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Upregulation of serum exosomal SUMO1P3 predicts unfavorable prognosis in triple negative breast cancer

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Abstract. – OBJECTIVE: Triple negative breast cancer (TNBC) is an aggressive subtype of breast cancer (BC) with poor prognosis. Identification of reliable biomarkers for predicting prognosis of TNBC contributes significantly to improve the clinical outcome and disease management. Long non-coding RNAs (LncRNAs) have been demonstrated to play a critical role in tumorigenesis of TNBC. In this study, we aimed to investigate the prognostic significance of serum exosomal lncRNA small ubiquitin-like modifier 1 pseudogene 3 (SUMO1P3) in TNBC.

PATIENTS AND METHODS: The expression level and clinical significance of tissue lncRNA SUMO1P3 in BC were analyzed using the public The Cancer Genome Atlas (TCGA) dataset. Then, the serum exosomal lncRNA SUMO1P3 levels were examined in patients with TNBC, patients with non-TNBC, patients with benign breast disease and healthy controls using the quantitative real-time PCR. The potential clinical significance of serum exosomal lncRNA SUMO1P3 was further evaluated.

RESULTS: Based on the TCGA data, tissue lncRNA SUMO1P3 was upregulated in BC tissues, and its upregulation was significantly correlated with poor survival. Our findings showed that the expression level of serum exosomal lncRNA SUMO1P3 was significantly higher in patients with TNBC compared to patients with non-TNBC, patients with benign breast disease and healthy controls. In addition, serum exosomal lncRNA SUMO1P3 was closely correlated with lymphovascular invasion, lymph node metastasis and histological grade. The serum exosomal lncRNA SUMO1P3 levels were markedly decreased in chemosensitive cases, while not in the chemoresistance cases. Moreover, patients in the high serum exosomal lncRNA SUMO1P3 group had worse overall survival than the patients in the low serum exosomal lncRNA SUMO1P3 group. The multivariate analysis showed that serum exosomal lncRNA SUMO1P3 was an independent prognostic factor for TNBC.

CONCLUSIONS: Collectively, serum exosomal lncRNA SUMO1P3 might be a reliable and robust prognostic biomarker for TNBC.

Key Words:
Triple negative breast cancer, SUMO1P3, LncRNA, Prognostic biomarker.

Introduction

Breast cancer (BC) is the one of the most frequently diagnosed cancers among women worldwide[1]. The progress in the therapy including surgery, radiation therapy, hormonal therapy, chemotherapy, and targeted therapy has greatly improved the survival of patients with BC. However, approximately 15-20% of BCs belong to the triple-negative BC (TNBC), which is defined by the absent expression of estrogen receptor (ER) and progesterone receptor (PR) and the lack pf amplification/overexpression of the human epidermal growth factor 2 receptor (HER2)[2]. The overall prognosis is poorer for TNBC compared to the other subtypes of BC[3,4]. Currently no effective prognostic biomarker is available for predicting the clinical outcome of TNBC, and development of new and reliable prognostic indicators is urgently needed to improve the clinical management of the disease.

Long non-coding RNAs (LncRNAs) is a class of non-coding RNA containing with more than 200 nucleotides in length. They actively regulate

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a variety of cellular processes like proliferation, growth, development and differentiation[5]. Abnormal expression of lncRNAs is closely involved in the initiation and progression of many cancer types including TNBC[6,7]. Of note, high expression lncRNA HOTAIR is strongly correlated with the positive lymph node metastasis and androgen receptor expression[8]. The expression of LINC01096 was significantly increased in TNBC tissues and cell lines. In addition, upregulation of LINC01096 was correlated with unfavorable outcome of TNBC. Knockdown of LINC01096 inhibited the cell viability, migration, invasion and promoted the apoptosis of TNBC cells by targeting miR-3130-3p[9], indicating that LINC01096 might play an oncogenic role in the progression of TNBC.

