

# Comprehensive Analysis of the Expression and Prognostic Significance of THBSs in Breast Cancer

**Jun Wang**

Wannan Medical College

**Xuebing Zhan**

Wannan Medical College

**Qian Luo**

Wannan Medical College

**Yunshu Kuang**

Wannan Medical College

**Xiao Liang**

Wannan Medical College

**Junjie Wang**

Wannan Medical College

**Xiangxiang Tao**

Wannan Medical College

**Entao Sun**

Wannan Medical College

**Bing Chen** (✉ [chenbing@wnmc.edu.cn](mailto:chenbing@wnmc.edu.cn))

Wannan Medical College

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## Research Article

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# Abstract

**Background:** Breast cancer is one of the most common tumors for women worldwide. Thrombospondins (THBSs) are reported to play important roles in various cellular processes and are involved in the occurrence and development of human cancers. However, the expression and prognostic value of THBSs family in breast cancer remain unclear.

**Methods:** In this study, we examined the genes and protein expression levels of THBSs and their prognostic value by synthesizing several mainstream databases, including Oncomine, Human Protein Atlas (HPA), UALCAN, and KM Plotter. We also analyzed THBS interaction networks, genetic alterations, functional enrichment, and drug sensitivity with several publicly accessible databases, including GEPIA, GeneMANIA, STRING, cBioPortal, Metascape and NCI-60 database.

**Results:** The results showed that the mRNA expression levels of THBS1, THBS2, THBS3, and THBS5 in breast cancer tissues were significantly higher than in normal tissues. The mRNA expression levels of THBS4 were different in different subtypes of breast cancer, and the protein expression levels of THBS1, THBS2, and THBS4 in breast cancer tissues were higher than in normal breast tissues. Survival analysis showed that breast cancer patients with high THBS1 gene expression showed worse overall survival (OS), relapse-free survival (RFS), and post-progression survival (PPS), and breast cancer patients with high THBS2 gene expression also showed worse RFS. Conversely, lower THBS3 levels predicted worse RFS, and lower THBS4 levels predicted worse OS, RFS, and distant metastasis-free survival (DMFS).

**Conclusions:** These results suggest that THBSs may be potential biomarkers for breast cancer.

## Background

Breast cancer has the highest incidence among all cancers affecting women. It is also one of the major causes of cancer death in women worldwide and one of the major public health problems in the current society[1, 2]. According to 2020 statistics, 30% of newly diagnosed cancers in women were breast cancer cases, accounting for 15% of all cancer deaths among women in the United States[1]. According to the expression characteristics of cell surface receptors, breast cancer can be divided into four subtypes: Luminal A, Luminal B, basal-like subtypes, and HER2 overexpressing subtypes[3]. Early menorrhoea, late menopause, postmenopausal obesity, alcohol, oral contraceptives and hormone therapy for menopause are associated with increased risk in women, and fertility, breastfeeding and physical activity may have protective effects[4]. At present, the main treatment methods for breast cancer include surgery, radiotherapy, and chemotherapy[5]. As biomarkers, ER (estrogen receptor), PR (progesterone receptor), and HER-2 (human epidermal growth factor receptor 2) have also been widely used as endocrine therapy or targeted therapy targets for breast cancer[6, 7]. In recent years, with the rapid development of many accessible online databases, members of the E2Fs, S100s, APOs, CBXs, AQPs, PAKs, and STATs family have been validated as new biomarkers for breast cancer[8–14]. Although many previous experiments and clinical studies have been conducted to find new treatment methods, the molecular mechanism of

breast cancer is still not clear due to its heterogeneity, and the 5-year survival rate of breast cancer patients needs to be further improved[15]. Therefore, the search for new highly specific and sensitive biomarkers and new protein families will help clarify the molecular mechanism of breast cancer patients, improve the prognosis of breast cancer patients and individualize patient treatment.

Thrombospondins (THBSs) are extracellular matrix proteins that were first discovered in human cells and include five homologous proteins. They are involved in angiogenesis, proliferation, apoptosis, NO-cGMP-dependent protein kinase pathway and transforming growth factor- $\beta$  (TGF- $\beta$ ) activity, embryonic development, wound healing, synaptogenesis and tumorigenesis[16, 17]. So far, five family members have been found, which can be divided into two subfamilies A and B, according to their structure and function. Subtype A includes THBS1 (TSP1) and THBS2 (TSP2). Subtype B includes THBS3 (TSP3), THBS4 (TSP4), and THBS5 (TSP5) or cartilage oligomeric matrix protein (COMP). Type A has thrombospondin type 1 repeat (TSRs), which consist of three prepared motif repeats and bind to transforming growth factor (TGF- $\beta$ ), and is considered to be the structural basis for THBS to inhibit endothelial cell proliferation and induce endothelial cell apoptosis[18]. THBS1 and THBS2 are usually present in specific developmental stages, tissue remodeling, and specific physiological processes of injury or inflammation and can bind with TGF- $\beta$  to affect angiogenesis, while THBS5 is a constituent of the skeletal and cartilage extracellular matrix[19].

Previous studies have shown that THBSs are overexpressed in many human cancers. For example, THBS1 expression is up-regulated in oral squamous cell carcinoma, glioblastoma, esophageal squamous cell carcinoma, colorectal cancer, liver cancer, breast cancer, and gastric cancer[20–26]. However, THBS1 is down-regulated in non-small-cell lung cancer (NSCLC), cervical cancer, and clear cell renal cell carcinoma[27–29], and PEDF can inhibit the invasive behavior of NSCLC by increasing the content of THBS1 [30]. Recent studies have shown that Hippo component YAP promotes focal adhesion and tumor invasion through transcriptional activation of the THBS1/FAK signaling pathway in breast cancer[31]. In addition, experiments have shown that ABT-510 is effective in reducing angiogenesis and inhibiting tumor growth in malignant glioma[32]. Similarly, THBS2 mRNA levels are upregulated in NSCLC, gastric cancer, colorectal cancer, oral squamous cell carcinoma, uveal melanoma, pancreatic ductal adenocarcinoma, and esophageal squamous cell carcinoma[27, 33–38] and downregulated in cervical and renal cancers [39, 40]. THBS2 and CA19-9 can be used as new markers to detect pancreatic ductal adenocarcinoma[41]. The second subtype of THBSs, THBS3 is highly expressed in osteosarcoma[42]. It has been shown that THBS4 be significantly increased in human cancers, including gastric, liver, prostate, and colorectal cancers[43–46]. Recent studies have shown that THBS4 can promote hepatocellular carcinoma (HCC) progression by regulating ITGB1 through the FAK/PI3K/ Akt pathway[47]. It was found that THBS5 is significantly upregulated in human cancers, including colon cancer, pancreatic cancer, and breast cancer[48–50].

Studies have shown differential expression of THBSs in different cancers. However, few studies have focused on the expression and prognostic value of the THBSs family in breast cancer. In this study, we comprehensively examined the transcriptional level of THBSs and investigated the prognostic value of

human cancers. In addition, we analyzed the interaction network, genetic alteration, functional enrichment, and drug sensitivity of THBSs.

## Methods

### 2.1 Oncomine analysis

Oncomine (<https://www.oncomine.org>) is a publicly accessible online cancer database, containing gene expression array data[51, 52]. In our study, Oncomine was used to analyze the mRNA expression level of the THBS members in different cancers. We compared the mRNA expression of THBS members in cancer and normal tissues using Student's t-test after set the thresholds as follows: P value: 0.05 and fold-change: 2.

### 2.2 GEPIA dataset analysis

GEPIA (<http://gepia.cancer-pku.cn/index.html>) is a newly developed analytical tool using a standard processing pipeline and consist of thousands of tumors and normal tissue samples data[53]. We identified 100 genes that were similar to each of the THBS family members.

### 2.3 Human protein atlas analysis

The Human Protein Atlas (<https://www.proteinatlas.org/>) is an online tool that contains transcriptome profiling data and immunohistochemistry profiling data for more than 8,000 patients and 17 major cancer types, allowing users to directly profile the protein expression patterns of specific genes in specified tumors[54]. In this study, we obtained immunohistochemical images in this tool to directly compare the protein expression of different THBSs family members in human healthy and breast cancer tissues and analyze the THBSs family protein expression patterns in depth.

