**Introduction**

CC has the fourth highest morbidity and mortality in cancers (1). This year has seen an increase of about 570,000 diagnoses and 310,000 deaths of CC cases in the world (2). Although CC treatment has been developed for the patients, the prognosis of CC patients is still unoptimistic (3). Hence, mechanism in CC is imperative to be understood so that the therapeutic method for CC could be improved.

Long non-coding RNA (lncRNA) is a newly proposed non-coding RNA (ncRNA) whose length is over 200 nucleotides, and is engaged in regulating cancer progression
For example, IncRNA HOXD-AS1 enhances the proliferative and invasive capacity of melanoma cells through inhibiting RUNX3 expression (6). Moreover, IncRNA SUMO1P3 facilitates tumor growth, distant metastasis and angiogenesis in colon cancer (7). ZEB1-AS1 shows oncogenic behaviors in gastric cancer and predicts an unfavorable prognosis (8). Numerous recent reports have demonstrated that IncRNA containing miRNA response element (MRE) elicit competitive endogenous RNA (ceRNA) function through sequestering target miRNAs at the MREs (9-11). Interestingly, various IncRNAs functioning as ceRNA are largely documented in CC. For instance, LINC00473 promotes CC tumorigenesis by targeting miR-34a and preventing ILF2 degradation (12). SNHG16, a sponge of miR-216-5p, contributes to the migration and invasion of CC cells through regulating ZEB1 (13). In addition, CASC2/miR-21/PTEN axis modulates cisplatin sensitivity of CC (14). It was discovered that RBMB antisense RNA 1 (RGMB-AS1) showed oncogenic property in cancers such glioma (15), laryngeal squamous carcinoma (16), and lung adenocarcinoma (17). However, no report has linked RGMB-AS1 to CC yet.

**The aim of this research was to inquire the biological role and possible mechanism of RGMB-AS1 in CC.** We identified marked upregulation of RGMB-AS1 in CC tissues and cells and demonstrated that RBMB-AS1 elicited promoting function in proliferation, apoptosis and invasion of CC cells via regulating microRNA-4428 (miR-4428)/PBX homeobox 1 (PBX1) axis.

**Methods**

**Human tissue samples**

All patients (n=32) were diagnosed with CC in the First Affiliated Hospital of Hebei North University Prior to this study, and these patients did not receive any other treatment. Fresh CC tissues and corresponding para-tumor tissues were gained from abovementioned participants hospitalized from May 2013 to December 2018. Fresh tissues were frozen in liquid nitrogen immediately after resection and stored at -80 °C. Written informed consent was signed by every patient and this experiment gained permission from ethics committee of the First Affiliated Hospital of Hebei North University (approval number: 1809036).

**Cell culture**

Human cervical epithelial cell (H8) and CC cells (HeLa, C-33A, SiHa and CaSki) were bought from Chinese Academy of Sciences (Beijing, China). A RPMI-1640 medium (Invitrogen, Carlsbad, USA) containing 10% FBS (Invitrogen) plus 1% penicillin/streptomycin (Sigma-Aldrich, Milan, Italy) was applied to culture above cells. A moist environment of 5% CO₂ at 37 °C was required for culturing. Besides, medium was replaced every 3 days.

**Cell transfection**

Specific shRNAs against RGMB-AS1 (sh-RGMB-AS1#1 and sh-RGMB-AS1#2) with corresponding negative control (sh-NC), and the pcDNA3.1 vector targeting RGMB-AS1 or PBX1 and with negative control vector, were gained from Genechem (Shanghai, China). Moreover, miR-4428 mimics and NC mimics were gained from GenePharma (Shanghai, China). Above plasmids were separately transfected into SiHa or CaSki cells utilizing Lipofectamine 3000 (Invitrogen, Carlsbad, USA).

**Quantitative real-time PCR (qRT-PCR)**

Isolation of cellular RNA was finished via TRIZOL (Invitrogen, Carlsbad, USA) and cDNA was synthesized by iScript™ Reverse Transcription Supermix (Bio-Rad, Hercules, CA, USA). Samples were sequentially analyzed with SYBR Green Master Mix (Vazyme, Nanjing, China) on ABI 7500 Realtime PCR system (Applied Biosystems, Foster City, CA, USA). Fold changes of target genes were calculated as per $2^{-\Delta\Delta Ct}$ method and GAPDH or U6 served as the endogenous calibrator control.