Exosomes are extracellular vesicles that contain biologically active cargo of proteins, lipids, and nucleic acids. They are widely and stably detected in various body fluids such as serum, plasma and saliva[10]. Exosomal lncRNAs are crucial players in BC tumorigenesis and abnormal expression of serum exosomal lncRNAs might serve as prognostic biomarkers of BC[11,12]. Ubiquitination is an important posttranslational modification process through which ubiquitin molecules are linked to protein substrates for protein degradation[13]. It plays a critical role in regulating various cellular processes such as signal transduction, receptor internalization, transcriptional regulation and cell-cycle progression[14]. Small ubiquitin-like modifier 1 pseudogene 3 (SUMO1P3) is a recently identified lncRNA that aberrantly expressed in various types of cancers including BC[15-17]. However, the relationship between serum exosomal lncRNA SUMO1P3 and BC, especially TNBC, is completely unknown. The aim of this study is to determine the prognostic efficacy of serum exosomal lncRNA SUMO1P3 in TNBC.

Patients and Methods

Ethics Statement

The study was approved by the Ethics Committee of Songgang People's Hospital of Baoan District, and written informed consents were obtained from all the participants.

Study Subjects

Serum samples were collected from 130 patients with TNBC, 60 patients with non-TNBC, 60 patients with benign breast disease and 50 healthy female individuals referred to our department. All the patients were pathologically confirmed. The exclusion criteria were as follows: (1) male patients, (2) patients had received any treatment prior to the collection of serum samples, (3) patients with other malignant diseases or/and systemic disease. The detailed information of the TNBC patients was provided in Table I. In addition, the paired serum samples were collected 1 month after two cycles of chemotherapy. Overall survival (OS) was defined as the time from last day of treatment to death or last follow-up

Serum Sample and Exosome Isolation

At least 5 mL of venous blood was withdrawn from each participant. The serum supernatant was obtained by centrifuging the blood samples 2000 × g for 15 min at 4°C, and transferred to a RNase-free tube. The exosomes were isolated from the serum samples with the Total Exosome Isolation Reagent (from serum) (Invitrogen, Carlsbad, CA, USA). Briefly, the Total Exosome Isolation reagent was mixed well with the serum samples. Followed by incubation at 4°C for 30 min, the mixture was centrifugated 10,000 × g for 10 min at room temperature. The supernatant was discarded and resuspended in PBS. The exosomes were stored at -80°C for further use.

Quantitative Real Ttime-PCR (qRT-PCR)

The total RNA from exosomes was extracted using TRIzol LS Reagent (Invitrogen, Carlsbad,

Table I. The clinicopathological parameters of patients with TNBC.

Clinicopathological parameter	Number
Age (year)	
> 50	62 (47.69%)
<=50	68 (52.31%)
Basal phenotype	
Yes	86 (66.15%)
No	44 (33.85%)
Tumor size (cm)	
>2	76 (58.46%)
<=2	54 (41.54%)
Lymphovascular invasion	
Yes	83 (63.85%)
No	47 (36.15%)
Lymph node metastasis	
Yes	74 (56.92%)
No	56 (43.08%)
Histological grade	
G2-G3	87 (66.92%)
G1	43 (33.08%)

CA, USA). The RNA concentration and purity were evaluated using a NanoDrop 2000 (Thermo Fisher Scientific, Waltham, MA, USA). The RNA was then transcribed into cDNA with the a PrimeScript RT Reagent Kit (Takara, Dalian, Liaoning, China). Quantitative real-time PCR was performed on the Applied Biosystems™ 7500 Real-Time PCR Systems (Applied Biosystems, Foster City, CA, USA) with SYBR Premix Ex Taq (Takara, Dalian, Liaoning, China). The glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was chosen as the internal control and the fold change in the expression of serum exosomal lncRNA SUMO1P3 was calculated with the $2^{-\Delta\Delta CT}$ method. The primer sequences were as follows: SUMO1P3 forward: 5′-ACTGGGAATGGAGGAAGA-3′, SUMO1P3 reverse: 5′-TGAGAAAGG-ATTGAGGGAAAAG-3′; GAPDH forward: 5′-TCCCATCACCATCTTCCA-3′, GAPDH reverse: 5′-CATCACGCCACAGTTTCC-3′

The Cancer Genome Atlas (TCGA) Dataset Analysis

The RNA-Seq data of breast cancer and the corresponding clinical data were downloaded from the TCGA database (https://tcga-data.nci.nih.gov/tcga/). The difference of lncRNA SUMO1P3 expression between normal tissue and BC samples was calculated with the Edger R package. The interactive body-map constructed based on the median expression of lncRNA SUMO1P3 in the tumor and normal samples as well as the survival difference in BC patients with high and low lncRNA SUMO1P3 expression were obtained using the GEPIA (http://gepia.cancer-pku.cn/).

