Bioinformatics analysis of UNC93B1 gene and prognostic value in breast cancer

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Abstract

**Background:** The Unc-93 homolog B1 (UNC93B1) is a transmembrane protein that is associated with immune diseases such as influenza, herpes simplex encephalitis, and systemic lupus erythematosus; however, the role of UNC93B1 in cancer (including human breast cancer) is known less. The analysis of the association between UNC93B1 expression and breast cancer survival is also unclear.

**Methods:** We used multiple online databases including Oncomine, GEPIA, bcGenExMiner v4.6 and PrognoScan, to conduct bioinformatics analysis of clinical parameters and survival data related to UNC93B1 in breast cancer patients.

**Results:** It was found that UNC93B1 was expressed in different subtypes of breast cancer compared to normal tissues. Scarff-Bloom-Richardson (SBR) classification, Nottingham prognostic index (NPI), estrogen receptor (ER) negative, progesterone receptor (PR) negative epidermal growth factor receptor-2 (HER2) positive and lymph node positive are positively correlated with the UNC93B1 level. We found that increased expression of UNC93B1 was associated with worse relapse-free survival, disease-specific survival, and overall survival. We also confirmed the positive correlation between UNC93B1 and ALDH3B1 gene expression.

**Conclusion:** The lower expression of UNC93B1 was correlated with better clinical prognostic parameters and clinical survival in breast cancer on the basis of the bioinformatic analysis.

Introduction

Breast cancer is the most common malignant tumor in women and the main cause of cancer deaths in women worldwide [1]. Although local surgery, conventional chemotherapy, precision radiotherapy, endocrine therapy and the application of monoclonal antibodies have significant benefits for the prognosis of breast cancer patients. Many patients face the threat of relapse and death. The prognosis of breast cancer is related to clinical, pathological and molecular characteristics [2]. UNC93 homolog B1 (UNC93B1), which encodes a protein that participates in innate and adaptive immune responses by regulating Toll-like receptor signaling. The encoded protein transports the nucleotide-sensitive toll receptor from the endoplasmic reticulum to the endolysosome. The lack of encoded protein is related to herpes simplex encephalitis. The combined sequence from multiple cDNA clones is 2282 bp. Comparison with the genome sequence shows that the gene contains 11 exons[3]. The upregulation of ER membrane protein UNC93B1 on human lupus B cells indicates that TLR9 and UNC93B1 play a part in the pathogenesis of SLE by inducing defective peripheral B cell tolerance[4]. Unc93b1 plays an important role in regulating the host’s inflammatory response to CVB3 infection, and also reveals the potential mediators of host tissue damage, which is worthy of further study in acute viral myocarditis[5]. UNC93B1 promotes tumor growth by controlling the secretion of human oral cancer granulocyte macrophage colony stimulating factor[6]. The important role of UNC93B1 in the occurrence and development of malignant tumors can be observed in terms of tumor occurrence and development, differential expression in tumors.
and related molecular mechanisms. The mutation of Unc93b1(3D / 3D) blocks TLR9 and TLR7 signal transduction, inhibits MYD88(L265P) B cell growth in vitro, and reduces the accumulation of plasma blasts [7]. TLR3 is expressed in metastatic intestinal epithelial cells, and TLR3 promotes the invasion of these cells. UNC93B1 modifies TLR3 expression and localization, which may influence cancer progression[8]. UNC93B1 may be a mutated gene for the cancer-related function of chronic myeloid leukemia (CML)[9]. However, the analysis of UNC93B1 in breast cancer is rare, and the relationship between UNC93B1 expression and the survival of breast cancer patients is still unknown in this study. various online analysis databases were used to evaluate the correlation between UNC93B1 expression and prognostic factors of breast cancer, and to explore the prognostic significance of UNC93B1 gene in breast cancer treatment. First, identify genes that are highly expressed in breast cancer from Oncomine, including unc93b1. Then, used GEPIA to identify genes that benefit from overall survival; used bc-GenExMiner v4.6 to analyze the unc93b1 gene expression and prognosis of various types of breast cancer.

