Long non-coding RNA BCAR4 promotes liver cancer progression by regulating proliferation, migration and invasion

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Abstract. Liver cancer (LC) is one of the primary contributors of cancer-associated death worldwide. Long non-coding RNAs (lncRNAs) have been shown to participate in almost every aspect of cell biology and serve fundamental roles in carcinogenesis and cancer progression, including in LC. However, the clinical significance and functional role of the lncRNA breast cancer anti-estrogen resistance 4 (BCAR4) in LC have not yet been identified. The present study measured the expression levels of BCAR4 in LC cells and tissues, and discovered that BCAR4 was upregulated in LC tissues compared with adjacent normal tissues. Furthermore, high BCAR4 expression was associated with the presence of multiple tumors and advanced Tumor-Node-Metastasis stages (III/IV). Survival analysis found that high BCAR4 expression indicated poor overall survival (OS) and progression-free survival (PFS). By analyzing the risk factors of poor OS and PFS using univariate analysis and multivariate analysis, high BCAR4 expression was revealed to be an independent risk factor of poor prognosis. In addition, the role of BCAR4 was further investigated *in vitro*, which revealed overexpression of BCAR4 to markedly promote the proliferation, migration and invasion of LC cells. Conversely, the loss of BCAR4 expression repressed the proliferation, migration and invasion of LC cells. In conclusion, BCAR4 is overexpressed in LC and is associated with LC progression. Therefore, BCAR4 may be used as a potential prognostic marker in LC.

Introduction

Liver cancer (LC), the third leading cause of cancer-associated mortality and the sixth most common cancer worldwide in 2016, represents a global health problem (1). The majority of patients with LC are diagnosed at advanced stages due to the insidious developmental process (2). Curative therapies such as resection or transplantation are not suitable for patients with advanced stage LC, and advanced LCs are resistant to the majority of standard chemotherapeutic regimens (3). Therefore, the overall survival (OS) of patients with LC is dismal, with a five-year survival rate of <15% in USA (3-5). Therefore, current research efforts are directed towards the discovery of biomarkers for early diagnosis, recognition of molecular subclasses of LC, correlation of molecular signatures with radiological/histological features, characterization of new therapeutic targets and personalization of therapies based on an individual's tumor biology (6). Further investigations into the mechanisms of carcinogenesis and development of LC are essential to achieve these goals.

In the past few decades, intensive investigations have focused on the role of protein-coding genes in the pathogenesis of LC (7-10). However, only 2% of the human genome is transcribed into protein-coding mRNAs, whereas 70-80% of the genome is actively transcribed into non-coding RNAs (11,12). Long non-coding RNAs (lncRNAs) are the non-coding RNAs, >200 nucleotides in length, without protein-coding ability (13). Accumulating evidence has suggested that lncRNAs affect all essential processes in living cells, including chromatin condensation, replication, transcription, splicing, and translational and post-translational modification of proteins and constituting the most abundant part of the transcribed genome (14). In addition, a number of lncRNAs have been discovered since the discovery and characterization of the first lncRNA H19, in 1991 (15). In addition, current evidence has identified lncRNAs as key regulators of cancer signaling networks and characteristic cancer behaviors (16). Some lncRNAs, such as H19, HOX transcript antisense intergenic RNA, metastasis associated lung adenocarcinoma transcript 1 and lncRNA associated with microvascular invasion in HCC, were suggested to be dysregulated in LC and may serve as prognostic markers and therapeutic targets in patients with LC (6,15,17-21). However, the clinical significance and functional role of breast cancer anti-estrogen resistance 4 (BCAR4) in LC have not yet been reported.

The present study aimed to investigate the expression levels of BCAR4 in LC cells and tissues. Furthermore, the clinical significance of BCAR4 in LC was explored, and its prognostic value was investigated, with the purpose of evaluating the functional role of BCAR4 in LC cells.

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Key words: BCAR4, hepatocellular cancer, prognosis

Materials and methods

Collection of clinical specimens and follow-up. Tumor tissues and paired normal adjacent tissues were collected from 188 patients with LC (65 males and 35 females; median age, 62.3 years; range, 39-86 years), who underwent surgical resection between April 2011 and May 2014 at The Jiangxi Provincial People's Hospital Affiliated with Nanchang University (Nanchang, China). Adjacent tissues were obtained ≥2 cm away from the border of tumor tissues. The final diagnosis of patients was confirmed by pathology. None of the patients received preoperative chemotherapy, radiotherapy or other anticancer treatments. The specimens were collected immediately following surgical resection, and the collected tissues were frozen in liquid nitrogen and stored at -80˚C until further use. The clinicopathological characteristics of the patients analyzed in the present study included details of age, sex, serum α-fetoprotein (AFP) level, alcoholism, hepatitis B virus (HBV) infection, liver cirrhosis, differentiation grade (American Joint Committee on Cancer) (22), tumor size, encapsulation, number of tumors, lymph-vascular space invasion (LVSI), and Tumor-Node-Metastasis (TNM) stage (American Joint Committee on Cancer) (22). Written informed consent was obtained from each patient and the present study was approved by The Ethnic Committee of Jiangxi Provincial People's Hospital Affiliated with Nanchang University.

