Up-regulated BCAR4 contributes to proliferation and migration of cervical cancer cells

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OBJECTIVES: Recently, thousands of long non-coding RNAs (lncRNAs) involved in development and metastasis of malignant tumors have been identified. Long non-coding RNA (lncRNA) breast cancer anti-estrogen resistance 4 (BCAR4) has been proved to promote proliferation and metastasis in multiple tumors. However, the function and significance of BCAR4 in cervical cancer are still unclear.

METHODS: In this study, we concentrated on the biological function and clinical significance of BCAR4 in cervical cancer. More specifically, BCAR4 expression was evaluated in cervical cancer tissues and cell lines by qRT-PCR. Moreover, the prognostic factors were assessed by Kaplan-Meier analysis and Cox proportional hazards models. Additionally, functional assays were conducted and the potential mechanism was explored.

RESULTS: Our study showed that BCAR4 expression was significantly up-regulated in cervical cancer tissue and cell lines. Moreover, patients with high BCAR4 expression showed worse survival outcomes and overexpression of BCAR4 might be an independent prognostic factor in cervical cancer. Furthermore, overexpression of BCAR4 remarkably promoted the proliferation, motility of cervical cancer cells and silencing BCAR4 significantly suppressed the proliferation, migration and invasion of cervical cancer cells. Additionally, overexpression of BCAR4 promoted epithelial-mesenchymal transition (EMT) process and silencing BCAR4 suppressed EMT process in cervical cancer cells.

CONCLUSIONS: The results indicated that BCAR4 might play a crucial role in cervical cancer progression and act as an underlying biomarker for the diagnosis and prognosis of cervical cancer.

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proliferation of gastric cancer cells through targeting miR-219-1 [7]. Some IncRNAs have showed potential diagnostic value and acted as prognostic factor. For example, SPRY4-IT served as a novel prognostic factor in thyroid cancer [8]. Moreover, PANDAR was widely overexpressed in a variety of tumors, including renal cell carcinoma, colorectal cancer, cholangiocarcinoma and other cancers and served as a biomarker in the diagnosis and treatment of several tumors [9]. Furthermore, a systematic review and meta-analysis showed that some IncRNAs such as HOTAIR had prognostic and diagnostic significance in cervical cancer [10]. Although an increasing number of research concentrated on IncRNAs, only a few IncRNAs have been identified with sufficient functional research.

LncRNA breast cancer anti-estrogen resistance 4 (BCAR4) was originally identified in breast cancer cells resistant to anti-estrogens. Moreover, BCAR4 was up-regulated in several tumors, including colon cancer [11], osteosarcoma [12], non-small cell lung cancer [13] and breast cancer [14]. Insufficient mechanism researches on BCAR4 were conducted in malignant tumors. For example, BCAR4 enhanced proliferation of colon cancer via the activation of Wnt/β-catenin signaling [11]. Moreover, BCAR4 promoted the proliferation and migration of chondrosarcoma cell via activating mTOR signaling pathway [15]. Furthermore, BCAR4 promoted non-small cell lung cancer cells proliferation, invasion and metastasis via affecting epithelial-mesenchymal transition [16]. However, the role of BCAR4 in cervical cancer has not been reported.

In this paper, we aimed to explore the role of BCAR4 in cervical cancer. Specifically, the biological function and clinical significance of BCAR4 were analyzed. The prognostic significance of BCAR4 was assessed in cervical cancer. Additionally, our findings provided a novel sight into the biological function of BCAR4 in cervical cancer.

2. Materials and methods

2.1. Ethics statement

All patients gave written informed consents prior to samples collection and the study was approved by the Ethics Committee of the first affiliated hospital of wenzhou medical university. The study was performed according to the Declaration of Helsinki.

2.2. Patients and tissue samples

128 cervical cancer tissues and adjacent non-cancerous normal tissues were collected from the first affiliated hospital of wenzhou medical university between February 2010 and January 2013. No patients had access to chemotherapy or radiotherapy before the surgery. All specimens were immediately preserved in liquid nitrogen until use.

