Significance of combined TGF-β1 and survivin expression on the prognosis of patients with triple-negative breast cancer

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Abstract. Compared with other types of breast cancer, triple-negative breast cancer (TNBC) has the characteristics of rapid progression, a lack of specific molecular targets for treatment and a poor prognosis. However, based on previously published studies, TGF-β1 and survivin are potentially meaningful for the prognosis of patients with TNBC. The present study was therefore designed to measure and compare the expression of transforming growth factor-β1 (TGF-β1) and survivin in tissue samples of TNBC and non-TNBC patients in order to evaluate their ability as prognostic indicators. In total, 90 TNBC and 52 non-TNBC tissue specimens were selected, following which immunohistochemistry was used to detect the expression of TGF-β1 and survivin in the cancer tissues. Subsequently, the potential association between the expression levels of these two proteins and the clinicopathological variables was analyzed. The expression levels of TGF-β1 and survivin in TNBC tissues were found to be significantly higher compared with those in the non-TNBC tissues. In addition, the results of the present study demonstrated that TGF-β1 expression was positively associated with survivin expression in the TNBC samples, but no significant correlation was found between TGF-β1 and survivin expression in the non-TNBC samples. Kaplan-Meier (K-M) analysis was performed to assess the levels of TGF-β1 and survivin in regard to patient survival, and univariate and multivariate Cox analyses of TGF-β1 and survivin protein expression were performed to analyze the overall survival (OS) and progression-free survival (PFS) rates of patients with TNBC and non-TNBC. Although multivariate Cox analysis demonstrated that neither TGF-β1 or survivin were independent prognostic predictors of TNBC or non-TNBC, results of the K-M curve revealed that patients with TNBC with TGF-β1- and survivin-positive breast cancer exhibited shorter OS and PFS times. Multivariate Cox analysis demonstrated that in patients with TNBC, the combined expression of TGF-β1 and survivin may yield additional prognostic information, compared with patients with non-TNBC.

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Introduction

Triple-negative breast cancer (TNBC) accounts for approximately 10-20% of all breast cancers, and is negative for estrogen receptor (ER), progesterone receptor (PR) and human epidermal growth factor receptor-2 (HER-2) expression (1,2). Compared with other types of breast cancer, TNBC is characterized by an increased likelihood of development in younger individuals, frequently higher grades, a more aggressive subtype and an increased likelihood to metastasize to distant organs (3,4). For the treatment of TNBC, due to high tumor heterogeneity and the lack of specific therapeutic molecular targets, chemotherapy continues to be the main method of systemic treatment at present (5). However, the majority of patients with TNBC are not sensitive to chemotherapy, such that most relapse following chemotherapy and have a poor prognosis (6,7). Therefore, it remains urgent to reveal the underlying mechanisms of TNBC progression and develop novel treatment strategies for this disease.

Transforming growth factor-β (TGF-β) is a cytokine that is a biologically active polypeptide and regulates the proliferation, apoptosis, migration and differentiation of various cancer cell types (8,9). There are currently three known subtypes of TGF-β, namely TGF-β1, TGF-β2 and TGF-β3, detected in humans (10). In terms of physiological functions, TGF-β1 regulates the development of mammary ducts and acini (11). In breast cancer, TGF-β1 signaling has a dual effect on tumor growth and metastasis (12,13). For instance, during the early stages of tumor progression, TGF-β1 may inhibit this process by inducing premature breast cancer stem cell aging (14). However, in the later stages, TGF-β1 can promote stem cell-like properties in breast cancer cells (15). In addition, high levels of TGF-β1 can enhance the invasive ability of cells and facilitate tumor growth by inducing epithelial-mesenchymal transition (EMT), interstitial blood vessel formation or by promoting evasion from immune surveillance (16-19). Kim *et al* previously demonstrated that downregulation of TGF-β1 significantly

increased the migratory ability of TNBC HCC1806 cells, and that patients with high levels of TGF-β1 expression exhibited a poor prognosis (20). In a TNBC mouse model, treatment with TGF-β1-neutralizing antibodies or receptor serine/threonine kinase inhibitors significantly inhibited the development of lung and bone metastasis (21). These previous data suggest that TGF-β1 may be involved in the progression of TNBC; thus, further research should focus on uncovering the potential value of TGF-β1 in TNBC prognosis.

Survivin, also known as baculoviral IAP repeat-containing protein 5, is a member of the apoptosis-inhibiting protein family (22). Survivin can promote cell proliferation by inhibiting caspase activation and stabilizing microtubules during cell mitosis to protect cells from apoptosis (23). Previous studies have found that survivin is upregulated in a variety of human malignancies (24-26) and is associated with poor prognosis (27-30), leading to proposals of survivin being used as a potential tumor marker and prognostic indicator. However, the significance of survivin in breast cancer progression remain controversial. Kennedy *et al* reported that survivin is an independent predictor, whereby patients with high survivin expression tend to have superior prognoses (31). In addition, Yamashita *et al* (32) and Hinnis *et al* (33) both demonstrated that survivin is an independent predictor, such that high expression levels of survivin are associated with poor prognosis. In contrast, Chu *et al* (34) documented that survivin is not an independent predictor or associated with the recurrence of breast cancer. Notably, molecular characteristics were not assigned based on ER/PR and HER-2 in the aforementioned studies. In terms of TNBC, Yamanaka *et al* found that inhibition of survivin expression can significantly suppress the metastasis of TNBC MDA-MB-231 cells, while also significantly prolonging the survival time of tumor-bearing mice (35). Furthermore, Shi *et al* (36) found that survivin expression was associated with the histological grade and TNM stage of patients with TNBC, where the survival rate of patients with survivin-positive TNBC was lower. Observations from these aforementioned studies suggest that survivin may be involved in the pathophysiological process of TNBC and therefore attention should be paid to the potential value of applying survivin as a prognostic indicator.

In the present study, tissue samples obtained from patients with TNBC and non-TNBC were used, and the expression of TGF-β1 and survivin was analyzed and compared between both types of breast cancer by immunohistochemistry (IHC). The correlation among the expression levels of these two proteins and the various clinicopathological parameters was recorded and analyzed. In addition, factors that can potentially affect prognosis were also investigated. The aim of the present study was to clarify the effects and significance of TGF-β1 and survivin protein expression, either alone or in combination, in regards to the prognosis of TNBC or non-TNBC.