### 2.4 UALCAN

UALCAN (<http://ualcan.path.uab.edu>) is an interactive web resource based on level 3 RNA-seq and clinical data of 31 cancer types from TCGA database. It can be used to analyze relative transcriptional expression of potential genes of interest between tumor and normal samples and association of the transcriptional expression with relative clinicopathologic parameters[55]. In this study, UALCAN was used to analyze the mRNA expressions of 5 THBSs family members in primary breast tissues and their association with clinicopathologic parameters and nodal metastasis status. Difference of transcriptional expression was compared by students' t test and  $p < 0.05$  was considered as statically significant.

### 2.5 Kaplan-Meier plotter analysis

The Kaplan-Meier Plotter (<http://www.kmplot.com>) is a tool to draw survival plots with gene expression data and survival information from GEO, EGA, and TCGA cancer microarray datasets[56]. We evaluated the relevance of the mRNA expression level of five THBS proteins to the clinical outcomes (OS, RFS, PPS,

and DMFS) of untreated breast cancer patients. This tool automatically calculates the best cutoff value, log-rank p-value, hazard ratio (HR), and 95% confidence intervals (CIs).

## 2.6 GeneMANIA analysis

GeneMANIA (<http://www.genemania.org>), an online system for network analysis, can be used for predicting and visualizing the protein-protein interaction (PPI) network and gene functional assays[57] and features several bioinformatics methods: physical interaction, gene co-expression, gene co-location, gene enrichment analysis, and website prediction. In our study, GeneMANIA was used to construct the gene networks and predict the functions of THBSs.

## 2.7 STRING analysis

STRING (<https://string-db.org/>) is a database of known and predicted protein–protein interactions (PPI) [58]. Herein, to detect the role of THBS family co-expressed genes, the online database of STRING was applied to analyze associations among the PPI network of THBS family co-expressed genes, and the species were set to Homo sapiens and a combined score of > 0.7 was considered statistically significant. The nodes meant proteins; the edges mean the interaction of proteins and we hide disconnected nodes in the network.

## 2.8 cBioPortal for cancer genomics analysis

cBioPortal (<http://www.cbioportal.org>) is an online open-access website resource for exploring, visualizing, and analyzing multidimensional cancer genomics data[59]. In this study, we analyzed the genomic profiles of 5 THBS family members, which contained mutations, putative copy-number alterations from GISTIC and mRNA Expression z-Scores (RNA Seq V2 RSEM) with a z-score threshold  $\pm 1.8$ .

## 2.9 Metascape analysis

Metascape (<http://metascape.org>) is a tool for gene annotation and gene list enrichment analysis[60]. In this study, the pathway and process enrichment of THBSs and similar genes were analyzed in Metascape.

## 2.10 Drug-Gene Interaction Network Analysis

The NCI-60 database, containing data from 60 cancer cell lines, was analyzed by the CELLMINER website (<https://discover.nci.nih.gov/cellminer/>)[61]. The expression status of THBSs and z-score for cell sensitivity data was downloaded from the website and assessed through Pearson correlation analysis to determine the correlation between THBSs expression and drug sensitivity.

## Results

### 3.1 The mRNA and protein expression of THBSs in breast cancer

We first used the Oncomine database to analyze transcription levels of THBSs in various cancer types and the corresponding normal tissues. The results showed that there were 465, 447, 436, 434, and 438 unique assays for THBS1, THBS2, THBS3, THBS4, and THBS5, respectively. In tumor tissue, THBS1 was significantly increased in 40 datasets and decreased in 33 datasets. THBS2 expression is elevated in tumor tissues compared to normal tissues, especially in breast cancer, colorectal cancer, gastric cancer, head and neck cancer, lung cancer, and pancreatic cancer. For THBS3 and THBS4, the expression increased in 8 and 25 datasets, and decreased in 9 and 15 datasets, respectively. In addition, high expression of THBS5 was observed in 64 datasets, while reduced levels were found in four datasets.

Oncomine analysis showed a significant increase in the THBS1 gene levels in breast cancer tissues. According to the TCGA Breast Dataset, compared to normal breast tissue, THBS1 is upregulated in invasive ductal and lobular carcinoma, mixed lobular and ductal breast carcinoma and male breast carcinoma. According to the Radvanyi Breast Dataset [62], the expression of THBS1 is upregulated in invasive ductal breast carcinoma (IDC). The transcriptional levels of THBS2 were significantly higher in eight data sets and significantly lower in two datasets. In the TCGA Breast Dataset, THBS2 is upregulated in invasive breast carcinoma, invasive lobular breast carcinoma, and IDC. In the Ma Breast Dataset[63], THBS2 is upregulated in situ in ductal breast carcinoma and IDC. In the Perou Breast Dataset[64], THBS2 is upregulated in lobular breast carcinoma. In the Turashvili Breast Dataset[65], THBS2 is upregulated in IDC and invasive lobular breast carcinoma (ILC). However, in the Sorlie Breast 2 Dataset[66], THBS2 expression is downregulated in fibroadenoma. There was no significant difference in THBS3 gene expression between breast cancer and normal breast tissue. For THBS4, the Finak Breast Dataset[67] shows that the expression level of THBS4 is upregulated in invasive breast carcinoma. Radvanyi Breast dataset[62] showed that its expression level was upregulated in invasive mixed breast carcinoma. In addition, in the Ma Breast 4 Dataset[63], Perou Breast Dataset[64], Sorlie Breast Dataset[3], and Sorlie 2 Breast Dataset[66], THBS4 is in ductal breast carcinoma. The expression is downregulated in breast carcinoma. Finally, THBS5 was significantly upregulated in all types of breast cancer in 30 datasets, including the Curtis Breast Dataset[68] and Karnoub[69]. All the results with corresponding p-values for statistical significance are summarized in Fig. 1 and Table 1.

We also compared THBS transcriptional levels in breast cancer and normal tissue using UALCAN analysis (Fig. 2). We found that THBS2, THBS3, and THBS5 were upregulated in tumor tissues, and THBS1 and THBS4 were not significantly different between tumor and normal tissues. In addition, the relationship between THBS gene levels and breast cancer tumor stage was also analyzed. The results showed that the expression of THBS1, THBS2, THBS3, and THBS5 were correlated with tumor stage (Fig. 3). Finally, we evaluated the association between THBSs and the lymph node metastatic status. The results showed that THBS1, 2, 3, and 5 were associated with lymph node metastatic state, while THBS4 was not associated with lymph node metastatic state (Fig. 4).

To further explore the expression of THBS proteins in breast cancer, we analyzed the immunohistochemical staining images in Human Protein Atlas (HPA). The results showed that THBS1, THBS2, and THBS3 were not expressed in normal breast tissue, while the protein expression levels of

THBS1, THBS2, and THBS3 in breast cancer tissue were high, middle, and not detected, respectively. The THBS4 protein levels are low in normal breast tissue and medium in breast cancer tissue (Fig. 5). Unfortunately, we did not find immunohistochemical images of THBS5 in breast cancer and normal breast tissue in the HPA. In conclusion, we found that the protein expressions of THBS1, THBS2 and THBS4 in breast cancer tissues were higher than in normal breast tissues.

## 3.2 Prognostic values of THBSs in breast cancer

Using KM plotter, we found that high THBS1 and THBS2 levels were associated with worse RFS (HR = 1.13, P = 0.006; HR = 1.13, P = 0.018) and that high expression of THBS3 and THBS4 was associated with better RFS (HR = 0.83, P = 2.7E-4; HR = 0.72, P = 3.30E-10). Patients with high THBS1 expression showed worse OS (HR = 1.22, P = 0.037) and PPS (HR = 1.4, P = 0.0048). In addition, patients with elevated THBS4 gene levels had better OS (HR = 0.76, P = 0.0035) and DMFS (HR = 0.79, P = 0.0022) (Table 2). However, THBS5 gene expression in breast cancer patients was not significantly correlated with OS, RFS, DMFS and PPS.

