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ORIGINAL ARTICLE

Assessment of circulating HISLA as a potential biomarker for breast cancer diagnosis and prognosis

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Abstract

Breast cancer (BC) is the most frequently encountered and aggressive type of malignant tumor and affects the health of females across the globe. Approximately 30% of patients that are newly diagnosed have a high risk of subsequent metastasis and relapse. HIF-1α-stabilizing long noncoding RNA (HISLA) packaged in exosome has been recently identified and revealed as an important oncogenic gene in promoting BC progress. Thus, we sought to investigate whether serum circulating HISLA was involved in dynamics underlying its applicability for the diagnosis and prognosis of BC. We assessed serum HISLA expression in 40 patients with BC and 20 healthy controls to investigate its roles in BC using quantitative real-time polymerase chain reaction (qRT-PCR). We also assessed measures of correlation of clinical and pathological parameters with prognoses of BC patients. Our findings suggested that serum HISLA expression in BC patients was significantly higher than in healthy controls. Furthermore, high expression of serum HISLA was positively associated with advanced stage lymph node metastasis. Expression of HISLA was reduced in postoperative BC patients' serum samples, compared with preoperative serum samples. Pearson correlation assessments indicated significant correlation between serum HISLA expression and the tissue sample HISLA expression in BC patients. Our findings suggested that serum HISLA may serve as newfound biomarker which could help to improve diagnoses and prognoses for BC-afflicted patients.

Keywords Breast cancer · Long noncoding RNA · HISLA · Diagnosis · Biomarker

Introduction

Breast cancer (BC) is the most common type of malignant tumor that affects females and accounts for 30% of all newly cancer globally for women [1, 2]. Despite that great advances have been achieved with respect to diagnosing and treating BC in recent decades, a relatively high proportion of patients are newly diagnosed with BC at advanced stages and subsequently have poor prognoses [3]. Tumor invasion and metastasis from the primary invasion sites to distant organs is one of the major causes of cancer-related mortality [4, 5]. Furthermore, BC still ranks eighth with respect to incidence and sixth with respect to cancer-related deaths on a global scale [6]. Therefore, identifying novel biomarkers to facilitate better early diagnosis and limit early progression of BC as well as facilitate better treatment options is still required [7].

LncRNAs appear to play pivotal roles in a variety of physiological and pathological diseases processes, as well as in cancers [8, 9]. LncRNAs, which were defined as longer than 200 nucleotides (nt) in length, lack protein-coding ability and are verified as involved in gene expression regulation across multiple levels thereby affecting tumorigenesis and tumor development [10]. Abnormal expression profiles and dysfunction of LncRNAs are common phenomena in malignant tumors and are involved in the dynamics underlying progression of multiple types of malignancies [11]. Indeed,

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several LncRNAs have been identified as potential biomarkers that can facilitate early diagnosis and better prognoses for some types of cancers [12]. For example, increased expression of serum HOTAIR was validated as a novel diagnostic and prognostic biomarker for esophageal squamous cell carcinoma and glioblastoma [13, 14].

HIF-1α-stabilizing long noncoding RNA (HISLA) packaged and transferred via extracellular vesicle (EV) derived from tumor-associated macrophages (TAMs) to breast cancer cells was upregulated and positively associated with poor prognoses for patients by way of accelerating aerobic glycolysis and apoptosis resistance in BC [15]. However, patterns of HISLA expression levels in serum of BC-afflicted samples, and whether or not these levels could be useful as a potential biomarker for BC diagnosis or prognosis lacked illustration. Thus, in the current study we sought to assess serum HISLA expression in BC-afflicted patients and to evaluate its diagnostic value for early identification of BC.

Materials and methods

Patients and blood samples

A total of 40 BC patients and 20 healthy control individuals were recruited for the study. BC-afflicted tissues and paired adjacent normal breast tissues as well as serum samples from BC-afflicted and healthy control individuals were obtained from the Shenzhen People's Hospital from March 2013 to September 2019. All tissues and serum samples were collected upon receipts of fully informed consents, which were in agreement with as well as approved by the Ethics Committee of the Shenzhen People's Hospital (China). All of the BC-afflicted patients were newly diagnosed, and none of these patients previously received any treatments prior to the collection of serum samples. Pathological diagnosed were verified by trained pathologists and clinicopathological characteristics were recorded.