2.3. Cell lines and culture conditions

Cervical cancer cell lines (HeLa, SiHa, Caski, C-4-1 and C-33A) were commercially obtained (ATCC, Manassas, VA, USA). All cell lines were maintained in RPMI-1640 medium (Gibco, Gaithersburg, MD, USA) with 10% fetal bovine serum (Gibco, Gaithersburg, MD, USA) and 1% penicillin/streptomycin (Invitrogen, Carlsbad, CA, USA) at 37 °C in a humidified atmosphere with 5% CO2.

2.4. Plasmid construction and cell transfection

The pcDNA3.1-BCAR4 plasmid containing BCAR4 was designed and synthesized by Invitrogen (USA). The pcDNA3-BCAR4 was transfected into HeLa and SiHa cells using Lipofectamine 3000 (Invitrogen, Carlsbad, CA, USA) to obtain the cells with over-expression of BCAR4. The shBCAR4 (5’-AGACTTGGATTTAGTG-GTGGCT-3’) was commercially purchased and cloned into pGPH1/Neo (Thermo Fisher Scientific, USA) as described in previous literature [17]. The pGPH1-shBCAR4 or negative control plasmid was transfected into HeLa and SiHa cells using Lipofectamine 3000 (Invitrogen, Carlsbad, CA, USA) according to the manufacturer’s instructions.

2.5. Quantitative real-time polymerase chain reaction (qRT-PCR) assay

Total RNA were extracted form tissue and cell lines by Trizol reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer’s instructions. Then a Reverse Transcription Kit (Takara, Dalian, China) was used to reversely transcribe total RNA into cDNA according to the manufacturer’s instructions. Real-time PCR was conducted by CFX96 Real-Time PCR Detection System (Bio-Rad, Shanghai, China) using SYBRGreen PCR Master Mix (Takara, Dalian, China) according to the manufacturer’s instructions. The sequences of the PCR primers (Invitrogen, Shanghai, China) were commercially designed as follows: BCAR4, forward 5′-ACAGCACCTTGTTGCTCATCT-3′ and reverse 5′-TTGCTTGGGCACTGTCCAC-3′; and GAPDH, forward 5′-CGAGTCACGGATTTGGTCGTATGGC-3′ and reverse 5′-GCTCTGAAGCATGGTATGGGATT-3′. The 2−ΔΔCt method was used to calculate the relative expression level and GAPDH expression served as an internal control.

2.6. Cell proliferation assay

Cell proliferation assay was carried out by Cell Counting kit-8 (CCK-8, Dojindo, Mashikimachi, Japan) according to the manufacturer’s instructions. Briefly, the transfected cells were incubated in 96-well plates and the absorbance values were detected at intervals of 24 h at 450 nm by SpectraMax M3 (Molecular Devices, LLC, Sunnyvale, CA, USA). The assays were performed in triplicate.

2.7. Colony formation assay

After transfection, HeLa and SiHa cells were trypsinized and cultivated in 12-well plates. After incubation in RPMI 1640 medium for 14 days, the cells were washed with PBS, fixed with methanol and stained with crystal violet, respectively. The number of colonies was counted as described in previous literature [18]. The assays were performed in triplicate.

2.8. Wound healing assay

Wound healing assay was used to evaluate the motility of cervical cancer cells. Briefly, the transfected cells were cultivated in 6-well plates by the time the cells reached 90% confluence. Subsequently, a pipette tip was used to create wounds. After the cells were washed with PBS, wound healing was measured by microscope every 12 h. The assays were performed in triplicate.

2.9. Transwell assay

Transwell assay was used to evaluate the migration and invasion of cervical cancer cells in Matrigel-coated (BD Biosciences, USA) transwell inserts (Corning, USA). The transfected cells were placed into the upper chamber filled with Dulbecco’s modified Eagle’s medium (DMEM) and the lower chambers were filled with DMEM with 20% FBS. After cultivating for 48 h, cells were fixed with methanol, stained with crystal violet and washed with PBS, respectively. Counts were performed in five random fields. The assays were performed in triplicate.