We further analyzed the prognostic significance of THBSs in these four intrinsic subtypes. Interestingly, high THBS1 expression was significantly associated with worse RFS (HR = 1.43, P = 0.0017) in the basal subtype and worse PPS (HR = 1.58, P = 0.012) in Luminal A. High THBS2 expression predicted worse OS (HR = 1.65, P = 0.0051), RFS (HR = 1.3, P = 0.004) and PPS (HR = 1.58, P = 0.034) in Luminal B subtype, significantly correlated with better RFS (HR = 0.67, P = 0.0051) and worse DMFS (HR = 2.39, P = 0.00085) in the HER2 + subtype. High expression of THBS3 was significantly associated with better RFS in the Luminal A and Luminal B subtypes (HR = 0.78, P = 0.0026; HR = 0.81, P = 0.017). High THBS3 expression in the HER2 + subtype was significantly associated with worse DMFS (HR = 2.93, P = 4.30E-05). High THBS4 expression predicted worse OS (HR = 1.78, P = 0.0038) and DMFS (HR = 1.45, P = 0.02) in the basal subtype. In contrast, the Luminal A subtype was significantly associated with better OS (HR = 0.54, p = 0.00019), RFS (HR = 0.74, p = 0.00023), and PPS (HR = 0.68, p = 0.031). Finally, high THBS5 expression predicted worse RFS in the HER2 + subtype (HR = 1.52, P = 0.021). The detailed results are summarized in Table 3. In addition, we analyzed the prognostic value of THBSs according to the ER/PR/HER2 status in Table 4. The results showed that high THBS1 expression was associated with worse RFS (HR = 1.16, P = 0.0094) and PPS (HR = 1.45, P = 0.0068) in the HER2- state. High THBS2 expression was associated with worse OS (HR = 2.07, P = 0.0042) and PPS (HR = 2.96, P = 0.0368) in PR- and worse RFS in ER- (HR = 1.4, P = 0.0006). High THBS3 expression was associated with better RFS in HER2- state (HR = 0.81, P = 0.0002) and worse RFS in the PR- state (HR = 1.32, P = 0.018), and worse DMFS in the ER- state (HR = 1.31, P = 0.018, P = 0.0424). High THBS4 expression was associated with better OS (HR = 0.69, P = 0.0008) and RFS (HR = 0.68, P = 6.20E-11) in the HER2- state, and better OS in the ER + state (HR = 0.67, P = 0.0109). Finally, high THBS5 expression predicted favorable RFS under HER2- state (HR = 0.85, P = 0.0041) but unfavorable PPS under PR- state (HR = 2, P = 0.0066).

## 3.3. Correlation analyses of THBSs in breast cancer

We used GeneMANIA to analyze the relationship of THBSs at the gene level (Fig. 6A). Results showed that all THBSs have shared protein domains, and physical interactions were found between THBS1 and THBS2, THBS1 and THBS3, and THBS3 and THBS4. Relationships have been found in the co-expression of THBS1 and THBS2, THBS2 and THBS5, THBS3 and THBS5, and THBS4 and THBS5. In addition, the relationships of THBS1 and THBS2, THBS2 and THBS3, THBS3 and THBS4, and THBS4 and THBS5 were also found in website predict.

We identified THBS interactions at the protein expression level using STRING (Fig. 6B). THBS2 interacts with THBS1 and THBS3 based on protein homology, co-expression data, experimentally determined data, and textmining. In addition, the relationship between THBS1 and THBS2 was only noticed on gene co-occurrence.

### 3.4. THBSs genetic alteration in breast cancer

We analyzed the alteration of the THBS genes in breast cancer using cBioPortal. An analysis of nine breast cancer databases, namely Metastatic Breast Cancer Project (Provisional, February 2020), Breast Cancer (METABRIC, Nature 2012 & Nat Commun 2016), Breast Invasive Carcinoma (TCGA, Cell 2015), Metastatic Breast Cancer (INSERM, PLoS Med 2016), Breast Cancer Xenografts (British Columbia, Nature 2015), Breast Invasive Carcinoma (Sanger, Nature 2012), Breast Invasive Carcinoma (Broad, Nature 2012), Breast Invasive Carcinoma (British Columbia, Nature 2012), and Breast Cancer (SMC 2018), showed that the percentage of THBSs genetic alterations were 35.86% (85/237), 23.33% (507/2173), 18.73% (153/817), 18.52% (40/216), 8.62% (10/116), 2.00% (2/100), 1.94% (2/103), 1.94% (1/103) and 1.08% (2/186), respectively (Fig. 7A). Based on the TCGA Provisional dataset, the alteration frequency of THBSs was (THBS1, 2.1%; THBS2, 1.4%; THBS3, 17%; THBS4, 0.9%; THBS5, 1.7%) (Fig. 7B).

## 3.5. Functional enrichment analysis of THBSs in breast cancer

To investigate the function of THBSs and their similar genes, we analyzed the GO and KEGG pathways using Metascape. First, we explored GEPIA to identify THBS-like genes in breast cancer tissues; we used the first 100 similar genes for each THBS gene (Additional table 1). The results showed that the first 20 GO pathways were enriched, namely collagen-containing extracellular matrix, vasculature development, collagen binding, collagen fibril organization, cell-substrate adhesion, integrin binding, positive regulation of cell migration, response to transforming growth factor beta, basement membrane, anchoring junction, response to wounding, skeletal system development, heart development, negative regulation of cell proliferation, endothelium development, extracellular matrix disassembly, actin filament-based process, calcium ion binding, circulatory system process, and platelet alpha granule (Fig. 8A). The top 10 KEGG pathways were: ECM-receptor interaction, protein digestion, and absorption, proteoglycans in cancer, hypertrophic cardiomyopathy (HCM), TGF-beta signaling pathway, regulation of actin cytoskeleton, AGE-RAGE signaling pathway in diabetic complications, leukocyte transendothelial migration, Wnt signaling

pathway, and hedgehog signaling pathway (Fig. 8B). Then, we carried out enrichment term network analysis of THBS genes and similar genes (Fig. 8C).

### 3.6. Drug sensitivity analysis

We evaluated the effect of THBSs on drug sensitivity using the NCI-60 database, which contributes to increase the precision of the treatments. Drug sensitivity was measured by a z-score, with a higher score indicating that the cells were more sensitive to drug therapy (Fig. 9). It is worth noting that the high expression of THBS1, THBS2, THBS3, and THBS4 is associated with the resistance of different cell lines to several chemotherapeutic drugs. Unfortunately, we failed to find data on the effect of THBS5 on drug sensitivity from the NCI-60 database. In addition, we noticed that different genes had similar associations with the same drug. For example, THBS1 and THBS3 are associated with reduced cell sensitivity to vinblastine, eribulin mesilate, and actinomycin D.

## Discussion

THBSs, which are overexpressed in various human cancers, can be involved in a series of cellular processes, such as angiogenesis in the tumor microenvironment[70]. However, little is known about the expression and prognosis role of THBSs in breast cancer. Therefore, our study comprehensively analyzed the transcriptional level and prognostic value of THBSs in breast cancer.