Statistical Analysis

All statistical analyses were performed with GraphPad 8.0 (GraphPad Software, Inc., La Jolla, CA, USA). The statistical differences in different groups were analyzed by Mann-Whitney test or Kruskal-Wallis test. Chi-square test was used to evaluate the association between serum exosomal lncRNA SUMO1P3 levels and the clinicopathological parameters of TNBC patients. The Kaplan-Meier method was used to constructed the survival curves, and the log-rank test was employed to compare the OS difference. The multivariate cox regression analysis was performed to identify the independent prognostic factors for TNBC. A p-value < 0.05 was considered statistically significant.

Results

Serum Exosomal LncRNA SUMO1P3 Was Significantly Increased in TNBC Patients

Based on the TCGA datasets, the median expression of lncRNA SUMO1P3 in the tumor and normal samples was expressed using the interactive bodymap. The red and green color indicated the relatively high and low expression, respectively. Our results the median expression of lncRNA SUMO1P3 was consistently higher in the tumor samples compared to the normal samples in different cancer types (Figure 1A). The tissue lncRNA SUMO1P3 level was significantly higher in BC samples than in normal samples (p<0.001) (Figure 1B). The survival analysis showed that BC patients with higher tissue lncRNA SUMO1P3 expression suffered worse OS than those with lower tissue lncRNA SUMO1P3 expression (p=0.0026) (Figure 1C). Our data showed that the expression level of serum exosomal lncRNA SUMO1P3 was markedly higher in patients with TNBC compared to patients with non-TNBC patients (p<0.001), patients with benign breast disease (p<0.001), and healthy volunteers (p<0.001). In addition, serum exosomal lncRNA SUMO1P3 level was higher in patients with non-TNBC patients than in patients with benign breast disease (p=0.008), and healthy volunteers (p=0.025) (Figure 1D).

The Correlation Between Serum Exosomal LncRNA SUMO1P3 and Clinicopathological Parameters of TNBC Patients

As shown in Figure 2, serum exosomal lncRNA SUMO1P3 levels were significantly higher in TNBC patients with positive lymphovascular invasion (p=0.002), or with positive lymph node metastasis (p=0.001), or with grade G2-G3 (p<0.001) compared to those without lymphovascular invasion, or without lymph node metastasis, or with grade G1. However, no association was found between serum exosomal lncRNA SUMO1P3 and other clinicopathological parameters such as age (p=0.284), basal phenotype (p=0.904) and tumor size (p=0.891).

Serum Exosomal LncRNA SUMO1P3 Was Associated with Therapeutic Responses

The serum exosomal lncRNA SUMO1P3 levels were compared between the pre-treated and post-treated blood samples. The results showed that the expression level of serum exosomal ln-