Materials and methods

Patients and tissue specimens. A total of 142 breast cancer paraffin-embedded specimens with complete clinicopathological and regular follow-up data were collected between January 1, 2010 and January 1, 2013 from the Affiliated Hospital of Beihua University (Jilin, China). The present study was approved by the Ethics Committee of the Affiliated Hospital of Beihua University and informed consent was obtained from each patient once the purpose and nature of the study had been fully explained.

The inclusion criteria for patients in the present study was as follows: i) all patients were female with TNBC or non-TNBC, and were diagnosed for the first time; ii) complete clinicopathological and routine follow-up data were available, and iii) no chemotherapy, radiotherapy or other antitumor treatments was performed prior to surgery. All patients underwent radical mastectomy, and there was no distant metastasis at the time of initial diagnosis. Post-operative chemotherapy was based on anthracyclines and paclitaxel drugs for 6-8 cycles. Patients with >3 axillary lymph node metastases received local radiotherapy. Patients with non-TNBC were selected as a control group. Notably, all patients in the control group exhibited no signs of distant metastasis, and received parallel surgical resection, chemotherapy and radiotherapy for axillary lymph node metastasis (as previously described). Among the patients, 90 were diagnosed with TNBC and 52 with non-TNBC using IHC. The main characteristics of patients with TNBC and non-TNBC are summarized in Table I. All patients were followed up until December 2016. The median age of the patients with TNBC was 48 years, ranging from 28 to 84 years. In total, 59 cases were defined as well and moderately differentiated, while 31 cases were defined as poorly differentiated. Breast tumors were histopathologically classified according to the WHO classification (37). The median age of the non-TNBC patients was 51.5 years, ranging from 38 to 70 years. Among them, 35 cases were defined as well and moderately differentiated, whereas 17 cases were categorized as poorly differentiated. Overall survival (OS) was defined as the period of time from surgical removal of the primary tumors to death or to the last follow-up. The range of OS time in patients with TNBC and non-TNBC in the present study was 10-70 and 15-65 months, respectively. The median survival time was 36.75 and 39.50 months, respectively. Progression-free survival (PFS) was defined as the time from primary tumor resection to deterioration (recurrence or metastasis) or death. TNBC or non-TNBC relapse and metastasis were diagnosed by clinical examination, breast ultrasonography, axillary and cervical lymph ultrasonography, chest computed tomography (CT), epigastric magnetic resonance imaging (MRI) or MRI scans. The range of PFS time of patients with TNBC and non-TNBC was 5-70 and 10-65 months, respectively. The median progression time was 25.25 and 35.50 months, respectively.

IHC assay. The paraffin blocks of TNBC and non-TNBC tissues were selected and sliced continuously to a thickness of 5 μm. Pathological diagnosis was performed using light microscopy after hematoxylin and eosin (H&E) staining (hematoxylin staining for 5 min, eosin staining for 20-30 sec, at room temperature). IHC staining (conventional streptavidin peroxidase method) was then used to detect the protein expression of TGF-β1 and survivin.

The staining protocol was performed as follows. First, the paraffin-embedded tissues were dewaxed with xylene and rehydrated with a descending series of ethanol (the concentration of gradient ethanol was 100, 95 and 80% respectively). Then, the slices were placed in a citric acid tissue antigen

Table I. Clinicopathological characteristics in patients with TNBC (n=90) and non-TNBC (n=52).

Characteristics	TNBC patients	Non-TNBC patients
Age, years		
Median	48.0	51.5
Range	28-84	38-70
Tumor size, n (%)		
T_2	27 (30.0)	12 (23.1)
T_3	63 (70.0)	40 (76.9)
Lymph node metastasis, n (%)		
N_0	40 (44.4)	26 (50.0)
N_I	11 (12.2)	5 (9.6)
N_2	34 (37.8)	20 (38.5)
N_3	5 (5.6)	1 (1.9)
TNM classification, n (%)		
Stage II	44 (48.9)	25 (48.1)
Stage III	46 (51.1)	27 (51.9)
Histological grade, n (%)		
Well	1 (1.1)	2 (3.8)
Moderate	58 (64.5)	33 (63.5)
Poor	31 (34.4)	17 (32.7)
ER status, n (%)		
Positive	0 (0.0)	39 (75.0)
Negative	90 (100.0)	13 (25.0)
PR status, n (%)		
Positive	0 (0.0)	21 (40.4)
Negative	90 (100.0)	31 (59.6)
HER-2 status, n (%)		
Positive	0 (0.0)	17 (32.7)
Negative	90 (100.0)	35 (67.3)
Ki67 index, n (%)		
<14%	0 (0.0)	7 (13.5)
≥14%	90 (100.0)	45 (86.5)
Treatment modality-Curative resection, n (%)		
Yes	90 (100.0)	52 (100.0)
No	0 (0.0)	0 (0.0)
Treatment modality-Postoperative chemotherapy, n (%)		
Yes	90 (100.0)	3 (5.8)
No	0 (0.0)	49 (94.2)
Treatment modality-Postoperative radiotherapy, n (%)		
Yes	47 (52.2)	25 (48.1)
No	43 (47.8)	27 (51.9)

TNBC, triple-negative breast cancer; TNM, Tumor, Node, Metastasis; ER, estrogen receptor; PR, progesterone receptor; HER-2, human epidermal growth factor receptor-2.

retrieval solution (100X; cat. no. mvs-0101; Fuzhou Maixin Biotechnology Co., Ltd.), boiled at 95˚C for 20 min and then cooled to room temperature. An endogenous peroxidase blocker (cat. no. kit-9707; Fuzhou Maixin Biotechnology Co., Ltd.) was added to block endogenous peroxidase, which required incubation at room temperature for 10 min to eliminate non-specific staining. The slides were incubated with non-immunized goat serum (ready-to-use; cat. no. kit-9707; Fuzhou Maixin Biotechnology Co., Ltd.) for 10 min at room temperature and the serum was removed. Subsequently, rabbit monoclonal anti-TGF-β1 antibody (1:100; cat. no. ab215715; Abcam) and rabbit monoclonal anti-survivin antibody (1:100; cat. no. ab76424; Abcam) were added to the slices and incubated at 4˚C overnight. After washing the slides three times with PBS, biotin-labeled goat anti-rabbit immunoglobulin (ready-to-use; cat. no. kit-9707; Fuzhou Maixin Biotechnology Co., Ltd.) was added and incubated for 10 min at room temperature. Then, in a wet box, streptavidin-biotin protein peroxidase (cat. no. kit-9707; Fuzhou Maixin Biotechnology Co., Ltd.) was added to the slices and incubated at room temperature for 10 min. After washing the sample with PBS, it was treated with 3,3'-diaminobenzidine (DAB; cat. no. DAB-0031; Fuzhou Maixin Biotechnology Co., Ltd.) for 5 min at room temperature and counterstained with hematoxylin at room temperature for 1 min.