Follow-up was performed by telephone interview and questionnaire every 3 months. The deadline of the follow-up was June 2017. OS was defined as the time interval between the date of diagnosis and the end of the follow-up, or the date at which the patient succumbed to the disease. Progression-free survival (PFS) was defined as the interval between the date of surgery and recurrence; if recurrence was not diagnosed, the patients were censored on the date of death or the last follow-up.

Cell culture and transfection. Liver cancer cell lines (HuH-6, Hep3B, HuH-7, HepG2, and HuH-1) were purchased from The Cell Bank of Type Culture Collection of the Chinese Academy of Sciences. HuH-6 and HuH-1 were cultured in RPMI-1640 medium (Invitrogen; Thermo Fisher Scientific, Inc.), whereas Hep3B, HuH-7 and HepG2 were cultured in DMEM (Invitrogen; Thermo Fisher Scientific, Inc.) supplemented with 10% FBS (Thermo Fisher Scientific, Inc.), 100 U/ml penicillin and 100 mg/ml streptomycin (Thermo Fisher Scientific, Inc.) in a humidified atmosphere containing 5% CO_2 at 37˚C.

The BCAR4 expression vector (pcDNA3.1-BCAR4), a negative control vector (pcDNA3.1-Vector), specific small interfering RNAs (siRNAs) targeting BCAR4 (siBCAR4-1 and siBCAR4-2; cat. no. A01002) and a scrambled negative control (siNC; cat. no. A06001) were purchased from Shanghai GenePharma Co., Ltd,. The overexpression and silencing of BCAR4 were conducted using pcDNA3.1-BCAR4 (800 ng) and the siRNAs (siBCAR4-1 and siBCAR4-2; 50 nM), respectively. Transfection was performed using the Lipofectamine® 2000 reagent (Thermo Fisher Scientific Inc.) according to the manufacturer's instructions. Cells were subjected to subsequent experimentation 48 h following transfection.

Reverse transcription-quantitative PCR assay (RT-qPCR). Total RNA from the tissue samples and the transfected cells were extracted using the TRIzol reagent (Invitrogen; Thermo Fisher Scientific, Inc.) according to the manufacturer's protocol. The concentration and purity of RNA were determined by measuring its optical density (OD) using a NanoDrop 2000 spectrophotometer (1.8<A260/280<2.0; Thermo Fisher Scientific, Inc.). Total RNA (1 μg) was reverse transcribed in a final volume of 20 μl, under standard conditions (37˚C for 15 min, then 85˚C for 5 sec), using PrimeScript RT Reagent kit (Takara Biotechnology Co., Ltd.). qPCR was performed using an ABI PRISM 7000 Fluorescent Quantitative PCR system (Applied Biosystems; Thermo Fisher Scientific, Inc.) according to the manufacturer's protocols. Briefly, reactions were loaded onto a 96-well plate in triplicate and the thermocycling conditions used were: 95˚C For 5 min; followed by 40 cycles of denaturation at 95˚C for 30 sec, 1 min of annealing at 60˚C and extension at 60˚C for 1 min. The average value of triplicate samples was used to calculate the relative expression of BCAR4 using the $2^{-\Delta\Delta Cq}$ method (23). The experiments were repeated at least three times. The primer sequences were as follows: BCAR4 forward, 5'-TACAACCACTGCACTACCTG-3' and reverse, 5'-TGGAATGCTTGAAGGCTGCT-3'; and GAPDH, forward, 5'-CGCTCTCTGCTCCTCCTGTTC-3' and reverse, 5'-ATCCGTTGACTCCGACCTTCAC-3'. GAPDH was used as the internal control.

MTT and colony-formation assays. A Cell Proliferation Reagent kit I (MTT) (Roche Diagnostics) was used to assess cell proliferation. Transfected cells ($2x10^3$ per well) were plated in each well of a 96-well plate, dimethyl sulfoxide was used to dissolve the purple formazan, and the optical density was assessed at specific time points (0, 24, 48, 72, and 96 h) at a wavelength of 490 nm, according to the manufacturer's protocol.