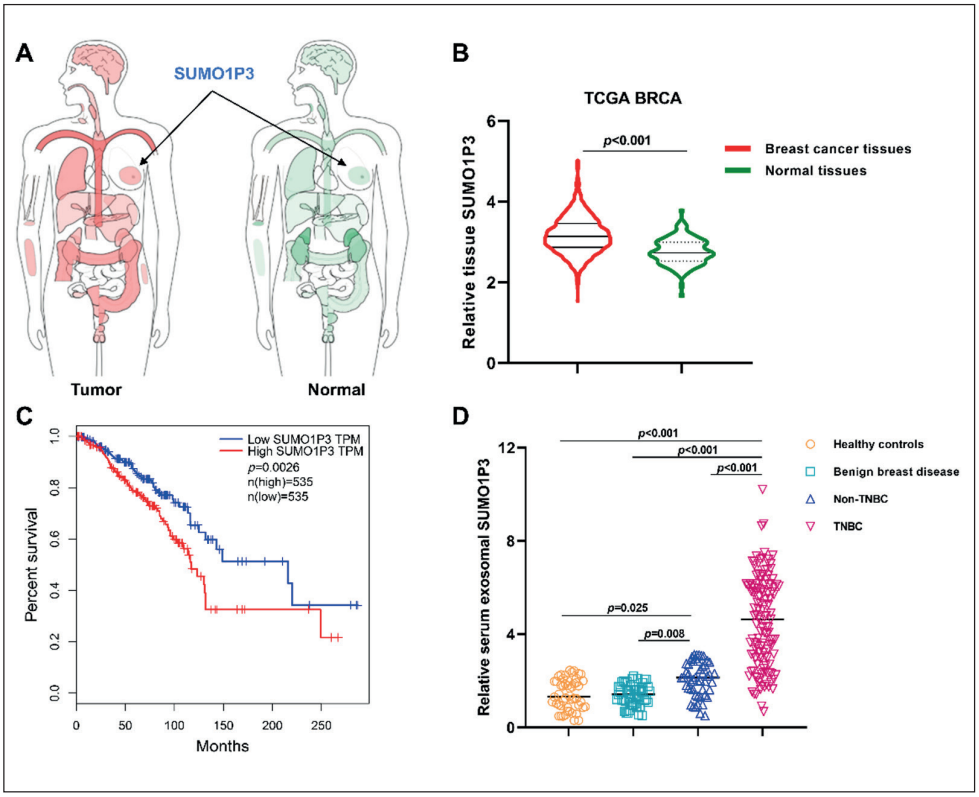

Figure 1. Serum exosomal lncRNA SUMO1P3 was significantly increased in TNBC patients. **A,** The median expression of tissue lncRNA SUMO1P3 was consistently higher in tumor samples compared to the normal samples in different types of cancer. **B,** The tissue lncRNA SUMO1P3 was significantly higher in BC specimens than in normal samples. **C,** The BC patients with higher tissue lncRNA SUMO1P3 had poorer OS. **D,** The expression level of serum exosomal lncRNA SUMO1P3 was significantly higher in patients with TNBC compared to patients with non-TNBC, patients with benign breast disease and healthy controls.

