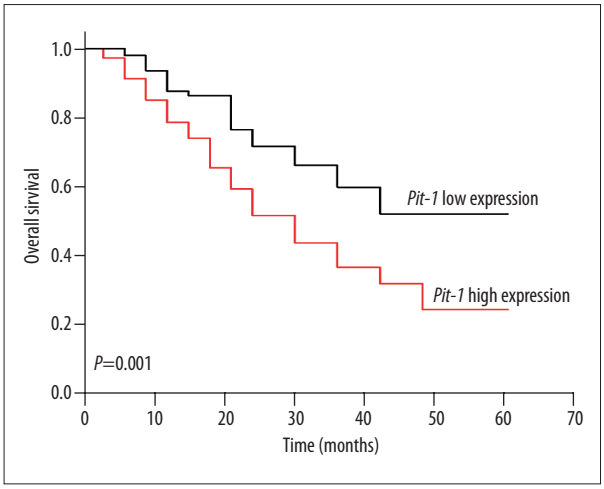

Figure 2. Overall survival of breast cancer patients. Patients with high levels of *Pit-1* had a shorter overall survival time (red line), while those with low levels of *Pit-1* showed a longer overall survival (black line). There was a significant difference between groups (log-rank test, *P*=0.001).

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Table 2. Cox regression analysis.

Characteristic	Univariate analysis			Multivariate analysis		
	HR	95%CI	*P*	HR	95%CI	*P*
Pit-1 (high *vs.* low)	2.033	1.313–3.148	0.001	1.955	1.259–3.035	0.003
Age (≥45 *vs.* <45)	1.116	0.740–1.685	0.600	1.032	0.673–1.582	0.365
Tumor size (≥5 *vs.* <5)	1.212	0.793–1.854	0.375	1.224	0.790–1.895	0.365
Clinical stage (III+IV *vs.* I+II)	1.293	0.855–1.594	0.223	1.229	0.799–1.890	0.349
Pathology (IDC *vs.* ILC)	0.901	0.597–1.362	0.622	1.106	0.724–1.690	0.640
Tumor grade (III *vs.* I+II)	1.224	0.811–1.848	0.336	1.240	0.813–1.893	0.318
Lymph node metastasis (yes *vs.* no)	1.347	0.888–2.044	0.161	1.326	0.869–2.022	0.191

results indicated that patients with low levels of *Pit-1* had a longer survival time than those with high levels of *Pit-1* (43.4 months *vs.* 32.7 months, log-rank test, *P*=0.001, Figure 2). Univariate analysis indicated that *Pit-1* was associated with breast cancer prognosis (*P*=0.001, Table 2) and multivariate analysis suggested that *Pit-1* was an independently prognostic factor in breast cancer (HR=1.955, 95% CI=1.259–3.035, *P*=0.003, Table 2).

Discussion

Pit-1 is one of the first-identified members of the homeodomain-containing proteins, which act as transcriptional regulators determining the fate of cells [16]. *Pit-1* was reported to take part in several types of cancer. Palmieri et al. reported that *Pit-1* plays a key role in pituitary gland development and physiology, acting as a transcriptional factor that regulates the gene expression of *Gh*, *Prl*, *Ghrhr,* and *Pit-1* itself [19]. In the study of Costoya et al., increased expression level of *Pit-1* was found in exponentially growing human myeloid leukemic cells, and the level was specifically associated with cell proliferation [16]. Recently, a number of studies have indicated that *Pit-1* plays an important role in breast cancer cells [12,13,17,20,21], but the clinical significance of *Pit-1* in breast cancer patients had rarely been reported.

Relative expression of *Pit-1* in tumor tissues and corresponding normal tissues of 106 breast cancer patients were detected in the present study. The results suggest that *Pit-1* is highly expressed in tumor tissues compared with normal tissues. The increased expression pattern suggests that *Pit-1* might play an oncogenic role in breast cancer. Moreover, the abnormal expression was associated with tumor size, tumor grade, clinical stage, and lymph node metastasis. These findings indicate that *Pit-1* is associated with breast cancer progression.

The molecular biomarkers for breast cancer prognosis have been reported in many studies. Yeh et al. reported that granulin is an important prognostic biomarker and potential therapeutic target in breast cancer [22]. Fu et al. indicated that SRY-related HMG-box (*SOX17*) is associated with breast cancer progression and can act as a useful prognostic biomarker [23]. Pelkonen et al. claimed that *hepsin* and *TMPRSS3* can serve as prognostic markers in breast cancer [24]. In addition, Jones et al. [25] explored the effects of increased estrogen levels as risk factors of breast cancer in African American women. In the study of Liang et al. [26], serum soluble E-cadherin was proved to be an independent prognostic factor in Asian breast cancer patients. Other molecules, such as karyopherin α-2 (*KPNA2*), *HER3*, *PTEN*, *p-HER2*, *P13K*, and *VEGFR-2,* have also emerged as prognostic factors in breast cancer [27,28]. In the present study, searched for additional molecular markers to better predict and improve the prognosis of breast cancer patients. Our results show that high levels of *Pit-1* are significantly associated with poor overall survival of breast cancer patients, and *Pit-1* could serve as an independent indicator for breast cancer prognosis.

Conclusions

Pit-1 expression is associated with breast cancer progression. *Pit-1* may be a potential predictor for breast cancer prognosis and a target for treatments.

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