Blood samples from 20 healthy control individuals, 40 BC preoperative patients, and 20 BC postoperative patients were collected. Then, blood samples were centrifuged for 15 min at 1500 r/min at 4 °C, and supernatant serum was collected and stored at − 80 °C. The 40 surgically resected BC-afflicted samples and corresponding 20 adjacent normal breast tissue samples were collected post-operations. Samples were immediately frozen in liquid nitrogen and then were stored at − 80 °C.

Quantitative real-time PCR (qRT-PCR)

TRIzol LS Reagent (Invitrogen Life Technologies) and TRIzol Reagent (Invitrogen Life Technologies) were used to extract total RNA from serum samples and tumor tissues, respectively. Reverse-transcription PCR was applied with the use of SuperScriptTM III Reverse Transcriptase (Invitrogen) kits following all manufacturer protocols. qRT-PCR facilitated examination HISLA expression using Gene Amp PCR System 9700 (Applied Biosystems, CA, USA) and 2 × PCR Master mix (TAKARA, Dalian, China). The qRT-PCR primer set for HISLA in our study was as follows: forward, 5′-TGAGTA GAAGAGAGTGGGGAGGG-3′; reverse, 5′-ACTGTG GCATGGTGATTGTTTGG-3′. β-actin was used as an internal control and the actin primer set was as follows: 5′-GGTGGCTTTTAGGATGGCAAG-3′; reverse, 5′-ACT GGAACGGTGAAGGTGACAG-3′. HISLA expression levels in BC-afflicted tissue and serum samples were normalized to the expression of β-actin, which were estimated using the calculations from the $2^{-\Delta\Delta C_t}$ method.

Statistical analyses

GraphPad Prism software (Version 7.0, San Diego, USA) was used for statistical analyses. Means ± standard deviations (SD) were used as measurements for data among groups. Differences between two groups were assessed by using student's t-tests. Pearson correlation analyses facilitated evaluation of associations between two variables. Receiver operating characteristics (ROC) curves were plotted to determine how well expression levels of serum HISLA could be used to help discriminate between tumor samples and healthy control samples. Overall, survival was compared using the Kaplan–Meier method and log-rank tests. $P < 0.05$ was considered as the level of statistical significance at which the null hypothesis of no differences between or among comparisons would be rejected.

Results

Upregulation of HISLA was associated with advanced stage of BC patients

At first, qRT-PCR was applied to facilitate examinations of HISLA expression in 40 BC-afflicted tissue and 20 adjacent normal breast tissue samples. Our results indicated that HISLA was remarkably upregulated in BC-afflicted tissues, compared to adjacent normal breast tissues (Fig. 1a, $P < 0.0001$). We next estimated correlation between HISLA expression levels and clinicopathological features. Results indicated that the higher expression of HISLA in BC-afflicted patients was significantly correlated with advanced stages of BC (Fig. 1b, $P < 0.050$) and lymph node metastasis (Fig. 1c, $P = 0.040$).