For the colony-formation assay, each well in a 6-well culture plate was seeded with $1x10^3$ cells and cultured for 7 days in DMEM (Invitrogen; Thermo Fisher Scientific, Inc.) supplemented with 10% FBS, 100 U/ml penicillin and 100 mg/ml streptomycin (all Thermo Fisher Scientific, Inc.). Subsequently, the adherent cells were washed with PBS, fixed with 10% paraformaldehyde for 10 min at room temperature and stained with 1% crystal violet for 5 min at room temperature. Images of culture plates were captured using a light microscope (magnification, x40), and the number of colonies was counted. Triplicate wells were measured in each treatment group.

Transwell migration and invasion assays. After 48 h of transfection, cells ($1x10^5$) were resuspended in fresh medium (200 μl) and added to the upper side of a Transwell chamber (8-μm pore size; BD Biosciences), uncoated (for the Transwell migration assay) or coated (for the Matrigel invasion assay) with 50 μl Matrigel (BD Biosciences). Medium (700 μl) containing 20% FBS was added to the lower chamber. After 24 h of incubation, the cells remaining on the upper membrane were removed using cotton wool. The migrated/invaded cells on the bottom side of the chamber were fixed with 4% paraformaldehyde for 30 min at room temperature and stained with Giemsa (Beijing Zhongsheng Ruitai Technology Co., Ltd.; 1:10 dilution) for 30 min at room temperature. Cell numbers were counted in 5 random fields of each chamber under the light microscope (magnification, x40).

