

Correlation of TBK1, AR, and other serum cancer-related biomarkers in breast cancer patients

An observational study

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Abstract

Breast cancer (BC) ranks first for incidence and mortality in gynecological malignant tumors. This study aims to investigate the diagnostic value of Tank-binding kinase 1 (TBK1) and its correlation with androgen receptor (AR) and other serum cancer-related biomarkers in BC patient. The present observational study included 451 female BC patients and 451 healthy controls. Serum levels of TBK1, AR and other cancer-related biomarkers were detected in all the patients and healthy controls. Patients' demographic data and clinical data including age, body mass index (BMI), tumor node Metastasis (TNM), pathological type, tumor size and lymph node metastasis were collected. The follow-up lasted for 5 years.

The deceased group had higher rate of patients with TNM III-IV, lymph node metastasis or tumor diameter >2. Deceased group had much higher rate of patients with negative ER and positive Ki67. Besides, increased TBK1 was found in BC patients with positive correlation with AR, CA15-3, CA125, CEA, and CA19-9. Serum TBK1 was associated with the clinic outcomes of BC patients and those with high TBK1 had lower 5-year survival rate. Moreover, cutoff value of 13.95 ng/mL TBK1 showed AUC of 0.981 (93.6% for sensitivity and 86.3% for specificity) for diagnosing BC, and cutoff value of 22.65 ng/mL TBK1 had AUC of 0.996 (97.7% for sensitivity and 96.3% for specificity) for diagnosing the death of BC patients. Serum TBK1 was positively correlated with AR and other serum cancer-related biomarkers. In addition, high TBK1 predicted the poor prognosis and might be used for the diagnosis of BC.

Abbreviations: AR = androgen receptor, BC = breast cancer, BMI = body mass index, ELISA = enzyme linked immunosorbent assay, IHC = immunohistochemistry, K-M curve = Kaplan-Meier curve, ROC curve = receiver operating characteristic curve, TBK1 = Tank-binding kinase 1, TNM = tumor node metastasis.

Keywords: AR, breast cancer, serum cancer-related biomarkers, TBK1

1. Introduction

Breast cancer (BC) ranks first for incidence and mortality in gynecological malignant tumors. Recent data shows more than 2.3 million cases are newly diagnosed as BC every year worldwide.^[1,2] In China, a total number of 248,620 new BC cases were identified each year with a mortality of 6.6 per 10,000, and the 5-year survival rate was estimated to be 88%, ranging from 58% to 90% in different areas.^[3] Current evidence shows a good prognosis for BC patients on early stage, however, the prognosis of patients on advanced stage with metastasis or patients with recurrence are not satisfied.^[4] The morbidity and mortality of BC have increased rapidly in last decades, thus, new diagnostic biomarker and therapeutic target are of great significance to improve the clinic outcomes.

Tank-binding kinase (TBK1) is a kind of atypical I κ B kinases interacting with multiple substrates.^[5] Numerous studies show that TBK1 plays important roles in the process of inflammatory response, autophagy, and cell death. TBK1 was found to be involved in autophagy and mitophagy, and mutations of TBK1 might result in impaired autophagy and induce amyotrophic lateral sclerosis.^[6] Zhao et al reported TBK1 regulated metabolism and inflammatory response in adipose tissue.^[7] Nozawa et al demonstrated TBK1 domain family member 9 (TBC1D9) mediated TBK1 expression in xenophagy and mitophagy via Ca²⁺ signaling in autophagy.^[8]

Circulating evidence shows TBK1 is involved in pathogenesis of different cancers.^[9] A previous review suggests TBK1 have become a novel promising target against cancers.^[10] Inhibition of TBK1 suppressed cell growth in VHL-deficient kidney cancer, besides, an in vivo study on orthotopic

QLM and LHM contributed equally to this work.

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

All data in this study can be obtained from the authors for reasonable use.

This study has obtained the approval from the ethic committee of Affiliated Hospital of ChengDe Medical College. Written informed consent was obtained from all participants. Declaration of Helsinki was obeyed in this study.

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xenograft model proved TBK1 deficiency alleviated kidney tumorigenesis.^[11] TBK1 is also found to facilitate the tumor progression and considered as a potential treatment target for BC.^[12] Deng et al reported that knockout of TBK1 inhibited cell growth in HER-2⁺ BC mouse model, and clinic data further confirmed this finding.^[13] However, up to now, the role of TBK1 in BC and its association with the prognosis of BC patients is still unclear.

