JBUON 2021; 26(3): 728-733
ISSN: 1107-0625, online ISSN: 2241-6293 • www.jbuon.com
Email: editorial_office@jbuon.com

ORIGINAL ARTICLE

Expression and clinical significance of lncRNA-SChLAP1 in breast cancer

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Summary

Purpose: *The purpose of this study was to investigate the expression of long non-coding RNA (lncRNA)-SChLAP1 in breast cancer (BCa) tissues and its clinical significance in the progression of this cancer.*

Methods: *100 pairs of surgically resected BCa tissue samples and normal breast tissues were collected from BCa patients, and SChLAP1 expression in above tissues was detected by quantitative real-time polymerase chain reaction (qRT-PCR). Meanwhile, the receiver operating curve (ROC) was plotted to analyze the efficacy of SChLAP1 in the diagnosis of BCa. In addition, the interplay between SChLAP1 expression and clinical features as well as the pathological features of BCa patients was analyzed.*

Results: *The expression level of SChLAP1 in BCa tissue samples was remarkably higher than that in normal ones. When the area under the ROC curve (AUC)=0.8639 and p<0.001, BCa could be diagnosed by SChLAP1; meanwhile, when the cut-off value was 3.26, the sensitivity was 80% and the specificity was 72%. Moreover, it was uncovered that the SChLAP1 expression was associated with patients' menarche, menopause, age of first pregnancy, or whether breastfeeding is administrated and whether oral contraceptives is taken; in addition, alcohol consumption, body mass index or tumor size and clinical stage were also the factors affecting the expression of SChLAP1.*

Conclusions: *SChLAP1 is highly expressed in BCa tissues and can serve as a potential biomarker for the diagnosis of this cancer.*

Key words: *breast cancer, SChLAP1, clinical significance, biomarkers*

Introduction

Breast cancer (BCa) is the most common malignant tumor that seriously affects women's health. The rapid development of molecular biology makes gene therapy of BCa become a research hotspot [1]. Exploring biomarkers related to BCa provides a new research direction for the diagnosis and treatment of BCa [2].

Long non-coding RNA (lncRNA) is considered as "transcriptional noise" in its early discovery because it cannot encode proteins. In recent years, with the expansion of the research on non-coding RNAs, lncRNAs have been recognized to play a significant role in more and more tumors [3,4]. Many scholars have confirmed that lncRNAs are closely related to the incidence of BCa. Qiu et al. [5] demonstrated that lncRNA LINC00668 promoted the progression of BCa by inhibiting apoptosis and accelerating cell cycle. Yang et al. [6] found through microarray analysis that the survival period of BCa patients was reduced when the expression of LncRNA HOTTIP was enhanced, and predicted that this lncRNA molecule could be used as a predictor for BCa patients.

LncRNA SChLAP1 (second chromosome locus associated with prostate-1), first discovered by Prensner et al. [7], is closely related to prostate cancer and located in the nucleus [8], and the

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Received: 23/11/2020; Accepted: 14/01/2021

high expression of SChLAP1 is more likely to trigger fatal prostate cancer [9]. It can also be used as an independent risk factor to predict biochemical recurrence of prostate cancer after radical resection, and the increased expression of SChLAP1 is particularly significant in metastatic prostate cancer, thus making SChLAP1 an ideal biomarker for predicting the prognosis of prostate cancer [8]. It has been reported that SChLAP1 may also play a role in the progression of bladder cancer [10]. However, currently, there is no report on the correlation between SChLAP1 and BCa. Therefore, this study aims to explore the relationship between SChLAP1 expression and BCa, so as to provide new guidance for the early diagnosis of BCa.

Methods

Clinical sample

BCa tissue samples and paracancerous normal breast tissues were collected from BCa patients undergoing surgery in our hospital, and transported to the hospital's central laboratory using liquid nitrogen tanks and stored at -80°C. BCa specimens were confirmed by pathological examination after operation. None of the patients received chemoradiotherapy. The study was approved by the Ethics Committee of The Second Affiliated Hospital of Fujian Medical University. Signed written informed consents were obtained from the patients and/or guardians.