Normal rabbit serum (ready-to-use; cat. no. AR0010; Wuhan Boster Biological Technology Co., Ltd.) was used as the negative control instead of rabbit monoclonal anti-TGF-β1 or rabbit monoclonal anti-survivin antibody. Breast cancer tissues previously confirmed to express TGF-β1 or survivin protein were used as positive controls and controls were used in each experiment. The stained specimens were observed under an optical microscope at x200 and x400 magnifications.

IHC evaluation. Each slice was evaluated by using a blind reading method. All IHC-stained sections were scored by at least three of four independent and experienced pathologists (NL, CY, DQ and JZ) who participated in the present study. They had no prior knowledge of the clinicopathological parameters or of clinical outcomes of the patients. In total, images taken from five high-power visual fields (magnification, x400) were randomly selected per section and evaluated. The staining results were scored according to the proportion of positive cells and staining intensity in each section, where a semi-quantitative analysis was performed by multiplying the staining intensity score by the positive cell rate score, generating the immunoreactive score (IRS) (38). The percentage of positive cells was assessed as follows: i) <10% was defined as 0 points; ii) 10-25% was scored as 1 point; iii) 26-50% was defined as 2 points; iv) 51-75% was scored as 3 points; v) >75% was scored as 4 points (39). Staining intensity was evaluated as follows: i) 0 points for no staining or light yellow; ii) 1 point for light brown; iii) 2 points for brown; iv) 3 points for dark brown. In view of the fact that the expression of survivin in both the cytoplasm and the nucleus is associated with cell proliferation or survival (40), Taubert *et al* also suggested that survivin expression in both the cytoplasm and the nucleus should be considered together to evaluate its impact on prognosis (41); therefore, we chose average fractions of the cytoplasm and nucleus for the evaluation of survivin expression.

Table II. Association analysis of clinicopathological parameters with TGF-β1 and survivin expression in patients with TNBC (n=90).

		TGF-β1				Survivin			
Variable	n	(-)	(+)	χ^2	P-value	(-)	(+)	χ^2	P-value
Total (TNBC)	90	24	66			19	71		
Age, years									
≤48	51	14	37	0.037[a]	0.847	10	41	0.160[a]	0.689
>48	39	10	29			9	30		
Tumor size									
T2	27	10	17	2.121[a]	0.145	10	17	5.874[a]	0.015[b]
T3	63	14	49			9	54		
Lymph node metastasis									
Positive	50	10	40	2.557[a]	0.110	8	42	1.765[a]	0.184
Negative	40	14	26			11	29		
TNM classification									
Stage II	44	15	29	2.426[a]	0.119	12	32	1.962[a]	0.161
Stage III	46	9	37			7	39		
Tumor differentiation									
Well/moderate	59	16	43	0.018[a]	0.894	12	47	0.061[a]	0.804
Poor	31	8	23			7	24		

[a]Pearson's χ^2 test. [b]Statistically significant. TGF, tumor growth factor; TNBC, triple-negative breast cancer; TNM, Tumor, Node, Metastasis.

If differential intensities were detected between the cytoplasm and nucleus, we used the average fraction of the cytoplasm and nucleus. The higher the IRS, the higher the protein expression level, which was rated as follows: i) 0, no staining; ii) 1-4, weak staining; iii) 5-8, moderate staining; iv) 9-12, strong staining. An IRS of <1 was considered to indicate negative staining for TGF-β1 or survivin.

Statistical analysis. SPSS 25.0 statistical software (IBM Corp.) was used for statistical analysis and processing. Mann-Whitney U test was used to compare the expression levels of TGF-β1 or survivin in TNBC or non-TNBC tissues. Pearson correlation analysis was used to test the correlation between TGF-β1 and survivin, while the association between TGF-β1 or survivin expression and clinicopathological parameters was tested by using a χ^2 test or continuous correction χ^2 test. Kaplan-Meier (K-M) method with log-rank test was used to construct the OS and PFS curves. Cox regression was used for the univariate and multivariate analyses of OS and PFS. P<0.05 was considered to indicate a statistically significant difference.

Results

TGF-β1 and survivin expression in TNBC and non-TNBC samples. Among the 90 cases of TNBC, 24 (26.7%) were tested negative for TGF-β1 expression, whereas 66 (73.3%) had positive TGF-β1 expression. By contrast, 19 (21.1%) tested negative for survivin expression and 71 (78.9%) had positive expression (Table II). Among the 52 cases of non-TNBC, 23 (44.2%) were negative for TGF-β1 and 29 (55.8%) were positive for TGF-β1. In addition, 14 (26.9%) were negative for survivin and 38 (73.1%) were positive for surviving (Table III). Positive TGF-β1 protein staining was mainly distributed in the cytoplasm, while positive survivin protein staining was detected both in the nucleus and cytoplasm (Fig. 1). Comparing the expression levels of these two proteins in TNBC and non-TNBC, TGF-β1 expression levels were significantly higher in TNBC compared with those in non-TNBC (z=-2.009; P=0.045). Similarly, the levels of survivin protein expression in TNBC were also significantly higher compared with those in non-TNBC (z=-4.417; P<0.001; Fig. 2).

According to the expression levels of TGF-β1 and survivin that were detected in each patient in this study (that is, the IRS value of these two proteins in each patient), the correlation between TGF-β1 and survivin in TNBC and non-TNBC samples was determined using Pearson correlation analysis. According to the Pearson correlation coefficient and the P-value, the expression of TGF-β1 was found to be positively correlated with that of survivin in TNBC (r=0.326; P=0.002), but no correlation was identified between TGF-β1 or survivin expression in non-TNBC (r=0.072; P=0.610; Fig. 3).