Androgen receptor (AR) is expressed in 3 main BC subtypes and AR-directed therapies for BC attracts more and more attentions.^[14] The ratio of AR/ER is an independent predictor of disease-free survival and disease specific survival; besides, AR inhibitor suppressed estradiol-mediated cell proliferation and enhanced cell apoptosis in ER+/AR+ BC.^[15] The expression rate of AR-positive was 72.9% in whole sections from primary BCs, and a significant correlation was found between AR and human epidermal growth factor receptor type 2 (HER-2) overexpression.^[16] A review also shows 60% to 80% positive expression of AR in BC and illustrates AR pathway is associated with key signaling pathways, such as PI3K/Akt/mTOR and MAPK pathways.^[17] However, no research focuses on the correlation between AR and TBK1 in BC.

Our research aims to study the role of TBK1, AR and serum cancer-related biomarkers in BC patients. It was found that TBK1 expression was positively correlated with the levels of AR and cancer-related biomarkers. High TBK1 expression was associated with poor prognosis of BC patients. Additionally, TBK1 showed diagnostic value for BC and the death in BC patients.

2. Objectives

Our research firstly aims to investigate the role of TBK1 in the progression of BC. Secondly, the association between TBK1 and prognosis of BC patients will be analyzed. Additionally, the association between TBK1, AR and biomarkers related to cancer will also be studied. Finally, the diagnostic value of TBK1 will be analyzed.

3. Methods and Materials

3.1. Patients

The present prospective observational study included 451 Asian cases of female BC patients who were admitted to our hospital from May 2013 to December 2015. All patients were diagnosed as BC by histological analysis. The cancer stage was defined by tumor, nodes, metastasis (TNM) stage. The inclusion criteria were patients were diagnosed as primary BC for the first time and no patient received any chemotherapy or radiotherapy before the study. The exclusion criteria were patients who had severe inflammation or other system diseases including renal, liver and cardiovascular diseases; patients with other cancers or metastasis; and patients with recurrent BC or ductal carcinoma in situ. Both tumor tissue samples and paracancerous tissues were collected and were restored at -80°C for the following experiments. Additionally, blood samples and medical records of 451 healthy female cases who came to our hospital for physical examination during the same period were collected.

3.2. Measurement of serum TBK1 and other serum cancer-related biomarkers

The measurement of serum TBK1 was performed by enzyme linked immunosorbent assay (ELISA) using Human TANK-binding kinase 1 ELISA Kit (MYBiosource, cat. No. MBS9330803, range 1.25–40 ng/mL). The serum levels of cancer-related biomarkers CA15-3, CA125, CEA, and CA19-9 were measured by chemiluminescence immunoassay as reported elsewhere.^[18]

3.3. Measurement of AR by immunohistochemistry

The AR expression in BC tissues were measured by immunohistochemistry (IHC). In brief, samples were fixed at 60°C for 1 hour and deparaffinized in xylene solutions, followed

Table 1

Basic characteristics of all participants.

Variables	All patients, n = 451	Survival, n = 322	Deceased, n = 129	Healthy, n = 451	P*
Age, yr	50.06 ± 14.27	49.34 ± 14.20	51.84 ± 14.34	49.55 ± 13.73	.096
BMI, kg/m ²	26.15 ± 3.70	26.25 ± 3.74	25.91 ± 3.63	25.74 ± 3.68	.382
TNM stage, n (%)					
I–II	349 (77.38)	202 (87.45)	56 (43.41)	–	<.001
III–IV	102 (22.62)	29 (12.55)	73 (56.59)		
Pathological type, n (%)					
Invasive ductal carcinoma	351 (77.83)	257 (79.81)	94 (72.87)		.109
Invasive lobular carcinoma	82 (18.18)	52 (16.29)	30 (23.26)		.077
Mucinous adenocarcinoma	18 (3.99)	13 (4.04)	5 (3.88)		.937
Triple negative breast cancer, n (%)	59 (13.08)	43 (13.35)	16 (12.40)		.787
Tumor diameter, n (%)					
>2 cm	351 (77.83)	232 (72.05)	119 (92.25)		<.001
≤2 cm	100 (22.17)	90 (27.95)	10 (7.75)		
Lymph node metastasis, n (%)	237 (52.55)	153 (47.52)	84 (65.12)		.001
ER, n (%)					
Negative	262 (58.09)	171 (53.11)	91 (70.54)	–	.001
Positive	189 (41.91)	151 (46.89)	38 (29.46)		
PR, n (%)					
Negative	213 (47.23)	154 (47.83)	59 (45.74)		.688
Positive	238 (52.77)	168 (52.17)	70 (54.26)		
HER, n (%)					
Negative	108 (28.38)	89 (27.64)	39 (30.23)		.581
Positive	323 (71.62)	233 (72.36)	90 (69.77)		
Ki67, n (%)					
Negative	98 (21.73)	82 (25.47)	16 (12.40)		.002
Positive	353 (78.27)	240 (74.53)	113 (87.60)		

BMI = body mass index, HER = human epidermal growth factor receptor, TNM = tumor node metastasis.