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2.10. Western blotting assay

After washing with PBS, the cells were lysed in RIPA buffer (Beyotime, China) complemented with protease inhibitors (Roche, Switzerland). Additionally, the BCA Protein Assay Kit (Beyotime Institute of Biotechnology, China) was used to measure the protein concentrations. Subsequently, 10% sodium dodecyl sulfate polyacrylamide gel electrophoresis was used to separate the proteins before the proteins were transferred onto polyvinylidene fluoride membranes (Millipore, USA). After incubated with primary antibodies for 24 h at 4 °C, the membranes were washed and incubated with secondary antibodies conjugated with horseradish peroxidase for 1 h. Furthermore, the immunoreactive bands were visualized by ECL chromogenic substrate (Amersham Biosciences, Sweden). Primary antibodies (Sigma, USA) were as follows: E-cadherin, vimentin, ZO-1, N-cadherin and β-actin. The β-actin antibody was used as an internal control. The assays were performed in triplicate.

2.11. Statistical analysis

SPSS 20.0 (SPSS Inc, USA) and Prism 7.00 (GraphPad Software, Inc., USA) were used to analyze the data and the data showed as the mean ± S.D. Moreover, differences between two groups were analyzed by student’s t-test and the multi-sample analysis was performed by one-way ANOVA. Additionally, Kaplan-Meier method was used for survival analysis. P values less than 0.05 were considered statistically significant.

3. Results

3.1. BCAR4 expression was up-regulated in cervical cancer

As shown in Fig. 1A, the expression of BCAR4 was measured in 64 cervical cancer tissues compared with 64 adjacent non-cancerous normal tissues by qRT-PCR and normalized to GAPDH. Specifically, BCAR4 expression was significantly increased in cervical cancer tissues compared with adjacent non-cancerous normal tissues (P < 0.001), indicating that the expression of BCAR4 might be linked to the pathogenesis of cervical cancer. As shown in Table 1, the relationship between BCAR4 expression and clinicopathological factors such as age, menopause, depth of invasion, CEA level, tumor size, lymph node metastasis and FIGO stage was assessed in cervical cancer. BCAR4 expression was assigned to two groups according to the median value of the relative expression of BCAR4 in cervical tissues.

Table 1

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>No. of patients (%)</th>
<th>Low expression</th>
<th>High expression</th>
<th>P</th>
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<tbody>
<tr>
<td>Age (years)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;50</td>
<td>56 (43.8%)</td>
<td>25</td>
<td>31</td>
<td>0.285</td>
</tr>
<tr>
<td>≥50</td>
<td>72 (56.3%)</td>
<td>39</td>
<td>33</td>
<td></td>
</tr>
<tr>
<td>Menopause</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>52 (40.6%)</td>
<td>29</td>
<td>23</td>
<td>0.2802</td>
</tr>
<tr>
<td>Yes</td>
<td>76 (59.4%)</td>
<td>35</td>
<td>41</td>
<td></td>
</tr>
<tr>
<td>Tumor size</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;4 cm</td>
<td>70 (54.7%)</td>
<td>42</td>
<td>28</td>
<td>0.0022**</td>
</tr>
<tr>
<td>≥4 cm</td>
<td>58 (45.3%)</td>
<td>22</td>
<td>36</td>
<td></td>
</tr>
<tr>
<td>Depth of invasion</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;2/3</td>
<td>75 (58.6%)</td>
<td>40</td>
<td>35</td>
<td>0.3696</td>
</tr>
<tr>
<td>≥2/3</td>
<td>53 (41.4%)</td>
<td>24</td>
<td>29</td>
<td></td>
</tr>
<tr>
<td>LNM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>78 (60.9%)</td>
<td>45</td>
<td>33</td>
<td>0.0100*</td>
</tr>
<tr>
<td>Positive</td>
<td>50 (39.1%)</td>
<td>19</td>
<td>31</td>
<td></td>
</tr>
<tr>
<td>FIGO stage</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I-II</td>
<td>62 (48.4%)</td>
<td>38</td>
<td>24</td>
<td>0.0007**</td>
</tr>
<tr>
<td>III-IV</td>
<td>66 (51.6%)</td>
<td>26</td>
<td>40</td>
<td></td>
</tr>
<tr>
<td>CEA</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>73 (57%)</td>
<td>30</td>
<td>43</td>
<td>0.1834</td>
</tr>
<tr>
<td>Positive</td>
<td>55 (43%)</td>
<td>34</td>
<td>21</td>
<td></td>
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</tbody>
</table>