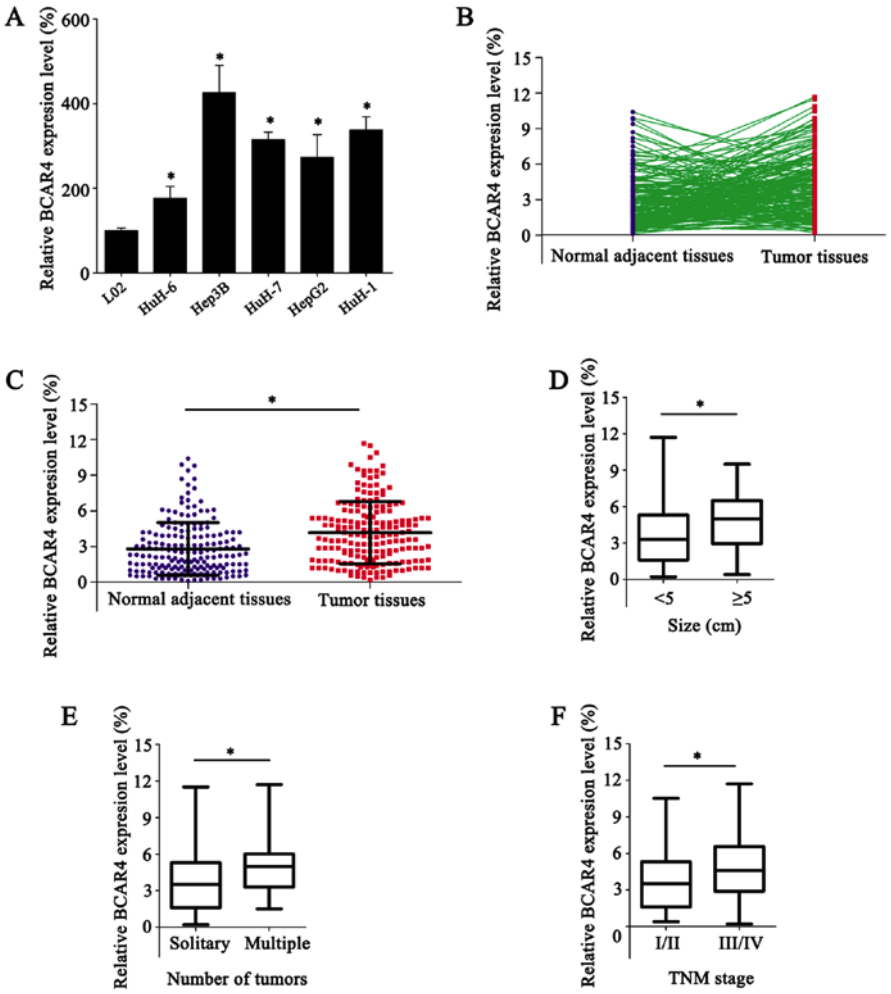

Figure 1. BCAR4 is overexpressed in LC and is associated with cancer progression. (A) Expression of BCAR4 was detected in five LC cell lines by RT-qPCR. (B) Expression of BCAR4 was measured in LC tissues and paired normal adjacent tissues (n=188) by RT-qPCR. *P<0.05. (C) The expression level of BCAR4 in LC tissues and paired normal adjacent tissues (n=188) was statistically analyzed. The expression levels of BCAR4 in LC patients with (D) tumor size ≥5 cm and <5 cm, (E) solitary tumor and multiple tumors, and (F) less advanced TNM stages (I/II) and advanced TNM stages (III/IV) were detected and calculated by RT-qPCR. *P<0.05. LC, liver cancer; BCAR4, breast cancer anti-estrogen resistance 4; RT-q, reverse transcription-quantitative; TNM, Tumor-Node-Metastasis.

Statistical analysis. All statistical analyses were performed using SPSS version 20.0 (IBM Corp.). The χ^2 test and Student's t-test were performed to examine the associations between BCAR4 expression level and clinical characteristics. Paired and unpaired Student's t-test were used to compare the difference between paired and independent samples, respectively. A one-way ANOVA with a post-hoc Dunnett's test was used to detect differences between multiple groups. The survival curves were plotted using the Kaplan-Meier method and a log-rank test was used to evaluate the differences between the survival curves. Hazard ratio (HR) and 95% confidence interval (95% CI) values were calculated using a Cox proportional hazard regression model to evaluate the association between BCAR4 expression and OS/PFS. Variables with a value of P<0.05 in univariate analysis were subjected to multivariate analysis on the basis of Cox regression analyses. P<0.05 was considered to indicate a statistically significant difference.

Results

BCAR4 is overexpressed in LC and is associated with cancer progression. Determining the expression levels of lncRNAs is a pivotal aspect in analyzing their potential role in cancer. In order to investigate the functional role of BCAR4 in LC, its expression levels in five LC cell lines (HuH-6, Hep3B, HuH-7, HepG2 and HuH-1) were measured by RT-qPCR. BCAR4 displayed varying expression levels in LC cells; Hep3B cells exhibited the highest expression level of the 4 LC cell lines, while HepG2 cells expressed the lowest levels, and the difference in expression between the aforementioned cell lines was significant (Fig. 1A). The expression of BCAR4 was detected in 188 LC tissues and paired normal adjacent tissues (Fig. 1B). LC tissues exhibited significantly higher levels of BCAR4 expression compared with the paired normal adjacent tissues (P<0.05; Fig. 1C). Additionally, patients with tumor sizes ≥5 cm, multiple tumors and advanced TNM stages (III/IV) showed significantly higher expression levels of BCAR4 compared with those with tumor size <5 cm (P<0.05; Fig. 1D), solitary tumors (P<0.05; Fig. 1E) and less advanced TNM stages (I/II) (P<0.05; Fig. 1F), respectively.

In order to determine the clinical significance of BCAR4 in LC, patients were divided into a low-BCAR4 expression group (n=100) and a high-BCAR4 expression group (n=88).

Table I. Association between BCAR4 expression and clinicopathological characteristics of hepatocellular carcinoma.

Parameters	No. of patients		P-value
	Low BCAR4 expression, n=100	High BCAR4 expression, n=88	
Age, years			0.970
<55	52	46	
≥55	48	42	
Sex			0.137
Male	65	66	
Female	35	22	
AFP, ng/ml			0.862
<20	51	46	
≥20	49	42	
Alcoholism			0.586
Negative	46	37	
Positive	54	51	
HBV			0.974
Negative	18	16	
Positive	82	72	
Liver cirrhosis			0.505
Present	44	43	
Absent	56	45	
Differentiation grade			0.320
Well + moderate	62	58	
Poor	38	30	
Tumor size, cm			0.089
<5	73	54	
≥5	27	34	
Encapsulation			0.784
No	48	44	
Complete	52	44	
Number of tumors			0.029
Solitary	81	59	
Multiple	19	29	
LVSI			0.122
Present	41	46	
Absent	59	42	
TNM stage			0.020
I/II	75	52	
III/IV	25	36	

AFP, α-fetoprotein; BCAR4, breast cancer anti-estrogen resistance 4; HBV, hepatitis B virus; LVSI, Lymph-vascular space invasion; TNM, Tumor-Node-Metastasis.