Association between TGF-β1 or survivin expression and clinicopathological parameters. To study the effect of TGF-β1 or survivin on patient prognosis, the potential association between TGF-β1 or survivin expression and the traditional prognostic markers, including age, tumor diameter, tumor histological grade and lymph node metastasis, was analyzed. In TNBC, TGF-β1 protein expression was not found to be associated with any of the clinicopathological parameters (P>0.05; Table II), whereas survivin protein expression was not associated with any of the clinicopathological parameters (P>0.05;

Table III. Association analysis of clinicopathological parameters with TGF-β1 and survivin expression in patients with non-TNBC (n=52).

		TGF-β1				Survivin			
Variable	n	(-)	(+)	χ^2	P-value	(-)	(+)	χ^2	P-value
Total (non-TNBC)	52	23	29			14	38		
Age, years									
≤51.5	26	13	13	0.702[a]	0.402	9	17	1.564[a]	0.211
>51.5	26	10	16			5	21		
Tumor size									
T2	12	5	7	0.042[a]	0.838	4	8	0.040[b]	0.842
T3	40	18	22			10	30		
Lymph node metastasis									
Negative	26	10	16	0.702[a]	0.402	6	20	0.391[a]	0.532
Positive	26	13	13			8	18		
TNM classification									
Stage II	25	9	16	1.322[a]	0.250	6	19	0.209[a]	0.647
Stage III	27	14	13			8	19		
Tumor differentiation									
Well/moderate	35	12	23	4.293[a]	0.038[c]	8	27	0.378[b]	0.538
Poor	17	11	6			6	11		

[a]Pearson's χ^2 test; [b]continuous correction χ^2 test. [c]Statistically significant. TGF, transforming growth factor; TNBC, triple-negative breast cancer; TNM, Tumor, Node, Metastasis.

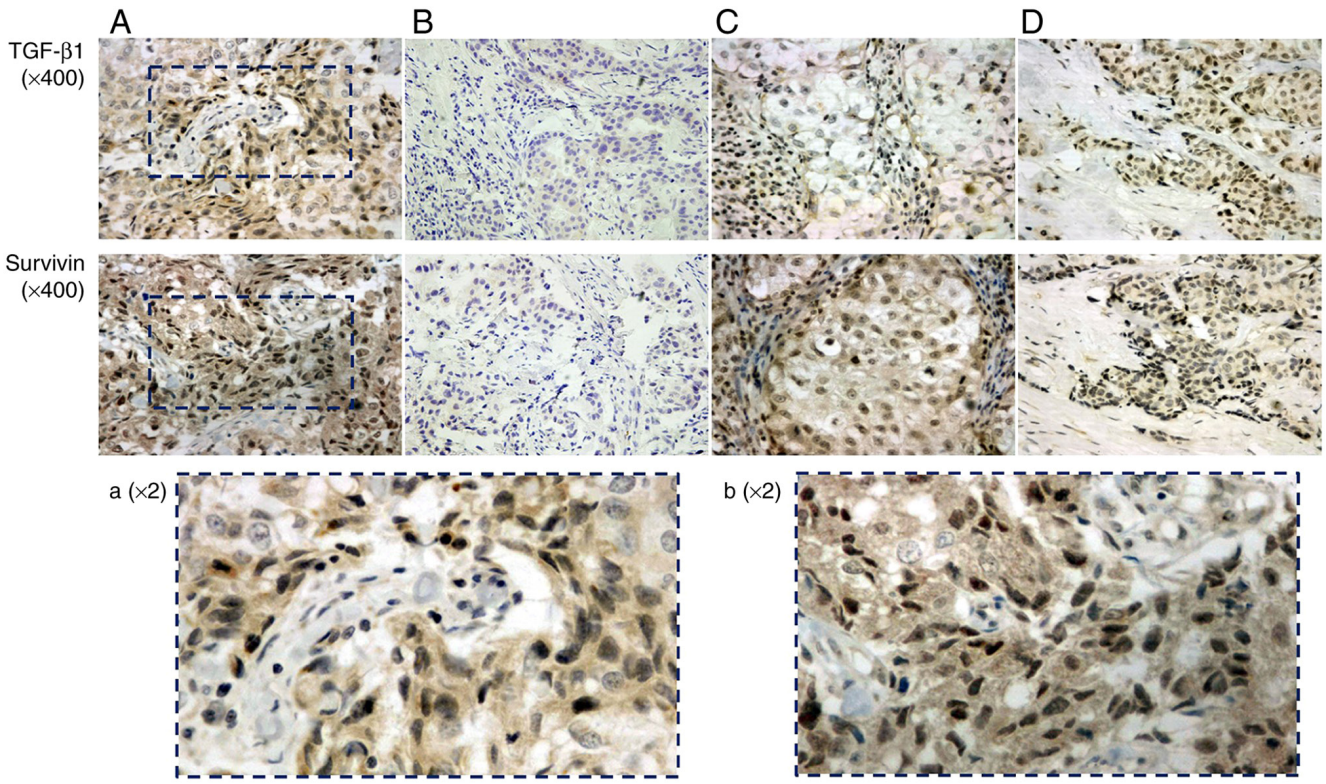

Figure 1. Analysis of TGF-β1 and survivin expression using immunohistochemical staining. (A-D) Representative immunohistochemical staining of TGF-β1 and survivin in four different TNBC specimens (magnification, x400). (A) The staining intensity of TGF-β1 and survivin was strong. (B) The expression of TGF-β1 and survivin was negative. (C) Expression of TGF-β1 was weak, while the expression of survivin was moderate. (D) Expression of TGF-β1 was moderate, while that of survivin was weak. (a) The area circled by the dotted line in figure A (TGF-β1) is enlarged (magnification, x2). (b) The area circled by the dotted line in figure A (survivin) is enlarged (magnification, x2). TGF-β1, transforming growth factor-β1; TNBC, triple-negative breast cancer.

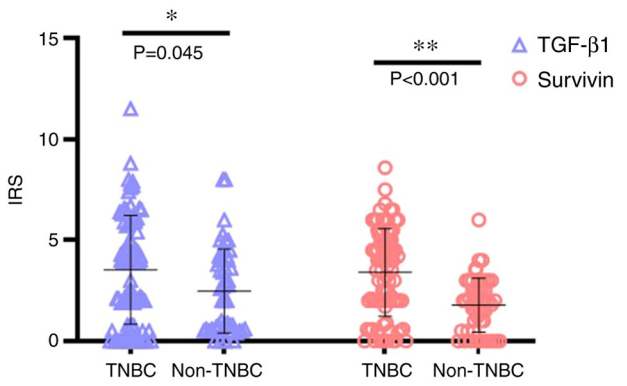

Figure 2. Comparison of TGF-β1 and survivin protein expression in patients with TNBC and non-TNBC. *P<0.05, **P<0.001, TNBC vs. non-TNBC. IRS, immunoreactive score; TGF-β1, transforming growth factor-β1; TNBC, triple-negative breast cancer.