*Comparison was made between survival and deceased patients.

by rehydration. Antigen retrieval in slides was conducted by T Cell Conditioning 1 (CC1) and incubated with 3% hydrogen peroxide for 30 minutes. After blocking with 5% goat serum, samples were incubated with primary AR antibody (ab133273, 1:100, Abcam) at 4°C overnight, followed with secondary goat anti-rabbit IgG H&L (HRP) antibody (ab205718, 1: 5000, Abcam) at 37°C for 30 minutes. Finally, after washing by PBS for 3 times and TBST for once, the slides were stained with diaminobenzidine (DAB). Bound antibody on the array was determined by an OptiView DAB detection kit. Counterstain of the samples were conducted using hematoxylin for 1 minute, followed with bluing by PBS. The pathologic scoring of AR was semiquantitatively evaluated. The score for stained area was defined as follows: 0 for none, 1 for <1/100, 2 for 1/100 to 1/10, 3 for 1/10 to 1/3, 4 for 1/3 to 2/3 and 5 for more than 2/3. IHC score was finally graded as negative for 0–2, weak for 3–4, moderate for 5–6, and strong for 7–8.

3.4. Clinical characteristics

Clinical characteristics of all patients at baseline were obtained, including age, BMI, TNM stage, pathological type, ratio of triple negative BC, tumor diameter, and the expressions of ER, PR, HER, and Ki67 were determined by histological analysis. The follow-up lasted for 5 years for all patients. The survival duration was determined from the admission to the last follow-up or the death.

3.5. Statistical analysis

All continuous data were analyzed by Kolmogorov–Smirnov analysis and proven to be normally distributed. Data were expressed as mean \pm SD. Student *t* test was conducted for the comparison between 2 groups. Kaplan–Meier (K–M) curve analysis was performed for 5-year overall survival. Receiver operating characteristic curve (ROC) was used for diagnostic analysis. All calculations were performed using SPSS 18.0 and graphed by Graphpad version 6.0. Statistical difference was defined as $P < .05$.

4. Results

4.1. Characteristics of all patients

This research included 451 female BC patients, including 351 cases of invasive ductal carcinoma, 82 cases of invasive lobular carcinoma and 18 cases of mucinous adenocarcinoma. The number of triple negative BC was 59 cases (13.08%). Compared to the survival cases, the deceased patients had significantly higher ratio of TNM III–IV, lymph node metastasis and tumor diameter >2 cm. Besides, the rates of patients with negative ER (70.54%, 91/129) and positive Ki67 (87.60%, 113/129) in deceased group were much higher than those in survival group ($P < .05$, Table 1). No obvious differences were found for the other parameters between survival and deceased patients or between BC patients and the healthy controls.

4.2. Serum TBK1 was increased in BC patients

Then, serum levels of TBK1 in all participants were measured. Figure 1A showed an obvious elevation of TBK1 in BC patients compared with healthy controls ($P < .05$). Besides, deceased patients showed significantly higher expression of TBK1 than the survival patients ($P < .05$, Fig. 1B). Patients with higher TNM stages (III–IV) also had increased TBK1 expression than patients with TNM I–II ($P < .05$, Fig. 1C). The findings indicated that TBK1 might be associated with the progression of BC.