The average BCAR4 expression level was used as the cut-off value. The association between BCAR4 expression levels and the clinicopathological characteristics of LC was statistically analyzed. Higher levels of BCAR4 expression were associated with the presence of multiple tumors and advanced TNM stages (III/IV) (Table I). Overall, high BCAR4 expression is associated with the clinical progression of LC.

High BCAR4 expression predicts poor prognosis in LC. A lack of prognostic markers with adequate efficiency and efficacy complicates individualized therapeutic regimens in clinical practice. The present study evaluated the prognostic value of high BCAR4 expression in LC. Patients with high BCAR4 expression exhibited poorer OS time (P<0.01; Fig. 2A) and PFS time (P<0.01; Fig. 2B) compared with patients with low BCAR4 expression.

Table II. Univariate analysis of clinicopathological features, OS and PFS of hepatocellular carcinoma patients.

Parameters	OS			PFS		
	HR	95% CI	P-value	HR	95% CI	P-value
Age, years (≥55 vs. <55)	1.373	0.950-1.984	0.092	1.332	0.925-1.918	0.124
Sex (male vs. female)	1.011	0.679-1.505	0.959	1.061	0.718-1.568	0.767
AFP, ng/ml (<20 vs. ≥20)	1.127	0.780-1.628	0.523	1.148	0.798-1.652	0.458
Alcoholism (negative vs. positive)	1.179	0.813-1.710	0.386	1.197	0.827-1.732	0.341
HBV (negative vs. positive)	1.045	0.639-1.711	0.860	0.957	0.596-1.535	0.855
Liver cirrhosis (present vs. absent)	1.082	0.749-1.563	0.674	1.074	0.746-1.547	0.699
Differentiation grade (poor vs. well + moderate)	0.865	0.58-1.271	0.461	0.833	0.568-1.222	0.350
Tumor size, cm (≥5 vs. <5)	1.623	1.109-2.374	0.013	1.587	1.087-2.316	0.017
Encapsulation (no vs. complete)	1.257	0.870-1.817	0.224	1.287	0.893-1.855	0.177
No. of tumors (multiple vs. solitary)	1.986	1.333-2.957	0.001	1.934	1.301-2.876	0.001
LVSI (present vs. absent)	2.036	1.401-2.959	<0.001	2.087	1.441-3.023	<0.001
TNM stage (III/IV vs. I/II)	3.333	2.289-4.853	<0.001	3.165	2.181-4.593	<0.001
BCAR4 (high vs. low)	1.157	1.076-1.244	<0.001	1.138	1.059-1.223	<0.001

OS, overall survival; PFS, progression-free survival; HR, hazard ratio; CI, confidence interval; AFP, alpha-fetoprotein; HBV, hepatitis B virus; LVSI, Lymph-vascular space invasion; TNM, Tumor-Node-Metastasis; BCAR4, breast cancer anti-estrogen resistance 4.