The results showed that the mRNA levels of THBS1, THBS2, THBS3, and THBS5 in breast cancer tissues were higher than those in normal tissues, while the mRNA levels of THBS4 were different in different subtypes of breast cancer. The protein expression levels of THBS1, THBS2, and THBS4 in breast cancer tissues were higher than those in normal breast tissues. It has been reported that THBS1 overexpression promotes melanoma invasion and a malignant phenotype by inducing epithelial-mesenchymal transformation of tumor cells[71]. THBS1 is highly expressed in invasive ductal carcinoma of the breast and promotes lymph node metastasis, and THBS-1 potentially could be a predictive marker for metastasis [72]. In our study, we found that high THBS1 expression was significantly associated with worse OS, RFS, and DMFS. In addition, high THBS1 expression was significantly associated with worse RFS in the basal subtypes. In the Lumina A subtypes, there was a significant correlation with worse PPS. Zhang et al. demonstrated that THBS1 mRNA expression was the highest in HER2 subtype and the lowest in Luminal B subtype, and taxol resistance gene 1 and thrombospondin 1 expression may vary according to the molecular subtypes of breast cancer [25]. In addition, the tRNA-derived fragment tRF-17-79MP9PP can reduce invasion and migration of breast cancer cells via the THBS1 /TGF- $\beta$ 1/Smad3 axis and THBS1 as a downstream target of tRF-17, and reduction of THBS1 expression also partially recovered the effects of tRF-17 inhibition breast cancer cell viability, invasion, and migration [73]. Recent studies have shown that THBS2 is a target of miR-20a, and THBS2 knockdown could eliminate the anti-proliferation, pro-apoptotic and anti-autophagy effects mediated by miR-20a inhibitors in cervical cancer cells[74]. We found that high THBS2 expression was associated with worse RFS and that high THBS2 expression was significantly associated with worse OS, RFS, and PPS in the Lumina B subtype and with

worse DMFS and better RFS in the HER2 + subtype. CD36 can mediate the N-terminal recombinant fragment of THBS2 to activate endothelial cell apoptosis and inhibit the growth and metastasis of breast cancer[75]. Schips et al. found that THBS3 enhances traumatic cardiomyopathy through intracellular integrin inhibition and myofibril instability, and transgene-mediated overexpression of  $\alpha 7\beta 1$  integrin in the heart ameliorates the predisposing disease effects of THBS3 by augmenting sarcolemmal stability [76]. In our report, patients with high THBS3 expression showed better RFS. In addition, high THBS3 expression predicted better RFS of the Lumina A and Lumina B subtypes, and worse DMFS of the HER2 + subtypes. Recently, Hou et al. found that THBS4 silencing regulates the cancer stem cell-like properties of prostate cancer by blocking the PI3K/Akt pathway, and the overexpression of THBS4 promoted self-renewal and proliferation, curbed the apoptosis of prostate cancer stem cells, and enhanced the in vivo tumorigenicity [77]. Patients with elevated THBS4 gene levels showed better OS, RFS, and DMFS. In the basal subtype, elevated THBS4 gene levels were associated with worse OS and DMFS; High expression of THBS4 in Lumina A subtype is associated with better OS, RFS, and PPS. In our study, the mRNA expression levels of THBS4 varied across different types of breast cancer, with high expression in invasive breast cancer and low expression in ductal and medullary breast cancer. Previous studies have shown that the mRNA level of THBS4 in breast cancer is variable, usually the highest in tumors with rich interstitial content (ILC, ER positive, low grade IDC, Luminal A, and normal-like subtypes)[78]. This is consistent with our study results, and THBS4 expression levels in ILC and IDC are not different; thus, THBS4 cannot be used as a biomarker to distinguish ILC and IDC[78]. Studies have shown that RvD1 inhibits the dry characteristics of CAFs-induced EMT and HCC cells by inhibiting the secretion of COMP[79]. In the HER2 + subtype, high expression of THBS5 is associated with worse RFS. Studies have shown that high expression of COMP in breast cancer cells is associated with a worse survival rate and reduced relapsed-free survival rate, moreover, due to the upregulation of matrix metalloproteinase-9, cells with high expression of COMP-expressing cells had a more invasive phenotype. Finally, in vitro experiments showed that compared with control cells, Comp-expressing cells showed better survival and higher protein synthesis rates when treated with brefeldin A[50].

In this study, GeneMANIA and STRING analysis showed that co-expression was only observed at the gene level for THBS1 and THBS2, THBS2 and THBS5, THBS3 and THBS5, and THBS4 and THBS5. The gene co-expression relationship was only observed at the protein expression levels for THBS1 and THBS2. Drug sensitivity analysis showed that THBS1 and THBS3 were associated with reduced cell sensitivity to vinblastine, eribulin mesylate, and actinomycin D.

There are some limitations to our study. First, all the data analyzed were obtained from different online databases, which may lead to background heterogeneity; further studies with larger sample sizes are needed to confirm our findings. Second, we did not conduct experiments to verify the results obtained from the bioinformatics analysis. Finally, we did not explore the molecular mechanisms of different THBSs in breast cancer. Subsequently, further in vitro and in vivo studies should be conducted to confirm our results.

# Conclusions

The gene and protein expression of THBSs in breast cancer and their prognostic significance were verified in this study. In addition, we analyzed THBSs considering their co-expression and interaction networks, genetic alteration, enrichment pathways, and drug sensitivity. The results showed that, compared with normal tissue, the expression levels of THBS1, THBS2, THBS3, and THBS5 were significantly upregulated in breast cancer tissue. In contrast, the expression levels of THBS4 were different in different subtypes of breast cancer, and the protein expression of THBS1, THBS2, and THBS4 proteins was higher in breast cancer tissue. Survival analysis revealed that breast cancer patients with high expression of THBS1 and low expression of THBS4 showed worse OS and RFS, and high expression of THBS2 and low expression of THBS3 were associated with worse RFS. In conclusion, THBSs may be novel prognostic biomarkers for breast cancer. Our findings will provide valuable clues to understanding the pathogenesis and progression of breast cancer in humans and contribute to developing of more effective clinical therapies in the future.

# Abbreviations

THBS/TSP: Thrombospondin; COMP: cartilage oligomeric matrix protein; HPA: Human Protein Atlas; KM Plotter: Kaplan-Meier plotter; OS: overall survival; RFS: relapse free survival; PPS: post progression survival; DMFS: distant metastasis free survival; ER: estrogen receptor; PR: progesterone receptor; HER-2: human epidermal growth factor receptor 2; TGF- $\beta$ : transforming growth factor- $\beta$ ; TSRs: thrombospondin type 1 repeats; NSCLC: non-small-cell lung cancer; HCC: hepatocellular carcinoma; PPI: protein-protein interaction; HR: Hazard Ratio; TCGA: the cancer genome atlas; GO: gene ontology; KEGG: Kyoto encyclopedia of genes and genomes; BP: biological processes; CC: cellular components; MF: molecular functions; HCM: Hypertrophic cardiomyopathy; ILC: Invasive Lobular Breast Carcinoma; IDC: Invasive Ductal breast Carcinoma.

# Declarations

## Acknowledgements

Not applicable.

## Authors' contributions

JW and XZ conceived and wrote the manuscript; QL and YK prepared the figures and data, and analyzed the data; XL, JW and XT contributed to discussions during the data analyses; ES and BC conceived the study and revised the manuscript.

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### **Availability of data and materials**

All data generated or analysed during this study are included in this published article [and its supplementary information files].

### **Ethics approval and consent to participate**

This article does not contain any studies with human participants or animals performed by any of the authors, therefore no ethic approval or consent is required. No administrative permission and/or licenses is acquired by this study to access the original data used in this research.

### **Consent for publication**

Not applicable.

### **Competing interests**

All authors declare no conflict of interest in this study.

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## Tables

Due to technical limitations, table 1-4 is only available as a download in the Supplemental Files section.