Quantitative real-time polymerase chain reaction (qRT-PCR) detection

Total RNA in tissues and cells was extracted with TRIzol (Invitrogen, Carlsbad, CA, USA), and the expression level of related genes was detected using a PCR detection kit. The primer sequences were as follows: SChLAP1: F 5'-CGGAGAGGATGGGCTCTGGCATT-3', R: 5'-AGGGACCCTTCAGGGTGGCTG-3'; glyceraldheyde 3-phosphate dehydrogenase (GAPDH) F: 5'-GGTGAAGGTCGGAGTCAACG-3', R: 5'-CCATGTAGTTGAGGTCAATGAAG-3'.

Follow-up

All patients were followed up from the time of surgery to 300 days, and the overall survival of the patients was recorded as the time from the day of surgery to the time of death due to any cause or the follow-up ended. All patients were followed up for complete data.

Statistics

SPSS 22.0 statistical software (IBM, Armonk, NY, USA) was used to perform data analysis. The measurement data were analyzed by independent sample t-test; and the correlations of gene expression level with clinical characteristics, postnatal habits and pathological characteristic parameters were analyzed. Besides, the receiver operating characteristic (ROC) curve was used to evaluate the diagnostic value of SChLAP1 for BCa. The difference was statistically significant at $p<0.05$. *$p<0.05$.

Results

High expression of SChLAP1 in BCa tissues

QRT-PCR results revealed that the expression level of SChLAP1 in BCa tissue specimens was remarkably higher than that in normal ones (Figure 1), which indicated that SChLAP1 expression may be associated with the occurrence and development of BCa.

Evaluation of the sensitivity and specificity of SChLAP1 to diagnose BCa

Since we found that SChLAP1 was differentially expressed in cancer and normal tissues, we plotted ROC curves to analyze whether SChLAP1 can be used as a potential biomarker for the diagnosis of BCa. The results indicated that in the diagnosis of BCa using SChLAP1, the area under the ROC curve (AUC) was 0.8639, and when the cut-off value was

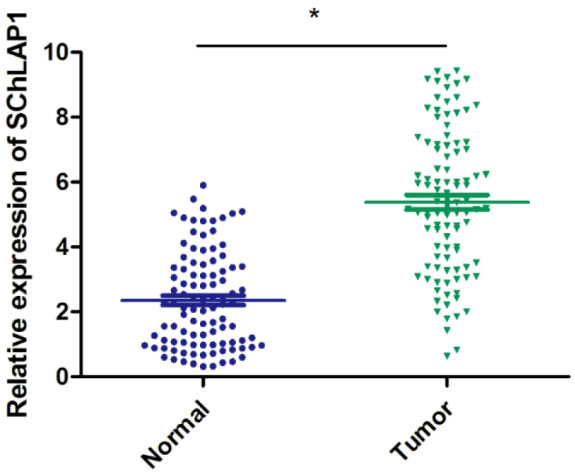

Figure 1. High expression of SChLAP1 in BCa tissues. QRT-PCR detected that SChLAP1 expression in BCa tissues was remarkably higher than that in normal tissues (*$p<0.05$).

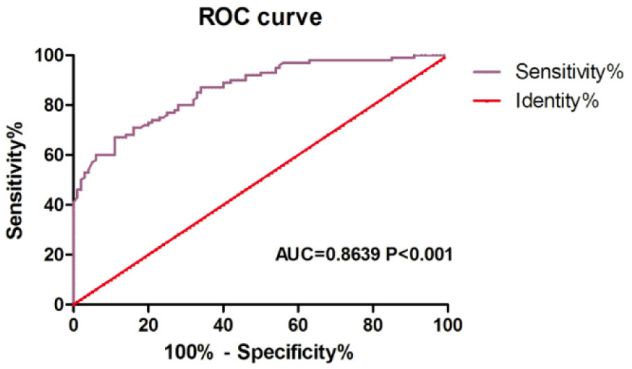

Figure 2. Sensitivity and specificity of SChLAP1 for BCa diagnosis. In the diagnosis of BCa using SChLAP1, AUC=0.8639 and $p<0.001$. When the cut-off value was 3.26, the sensitivity was 80% and the specificity was 72%.

