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

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Source of support: Departmental sources

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

Full-text PDF: http://www.medscimonit.com/abstract/index/idArt/896107
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 gland 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.

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,
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).

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

| Characteristics          | Total number (n) | Pit-1 expression | \(\chi^2\) | 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     |         |         |
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, Pri, Ghhr, 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 TMPRSS5 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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