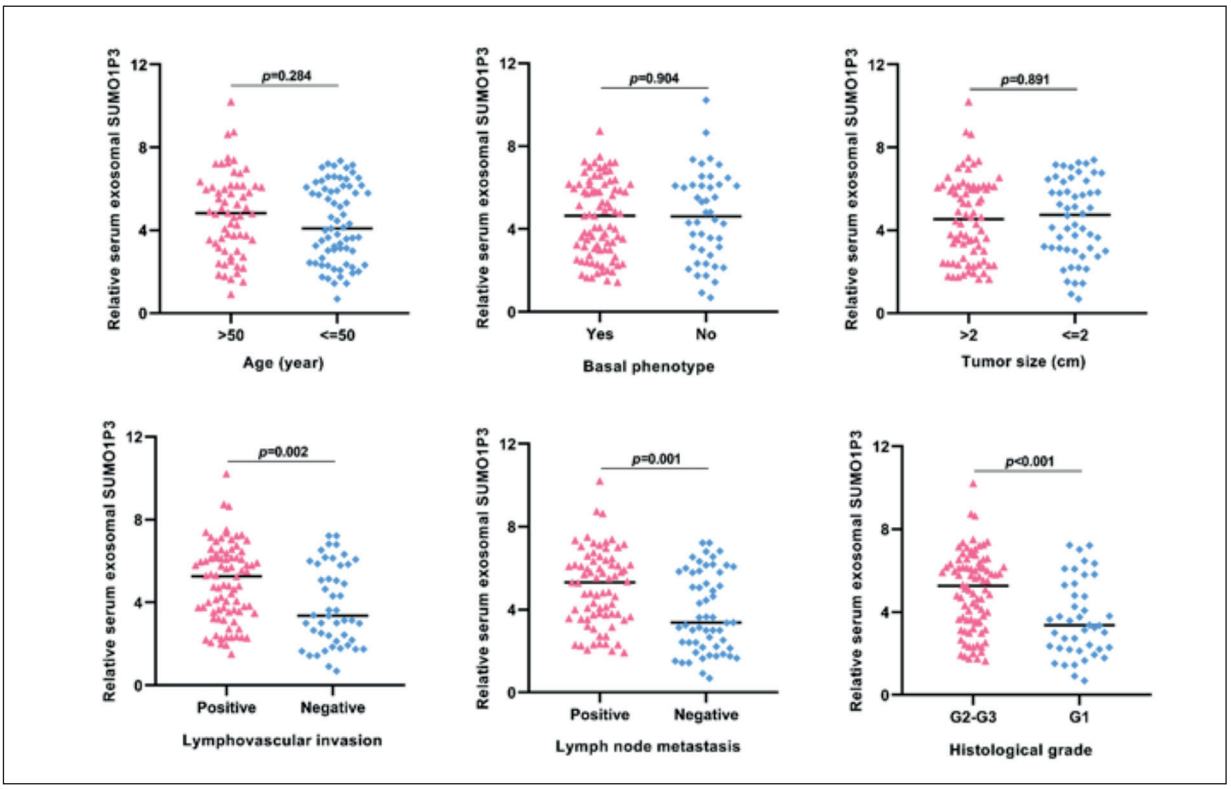

Figure 2. Serum exosomal lncRNA SUMO1P3 level was closely associated with lymphovascular invasion, lymph node metastasis and histological grade of TNBC.

cRNA SUMO1P3 was significantly lower following the chemotherapy (p<0.001) (Figure 3A). There were 31 chemoresistant cases and 99 chemosensitive cases in the TNBC cohort. Interestingly, no significant difference in serum exosomal lncRNA SUMO1P3 was found between the pre-treated and post-treated blood samples for the chemoresistant cases (p=0.297) (Figure 3B). However, the serum exosomal lncRNA SUMO1P3 levels dropped significantly in the chemosensitive cases following the chemotherapy (p<0.001) (Figure 3C).

High Serum Exosomal LncRNA SUMO1P3 Predicted Poor Prognosis in TNBC

The median expression of serum exosomal lncRNA SUMO1P3 in TNBC cohort was used as the cutoff value to split the TNBC patients into the high serum exosomal lncRNA SUMO1P3 group and low serum exosomal lncRNA SUMO1P3 group. The survival analysis showed that the TNBC patients in the high serum exosomal lncRNA SUMO1P3 group had significant shorter OS than the patients in the low serum exosomal lncRNA SUMO1P3 group (p=0.0007) (Figure 4A). The multivariate cox regression analysis showed that serum exosomal lncRNA SUMO1P3 (p=0.038, HR=1.972, 95% CI=1.039-3.746) and histological grade (p=0.005, HR=3.526, 95% CI=1.475-8.426) were independently associated with OS (Figure 4B).

Discussion

In this study, our results showed that the expression level of serum exosomal lncRNA SUMO1P3 was significantly upregulated in patients with TNBC compared to patients with non-TNBC, benign breast disease and healthy volunteers. In addition, high serum exosomal lncRNA SUMO1P3 level was strongly correlated with unfavorable clinicopathological parameters, chemoresistance and poor survival. The multivariate analysis revealed that serum exosomal lncRNA SUMO1P3 was an independent prognostic factor for TNBC. Taken together, these data suggest that serum exosomal lncRNA SUMO1P3 might be a reliable and novel biomarker for predicting the prognosis of TNBC.