3.26, the sensitivity was 80% and the specificity was 72% (Figure 2), suggesting that SChLAP1 has potential to be a biomarker for diagnosing BCa.

Relationship between SChLAP1 expression and clinical features of patients

In order to clarify the association between SChLAP1 and the related factors of patients' clinical characteristics, we took the cut-off value of ROC curve analysis of 3.26 as the boundary, and divided the expression level of SChLAP1 lower than 3.26 into the low expression group, and the expression level of SChLAP1 higher than 3.26 into the high expression group. Moreover, the results of x^2 test showed that there was no statistically significant difference in SChLAP1 expression among patients with different age, number of pregnancies and number of children ($p>0.05$), but in patients with different age of menarche, menopause, first-child born, breastfeeding and oral contraceptives, SChLAP1 expression showed a significant difference ($p<0.05$). The expression level of SChLAP1 was higher in the tissues of patients who had menarche age < 13, menopause age < 50, first birth age < 20, who had performed breastfeeding, and who had ever taken oral contraceptives (Table 1).

Relationship between relative expression of SChLAP1 and patient habits

Subsequently, x^2 test was performed to further elucidate the interplay between SChLAP1 and patients' lifestyle habits; as a result, SChLAP1 expression level showed no significant association with patients' smoking history ($p>0.05$), but with drinking history and body mass index ($p<0.05$). Specifically, SChLAP1 expression was remarkably increased in subjects who occasionally drink and who are overweight or obese (Table 2). The above results indicated that occasional alcohol consumption, overweight and obesity are relevant to the elevated SChLAP1 expression.

Relationship between SChLAP1 expression and pathological features of patients

Finally, we further explored the relationship between SChLAP1 expression and the factors associated with pathological features of patients

Table 1. Relationship between SChLAP1 related expression and clinical features of patients

Variables	*n*	*SChLAP1*		x^2	*p*
		High level (n=80)	*Low level (n=20)*		
Age, years					
<60	49	37	12	1.21	0.323
≥60	51	43	8		
Age at menarche, years					
<13	67	65	2	36.74	<0.001
≥13	33	15	18		
Age at Menopause, years					
<50	59	53	6	8.69	0.005
≥50	41	27	14		
Pregnancies (n)					
<3	43	31	12	2.95	0.129
≥3	57	49	8		
Children (n)					
<3	60	45	15	2.34	0.201
≥3	40	35	5		
Age at first birth, years					
<20	61	54	7	7.10	0.011
≥20	39	26	13		
Breastfeeding					
No	49	30	19	21.17	<0.001
Yes	51	50	1		
Oral contraceptive					
No	28	18	10	6.00	0.024
Yes	72	62	10		

Table 2. Relationship between relative expression of SChLAP1 and patient's habits

Variables	*n*	*SChLAP1*		x^2	*p*
		High level (n=80)	*Low level (n=20)*		
Smoking					
No	61	48	13	0.17	0.800
Yes	39	32	7		
Drinking					
No	45	35	10	16.18	<0.001
Sporadically	40	38	2		
Daily	15	7	8		
Body mass index					
Underweight	13	5	8	16.41	0.001
Normal	10	8	2		
Overweight	44	38	6		
Obese	33	29	4		

Table 3. Relationship between SChLAP1 related expression and pathological features of patients