## Figures

Analysis Type by cancer	Cancer vs. Normal		Cancer vs. Normal		Cancer vs. Normal		Cancer vs. Normal		Cancer vs. Normal	
	THBS1		THBS2		THBS3		THBS4		THBS5	
Bladder Cancer		2	1	2						
Brain and CNS Cancer	3	1	2	1	2		2		1	2
Breast Cancer	4		8	2			2	5	30	
Cervical Cancer										
Colorectal Cancer		3	12					1	10	
Esophageal Cancer	5		4						1	
Gastric Cancer	8		11				5		5	
Head and Neck Cancer	3		8					1	2	1
Kidney Cancer		9		3	2					
Leukemia	2	4	1		1	1	2	2		
Liver Cancer	1	2	4				1			
Lung Cancer		1	16				3	2	5	
Lymphoma	6		5		2		2			
Melanoma	1	1		1						
Myeloma			1							1
Other Cancer	3	1	5			3		3	1	
Ovarian Cancer	1	2		1					1	
Pancreatic Cancer	3		6		1		1		3	
Prostate Cancer		2	1				8	1	4	
Sarcoma		6	1		2	3	1		1	
Significant Unique Analysis	40	33	86	10	8	9	25	15	64	4
Total Unique Analysis	465		447		436		434		438	

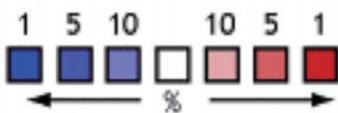
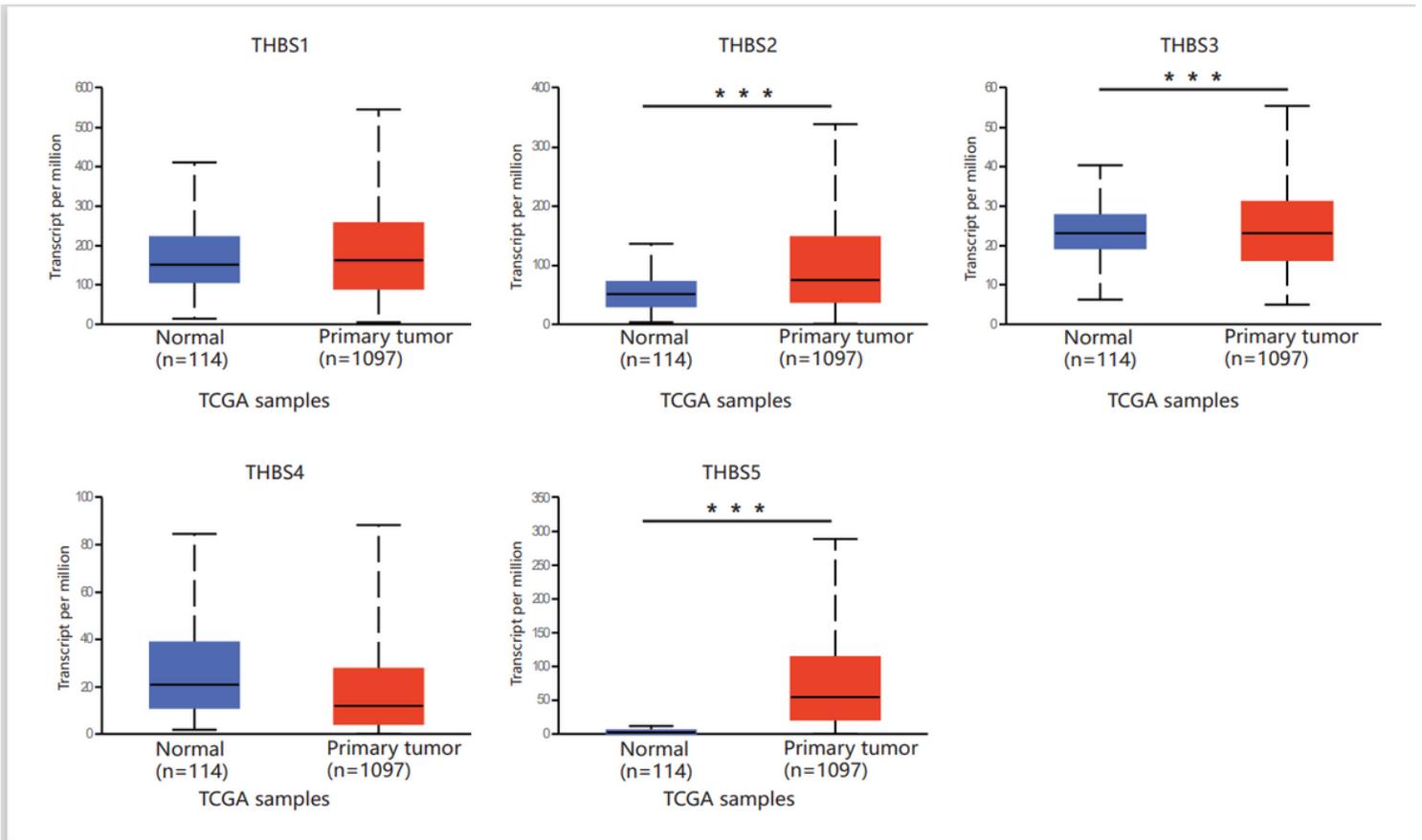


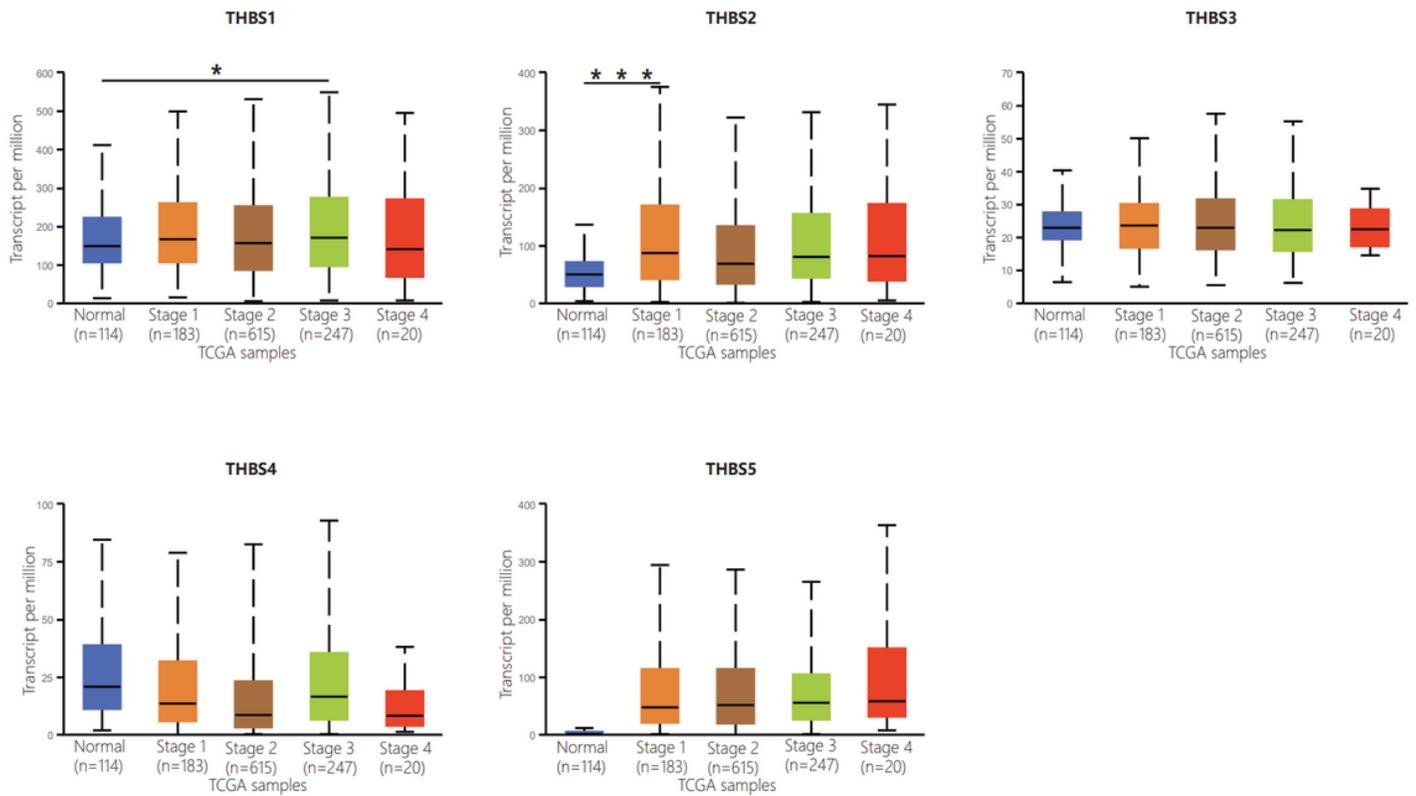
Figure 1

Oncomine analysis of the mRNA expression levels of THBSs in different cancers. The differences in expression levels of the genes between tumor and normal tissues are summarized. The thresholds (p-value  $\leq 0.05$ ; fold change  $\geq 2$ ; gene rank  $\leq 10\%$ ; data type: mRNA) are indicated in the colored cells. Red cells represent overexpression of the target gene in tumor tissues compared to normal tissues, whereas blue cells indicate downregulation of the gene. Gene rank is depicted by the color depth in the cells.