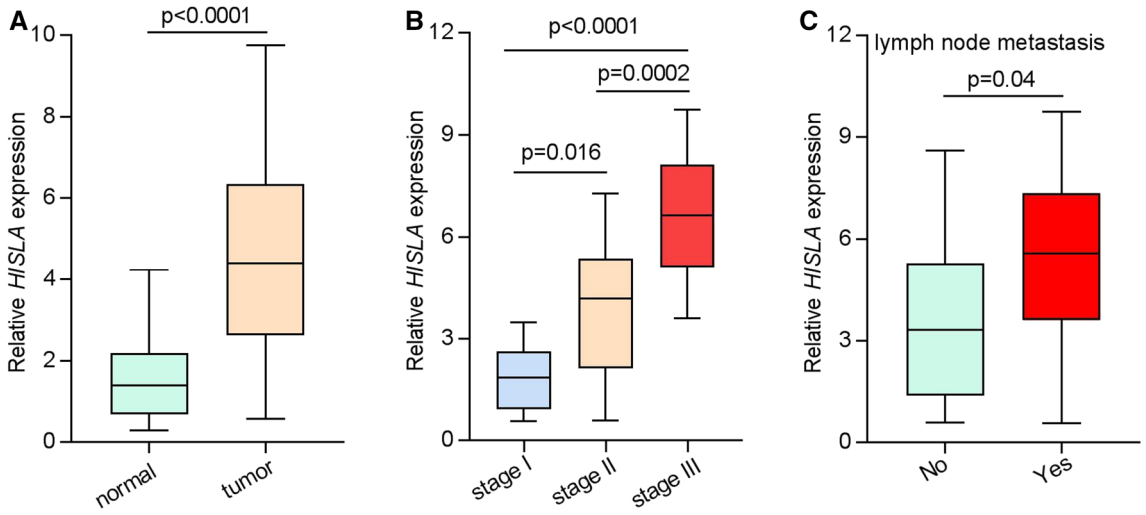

Fig. 1 Upregulation of HISLA was associated with the BC progression. **a** qRT-PCR was applied to examine the HISLA expression in BC tissues and adjacent normal breast tissues. **b** High expression level of HISLA in TNM stages of BC. **c** Upregulation of HISLA in BC was associated with lymph node metastasis

Serum HISLA expression was upregulated in BC patients

We further compared serum HISLA expression levels between 40 BC-afflicted patients and 20 healthy control individuals. Serum HISLA expression was also significantly over-expressed in BC-afflicted patients compared to respective levels in healthy controls (Fig. 2a, $P < 0.0001$). We next examined the correlation between HISLA expression levels and clinicopathological features. As shown in Table 1, our data also suggested that upregulation of HISLA was associated with histological grade, TNM stage, distant organ metastasis, and HER2 subtype. However, there were no significant correlations between serum HISLA levels and age and primary tumor size.

Serum HISLA expression level of patients was positively correlated with the expression of HISLA in BC-afflicted tissues

We also analyzed the correlation of HISLA expression between within-tissue and serum-derived samples of BC-afflicted patients. As shown in Fig. 2b, the results indicated that BC-afflicted tissues had significantly increased HISLA expression compared with the expression levels in serum ($P = 0.007$). Meanwhile, there was a positive correlation of

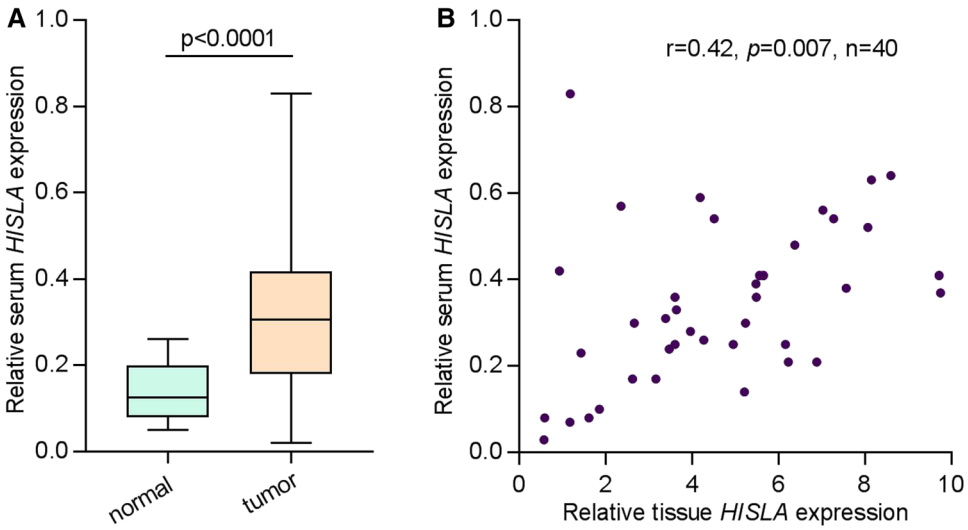

Fig. 2 The expression of serum HISLA was upregulated in BC patients. **a** qRT-PCR was performed to detect the serum HISLA expression between BC patients and healthy control individuals. **b** Pearson analysis was carried out to assess the correlation of HISLA expression between in tissue and in serum of BC

Table 1 Correlation between serum *HISLA* expression and clinicopathological characteristics in 40 breast cancer patients

Characteristics	*HISLA* expression level		*P* value
	Low expression (n = 19)	High expression = 21	
Age			0.75
< 48	10 (52.6)	10 (47.6)	
≥ 48	9 (47.4)	11(52.4)	
Primary tumor size (cm)			0.343
> 2	8 (26.3)	12 (57.1)	
≤ 2	11 (73.7)	9 (42.9)	
Histopathological grade			0.028
I	12 (63.2)	6 (28.6)	
II/III	7 (36.8)	15(71.4)	
TNM stage			0.01
I	14 (73.7%)	7 (33.3%)	
II/III	5 (26.3%)	14 (66.7%)	
Distant metastasis			0.026
Yes	6 (31.6)	14 (66.7)	
No	13 (68.4)	7 (33.3)	
Subtype			0.024
ER+HER2−	14	3	
ER−HER2−	3	5	
Her2+	9	6	

HISLA expression between the within serum samples and BC-afflicted tissue samples ($r = 0.42$, $P = 0.007$).