TNBC cells might synthesize abundance of lncRNA SUMO1P3, which is packaged into the TNBC cells derived exosomes or extracellular vesicles. The exosomes are then secreted into the bloodstream. In addition, the cancer cells can secrete much more exosomes than the non-malignant cells[18,19]. These two factors might account for the increased level of serum exosomal lncRNA SUMO1P3 in patients with TNBC. Consistent with our findings, the expression level of lncRNA SUMO1P3 was higher in BC tissues and cell lines. In addition, upregulation of tissue lncRNA SUMO1P3 was correlated with worse prognosis of TNBC. Downregulation of lncRNA SUMO1P3 suppressed the proliferation, migration,

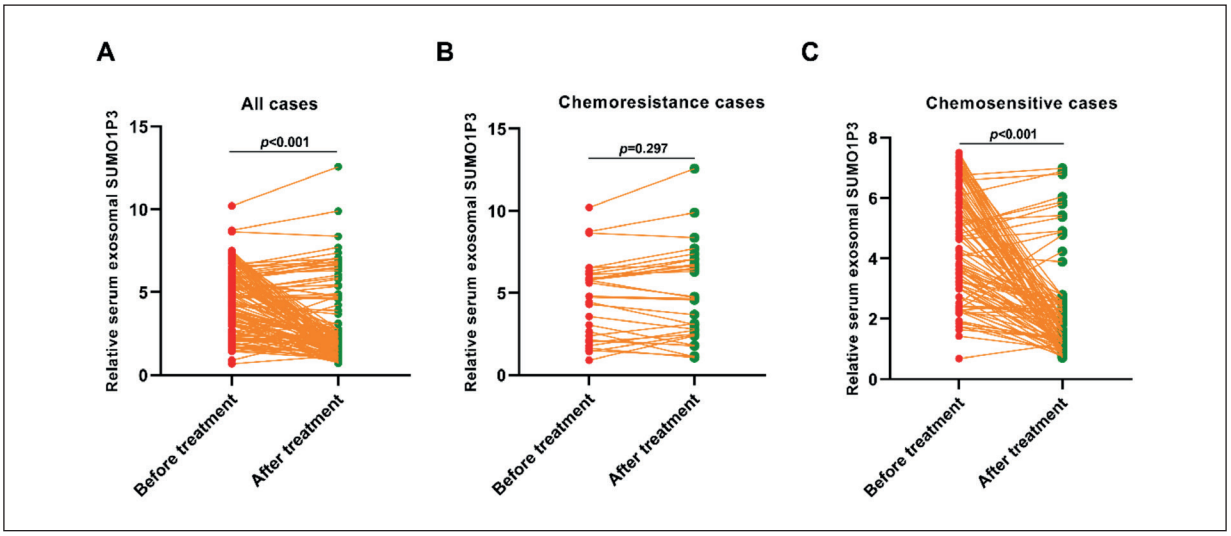

Figure 3. Serum exosomal lncRNA SUMO1P3 was associated with therapeutic responses. (A) Serum exosomal lncRNA SUMO1P3 level was markedly decreased in TNBC patients after receiving the chemotherapy. (B) The expression level of serum exosomal lncRNA SUMO1P3 changed little in the chemoresistance cases. (C) The serum exosomal lncRNA SUMO1P3 dropped significantly in the chemosensitive cases.