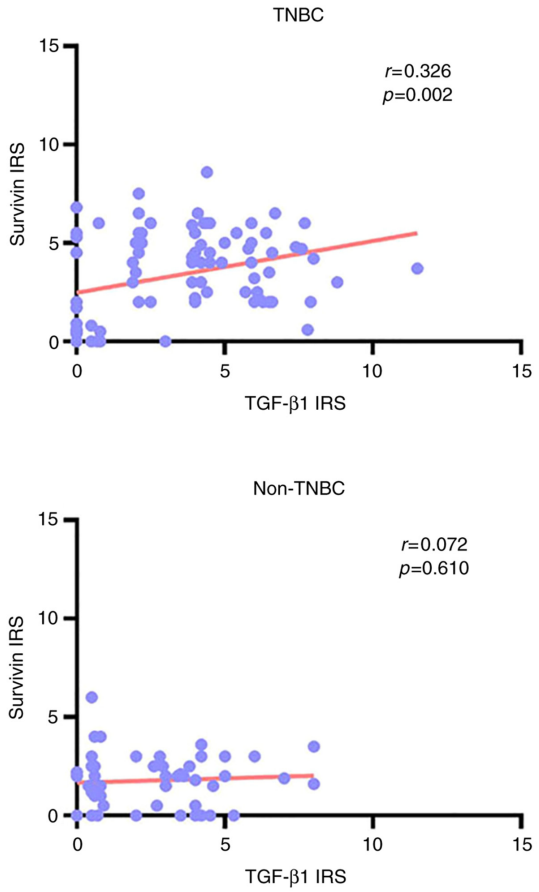

Figure 3. Correlation analysis of TGF-β1 and survivin expression in patients with TNBC and non-TNBC. IRS, immunoreactive score; TGF-β1, transforming growth factor-β1; TNBC, triple-negative breast cancer.

Table II) except for tumor diameter (P=0.015; Table II). In the non-TNBC cases, TGF-β1 protein expression was not associated with any of the clinicopathological parameters (P>0.05; Table III), except for histological tumor grade (P=0.038; Table III), while survivin protein expression was not associated with any of the clinicopathological parameters (P>0.05; Table III).

Association between TGF-β1 or survivin expression levels and the survival of patients with TNBC and non-TNBC. K-M analysis showed that the OS time (P=0.001) and PFS time (P=0.003) of patients with TGF-β1-positive TNBC was significantly shortened compared with that in patients with TGF-β1-negative TNBC (Fig. 4A). In addition, both the OS (P<0.001) and PFS (P=0.003) times of patients with survivin-negative TNBC were significantly longer compared with those of patients with survivin-positive TNBC (Fig. 4A). By contrast, in patients with non-TNBC, neither TGF-β1 nor survivin protein expression levels conferred significant differences in OS time (P=0.284 for TGF-β1 and P=0.819 for survivin; Fig. 4B) and PFS time (P=0.216 for TGF-β1 and P=0.363 for survivin; Fig. 4B).

Univariate and multivariate analysis of the prognostic variables in patients with TNBC or non-TNBC. Univariate analysis revealed that in patients with TNBC, poorly differentiated tumors, TGF-β1-positive and survivin-positive expression significantly predicted a shorter OS times (P=0.008, P=0.001 and P=0.001, respectively; Table IV) and increased risks of disease progression (P=0.001, P=0.004 and P=0.004, respectively; Table IV). However, the status of tumor differentiation, levels of TGF-β1 and survivin expression in patients with non-TNBC were not found to predict OS time (P=0.195, P=0.295 and P=0.821, respectively; Table IV) or PFS time (P=0.105, P=0.225 and P=0.370; respectively; Table IV). Subsequently, since the levels of TGF-β1 protein expression were found to be positively correlated with those of survivin protein expression in patients with TNBC, the patients were divided into four subgroups according to their expression profiles of TGF-β1 and survivin: TGF-β1/survivin co-negative group, TGF-β1/survivin co-positive group, TGF-β1-negative/survivin-positive group and TGF-β1-positive/survivin-negative group. Univariate analysis showed that TGF-β1/survivin co-expression (co-negative vs. co-positive) was significantly associated with OS time (P=0.001, Table IV) and PFS time (P=0.003, Table IV), while neither TGF-β1-negative/survivin-positive expression (vs. TGF-β1/survivin co-negative expression) nor TGF-β1-positive/survivin-negative expression (vs. TGF-β1/survivin co-negative expression) were associated with OS time (P=0.077 and P=0.417 Table IV) and PFS time (P=0.412 and P=0.960 p>0.05; Table IV) in patients with TNBC. In patients with non-TNBC, none of the four subgroups were associated with OS time (P>0.05; Table IV) or PFS time (P>0.05; Table IV). Other clinicopathological parameters, including age, tumor size and lymph node metastasis, were not found to be significantly associated with the OS time (P>0.05; Table IV) or PFS time (P>0.05; Table IV) in both patients with TNBC and patients with non-TNBC. Multivariate Cox regression analysis showed that in patients with TNBC, expression of either TGF-β1 or survivin alone was not an independent predictor of prognosis (P=0.572 and P=0.059, respectively; Table V) in OS time or progression of deterioration (P=0.365 and 0.126, respectively; Table V) in PFS time. However, the status of tumor differentiation remained to be a significant independent predictor (P=0.003 and P<0.001, respectively; Table V), whether in OS or PFS time. After the comprehensive consideration of the TGF-β1/survivin co-expression profile and tumor differentiation, both were found to be