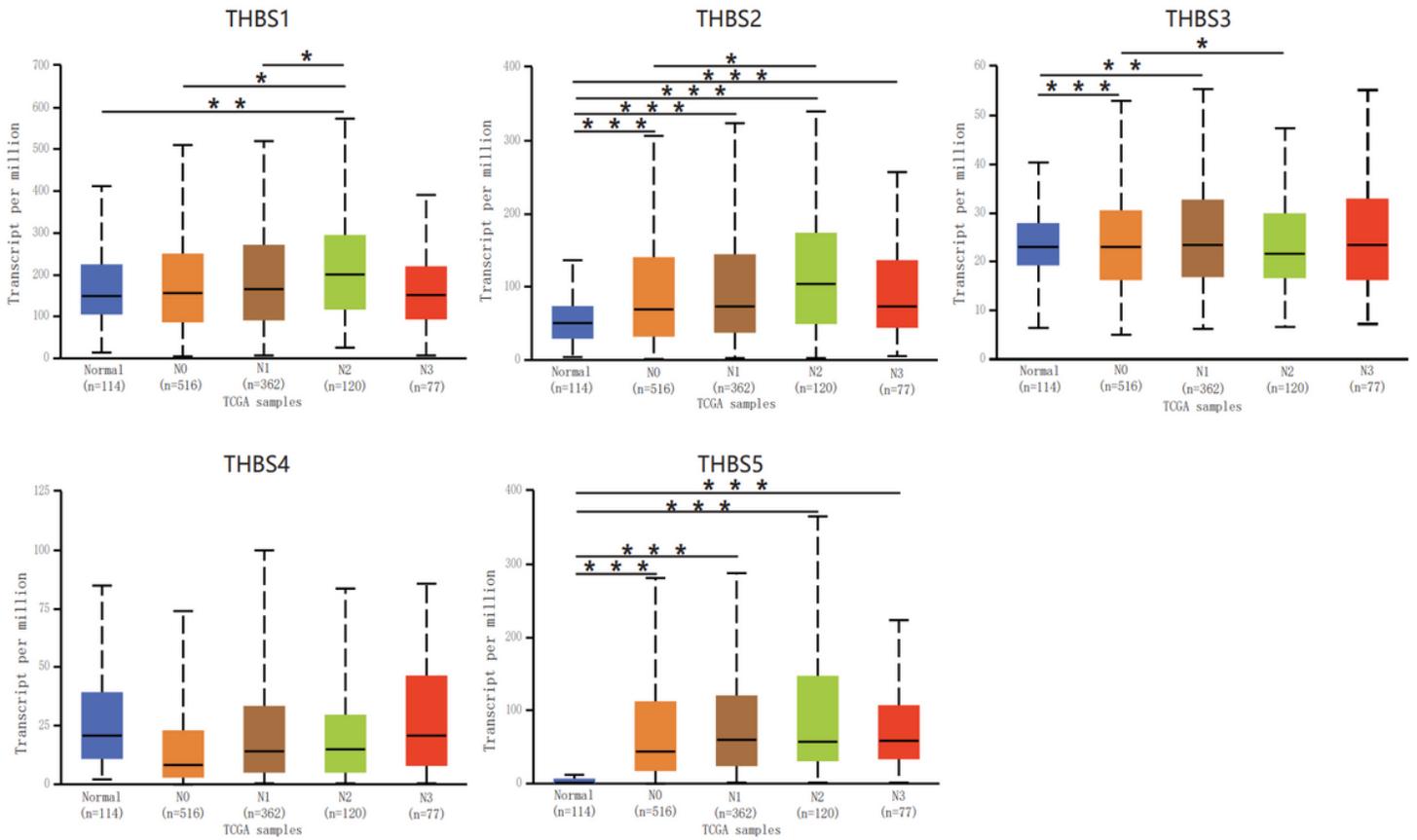
**Figure 2**

mRNA expression of THBSs: mRNA expression of different THBSs in breast cancer and normal tissues (UALCAN, \*P < 0.05, \*\*P < 0.01, and \*\*\*P < 0.001).



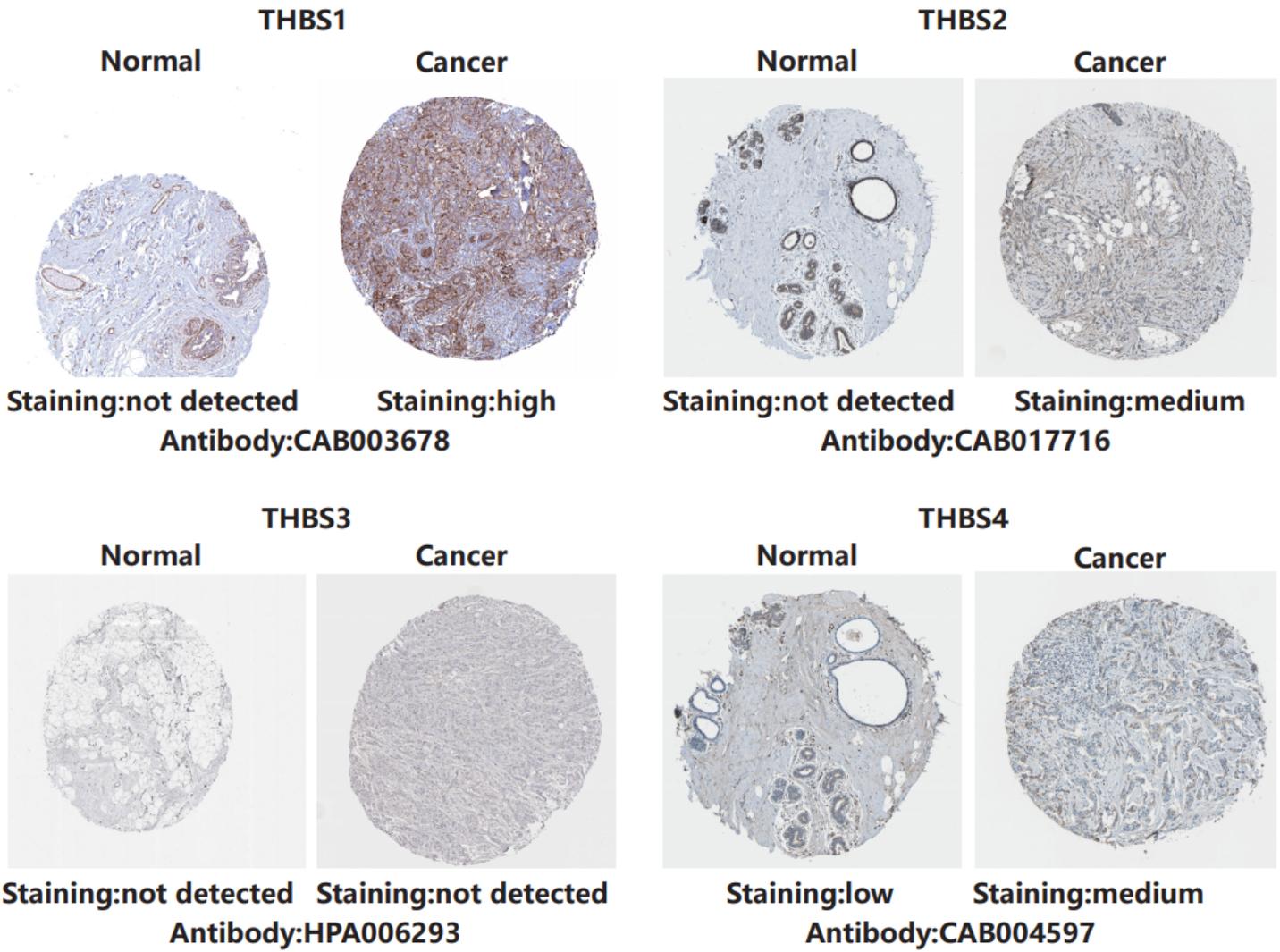
**Figure 3**

Clinicopathological parameters and THBSs mRNA levels in breast cancer patients (UALCAN): correlation between expression of THBSs and individual cancer stage in breast cancer patients (UALCAN,  $P < 0.05$ ,  $P < 0.01$ , and  $P < 0.001$ ).