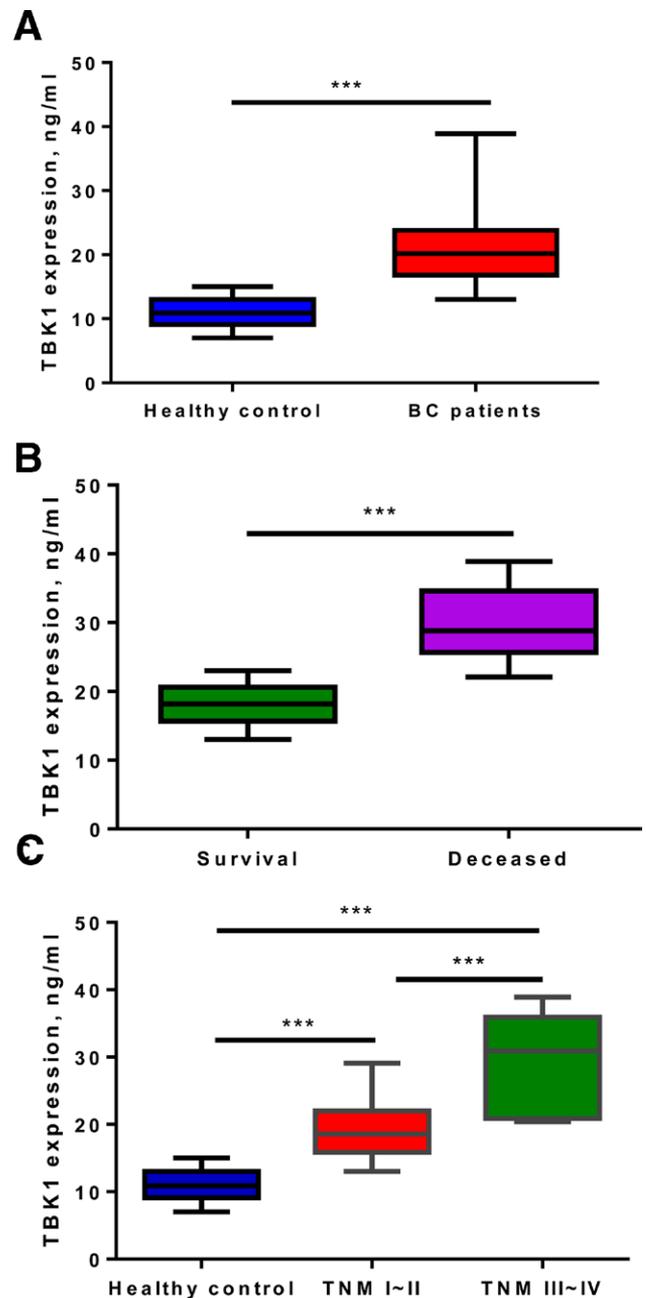


Figure 1. Serum TBK1 expression in all participants. (A) Detection of serum TBK1 in BC patients and healthy control. (B) Detection of serum TBK1 in the survived and deceased patients. (C) Detection of serum TBK1 in healthy control, patients with TNM I–II and TNM III–IV. BC = breast cancer, TBK1 = Tank-binding kinase 1, TNM = tumor node metastasis.

4.3. Serum TBK1 was positively correlated with AR and cancer biomarkers in BC patients

To further investigate the role of TBK1 in BC, all patients were divided into TBK1 high/low group according to the mean value of 21.43 ng/mL for serum TBK1. The levels of AR in BC tissues and cancer biomarkers in different groups were determined. As shown in Table 2 and Figure 2, TBK1 high group had higher ratio of AR-positive expression ($P < .05$). Meanwhile, TBK1 high group showed remarkably higher expression of CA15-3, CA125, CEA, and CA19-9 than TBK1 low group ($P < .05$). Further Pearson analysis confirmed positive correlations among serum TBK1 and AR, CA15-3, CA125, CEA, and CA19-9 (Table 3).

Table 2

Expression of AR and serum levels of CA153, CA125, CEA, and CA199 in breast cancer patients with different expression of TBK1.

Variables	TBK1 high, n = 174	TBK1 low, n = 277	P
AR, n (%)			
4–8	116 (66.67)	91 (32.85)	<.001
0–4	58 (33.33)	186 (67.15)	
CA153, U/mL	55.66 ± 8.36	39.74 ± 2.96	<.001
CA125, U/mL	64.97 ± 5.51	54.13 ± 3.58	<.001
CEA, ng/mL	7.68 ± 1.51	5.26 ± 0.47	<.001
CA199, U/mL	74.87 ± 5.89	62.24 ± 7.29	<.001

TBK1 = Tank-binding kinase 1.

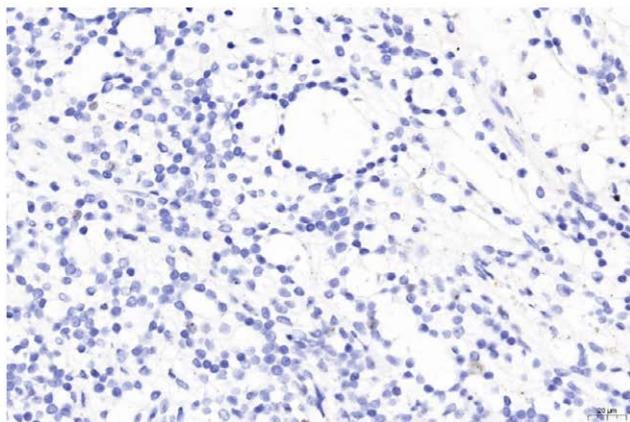
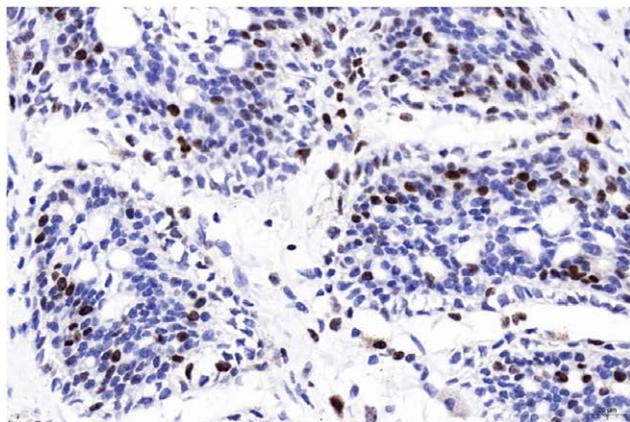
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Figure 2. AR expression in tissue samples of BC patients with low and high expression of TBK1. AR = androgen receptor, BC = breast cancer, TBK1 = Tank-binding kinase 1.