Methods And Materials

Oncomine (http://www.oncomine.org), The cancer microarray database and web-based data mining platform are designed to compare transcriptome data with normal tissues of most major cancer types [10]. UNC93B1 gene expression level was analyzed by Oncomine. We compared the UNC93B1 mRNA level of each microarray data between normal individuals and breast cancer patient tissues. The design parameters include 2-fold change, P value ≤ 1E-4, and top 10% gene grade. T test is used to analyze the expression difference between different breast cancer pathological types and normal tissues in the UNC93B1 gene overexpression data set. In addition, the co-expressed genes of UNC93B1 were also analyzed.

GEPIA (http://gepia.cancer-pku.cn/index.html) is a newly developed interactive web server for analyzing the RNA sequencing expression data of 9,736 tumors and 8,587 normal samples from the TCGA and the GTEx projects, using a standard processing pipeline. Using GEPIA identify UNC93B1 that benefit from os in breast cancer.

Breast Cancer Gene-Expression Miner v4.6 (bc-GenExMiner v4.6)

bc-GenExMiner v4.6, a statistical mining tool, has published annotated breast cancer transcriptome data (DNA microarray [n = 11 359] and RNA-seq [n = 4 712]). It offers the possibility of exploring the gene expression of genes of interest in breast cancer. Bc-GenExMiner v4.6 open access database to analyze the relationship between the UNC93B1 gene breast cancer-specific mRNA expression level and breast cancer-specific clinicopathological characteristics (including age, Scarff-Bloom-Richardson (SBR) grade, Nottingham) prognostic index (NPI), estrogen receptor (ER), progesterone receptor (PR), epidermal growth factor receptor 2 (HER2), lymph node status, triple negative status and basal-like status). Use this software for survival analysis. In addition, the database was used to analyze the relationship between UNC93B1 co-expressed genes. Update April 8, 2021
PrognoScan

PrognoScan (http://dna00.bio.kyutech.ac.jp/PrognoScan/index.html) is the microarray database of the biological relationship between gene expression and clinical prognosis in various cancers [11]. We utilized PrognoScan database to verify the correlation between mRNA level of UNC93B1 expression and survival with the adjusted cox P value <0.05 in breast cancer.

Results

The overexpression of UNC93B1 gene in breast cancer patients.

The online tumor database Oncomine was used to detect the expression of UNC93B1 gene compared with normal people and cancer patients in 20 common cancers. Up-regulation of UNC93B1 gene expression was found in breast, colon, kidney, lung, and lymphoma, while down-regulation of UNC93B1 gene expression was detected in bladder, cervical, esophageal, and head and neck cancers. Four out of ten reached the threshold, and they were 10 datasets from a total of 43 breast cancer datasets with overexpression of the UNC93B1 gene level (Figure 1). Compared with normal individuals, UNC93B1 gene expression levels are in breast tumors, medullary breast cancer, ductal breast cancer in situ, invasive ductal breast cancer, invasive breast cancer, breast cancer, mucinous breast cancer, invasive lobular breast cancer, Invasive duct and invasive, lobular breast cancer, tubular breast cancer (Figure 2A-I, P=6.61E-38, 3.32E-11, 3.83E-4, 4.82E-56, 5.52E-5, 3.36E-4, 3.34E-10, 3.33E-16, 5.65E-10, 0.010).