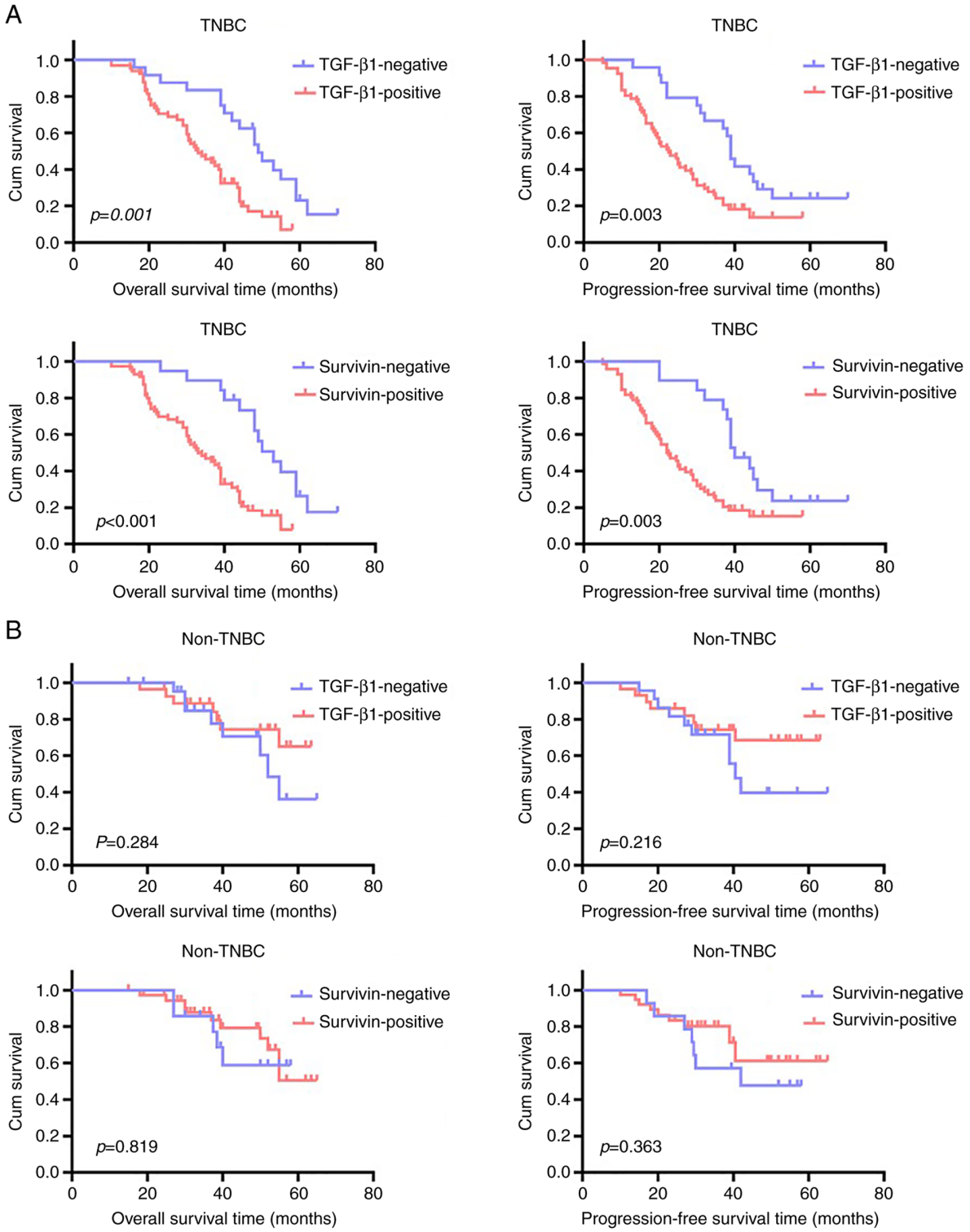

Figure 4. Effects of TGF-β1 and survivin expression on OS and PFS in non-TNBC and TNBC patients. (A) OS and PFS of patients with TNBC as stratified according to TGF-β1 or survivin expression. (B) OS and PFS of patients with non-TNBC as stratified according to TGF-β1 or survivin expression. TGF-β1, transforming growth factor-β1; TNBC, triple-negative breast cancer; OS, overall survival; PFS, progression-free survival.

independent predictors of OS (P=0.004 and P=0.002 respectively; Table V) and PFS (P=0.006 and P<0.001 respectively; Table V) in patients with TNBC. In patients with non-TNBC, neither the tumor differentiation status nor the expression profile of TGF-β1 and survivin, either alone or in combination, were significant predictors for OS (P>0.05; Table V) and PFS (P>0.05; Table V).

Discussion

The present study found that the expression levels of transforming growth factor-β1 (TGF-β1) protein in triple-negative breast cancer (TNBC) tissues were significantly higher compared with those in their non-TNBC counterparts. A previous study performed in 1,881 breast cancer tissue samples

Table IV. Univariate analysis of clinicopathological parameters for overall (OS) and progression-free survival (PFS) in patients with TNBC and non-TNBC.

A, OS

Variable	TNBC univariate Cox analysis HR (95% CI)	P-value	Non-TNBC univariate Cox analysis HR (95% CI)	P-value
Age[a]	1.118 (0.689-1.814)	0.652	1.370 (0.495-3.788)	0.544
Tumor size[b]	1.317 (0.784-2.211)	0.298	1.509 (0.472-4.820)	0.487
Tumor differentiation[c]	1.950 (1.189-3.198)	0.008[d]	1.977 (0.705-5.544)	0.195
Lymph node metastasis[e]	0.946 (0.583-1.537)	0.824	1.641 (0.583-4.623)	0.349
TGF-β1[e]	2.685 (1.467-4.913)	0.001[d]	0.579 (0.209-1.608)	0.295
Survivin[e]	3.221 (1.635-6.348)	0.001[d]	0.883 (0.302-2.586)	0.821
TGF-β1/survivin co-expression		0.007[d]		0.741
TGF-β1(-)/survivin(-)	1		1	
TGF-β1(-)/survivin(+)	2.688 (0.899-8.035)	0.077	0.986 (0.199-4.890)	0.986
TGF-β1(+)/survivin(-)	2.149 (0.269-17.182)	0.417	0.711 (0.118-4.272)	0.709
TGF-β1(+)/survivin(+)	3.512 (1.720-7.169)	0.001[d]	0.500 (0.091-2.743)	0.425