4.4. Correlation between serum TBK1 and prognosis in BC patients

We further compared the clinic characteristics and overall survival between TBK1 high group and low group. The data suggested patients with high TBK1 expression had higher ratio of TNM III-IV, tumor diameter >2cm and Ki67 positive expression (Table 4, $P < .05$). Compared with TBK1 low group, TBK1 high group had shorter 5-year overall survival ($P < .05$, Fig. 3). The mortality of TBK1 high group (90/174, 51.72%) was also higher than that of TBK1 lower group (39/277, 14.08%, $P < .05$). These results illustrated that TBK1 expression was associated with clinic outcomes and 5-year overall survival.

Table 3

Correlation among TBK1 and AR, CA153, CA125, CEA, and CA199 in breast cancer patients.

	Pearson correlation	P
AR	0.341	<.001
CA153	0.649	<.001
CA125	0.593	<.001
CEA	0.572	<.001
CA199	0.514	<.001

TBK1 = Tank-binding kinase 1.

Table 4

Basic characteristics of all patients with breast cancer.

Variables	TBK1 high, n = 174	TBK1 low, n = 277	P
Age, yr	51.18 ± 14.40	49.35 ± 14.17	.184
BMI, kg/m ²	25.74 ± 3.68	26.40 ± 3.71	.064
TNM stage, n (%)			
I–II	93 (53.45)	256 (92.42)	<.001
III–IV	81 (46.55)	21 (7.58)	
Pathological type, n (%)			
Invasive ductal carcinoma	130 (74.71)	221 (79.78)	.207
Invasive lobular carcinoma	35 (20.11)	49 (17.69)	.520
Mucinous adenocarcinoma	9 (5.17)	7 (2.53)	.190
Triple negative breast cancer, n (%)			
Tumor diameter, n (%)			
>2 cm	146 (83.91)	205 (74.01)	.014
≤2 cm	28 (16.09)	72 (26.99)	
Lymph node metastasis, n (%)	139 (79.89)	98 (35.38)	<.001
ER, n (%)			
Negative	104 (59.77)	109 (39.35)	<.001
Positive	70 (40.23)	168 (60.65)	
PR, n (%)			
Negative	87 (50.00)	126 (45.49)	.350
Positive	87 (50.00)	151 (54.51)	
HER, n (%)			
Negative	48 (27.59)	60 (21.66)	.151
Positive	126 (72.41)	217 (78.34)	
Ki67, n (%)			
Negative	27 (15.52)	71 (25.63)	.011
Positive	147 (84.48)	206 (74.37)	

BMI = body mass index, HER = human epidermal growth factor receptor, TNM = tumor node metastasis.

4.5. Diagnostic value of serum TBK1 in BC patients

The diagnostic value of serum TBK1 in BC patients was also analyzed. ROC curve showed a cutoff value of 13.95 ng/mL TBK1 for diagnosing BC, with AUC of 0.981, sensitivity of 93.6% of and specificity of 86.3% (Fig. 4A, $P < .001$). Additionally, cutoff value of 22.65 ng/mL TBK1 showed an AUC of 0.996 for predicting the death in BC patients with a sensitivity of 97.7% and specificity of 96.3% (Fig. 4B, $P < .001$). These results suggested that TBK1 expression might be considered as a potential diagnostic biomarker for BC and the death in BC patients.