Expression of UNC93B1 gene in different types of breast cancer

Bc-GenExMiner v4.6 software was used to evaluate the expression of UNC93B1 gene in breast cancer patients and several clinical parameters. There was no significant difference in the expression of UNC93B1 between the <51-year-old group and the >51-year-old group (Figure 3A, P=0.7626, Table 2). The Scarff-Bloom-Richardson grading system (SBR Grade) is based on histological grade or negative based on tumor size (<2 cm, 2-5 cm, ≥5 cm), lymph node status (positive or negative) and vascular invasion status (positive) Breast cancer [9] Nottingham prognostic index. (NPI) Based on histopathological factors (tumor size, lymph node stage and tumor grade) [12]. The expression of UNC93B1 gene increases with the advanced SBR grade and NPI of breast cancer in cancer patients (Figure 3B, P<0.0001, Figure 3C, P<0.0001). UNC93B1 gene expression is higher in ER-negative breast cancer patients (Figure 3D, P<0.0001, Table 2). The expression of UNC93B1 gene is higher in PR-negative breast cancer patients (Figure 3E, P<0.0001, Table 2). The UNC93B1 gene is highly expressed in HER2-positive breast cancer patients (Figure 3F, P<0.0001, Table 2). Compared with lymph node-negative patients, UNC93B1 gene expression increased in lymph node-positive patients (Figure 3G, P<0.0001, Table 2). In addition, UNC93B1 was significantly higher in patients with triple-negative and basal breast cancer than in patients with non-triple-negative and non-basal breast cancer (Figure 3H, P<0.0001, Figure 3I, P<0.0001, Table 2).
Use the survival meta-analysis software PrognoScan to draw survival curves with different survival information, including overall survival rate, recurrence-free survival rate and disease-specific survival rate. Breast cancer patients with UNC93B1 (blue) were positively correlated with overall survival (Figure 4A, P=0.000572, Table 3). The lower UNC93B1 expression group with the blue curve has a better recurrence-free survival rate (Figure 4B, P=0.011658, Table 3). Lower UNC93B1 gene expression (blue) is associated with good disease-specific survival (Figure 4C, P=0.030739, Table 3). We also analyzed the overall survival rate and metastasis-free recurrence survival rate of Bc-GenExMiner v4.6 with the same trend. The expression of UNC93B1 (purple) was positively correlated with metastatic recurrence-free survival (Figure 4D, P<0.0001), and the increase in UNC93B1 expression presented a poorer overall survival (Figure 4E, P=0.0009). We also analyzed the overall survival rate of GEPIA with the same trend. The expression of UNC93B1 (blue) was positively correlated with overall survival (Figure 4F, P=0.015).

**Correlation between UNC93B1 gene and ALDH3B1 gene**

Analyze the Oncomine database to evaluate co-expressed genes related to the UNC93B1 gene. In the Oncomine database, a total of 13,363 samples (111 data sets) were searched to find the co-expressed genes of UNC93B1. Co-express. In the profile of UNC93B1, large clusters of 19273 measured genes were identified in 1556 invasive breast cancers, and aldehyde dehydrogenase 3 family member B1 (ALDH3B1) was the main co-expressed gene (Figure 5A). According to Bc-GenExMiner v4.6, the co-expressed gene ALDH3B1 is positively correlated with UNC93B1 (Figure 5B, P<0.0001).

**Discussion**

The present study showed that UNC93B1 was up-regulated in breast cancer patients. According to the Oncomine database, normal individuals are respected. The high expression of UNC93B1 is associated with poor clinicopathological characteristics of breast cancer. At present, the results show that the high expression of UNC93B1 is located in different histological classifications of breast cancer, including invasive breast cancer, invasive ductal breast cancer, invasive lobular breast cancer, ductal breast cancer, medullary breast cancer, invasive ductal and infiltrating breast cancer Breast cancer. Lobular breast cancer and tubular breast cancer. The age in the analysis was not statistically significant. It has been reported that SBR grade and NPI are prognostic factors for breast cancer. The expression of UNC93B1 gene was significantly increased in SBR Grade, NPI, ER and PR negative, HER2 positive, lymph node positive, triple negative and basal-like states, and the results showed that they were associated with poor prognosis of breast cancer. Therefore, the overexpression of UNC93B1 gene may be a new biomarker factor related to poor prognosis of breast cancer. Survival curves of different data sets based on UNC93B1 gene expression were used to analyze the prognostic value of PrognoScan for breast cancer. Based on the meta-analysis of survival curve data, ten data sets with clinically statistical significance are proposed. Breast cancer patients with increased UNC93B1 gene show poor recurrence-free survival and disease-specific survival. UNC93B1 is positively correlated with the survival rate without distant metastasis. Similarly, this trend also confirmed that the increase in UNC93B1 gene expression showed a
poor overall survival rate, and that UNC93B1 was positively correlated with the metastasis-free survival rate in Bc-GenExMiner v4.6.