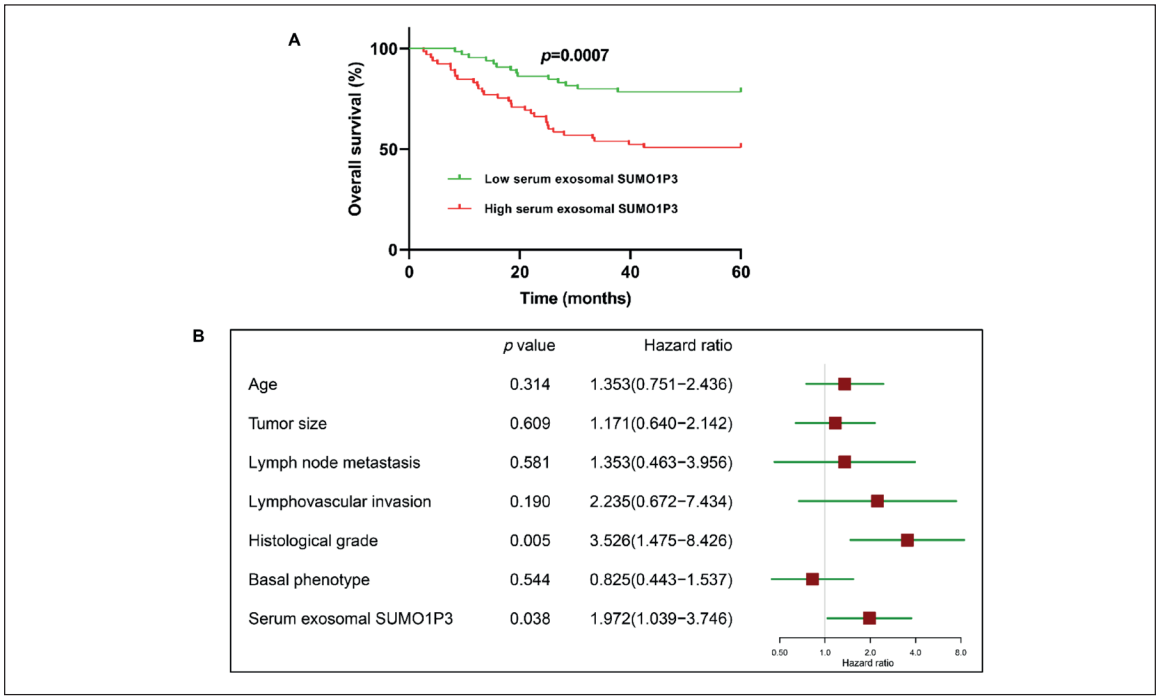

Figure 4. High serum exosomal lncRNA SUMO1P3 predicted poor prognosis in TNBC. **A,** The TNBC patients in the high serum exosomal lncRNA SUMO1P3 group had worse OS than those in the low serum exosomal lncRNA SUMO1P3 group. **B,** Serum exosomal lncRNA SUMO1P3 was an independent prognostic factor for TNBC.

and invasion of breast cancer cells by targeting miR-320a, suggesting lncRNA SUMO1P3 plays an oncogenic role in BC[17].

The role of lncRNA SUMO1P3 has also been evaluated in many other types of cancer. In particular, the expression level of lncRNA SUMO1P3 was significantly increased in hepatocellular carcinoma (HCC) tissues and cell lines. Inhibition of lncRNA SUMO1P3 suppressed proliferation, colony formation, and invasion as well as promoted apoptosis and radiosensitivity of HCC cells[20]. Similarly, lncRNA SUMO1P3 was markedly increased in gastric cancer tissues compared to the adjacent normal tissues. In addition, upregulation of lncRNA SUMO1P3 was associated with various clinical parameters including tumor size, differentiation, lymphatic metastasis, and invasion[21]. The lncRNA SUMO1P3 expression was significantly higher in colon cancer tissues and cell lines. Increased SUMO1P3 level was strongly correlated with unfavorable prognosis of patients with colon cancer. In addition, knockdown of lncRNA SUMO1P3 suppressed the malignant activities of colon cancer cells both *in vitro* and *in vivo*, indicating that lncRNA SUMO1P3 played a tumor promoting role in colon cancer[22]. Zhang et al[23] showed that lncRNA SUMO1P3 was overexpressed in non-small cell lung cancer (NSCLC) tissues, especially those with lymph node metastasis. High lncRNA SUMO1P3 expression was also significantly correlated with unfavorable clinical characteristics.

As we discussed above, in addition to TNBC, tissue lncRNA SUMO1P3 is also deregulated in many other types of cancers. Further studies are warrant to investigate whether serum exosomal lncRNA SUMO1P3 is also abnormally expressed in these cancers. In addition, the clinical significance of serum exosomal lncRNA SUMO1P3 needs further validation with independent cohorts. Moreover, since serum exosomal lncRNA SUMO1P3 might be also deregulated in other human diseases, it should be combined with other prognostic biomarkers and clinicopathological parameters to enhance the prognostic efficacy.

Conclusions

Cumulatively, to the best of our knowledge, this is the first study to demonstrate that serum exosomal lncRNA SUMO1P3 is significantly upregulated in TNBC. In addition, increased

serum exosomal lncRNA SUMO1P3 is strongly associated with treatment failure and unfavorable prognosis. These novel findings indicate that serum exosomal lncRNA SUMO1P3 is a promising prognostic biomarker for TNBC, which might vastly improve individual patient outcomes.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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