5. Discussion

Despite rapid developments of therapeutic methods, such as surgery and chemotherapy, the prognosis of BC patients is still poor, especially for those with tumor metastasis and recurrence.^{119,201} Although there are numerous studies on BC, new research targets are always needed. Since BC patients with metastasis and recurrence usually have a 5-year survival rate of no >20%,²¹¹ early diagnostic methods especially novel biomarkers are of great significance. Our research suggested an upregulation of serum TBK1 in BC patients and a positive correlation with AR, CA15-3, CA125, and CEA. TBK1 expression affected clinic

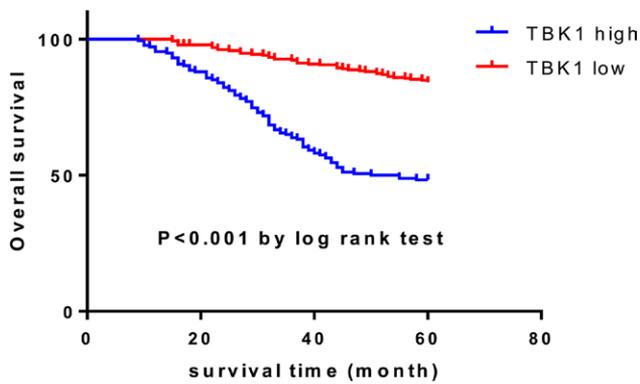


Figure 3. K–M curves for 5-year overall survival for all patients with different expression of TBK1. K–M curve = Kaplan–Meier curve, TBK1 = Tank-binding kinase 1.

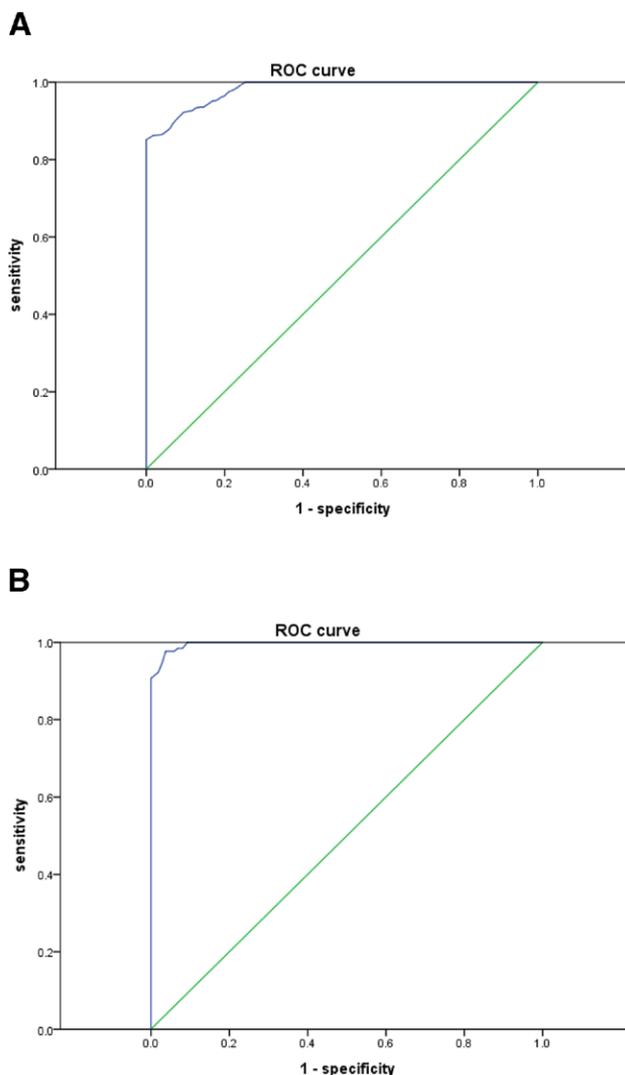


Figure 4. ROC curves for diagnosis of BC (A) and mortality of BC patients using TBK1 (B). BC = breast cancer, ROC curve = receiver operating characteristic curve, TBK1 = Tank-binding kinase 1.

outcomes and 5-year overall survival. Besides, serum TBK1 might be a promising diagnostic biomarker for BC.

The role of TBK1 in cancer development has been illustrated in different researches. Generally, TBK1 is considered as a

cancer promoter in most reported studies. It was found TBK1 mediated the activation and function of AKT/mTORC1 pathway via the upstream and downstream of mTOR kinase itself in Ras-mutant lung cancer.^[22] Jin et al reported TBK1 interacted with mTOR and suppressed its function, meanwhile, TBK1 knockdown reduced stem-like cell proliferation in vitro and in vivo.^[23] The association between TBK1 and BC progression was also demonstrated. Downregulated TBK1 decreased the levels of epithelial markers and elevated the levels of mesenchymal markers in ER α -positive BC cells, in addition, an in vivo study indicated TBK1 promoted tumor growth and lung metastasis in a ER α expression-dependent manner in BC.^[24] The study of Jiang et al revealed that knockout of TBK1 alleviated cell growth in human HER-2-positive BC and aggravated cellular senescence. Moreover, xenograft model of HER-2-positive BC showed EGFR/HER-2 inhibitor enhanced tumor cell apoptosis and inhibited tumor growth.^[25] In the present research, we also demonstrated that TBK1 was upregulated in BC patients. Besides, higher TBK1 expression was observed to be associated with clinic outcomes and predicted poor prognosis. These results were consistent with most previous studies that TBK1 serve as a cancer promoter in BC.