LNM: Lymph node metastasis; *P < 0.05; **P < 0.01.
cancer tissues. A significant association between BCAR4 expression and clinicopathological factors such as tumor size (P = 0.0022), lymph node metastasis (P = 0.0100) and FIGO stage (P = 0.0007) was found in cervical cancer tissues. However, no significant association between BCAR4 expression and clinicopathological factors such as age (P = 0.2850), menopause (P = 0.2800), depth of invasion (P = 0.3696) and CEA level (P = 0.1834) was found. Accordingly, the expression of BCAR4 was further explored in cervical cancer cells (HeLa, SiHa, Caski, C4-1 and C-33A) and immortalized cervical epithelial (ICE) cells by qRT-PCR in Fig. 1B and the result showed that a higher expression of BCAR4 was showed in HeLa cells and a lower expression of BCAR4 was showed in SiHa cells compared with immortalized cervical epithelial cells. Therefore, HeLa and SiHa cells were applied for the follow-up assays.

3.2. BCAR4 expression indicated a poor prognosis in cervical cancer

To assess the correlation between BCAR4 expression and the prognosis of cervical cancer, Kaplan-Meier analysis and log-rank test were used. Both overall survival (P = 0.009) (Fig. 2A) and progression-free survival (P = 0.010) (Fig. 2B) were significantly decreased in the patients with a higher BCAR4 expression. As shown in Table 2, the univariate Cox regression analysis of overall survival in cervical cancer revealed that tumor size (P = 0.003), lymph node metastasis (P = 0.003), FIGO stage (P = 0.001), depth of invasion (P = 0.028) and BCAR4 expression (P = 0.010) could be regarded as prognostic factors, consistent with the univariate Cox regression analysis of progression-free survival in cervical cancer (Table 3). Moreover, the multivariate Cox regression analysis of overall survival showed that BCAR4 expression (P = 0.020) served as the significant predictor of poor survival in cervical cancer, consistent with the multivariate Cox regression analysis of progression-free survival in cervical cancer (Table 3). Altogether, the results indicated that BCAR4 expression might play an important part in the occurrence and development of cervical cancer.

Table 2

<table>
<thead>
<tr>
<th>Risk factors</th>
<th>Univariate analysis</th>
<th>Multivariate analysis</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>HR</td>
<td>95%CI</td>
</tr>
<tr>
<td>Age (&lt;50 vs. ≥50)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Menopause (No vs. Yes)</td>
<td>1.440</td>
<td>0.553–3.753</td>
</tr>
<tr>
<td>CEA (Negative vs. Positive)</td>
<td>1.091</td>
<td>0.420–2.837</td>
</tr>
<tr>
<td>Tumor size (&lt;4 cm vs. ≥4 cm)</td>
<td>3.902</td>
<td>1.466–10.390</td>
</tr>
<tr>
<td>Depth of invasion (&lt;2/3 vs. ≥2/3)</td>
<td>2.817</td>
<td>1.065–7.452</td>
</tr>
<tr>
<td>LNM (Negative vs. Positive)</td>
<td>4.618</td>
<td>1.695–12.580</td>
</tr>
<tr>
<td>FIGO stage (I-II vs. III-IV)</td>
<td>4.843</td>
<td>1.836–12.770</td>
</tr>
<tr>
<td>BCAR4 expression (Low vs. High)</td>
<td>3.207</td>
<td>1.230–8.358</td>
</tr>
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</table>

LNM: Lymph node metastasis; *P < 0.05; **P < 0.01.