B, PFS

Variable	TNBC univariate Cox analysis HR (95% CI)	P-value	Non-TNBC univariate Cox analysis HR (95% CI)	P-value
Age[a]	1.139 (0.711-1.827)	0.588	1.104 (0.437-2.788)	0.834
Tumor size[b]	1.271 (0.761-2.122)	0.360	1.054 (0.372-2.982)	0.922
Tumor differentiation[c]	2.256 (1.386-3.673)	0.001[d]	2.151 (0.851-5.435)	0.105
Lymph node metastasis[e]	0.869 (0.543-1.391)	0.558	1.780 (0.690-4.595)	0.233
TGF-β1[e]	2.284 (1.309-3.983)	0.004[d]	0.561 (0.221-1.428)	0.225
Survivin[e]	2.429 (1.332-4.431)	0.004[d]	0.648 (0.251-1.673)	0.370
TGF-β1/survivin co-expression		0.020[d]		0.489
TGF-β1(-)/survivin (-)	1		1	
TGF-β1(-)/survivin (+)	1.542 (0.548-4.344)	0.412	0.736 (0.190-2.851)	0.657
TGF-β1(+)/survivin (-)	1.053 (0.137-8.099)	0.960	0.682 (0.152-3.049)	0.616
TGF-β1(+)/survivin (+)	2.615 (1.393-4.910)	0.003[d]	0.334 (0.075-1.496)	0.152

[a]≤median age vs. >median age; [b]T2 vs. T3; [c]well/moderate vs. poor; [d]Statistically significant; [e]negative vs. positive. TNBC, triple-negative breast cancer; TGF, transforming growth factor; HR, hazard ratio; CI, confidence interval.

by Hachim *et al* also found that the expression level of TGF-β1 mRNA in patients with TNBC was significantly higher compared with that in other types of breast cancer (42). In addition, Kim *et al* found that TGF-β1 mRNA expression and the invasive abilities of TNBC cells (MDA-MB-231, Hs578T and HCC1806) were significantly higher compared with those of non-TNBC cells (BT474, ZR75-1 and SKBR3) (43). The authors also revealed that treatment with the dual selective TGF-β1 receptor (RI/RII) inhibitor LY2109761 completely inhibited the invasiveness of the TNBC cells (43). However, the molecular mechanism underlying the differential expression profile of TGF-β1 in TNBC and non-TNBC remains unclear. It has been found that expression of the circular RNA ankyrin repeat and sterile α motif domain containing 1b (circANKS1B) was closely correlated with the invasion, metastasis and poor prognosis of breast cancer (including all subtypes of breast cancer) (44); in addition, circANKS1B expression was found to be low in non-TNBC tissues or cells (MCF-7), but was significantly higher in TNBC tissues or cells (MDA-MB-231) (44). Increased circANKS1B expression was found to increase the expression of the transcription factor upstream transcription factor 1, to upregulate the expression of TGF-β1 by directly binding to the promoter. Thus, TGF-β1/Smad signaling is activated to enhance epithelial-mesenchymal transition (EMT) and metastasis (44). This may partially explain the differential expression of TGF-β1 in TNBC and non-TNBC tissues found in the present study. Compared with those in patients with TGF-β1-positive TNBC, patients with TGF-β1-negative expression exhibited longer overall survival (OS) and progression-free survival (PFS) times, in addition to a better prognosis, although multivariate Cox analysis revealed that TGF-β1 expression was not an

Table V. Multivariate analysis of clinicopathological parameters for overall (OS) and progression-free survival (PFS) in patients with TNBC and non-TNBC.

A, OS-Comprehensive consideration of tumor differentiation, TGF-β1 and survivin

Variable	TNBC Multivariate Cox analysis HR (95% CI)	P-value	non-TNBC Multivariate Cox analysis HR (95% CI)	P-value
Tumor differentiation	2.127 (1.289-3.510)	0.003[a]	1.708 (0.534-5.464)	0.367
TGF-β1	1.308 (0.516-3.321)	0.572	0.726 (0.229-2.299)	0.586
Survivin	2.782 (0.961-8.051)	0.059	0.850 (0.289-2.503)	0.769

B, OS-comprehensive consideration of tumor differentiation and TGF-β1/survivin co-expression

Variable	TNBC Multivariate Cox analysis HR (95% CI)	P-value	non-TNBC Multivariate Cox analysis HR (95% CI)	P-value
Tumor differentiation	2.197 (1.322-3.652)	0.002[a]	1.740 (0.543-5.570)	0.351
TGF-β/survivin co-expression		0.004[a]		0.924
TGF-β1(-)/survivin(-)	1		1	
TGF-β1(-)/survivin(+)	3.388 (1.116-10.290)	0.031[a]	1.060 (0.212-5.287)	0.944
TGF-β1(+)/survivin(-)	3.319 (0.404-27.297)	0.264	0.971 (0.144-6.532)	0.976
TGF-β1(+)/survivin(+)	3.855 (1.876-7.922)	<0.001[a]	0.673 (0.111-4.093)	0.667

C, PFS-Comprehensive consideration of tumor differentiation, TGF-β1 and survivin

Variable	TNBC Multivariate Cox analysis HR (95% CI)	P-value	non-TNBC Multivariate Cox analysis HR (95% CI)	P-value
Tumor differentiation	2.656 (1.608-4.388)	<0.001[a]	1.768 (0.621-5.037)	0.286
TGF-β1	1.493 (0.627-3.556)	0.365	0.690 (0.242-1.967)	0.488
Survivin	2.099 (0.812-5.423)	0.126	0.662 (0.253-1.733)	0.401

D, PFS-comprehensive consideration of tumor differentiation and TGF-β1/survivin co-expression

Variable	TNBC Multivariate Cox analysis HR (95% CI)	P-value	non-TNBC Multivariate Cox analysis HR (95% CI)	P-value
Tumor differentiation	2.675 (1.606-4.455)	<0.001[a]	1.857 (0.651-5.293)	0.247
TGF-β/survivin co-expression		0.006[a]		0.727
TGF-β1(-)/survivin(-)	1		1	
TGF-β1(-)/survivin(+)	2.180 (0.756-6.291)	0.149	0.885 (0.222-3.525)	0.862
TGF-β1(+)/survivin(-)	1.733 (0.22-13.644)	0.602	1.010 (0.197-5.161)	0.991
TGF-β1(+)/survivin(+)	3.160 (1.663-6.007)	<0.001[a]	0.491 (0.097-2.498)	0.392

[a]Statistically significant. TNBC, triple-negative breast cancer; TGF, transforming growth factor; HR, hazard ratio; CI, confidence interval.

independent predictor in patients with TNBC, whether in OS or PFS time. In addition, no significant difference was found in OS or PFS between patients with TGF-β1-positive non-TNBC and patients with TGF-β1-negative non-TNBC. This suggest that TGF-β1 expression may be more beneficial in predicting the prognosis of patients with TNBC, in which TGF-β1 may serve as a key signaling component. Similarly, although survivin was also not observed to be an independent predictor of OS and PFS in patients with TNBC, the survival rate of patients with survivin-negative expression was significantly higher compared with that with survivin-positive expression. However, in patients with non-TNBC, survivin protein expression was not associated with OS or PFS. Therefore, the expression of survivin appeared to be of higher importance for the malignant progression of TNBC instead of non-TNBC, where patients with TNBC showing higher expression levels of survivin tended to exhibit more severe malignancy, resulting in poorer prognoses.