We used Oncomine database to mine the co-expression relationship genes of UCN93b1 gene and verified them in BC-Genexminer V4.6. Bc-genexminer V4.6 analysis showed that UNC93B1 gene was closely related to ALDH3B1 gene. ALDH3B1 was expressed only in hepatocytes, proximal convoluted tubule cells, cerebellar astrocytes, bronchiolar cilia cells, testicular efferent tubule cilia cells and histiocytes[13]. Aldehyde dehydrogenase 3B1 is an independent prognostic marker for poor prognosis in lung adenocarcinoma. Postoperative review of ACE aldehyde dehydrogenase 3B1 can guide individualized treatment[14]. ALDH3B1 has been associated with BRAF mutations or thyroid cancer[15]. ALDH3B1 is closely related to pyruvate metabolism, glycolysis/gluconeogenesis and tyrosine metabolism[16]. Bone metastasis of breast cancer was significantly correlated with the expression levels of ALDH3B1 and estrogen receptor[17].

Therefore, the positive correlation between ALDH3B1 and UNC93B1 gene is reliable. Human UNC-93B forms 12 transmembrane domains and is localized to the endoplasmic reticulum, with the highest expression in specialized antigen presenting cells such as dendritic cells and macrophages[18]. UNC93B1 promotes the growth of oral squamous cell carcinoma by controlling the secretion of GM-CSF[6]. Activation of endogenous IFN-β by UNC93B1 plays an important role in the immune monitoring and control of tumors[19]. The high expression of UNC93B1 may affect the poor prognosis, which is worthy of further study.

In conclusion, according to the analysis of various bioinformatics tools, UNC93B1 overexpression was negatively correlated with recurrence-free survival, disease-specific survival and overall survival in breast cancer patients. UNC93B1 was positively correlated with metastatic recurrence-free survival and distant metastasis-free survival. Downregulation of UNC93B1 was positively correlated with clinical parameters with good prognosis, such as lower UNC93B1 levels in patients with ER positive, lymph node status negative, non-triple negative, non-basal-like status, SBR grade and NPI. It is suggested that UNC93B1 is a potentially useful prognostic molecular biomarker of poor prognosis in BC and may play an important regulatory role in the progression of cancer. However, further investigations are needed to testify the biological effect of UNC93B1.

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Tables

Table 1 Different datasets to analyze UNC93B1 gene expression in pathological classification of breast cancer
| Breast cancer subtype                          | P value    | T test | Fold change | Sample |
|------------------------------------------------|------------|--------|-------------|--------|
| Being Breast Neoplasm                          | 6.61E-38   | 21.057 | 1.327       | 3      |
| Medullary Breast Carcinoma                     | 3.32E-11   | 9.220  | 9.220       | 32     |
| Ductal Breast Carcinoma                        | 3.83E-4    | 4.816  | 1.587       | 10     |
| Ductal Breast Carcinoma in situ                |            |        |             |        |
| Invasive Ductal Breast Carcinoma                | 4.82E-56   | 21.024 | 1.555       | 1556   |
| Invasive Breast Carcinoma                      | 5.52E-5    | 4.684  | 1.299       | 21     |
| Breast Carcinoma                               | 3.36E-4    | 4.370  | 1.655       | 14     |
| Mucinous Breast Carcinoma                      | 3.34E-10   | 7.474  | 1.579       | 46     |
| Invasive Lobular Breast Carcinoma              | 3.33E-16   | 8.674  | 1.373       | 148    |
| Invasive Ductal and Invasive Lobular Breast Carcinoma | 5.65E-10 | 6.551  | 1,206       | 90     |
| Tubular Breast Carcinoma                       | 0.010      | 2.343  | 1.093       | 67     |