Variables	*n*	*SChLAP1*		x^2	*p*
		High level (n=80)	*Low level (n=20)*		
Size of the tumor, cm					
<2	43	29	14	7.44	0.011
≥2	57	51	6		
Lymph node invasion					
No	59	44	15	2.646	0.131
Yes	41	36	5		
Pathological grade					
Ductal carcinoma *in situ*	7	4	3	3.24	0.356
Invasive carcinoma NOS	69	56	13		
Invasive lobular carcinoma	13	10	3		
Invasive lobular and ductal carcinoma	11	10	1		
Stage					
I +II	41	26	15	11.94	0.001
III +IV	59	54	5		

through the x^2 test. The results revealed that it was not associated with lymph node metastasis and pathological classification ($p>0.05$), but with tumor size and clinical stage ($p<0.05$). Specifically, the expression of SChLAP1 in patients with tumor ≥2cm or in III+IV clinical stage was remarkably higher than those with tumor <2 cm or in I + II (Table 3). The above results suggested that tumor size and clinical stage may have certain effect on the expression of SChLAP1.

Discussion

BCa is the most common female tumor. Every year, 1.677 million new female patients (52.7 per 100,000 people) are diagnosed worldwide, causing about 522,000 deaths (13.2 per 100,000 people) [1]. Although the prevalence rate (37.9/100,000) and mortality rate (9.2/100,000) of BCa in China are lower than the world average [11], a number of epidemiological surveys show that the incidence of BCa in China is on the rise [12], posing a serious threat to women's health in China.

LncRNAs are a class of non-coding RNAs with a length of about 200 nucleotides, accounting for the highest proportion in non-coding RNAs and widely existing in normal human tissues [13,14]. It has many functions, such as regulating transcription, translation, cell differentiation, regulation of gene expression, regulation of cell cycle, chromatin modification and transport between nucleus and cytoplasm, and plays a very significant role in tu-

mor formation, prognostic markers and risk prediction of treatment mode [15]. Recent studies [16-18] have shown that more and more lncRNAs, such as GACAT3, XIST, DANCR, etc. are relevant to the progression of BCa, involving various molecules and signaling pathways in cells.

The emerging LncRNA-SChLAP1, also known as LINC00913, has been named as the second chromosome loci associated with prostage-1, which is an important mediator of tumor invasion and hematopoietic metastasis [19]. *In vitro* and *in vivo* functional acquisition and functional deficiency experiments showed that SChLAP1 resisted the genomic localization and regulation of SWI/SNF chromatin modification, inhibited the anti-cancer function of SWI/SNF complex, promoted the invasion and metastasis of cancer cells, and thus led to the development of lethal cancer [20]. By disrupting the SWI/SNF complex, SChLAP1 can lead to hundreds of changes in gene expression and therefore enhance the risk of tumor progression through promoting a comprehensive metastatic cascade rather than a single signaling pathway [21].

In this study, SChLAP1 expression was found remarkably increased in the collected BCa tissue specimens compared to the normal ones. Subject performance curve (ROC) analysis indicated that SChLAP1 could be used as a potential biomarker for the diagnosis of BCa. SChLAP1 expression was correlated with patients' menarche, menopause, age of first birth, breastfeeding and oral contraceptives; meanwhile, alcohol consumption and body mass index as well as tumor size ad tumor clinical stage were also confirmed to have some relevance to the expression of SChLAP1.

Consequently, we concluded that SChLAP1 expression in the tissues of patients can be affected by related clinical features, lifestyle and pathological features of BCa patients, which may provide a new idea for SChLAP1's research in BCa.

Conclusions

Consequently, SChLAP1 expression in the tissues of patients can be affected by related clinical features, lifestyle and pathological features of BCa patients, which may provide a new idea for SChLAP1's research in BCa.

Conflict of interests

The authors declare no conflict of interests.