Table 3

<table>
<thead>
<tr>
<th>Risk factors</th>
<th>Univariate analysis</th>
<th>Multivariate analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HR</td>
<td>95%CI</td>
</tr>
<tr>
<td>Age (&lt;50 vs. ≥50)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Menopause (No vs. Yes)</td>
<td>1.066</td>
<td>0.371–3.058</td>
</tr>
<tr>
<td>CEA (Negative vs. Positive)</td>
<td>0.695</td>
<td>0.242–1.996</td>
</tr>
<tr>
<td>Tumor size (&lt;4 cm vs. ≥4 cm)</td>
<td>3.904</td>
<td>1.334–11.420</td>
</tr>
<tr>
<td>Depth of invasion (&lt;2/3 vs. ≥2/3)</td>
<td>2.799</td>
<td>0.957–8.186</td>
</tr>
<tr>
<td>LNM (Negative vs. Positive)</td>
<td>4.784</td>
<td>1.587–14.420</td>
</tr>
<tr>
<td>FIGO stage (I-II vs. III-IV)</td>
<td>5.097</td>
<td>1.763–14.740</td>
</tr>
<tr>
<td>BCAR4 expression (Low vs. High)</td>
<td>3.507</td>
<td>1.219–10.090</td>
</tr>
</tbody>
</table>

LNM: Lymph node metastasis; *P < 0.05; **P < 0.01.

Fig. 2. The relationship between BCAR4 expression and survival analysis in cervical cancer. A. Overall survival (OS) was analyzed by Kaplan-Meier survival curve according to BCAR4 expression. B. Progression-free survival (PFS) was analyzed by Kaplan-Meier survival curve according to BCAR4 expression. Data showed as the mean ± standard deviation (SD). Bold text indicates statistical significance: *P < 0.05, **P < 0.01.
3.3. Overexpression of BCAR4 enhanced the proliferation and migration of cervical cancer cells

To study the effect of BCAR4 on cervical cancer cells, the pcDNA3.1-BCAR4 plasmid was constructed to up-regulate the expression of BCAR4. As shown in Fig. 3A, the BCAR4 expression was up-regulated in HeLa and SiHa cells transfected with pcDNA3.1-BCAR4 plasmid. Moreover, cell proliferation assay revealed that overexpression of BCAR4 in HeLa and SiHa cells enhanced proliferation of HeLa and SiHa cells (Fig. 3B). Furthermore, overexpression of BCAR4 promoted colony formation in Fig. 3C. Subsequently, wound healing assay (Fig. 3D) revealed that overexpression of BCAR4 promoted the HeLa and SiHa cells motility. Generally, BCAR4 promoted the proliferation and migration of cervical cancer cells.

3.4. Knockdown of BCAR4 inhibited cells proliferation, migration and invasion

To further study the effect of BCAR4 on cervical cancer cells, we knocked down BCAR4 in HeLa and SiHa cells. As shown in Fig. 4A, the silencing efficiency of BCAR4 was confirmed by qRT-PCR in HeLa and SiHa cells. Additionally, silencing BCAR4 inhibited the proliferation of HeLa and SiHa cells in cell proliferation assay (Fig. 4B and C). Moreover, the colony formation assay showed that silencing BCAR4 reduced the colony numbers, compared with control groups in Fig. 4D. Additionally, wound healing assay (Fig. 4E) revealed that silencing BCAR4 suppressed HeLa and SiHa cells motility. Consistently, transwell assay (Fig. 4F and G) showed that silencing BCAR4 restrained HeLa and SiHa cells migration and invasion. Altogether, knockdown of BCAR4 inhibited cervical cancer cells proliferation, migration and invasion.

3.5. BCAR4 promoted epithelial-mesenchymal transition

It was well known that epithelial-mesenchymal transition (EMT) affected the mobility and invasiveness of cervical cancer cells. To further study the relationship between BCAR4 and EMT in cervical cancer, the expression of EMT-related proteins such as E-cadherin, vimentin, ZO-1 and N-cadherin was measured in HeLa and SiHa cells. As shown in Fig. 5A, expression of vimentin and N-cadherin were increased and expression of E-cadherin and ZO-1 were decreased in BCAR4-overexpressing HeLa and SiHa cells, while expression of vimentin and N-cadherin were decreased and expression of E-cadherin and ZO-1 were increased in BCAR4-silenced HeLa and SiHa cells (Fig. 5B). The results indicated that BCAR4 might play an important part in the development of cervical cancer via EMT.

4. Discussion

Cervical cancer has become a major health problem for women worldwide and patients with an advanced cervical cancer had worse survival [19]. Therefore, a novel biomarker for diagnosis and treatment of cervical cancer is imperative.