Shi *et al* also drew a similar conclusion in a previous study with TNBC, whereby survivin-positive patients tended to have shorter OS and disease-free survival times (36), but Dogu *et al* and Jha *et al* did not observe any effects exerted by survivin on the prognosis of patients with TNBC (45,46). These discrepancies may be attributed to the different antibodies used, distinct sample groups or the cut-off criteria set prior to evaluation. Although some studies found that survivin was a prognostic marker of breast cancer (47,48), further research is required with regards to its significance in the progression of TNBC.

Another finding in the present study was that there was a positive correlation between the expression levels of TGF-β1 and survivin in TNBC, but not in non-TNBC. Therefore, further study on the significance of both TGF-β1 and survivin expression combined with the prognosis of patients with TNBC and patients with non-TNBC was performed. In this subsequent study, it was found that the survival time of patients with TGF-β1/survivin co-positivity was significantly shorter compared with that of patients who were negative for both TGF-β1 and survivin expression in TNBC. This suggests that the positive expression of TGF-β1 and survivin combined can be used as an independent predictor of prognosis for patients with TNBC. However, in non-TNBC, combined TGF-β1 and survivin expression was not associated with the OS or PFS of the patients, which may mean that the combined expression profile of TGF-β1 and survivin can mediate different effects in TNBC and non-TNBC, such that the combined positivity of TGF-β1 and survivin can predict the prognosis in patients with TNBC, but not in patients with non-TNBC.

Results of a previous study demonstrated that TGF-β1 was a negative regulator of survivin in a healthy prostatic epithelial cell line and in malignant tumors during the early stages of development (49). TGF-β1 activates retinoblastoma (Rb) by inducing the low phosphorylation of Rb, causing Rb to bind to E2F transcription factor 4 to form a repressor complex on the survivin promoter. Simultaneously, the TGF-β1/survivin regulatory axis remains intact to ensure sensitivity to apoptotic signals (50). Thus, TGF-β1 inhibits the expression of survivin in the non-TNBC MCF-7 cell line (51). Results of a further previous study demonstrated that TGF-β1 could not inhibit survivin expression; however, TGF-β1 may promote survivin expression in glioblastoma (52). TGF-β can induce survivin gene expression by activating the NF-κB subunit p65/RelA in mouse 4T1 TNBC cells (53), while in human MDA-MB-231 TNBC cells, TGF-β1 can upregulate survivin expression by activating the Wnt/β-catenin pathway (54,55). Indeed, upregulation of Wnt/β-catenin signaling in TNBC compared with that in non-TNBC and normal healthy tissues has been frequently observed (56-58). This may be the reason underlying the observation that the expression levels of survivin in TNBC were higher compared with those in non-TNBC in the present study. The regulatory relationship between TGF-β1 and survivin may be bidirectional. Although the specific mechanism underlying this phenomenon was not found, it may facilitate the understanding of the reason behind the prognosis of patients positive for both TGF-β1 and survivin expression being worse than that of patients negative for both TGF-β1 and survivin expression in the present study. In this patient subgroup, TGF-β1 may serve as the initiator of survivin expression, which may then inhibit cell apoptosis by promoting survivin expression and the proliferation of tumor cells. This may also transform TGF-β1 from a tumor suppressor into a tumor promoter. However, it should be noted that no associations between combined TGF-β1 and survivin expression and prognosis were found in patients with non-TNBC, suggesting that the TGF-β1/survivin signaling pathway serves a greater role in the malignant progression of TNBC.

In conclusion, the results of the present study showed that for patients with TNBC, the prognosis of those that tested negative for TGF-β1 or survivin expression was superior compared with that in patients positive for TGF-β1 or survivin expression. Although the expression levels of either TGF-β1 or survivin alone could not be used as an independent predictor, there was an interesting finding whereby the expression of TGF-β1 and survivin was positively correlated in the tissue samples of patients with TNBC. However, this was not found in the samples of patients with non-TNBC. According to the results of the present study, the combined levels of TGF-β1/survivin expression can be used as an independent prognostic factor for patients with TNBC, but not in their non-TNBC counterparts. Since TNBC is highly malignant with no specific treatment options available, analysis of TNBC and non-TNBC samples is of importance for the optimization of clinical treatment regimens, which may help to avoid improper or excessive treatment. Thus, further attention should be paid to the combined expression levels of TGF-β1 and survivin in patients with TNBC. Of course, the present study had its limitation as it detected the expression of proteins in paraffin samples only by immunohistochemistry, and future research will attempt to confirm the results of the present study by analyzing TGF-β1 and survivin mRNA and protein levels in fresh TNBC and non-TNBC samples.

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Availability of data and materials

The datasets used and/or analyzed during the present study are available from the corresponding author on reasonable request.

Authors' contributions

NL and CY contributed to the study design. DQ, CY and NL contributed to data analysis. NL, JZ and JJ contributed to the collection of the tissue samples and patient data. NL and CY wrote the manuscript. NL and CY confirm the authenticity of all the raw data. All authors read and approved the manuscript and agree to be accountable for all aspects of the research in ensuring that the accuracy or integrity of any part of the

work (including the data) are appropriately investigated and resolved.

Ethics approval and consent to participate

The study was approved by the Ethics Committee of the Affiliated Hospital of Beihua University [grant no. (2018)10], and informed consent was obtained from each patient once the purpose and nature of the study had been fully explained.

Patient consent for publication

Informed consent was obtained from all patients regarding the publication of the data and associated images.

Competing interests

The authors declare that they have no competing interests.

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