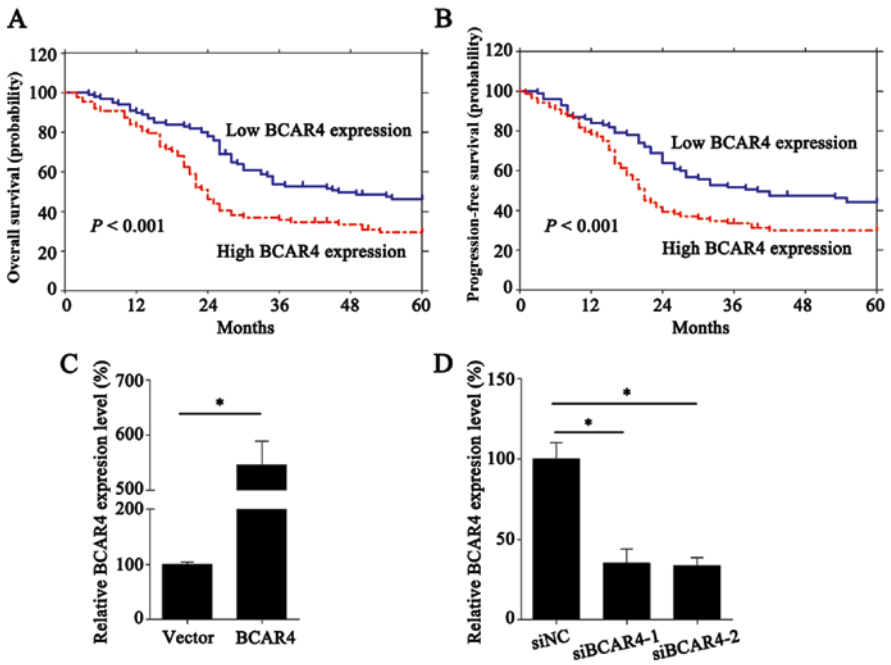

Figure 2. Overexpression of BCAR4 predicts poor prognosis in LC. (A) Overall survival and (B) progression-free survival of patients with LC in the low-BCAR4 expression group and the high-BCAR4 expression group are shown as Kaplan-Meier curves and compared using a log-rank test. (C) RT-qPCR was used to detect the expression of BCAR4 in HuH-6 cells overexpressing BCAR4 and (D) in Hep3B cells with BCAR4 knocked down. *P<0.05. LC, liver cancer; BCAR4, breast cancer anti-estrogen resistance 4; RT-q, reverse transcription-quantitative; si, small interfering RNA; NC, negative control.

Univariate analysis and multivariate analysis were performed to identify the risk factors of poor OS. Five parameters (tumor size ≥5 cm, multiple tumors, presence of LVSI, TNM stages III/IV and high BCAR4 expression) were shown to be risk factors of poor OS and PFS in the univariate analysis (Table II). Further analysis of these factors using multivariate analysis found that the presence of LVSI (P=0.001; HR, 1.943; 95% CI, 1.319-2.862), TNM stages III/IV (P<0.001; HR, 3.418; 95% CI, 2.156-5.420) and high BCAR4 expression (P=0.002; HR, 1.126; 95% CI, 1.046-1.212,) were independent risk factors of poor OS (Table III). In addition, the presence of LVSI (P<0.001; HR, 2.009; 95% CI, 1.372-2.941), TNM stage III/IV (P<0.001; HR, 3.253; 95% CI, 2.061-5.137) and high BCAR4 expression (P=0.007; HR, 1.105; 95% CI, 1.027-1.189) were also demonstrated to be independent risk factors of poor PFS

Table III. Multivariate analysis of clinicopathologic features and OS and PFS of patients with hepatocellular carcinoma.

	OS			PFS		
Parameters	HR	95% CI	P-value	HR	95% CI	P-value
Tumor size, cm (≥5 vs. <5)	0.889	0.554-1.427	0.626	0.889	0.556-1.421	0.622
No. of tumors (multiple vs. solitary)	0.836	0.491-1.423	0.509	0.857	0.506-1.450	0.564
LVSI (present vs. absent)	1.943	1.319-2.862	0.001	2.009	1.372-2.941	<0.001
TNM stage (III/IV vs. I/II)	3.418	2.156-5.420	<0.001	3.253	2.061-5.137	<0.001
BCAR4 (high vs. low)	1.126	1.046-1.212	0.002	1.105	1.027-1.189	0.007

OS, overall survival; PFS, progression-free survival; HR, hazard ratio; CI, confidence interval; LVSI, Lymph-vascular space invasion; TNM, Tumor-Node-Metastasis; BCAR4, breast cancer anti-estrogen resistance 4.