There are already lots of cancer biomarkers reported to be associated with clinic outcomes and prognosis of BC patients. Currently, the biomarkers of ER, PR, HER, and Ki67 are widely used for the diagnosis of BC in clinic. It was demonstrated that ER, PR, HER-2, and Ki67 showed potentials for evaluating tumor mutation burden as well as the predicted the sensitivity of immunotherapy in BC patients.^[26] A close association was found between HER-2 subtype and high nodal invasion in Thai women with BC.^[27] Yan et al illustrated Ki67 expression was correlated with that of ER, PR, HER-2, EGFR, and TOP- α , and affected the lymph node metastasis, tumor grade, and lymphovascular invasion in invasive ductal carcinoma. Besides, increased Ki67 was a risk factor for tumor recurrence, and it accelerated tumor progression and predicted a poor prognosis for BC patients.^[28] Except for the above factors, AR is also found to be correlated with poor prognosis of BC patients.^[29,30] A meta-analysis suggested that BC females with AR-positive tumor had improved overall survival and disease-free survival at both 3 and 5 years.^[31] Our result showed that deceased patients and patients with higher TBK1 had higher ratio of negative ER or positive Ki67. Despite common application of those factors, serum cancer-related biomarkers provided another option for BC diagnosis, such as CA15-3, CA125, CEA, and CA19-9.^[32–34] We also observed increases of CA15-3, CA125, CEA, and CA19-9 in BC patients with positive correlations with TBK1 expression, suggesting the potentials to diagnose BC.

6. Limitations

Some limitations also should be noticed. The sample size of this study is limited. In addition, the molecular mechanism of TBK1 in BC needs further investigation.

7. Conclusion

In conclusion, the present study found that the upregulated TBK1 predicted poor clinical outcomes and prognosis of BC patients. Besides, TBK1 could be a potential biomarker for diagnosing BC. This investigation might bring a novel research target for BC in future clinical research.

Author contributions

H.C.L. conducted most of the experiments, H.M.L. and J.Z. wrote the manuscript; Q.L.M. performed the experiments; S.D. analyzed the data; and L.H.M. designed the study and revised the manuscript.