Serum HISLA expression level in the diagnosis and prognosis of BC patients

We further analyzed ROC curves for serum HISLA levels to facilitate assessment of its diagnostic value (Fig. 3a). We found that serum HISLA levels could be used to help differentiate BC-afflicted patients from healthy control patients, with an AUC of 0.83 (95% CI: 0.73 to 0.93, $P < 0.0001$). Next, we analyzed the prognostic value of circulating HISLA levels in BC-afflicted patients using Kaplan–Meier analyses and log-rank tests. Our results exemplified a significant correlation between circulating HISLA expression and overall survival of BC patients, which suggested that elevated circulating HISLA could be used to help accurately predict a worse prognosis for BC-afflicted patients (Fig. 3b, $P = 0.04$).

Serum HISLA expression level compared in pre-operative and post-operative BC patients

To facilitate comparisons of serum HISLA expression levels and their changes before and after surgery, we assessed 20 paired pre-operative and postoperative serum samples from BC-afflicted patients. Expression levels of serum HISLA in postoperative samples decreased significantly compared with levels for preoperative ones (Fig. 4a, $P = 0.0027$; Fig. 4b, $P < 0.0001$).

Discussion

BC is the most common of aggressive types and is the second leading cause of morality-related tumors in women worldwide; these effects are subsequent to the rapid growth rate and high opportunity of regional and distant organ metastasis that is attributable to BC [16, 17]. Indeed, the incidence and mortality rate of BC increased rapidly in China in recent decades, and this disease accounted for 15% of newly diagnosed cancer cases for women in 2015 [18, 19]. Despite that over recent decades remarkable advances have been achieved in personalized treatments for BC, the occurrence of metastasis is often unavoidable and remains as the leading cause of cancer-related morality for most

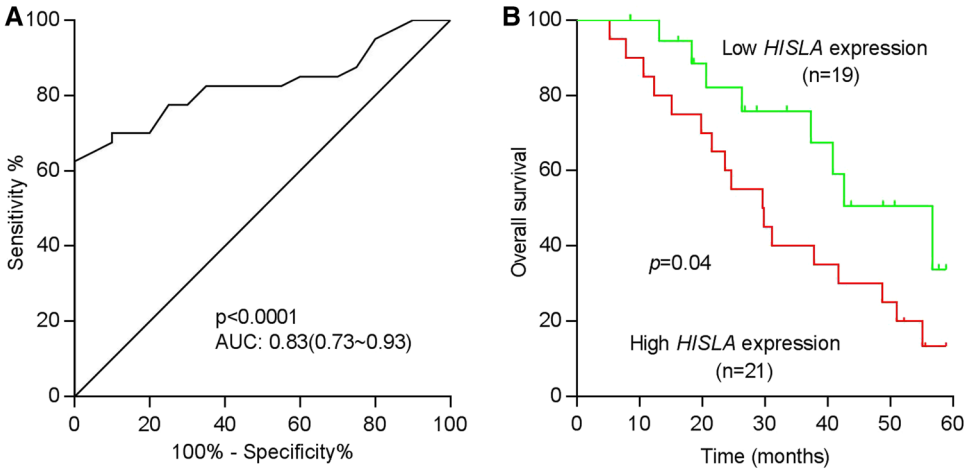

Fig. 3 Serum HISLA expression level in the diagnosis and prognosis of BC patients. **a** ROC curve analysis for serum HISLA in the diagnosis of BC. **b** Kaplan–Meier analysis of the overall survival of BC patients, and a log-rank test was used to assess the statistical significance between the low and the high HISLA expression in serum of BC patients