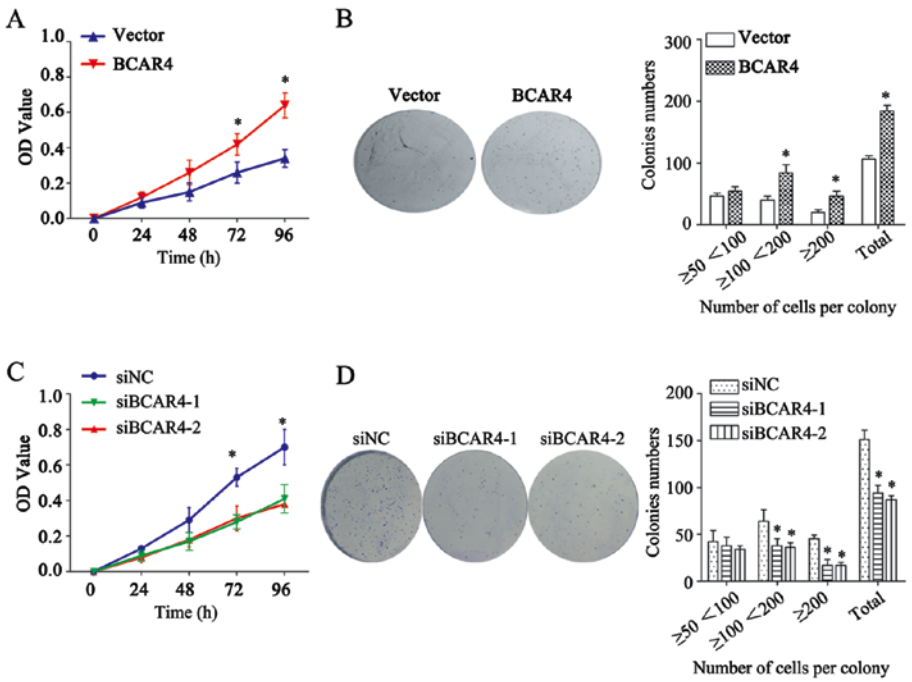

Figure 3. BCAR4 promotes proliferation of LC. (A) MTT and (B) colony-formation assays were conducted to evaluate the proliferation ability of HuH-6 cells overexpressing BCAR4. *P<0.05. (C) MTT and (D) colony-formation assays were performed to detect the proliferative ability of Hep3B cells with BCAR4 knocked down. *P<0.05. LC, liver cancer; BCAR4, breast cancer anti-estrogen resistance 4; si, small interfering RNA; NC, negative control; OD, optical density.

(Table III). Collectively, high BCAR4 expression predicts poor prognosis and serves as an independent risk factor of poor OS and PFS in LC.

BCAR4 promotes proliferation, migration and invasion in LC. HuH-6 and Hep3B cells had the lowest and highest BCAR4 expression levels among the 5 LC cell lines used in this study, respectively (Fig. 1A). BCAR4 was ectopically overexpressed in HuH-6 cells (Fig. 2C) and silenced in Hep3B cells (Fig. 2D) for the functional assays. The proliferative abilities of LC cells with BCAR4 overexpression and knockdown were evaluated using MTT and colony-formation assays. Overexpression of BCAR4 resulted in increased OD values after 72 and 96 h (P<0.05; Fig. 3A) and number of colonies (P<0.05; Fig. 3B) in HuH-6 cells compared with the empty vector control; whereas BCAR4 knockdown significantly decreased the OD value (P<0.05; Fig. 3C) and the colony number (P<0.05; Fig. 3D) in Hep3B cells compared with siNC transfected cells. Moreover, overexpression of BCAR4 in HuH-6 cells significantly promoted migration (P<0.05; Fig. 4A) and invasion (P<0.05; Fig. 4B) compared with the empty vector control. Knockdown of BCAR4 in Hep3B cells resulted in reduced migration (P<0.05; Fig. 4C) and invasion (P<0.05; Fig. 4D) compared with cells transfected with siNC. Overall, these results suggest that BCAR4 promotes the proliferation, migration and invasion of LC cells.

Discussion

Several canonical signaling pathways have been indicated to mediate lncRNA-regulated behaviors in LC, such as Wnt signaling (24,25) and STAT3 signaling (26-29). In addition,