References

- [1] Fahad Ullah M. Breast cancer: current perspectives on the disease status. *Adv Exp Med Biol.* 2019;1152:51–64.
- [2] Lei S, Zheng R, Zhang S, et al. Global patterns of breast cancer incidence and mortality: a population-based cancer registry data analysis from 2000 to 2020. *Cancer Commun (Lond).* 2021;41:1183–94.
- [3] Li T, Mello-Thoms C, Brennan PC. Descriptive epidemiology of breast cancer in China: incidence, mortality, survival and prevalence. *Breast Cancer Res Treat.* 2016;159:395–406.
- [4] Coughlin SS, Cypel Y. Epidemiology of breast cancer in women. In: Ahmad A. (eds) *Breast Cancer Metastasis and Drug Resistance.* New York, NY: Springer; 2013:19–34.
- [5] Richter B, Sliter DA, Herhaus L, et al. Phosphorylation of OPTN by TBK1 enhances its binding to Ub chains and promotes selective autophagy of damaged mitochondria. *Proc Natl Acad Sci USA.* 2016;113:4039–44.
- [6] Oakes JA, Davies MC, Collins MO. TBK1: a new player in ALS linking autophagy and neuroinflammation. *Mol Brain.* 2017;10:5.
- [7] Zhao P, Wong KI, Sun X, et al. TBK1 at the crossroads of inflammation and energy homeostasis in adipose tissue. *Cell.* 2018;172:731–743. e12.
- [8] Nozawa T, Sano S, Minowa-Nozawa A, et al. TBC1D9 regulates TBK1 activation through Ca²⁺ signaling in selective autophagy. *Nat Commun.* 2020;11:770.
- [9] Durand JK, Zhang Q, Baldwin AS. Roles for the IKK-related kinases TBK1 and IKKε in cancer. *Cells.* 2018;7:139.
- [10] Revach OY, Liu S, Jenkins RW. Targeting TANK-binding kinase 1 (TBK1) in cancer. *Expert Opin Ther Targets.* 2020;24:1065–78.
- [11] Hu L, Xie H, Liu X, et al. TBK1 is a synthetic lethal target in cancer with VHL loss. *Cancer Discov.* 2020;10:460–75.
- [12] Yu T, Yang Y, Yin DQ, et al. TBK1 inhibitors: a review of patent literature (2011 - 2014). *Expert Opin Ther Pat.* 2015;25:1385–96.
- [13] Deng T, Liu JC, Chung PE, et al. shRNA kinome screen identifies TBK1 as a therapeutic target for HER2+ breast cancer. *Cancer Res.* 2014;74:2119–30.
- [14] Chia K, O'Brien M, Brown M, et al. Targeting the androgen receptor in breast cancer. *Curr Oncol Rep.* 2015;17:4.
- [15] Cochrane DR, Bernales S, Jacobsen BM, et al. Role of the androgen receptor in breast cancer and preclinical analysis of enzalutamide. *Breast Cancer Res.* 2014;16:R7.
- [16] Park S, Koo J, Park HS, et al. Expression of androgen receptors in primary breast cancer. *Ann Oncol.* 2010;21:488–92.
- [17] Kono M, Fujii T, Lim B, et al. Androgen receptor function and androgen receptor-targeted therapies in breast cancer: a review. *JAMA Oncol.* 2017;3:1266–73.
- [18] Zhang J, Wang Y, Zhao T, et al. Evaluation of serum MUC5AC in combination with CA19-9 for the diagnosis of pancreatic cancer. *World J Surg Oncol.* 2020;18:31.
- [19] Waks AG, Winer EP. Breast cancer treatment: a review. *JAMA.* 2019;321:288–300.
- [20] Carioli G, Malvezzi M, Rodriguez T, et al. Trends and predictions to 2020 in breast cancer mortality in Europe. *Breast.* 2017;36:89–95.
- [21] Coventry BJ, Morton J. CD1a-positive infiltrating-dendritic cell density and 5-year survival from human breast cancer. *Br J Cancer.* 2003;89:533–8.
- [22] Zaman A, Bodemann BO, Makkar G, et al. TBK1 provides context-selective support of the activated AKT/mTOR pathway in lung cancer. *Cancer Res.* 2017;77:5077–94.
- [23] Kim JK, Jung Y, Wang J, et al. TBK1 regulates prostate cancer dormancy through mTOR inhibition. *Neoplasia.* 2013;15:1064–74.
- [24] Yang KM, Jung Y, Lee JM, et al. Loss of TBK1 induces epithelial-mesenchymal transition in the breast cancer cells by ERα downregulation. *Cancer Res.* 2013;73:6679–89.
- [25] Jiang Z, Liu JC, Chung PE, et al. Targeting HER2(+) breast cancer: the TBK1/IKKε axis. *Oncoscience.* 2014;1:180–2.
- [26] Xu J, Guo X, Jing M, et al. Prediction of tumor mutation burden in breast cancer based on the expression of ER, PR, HER-2, and Ki-67. *Onco Targets Ther.* 2018;11:2269–75.
- [27] Chuthapisith S, Permsapaya W, Warnissorn M, et al. Breast cancer subtypes identified by the ER, PR and HER-2 status in Thai women. *Asian Pac J Cancer Prev.* 2012;13:459–62.
- [28] Yan J, Liu XL, Han LZ, et al. Relation between Ki-67, ER, PR, Her2/neu, p21, EGFR, and TOP II-α expression in invasive ductal breast cancer patients and correlations with prognosis. *Asian Pac J Cancer Prev.* 2015;16:823–9.
- [29] Giovannelli P, Di Donato M, Galasso G, et al. The androgen receptor in breast cancer. *Front Endocrinol (Lausanne).* 2018;9:492.
- [30] Gerratana L, Basile D, Buono G, et al. Androgen receptor in triple negative breast cancer: a potential target for the targetless subtype. *Cancer Treat Rev.* 2018;68:102–10.
- [31] Vera-Badillo FE, Templeton AJ, de Gouveia P, et al. Androgen receptor expression and outcomes in early breast cancer: a systematic review and meta-analysis. *J Natl Cancer Inst.* 2014;106:319.
- [32] Wang W, Xu X, Tian B, et al. The diagnostic value of serum tumor markers CEA, CA19-9, CA125, CA15-3, and TPS in metastatic breast cancer. *Clin Chim Acta.* 2017;470:51–5.
- [33] Wang XF, Wu YH, Wang MS, et al. CEA, AFP, CA125, CA153 and CA199 in malignant pleural effusions predict the cause. *Asian Pac J Cancer Prev.* 2014;15:363–8.
- [34] Zhao S, Mei Y, Wang Y, et al. Levels of CEA, CA153, CA199, CA724 and AFP in nipple discharge of breast cancer patients. *Int J Clin Exp Med.* 2015;8:20837–44.