Recently, accumulated evidences have showed that the abnormal expression of long non-coding RNAs (lncRNAs) played an important role in the tumorigenesis of cervical cancer, including transcriptional regulation, post-transcriptional regulation, epigenetic regulation and so on [10,20]. For example, XLOC_008466...
promoted cells proliferation and inhibited cells apoptosis in cervical cancer cells [21]. Moreover, XLOC_010588 was significantly down-regulated in cervical cancer and inhibited the proliferation of cervical cancer cells via down-regulation of c-Myc [22]. Additionally, functional tests showed that knockdown of HOTAIR in Hela cells inhibited cells proliferation and migration by activating microRNA-326 [23]. Furthermore, LINC00473 as a target of microRNA 34a promoted the proliferation of cervical cancer cells via inhibiting ILF2 degradation [24]. The abnormal expression BCAR4 has been found in colon cancer [11], gastric cancer [25], non-small cell lung cancer [12,26], chondrosarcoma [15], osteosarcoma [12,17] and breast cancer [14]. However, the function of BCAR4 in cervical cancer is still unknown. In this study, we concentrated on the role of BCAR4 in cervical cancer. Specifically, the expression of BCAR4 was significantly increased in cervical cancer tissues and cells. Moreover, the expression of BCAR4 had a close relationship with clinicopathological factors such as tumor size, lymph node metastasis and FIGO stage. Furthermore, a higher BCAR4 expression showed a worse OS and PFS, indicating that BCAR4 could serve as an independent prognostic factor in cervical cancer. Additionally, BCAR4 expression enhanced the proliferation and motility of cervical cancer cells. Consistently, knockdown of BCAR4 inhibited cervical cancer cells proliferation, invasion and migration.

Epithelial–mesenchymal transition (EMT) occurred in the process of cancer progression and metastasis. It has been reported that inducing EMT is the primary mechanism by which epithelial cancer cells obtain the phenotypes of malignant tumor and the capability of metastasis [27]. Emerging research suggested that lncRNAs played a role in the tumourgenesis via EMT. For example, CRNDE promoted the proliferation, invasion and migration of osteosarcoma cells by regulating Notch1 signaling and epithelial-mesenchymal transition [28]. Moreover, knockdown of MALAT1 inhibited the invasion and metastasis of cervical cancer cells via inhibiting EMT [26]. Additionally, overexpression of PVT1 increased H3K27me3 levels in the promoter region of miR-195 and improved the sensitivity of cervical cancer cells to PTX via modulating EMT [29]. In this study, we investigated for the first time that the relationship between BCAR4 and EMT in cervical cancer and the results showed that overexpression of BCAR4 promoted EMT and silencing BCAR4 inhibited EMT, indicating that BCAR4 promoted the proliferation and migration via regulating EMT in cervical cancer. More in-depth mechanism research on the effect of BCAR4 on the proliferation and migration of cervical cancer cells is still needed in the further study.

In this study, the results showed that BCAR4 expression was upregulated in cervical cancer tissues and cells. Moreover, BCAR4 expression might serve as an independent prognostic factor in cervical cancer. Additionally, BCAR4 induced cells proliferation, promoted cells migration via regulating epithelial–mesenchymal transition (EMT). This study supplied some new views on the function of BCAR4 in the tumorigenesis of cervical cancer, indicating that BCAR4 might be a potential prognostic and diagnostic biomarker in cervical cancer.
Fig. 5. BCAR4 promoted epithelial-mesenchymal transition (EMT) in HeLa and SiHa cells. A. The expression of EMT-related proteins such as E-cadherin, vimentin, ZO-1 and N-cadherin was measured in HeLa and SiHa cells transfected with pcDNA-BCAR4 or pcDNA. B. The expression of EMT-related proteins such as E-cadherin, vimentin, ZO-1 and N-cadherin was measured in HeLa and SiHa cells transfected with shBCAR4 or shCtrl. The β-actin antibody was used as an internal control.

Conflicts of interest

The authors declare no conflict of interest.

Ethics statement

All patients gave written informed consents prior to samples collection and the study was approved by the Ethics Committee of the first affiliated hospital of Wenzhou Medical University. The study was performed according to the Declaration of Helsinki.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.suronc.2018.05.013.

References