References

1. Torre LA, Bray F, Siegel RL, Ferlay J, Lortet-Tieulent J, Jemal A. Global cancer statistics, 2012. CA Cancer J Clin 2015;65:87-108.
2. Gagan J, Van Allen EM. Next-generation sequencing to guide cancer therapy. Genome Med 2015;7:80.
3. Liang Y, Li Y, Song X et al. Long noncoding RNA LINP1 acts as an oncogene and promotes chemoresistance in breast cancer. Cancer Biol Ther 2018;19:120-31.
4. Wang H, Zhang M, Sun G. Long non-coding RNA NEAT1 regulates the proliferation, migration and invasion of gastric cancer cells via targeting miR-335-5p/ROCK1 axis. Pharmazie 2018;73:150-5.
5. Qiu X, Dong J, Zhao Z, Li J, Cai X. LncRNA LINC00668 promotes the progression of breast cancer by inhibiting apoptosis and accelerating cell cycle. Onco Targets Ther 2019;12:5615-25.
6. Yang Y, Qian J, Xiang Y, Chen Y, Qu J. The prognostic value of long noncoding RNA HOTTIP on clinical outcomes in breast cancer. Oncotarget 2017;8:6833-44.
7. Prensner JR, Iyer MK, Sahu A et al. The long noncoding RNA SChLAP1 promotes aggressive prostate cancer and antagonizes the SWI/SNF complex. Nat Genet 2013;45:1392-8.
8. Prensner JR, Zhao S, Erho N et al. RNA biomarkers associated with metastatic progression in prostate cancer: a multi-institutional high-throughput analysis of SChLAP1. Lancet Oncol 2014;15:1469-80.
9. Mehra R, Udager AM, Ahearn TU et al. Overexpression of the Long Non-coding RNA SChLAP1 Independently Predicts Lethal Prostate Cancer. Eur Urol 2016;70:549-52.
10. Zhang J, Shi Z, Nan Y, Li M. Inhibiting malignant phenotypes of the bladder cancer cells by silencing long noncoding RNA SChLAP1. Int Urol Nephrol 2016;48:711-6.
11. Zheng R, Zeng H, Zhang S, Chen T, Chen W. National estimates of cancer prevalence in China, 2011. Cancer Lett 2016;370:33-8.
12. Chen W, Zheng R, Zeng H, Zhang S, He J. Annual report on status of cancer in China, 2011. Chin J Cancer Res 2015;27:2-12.
13. Zhang G, Cai J. Evaluation of prognostic value of lncRNA BANCR in tumor patients: A systematic review and meta-analysis. J BUON 2019;24:2553-9.
14. Zhuang H, Cao G, Kou C, Li D. Overexpressed lncRNA CDKN2B-AS1 is an independent prognostic factor for liver cancer and promotes its proliferation. J BUON 2019;24:1441-8.
15. Xia E, Bhandari A, Shen Y et al. LncRNA CCND2-AS1 promotes proliferation, migration, and invasion in pap-

illary thyroid carcinoma. Biochem Biophys Res Commun 2018;496:628-32.

16. Hu H, Wang Y, Zhang T et al. Association of LncRNA-GACAT3 with MRI features of breast cancer and its molecular mechanism. J BUON 2019;24:2377-84.
17. Zheng R, Lin S, Guan L et al. Long non-coding RNA XIST inhibited breast cancer cell growth, migration, and invasion via miR-155/CDX1 axis. Biochem Biophys Res Commun 2018;498:1002-8.
18. Tao W, Wang C, Zhu B, Zhang G, Pang D. LncRNA DANCR contributes to tumor progression via targetting miR-216a-5p in breast cancer: lncRNA DANCR contributes to tumor progression. Biosci Rep 2019;39:BSR20181618.
19. Atala A. Re: The long noncoding RNA SChLAP1 promotes aggressive prostate cancer and antagonizes the SWI/SNF complex. J Urol 2014;192:613.
20. Sun X, Chuang JC, Kanchwala M et al. Suppression of the SWI/SNF Component Arid1a Promotes Mammalian Regeneration. Cell Stem Cell 2016;18:456-6.
21. Raab JR, Smith KN, Spear CC, Manner CJ, Calabrese JM, Magnuson T. SWI/SNF remains localized to chromatin in the presence of SCHLAP1. Nat Genet 2019;51:26-9.