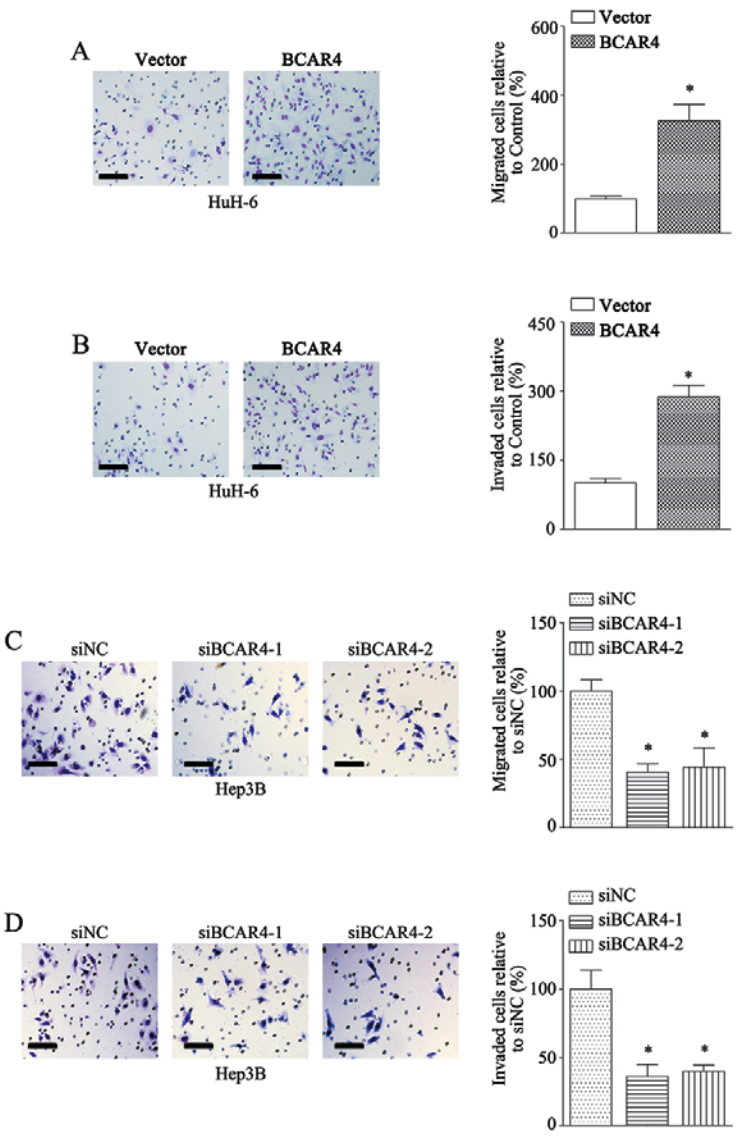

Figure 4. BCAR4 promotes migration and invasion in LC. (A) Migratory and (B) invasive abilities of HuH-6 cells overexpressing BCAR4 were measured using Transwell migration and Matrigel invasion assays. *P<0.05. (C) Migration and (D) invasion of Hep3B cells with BCAR4 knocked down, were detected using Transwell migration and Matrigel invasion assays. Scale bars, 50 μm. *P<0.05. LC, liver cancer; BCAR4, breast cancer anti-estrogen resistance 4; si, small interfering RNA; NC, negative control.

lncRNAs are involved in the determination and maintenance of hepatic cancer stem cell characteristics (30,31) and the regulation of epithelial-to-mesenchymal transition (32-34). Furthermore, one study evaluated lncRNAs alone and in combination as candidate biomarkers for LC diagnosis, with reported sensitivities ranging from 91.4 to 92.7% and specificity ranging from 82.1 to 88.6% (18). lncRNAs have been considered as potential early diagnostic biomarkers in LC (35,36). Additionally, some studies suggested that lncRNAs may be potential therapeutic targets in LC, owing to their tissue-specific expression patterns (19,37-39). Therefore, lncRNAs serve pivotal roles in LC biology and may shed new light on the early diagnosis and therapy for patients with LC.

In 2006, BCAR4 expression was first found to positively correlate with tamoxifen resistance, and ectopic expression of BCAR4 was shown to induce anti-estrogen resistance and anchorage-independent transformation of breast cancer (40); and this result was confirmed by additional studies (41-43). Further investigations performed by Godinho *et al* (44) found that BCAR4-positive breast tumors were driven by homologue 2/homologue 3 signaling. Therefore, BCAR4 was regarded as a suitable target for treating anti-estrogen-resistant breast cancer (42,43). In addition, the oncogenic role of BCAR4 has been defined in osteosarcoma (45), colorectal cancer (46), non-small cell lung cancer (47), chondrosarcoma (48), gastric cancer (49) and glioma (50). Studies have revealed that BCAR4 promotes cancer progression through a number of mechanisms, such as increasing the metastatic behaviors of cells, proliferation, chemoresistance and epithelial-to-mesenchymal transition (45,47-50). Several signaling pathways, such as Wnt signaling and mTOR signaling, have been shown to mediate the functional role of BCAR4 in accelerating cancer progression (48,51). The prognostic value of BCAR4 upregulation was reported in osteosarcoma, gastric cancer and colorectal cancer (45,46,49). These findings lay a foundation for utilizing BCAR4 as a prognostic marker and therapeutic target in different types of cancer. However, the detailed mechanisms

underlying the oncogenic functions of BCAR4 in LC are yet to be elucidated. Besides, the samples used in the present study were collected from a single medical center and the population size was limited; thus, more samples from multiple sources need to be analyzed to validate the present results.

The present study verified the upregulation of BCAR4 in LC, and its association with LC progression. Statistical analysis demonstrated that high BCAR4 expression indicated poor OS and PFS times. Furthermore, Cox regression analyses revealed high BCAR4 expression as an independent risk factor of poor OS and PFS. Furthermore, *in vitro* assays validated the effects of BCAR4 in promoting LC proliferation, migration and invasion. The present study indicates the potential of BCAR4 as a prognostic biomarker in LC.

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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

ZZ and AW designed the study and revised the manuscript. JM, ZZ AW and HL performed the experiments. JM and HL drafted the manuscript. JM and CL performed statistical analysis of the data and conducted follow-up of the patients. All authors approved the final manuscript.

Ethics approval and consent to participate

Written informed consent was obtained from each patient and the present study was approved by The Ethnic Committee of Jiangxi Provincial People's Hospital Affiliated with Nanchang University.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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