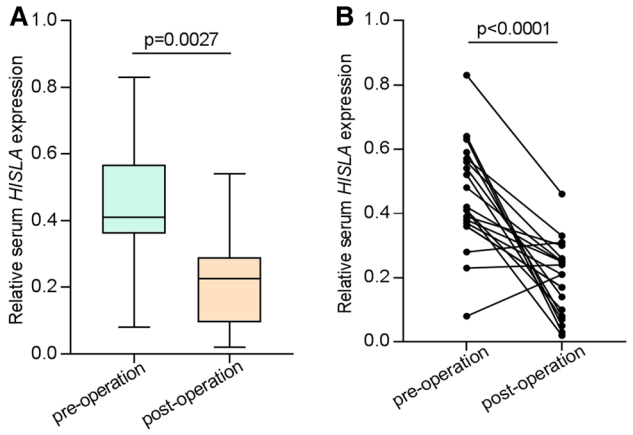

Fig. 4 Comparison of circulating HISLA expression between pre-operative and post-operative serum samples of BC patients. **a**, **b** Serum HISLA expression was significantly decreased in post-operative serum samples, compared with those in pre-operative ones

BC-afflicted patients [20, 21]. Given that BC during early stages often appears with non-specific symptoms, advanced or terminal staged diagnoses may often occur by the time symptoms develop, with subsequently poor prognoses and poor treatment effects [22]. Therefore, it is urgently necessary to reveal otherwise novel molecular mechanisms that control BC progression and metastasis and to assess if these can be used as biomarkers to help predict biological behavior and clinical outcomes [23]. Ideally, such biomarkers could also help to improve the designs and outcomes of treatment protocols and to develop additional and related novel therapeutic candidates for treating patients afflicted with BC [24].

During recent past decades, increasing lines of evidence have demonstrated that LncRNAs play crucial roles in various processes underlying tumor progression, such as proliferation, apoptosis, invasion, and metastasis [25, 26]. Meanwhile, abnormally expressed and dysfunctional LncRNAs were characterized as role players in multiple types of malignant tumors [27–29]. Furthermore, differential expression patterns of LncRNA have been suggested as potential effective biomarkers for diagnostic and prognostic assessments in patients afflicted with tumors [30]. As a convenient and non-invasive method, assessment of circulating serum LncRNAs expression levels was found to have provided new insights into tumor diagnosis and several circulating LncRNA expression signatures have been recently associated with tumor diagnosis and prognosis [31, 32]. For example, expression levels of circulating HOTAIR were significantly upregulated in ESCC and glioblastoma-afflicted patients [13, 14]. This indicated there was a high sensitivity and specificity with respect to the prediction of cancer progression. Circulating LncUEGC1 was believed to be a highly sensitive, stable, and non-invasive biomarker for early-stage gastric cancer [33]. In previous clinical research, the expression of HISLA in TAMs was associated with glycolysis, poor chemotherapeutic response, and shorter survival of BC patients. In the current study, our results indicated that the serum HISLA expression levels could be a novel diagnostic biomarker of BC.

Herein, we first examined and compared expression levels of HISLA in BC-afflicted tissues and adjacent normal breast tissues. HISLA expression was upregulated in BC-afflicted tissues compared with normal breast tissues. Meanwhile, high expression of HISLA was associated with advanced stages and grades of BC-afflicted patients. Furthermore, compared with healthy controls, serum HISLA expression was elevated in the serum of BC patients. Furthermore, serum HISLA expression levels were positively associated with the tissue-based expression levels of HISLA in BC-afflicted patients. Moreover, our data also suggested that serum HISLA expression was significantly correlated with histological grade, TNM stage, and distant organ metastasis. ROC curve analyses indicated that serum HISLA could be used to help differentiate BC-afflicted patients from healthy controls, and that high levels of expression of HISLA were also closely correlated with poor overall survival. These findings implied that serum HISLA levels could be used as a diagnostic and prognostic predictor for BC-afflicted patients.

Serum HISLA expression was remarkably decreased in postoperative specimens compared with preoperative samples. This finding indicated that serum HISLA was downregulated after tumor surgical resection. Therefore, serum HISLA expression levels could serve as an indicator for cancer recurrence of BC-afflicted patients who undergo tumor resection. However, there were some limitations in our study. For example, the small population of recruited BC patients and healthy controls might lead to deviations in the final results. Therefore, a larger research cohort should be enrolled to evaluate the estimates of diagnostic and prognostic significance of serum HISLA in BC that we completed herein. In conclusion, our study suggested that the serum HISLA expression might be a novel and useful diagnostic and prognostic biomarker for BC patients.

Author contributions CY conceived and designed the project; HH performed the experiment; JH collected the clinical samples and analysis; YY data statistical analysis; HH and WZ wrote the manuscript. All authors read and approved the final manuscript.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethics approval This study was approved by the Ethics Committee of The Shenzhen People's Hospital, and all of the participants provided informed consent.

Consent for publication Written informed consent for publication was obtained from each participant.

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