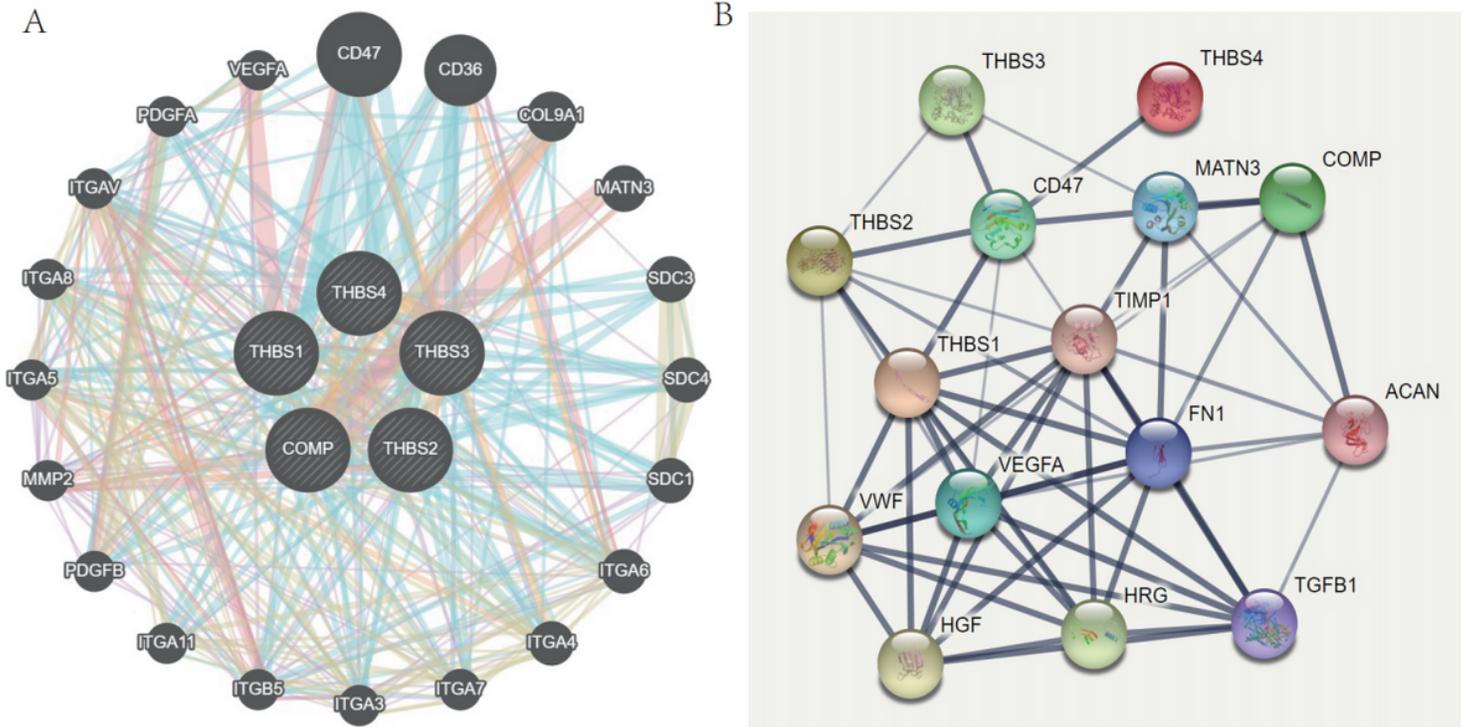
**Figure 4**

Clinicopathological parameters and THBSs mRNA levels in STAD patients (UALCAN): correlation between the expression level of THBSs and nodal metastatic status in breast cancer patients (UALCAN,  $P < 0.05$ ,  $P < 0.01$ , and  $P < 0.001$ ).



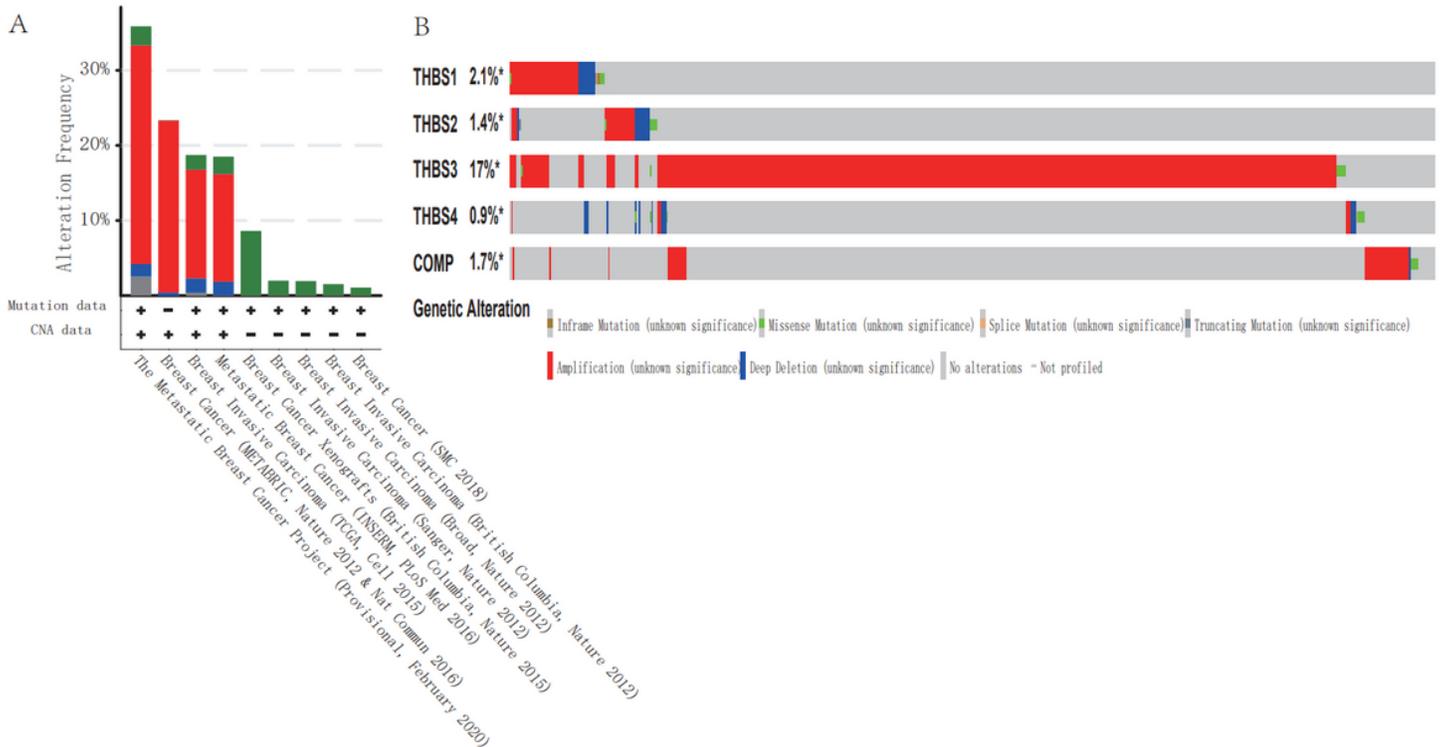
**Figure 5**

Immunohistochemistry staining images from Human Protein Atlas showed the protein expression levels of THBSs in breast cancers.