Table 2 UNC93B1 gene expression analysis in different clinical parameters of breast cancer with Bc-GenExMiner v4.6
| Variables             | No. of patients | UNC93B1mRNA | P value* |
|-----------------------|----------------|------------|----------|
| **Age**               |                |            | 0.7626   |
| ≤51                   | 2756           | -          |          |
| >51                   | 4572           | -          |          |
| **ER**                |                |            | <0.0001  |
| Negative              | 2330           | Increased  |          |
| Positive              | 6595           |            |          |
| **PR**                |                |            | <0.0001  |
| Negative              | 2422           | Increased  |          |
| Positive              | 3150           |            |          |
| **HERR-2**            |                |            | <0.0001  |
| Negative              | 4581           |            |          |
| Positive              | 778            | Increased  |          |
| **Nodal status**      |                |            | <0.0001  |
| Negative              | 4321           |            |          |
| Positive              | 3389           | Increased  |          |
| **Triple-negative status** |            |            | <0.0001  |
| Non-triple-negative   | 7098           |            |          |
| Triple-negative       | 897            | Increased  |          |
| **Basal-like status** |                |            | <0.0001  |
| Non-basal-like        | 7551           |            |          |
| Basal-like            | 1950           | Increased  |          |

*Statistical significance was determined by the Welch's test

Table 3 Different datasets to analyze the prognosis of UNC93B1 gene expression in breast cancer
| Dataset            | Probe ID       | End point              | No.  | Cox P-value     | HR          |
|--------------------|----------------|------------------------|------|----------------|-------------|
| GSE9893            | 9202           | Overall Survival       | 155  | 0.000572       | 1.75 [1.33–2.41] |
| GSE1456-GPL97      | 225869-s-      | Relapse Free Survival  | 159  | 0.011652       | 1.53 [1.10–2.13] |
| GSE-1456-GPL97     | 225869-s-      | Disease-Specific Survival | 159  | 0.030739       | 1.7 [1.12–2.56] |

Figures
Figure 1

Expression of unc93b1 gene in 20 common tumors compared with paired normal tissues Oncomine database was designed with fold change ≥ 2, P value ≤ 1E-4 and gene rank ≥ top 10%. The graphic represents the numbers of datasets with statistically significant (P)
Figure 2

Box plots of normal and tumor differentially expression of UNC93B1 gene in different subtypes of breast cancer (A) Being Breast Neoplasm, (B) Medullary Breast Carcinoma, (C) Ductal Breast Carcinoma in situ, (D) Invasive Ductal Breast Carcinoma, (E) Invasive Breast Carcinoma, (F) Breast Carcinoma, (G) Mucinous Breast Carcinoma, (H) Invasive Lobular Breast Carcinoma, (I) Invasive Ductal and Invasive Lobular Breast Carcinoma, (J) Tubular Breast Carcinoma

Figure 3

Bc-GenExMiner v4.6 to evaluate UNC93B1 gene expression with Violin plot according to clinical parameters in breast cancer patients (A) Age, (B) SBR grade, (C) NPI, (D) ER, (E) PR, (F) HER-2, (G) nodal status, (H) triple-negative status and (I) basal-like status

Figure 4

The survival curve of different datasets based on the expression of UNC93B1 gene was used to analyze the prognostic value in breast cancer (A) Overall Survival, (B) Relapse-Free Survival, (C) Distant Metastasis-Free Survival, (D) (E) analyzed the overall survival rate and metastasis-free recurrence survival rate of Bc-GenExMiner v4.6, (F) analyzed the overall survival rate of GEPIA

Figure 5

Co-expression analysis of gene UNC93B1 and other genes by Oncomine database analysis (A) Co-expression analysis of gene UNC93B1 in 111 data sets invasive breast cancer with Oncomine database. (B) The co-expression gene ALDH3B1 related to UNC93B1 by bc-GenExMiner software