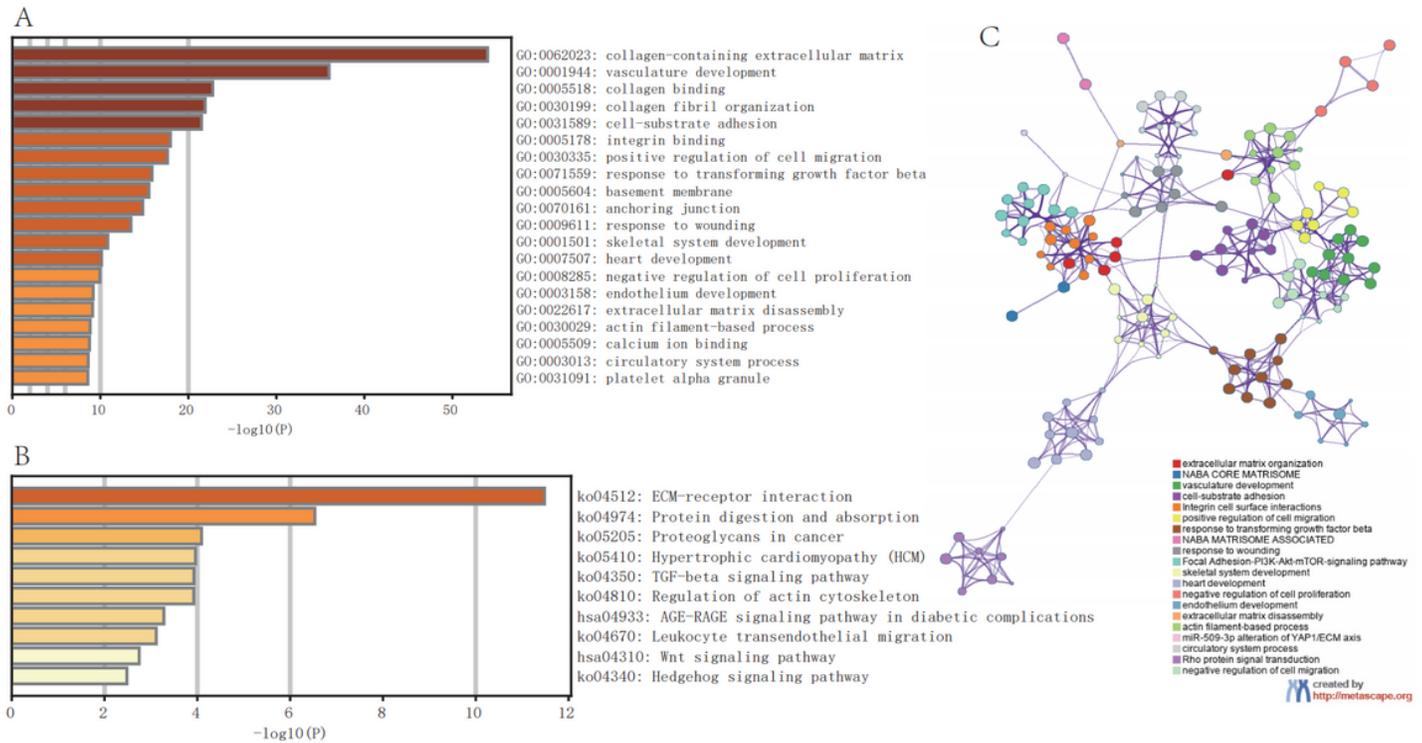
**Figure 6**

Co-expression and interaction of THBSs at the gene and protein levels in breast cancer patients. (A) Gene-gene interaction network among THBSs in the GeneMANIA dataset. (B) protein-protein interaction network among THBSs in the STRING dataset.



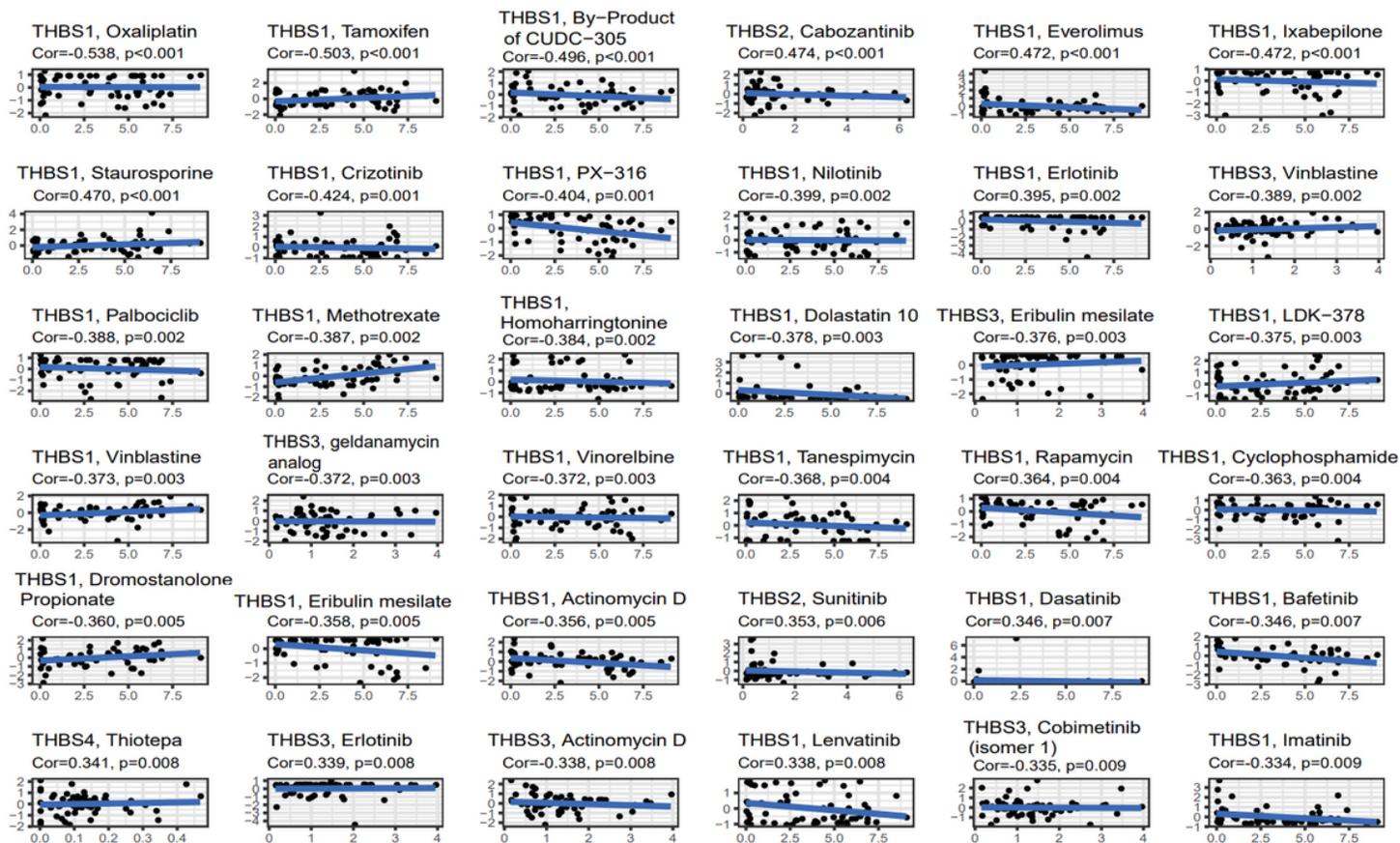
**Figure 7**

Alteration frequency of THBSs in breast cancer (cBioPortal). (A) THBSs genetic alteration in several datasets. (B) Alteration frequency of THBSs based on the TCGA Provisional dataset.



**Figure 8**

The enrichment analysis of THBSs and similar genes in breast cancer patients. (A) Top 20 Gene Ontology (GO) enrichment. (B) Top 10 of Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment. (C) Network of enriched terms of THBSs genes and similar genes.



**Figure 9**

The scatter plot indicates the correlation between THBSs expression and drug sensitivity (the z-score of the CellMiner interface) for the Pearson correlation test using NCI-60 cell line data. Top 36 associations are shown, ordered by P-value.

## Supplementary Files

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