**Cell counting kit-8 (CCK-8) assay**

Cell proliferation was explored with CCK-8 (Dojindo, Kumamoto, Japan). Transfected SiHa or CaSki cells were inoculated into 96-well plates, and cultured overnight. Upon this, before detection, each well was supplemented with 10 µL CCK-8 solutions. Values at 450 nm were examined through the Epoch Microplate Spectrophotometer (Bio Tek, Winooski, VT, USA) upon culture for 0, 24, 48, 72 and 96 h, respectively.
Western blot

Total protein was obtained from transfected SiHa or CaSki cells which were lysed by radio immunoprecipitation assay (RIPA) lysis buffer (Beyotime, Jiangsu, China). Subsequently, protein was quantified by using the BCA assay kit (Beyotime). The dodecyl sulfate, sodium salt (SDS)-polyacrylamide gel electrophoresis (PAGE) was performed on proteins from lysates which were then moved to polyvinylidene fluoride membranes (PVDF; Millipore, Bedford, MA, USA). Membranes were cultured with primary antibodies overnight at 4℃, and antibodies used were as follows: anti-Bcl-2 (ab32124, Abcam, Cambridge, USA), anti-Bax (ab32503, Abcam), anti-MMP2 (ab215986, Abcam), anti-MMP9 (ab219372, Abcam), anti-PBX1 (ab97994, Abcam) and anti-GAPDH (ab97994, Abcam). Then, secondary antibodies were added for cultivation for 1 h in dark room. Finally, the proteins were detected by an Enhanced Chemiluminescence Detection Kit (Millipore, Bedford, MA, USA).

Terminal deoxynucleotidyl transferase (TdT)-mediated dUTP nick end labeling assay

The apoptosis of transfected SiHa or CaSki cells was assessed via a TUNEL Apoptosis Kit (Invitrogen, Carlsbad, USA). Cells were dyed with DAPI (Koritai, Beijing, China). Then, cells were observed and images were captured by using a fluorescence microscopy (Olympus, Tokyo, Japan).

5-ethynyl-2’-deoxyuridine (EdU) assay

Transfected CaSki or SiHa cells were seeded into fresh 96-well plates for cultivation. After incubation with EdU (Sigma-Aldrich, St. Louis, MO, USA) for 2 h, the cells were fixed by using 4% PFA (Solarbio, Beijing, China) and dyed in Apollo Dye Solution (RiboBio, Guangdong, China). Nucleic acid was stained with Hoechst 33342 stain (Invitrogen, Carlsbad, USA). Images were captured under an inverted fluorescence microscope (Carl Zeiss, Jena, Germany) and the proportion of EdU-positive cells was determined.

Cell invasion assay

Cell invasion was studied utilizing the 24-well Transwell system (Corning Costar, Cambridge, MA, USA). Transfected SiHa or CaSki cells were trypsinized and suspended in serum-free medium and subsequently added to the upper chamber of the Transwell system with Matrigel (BD Biosciences, San Jose, CA, USA). A 0.6 mL medium with additional 10% FBS was plated to the lower chamber. Cells were cultured for 24 h for invasion test. Invasive cells were fixed and stained utilizing crystal violet (Richard-Allan Scientific, San Diego, CA, USA), followed by washed twice using PBS (Sigma-Aldrich). Stained cells were eventually observed by an inverted microscope (Olympus Co., Tokyo, Japan).

Subcellular fractionation

For separating nuclear and cytoplasmic fraction, RNAs from SiHa or CaSki cells were isolated with the PARIS kit (Thermo Fisher Scientific, Waltham, MA, USA). The RNA expression level of RGMB-AS1 in nuclear and cytoplasmic fractions was explored using qRT-PCR.

Luciferase reporter assay

RGMB-AS1-WT/Mut or PBX1-WT/Mut was sub-cloned into the pmirGLO dual-luciferase vector (Promega, Madison, WI, USA) so as to generate pmirGLO-RGMB-AS1-WT/Mut or pmirGLO-PBX1-WT/Mut. The pmirGLO-RGMB-AS1-WT/Mut was co-transfected into SiHa or CaSki cells with miR-4428 mimics or NC mimics. The pmirGLO-PBX1-WT/Mut was co-transfected into SiHa or CaSki cells with miR-4428 mimics or miR-4428 mimics + pcDNA3.1/RGMB-AS1 or NC mimics. At 48 h post-transfection, dual luciferase reporter assay system (Promega, Madison, WI, USA) was applied to examine luciferase activities.

RNA pull-down

SiHa or CaSki cells were lysed using lysis buffer and incubated with Bio-miR-4428 WT/Mut or Bio-NC, followed by incubation with streptavidin-coupled magnetic beads (Invitrogen). RGMB-AS1 and PBX1 levels were individually studied by qRT-PCR.

RNA immunoprecipitation (RIP)

RIP analysis was conducted in SiHa or CaSki cells using the Magna RIP RNA-Binding Protein Immunoprecipitation Kit (Millipore, Bedford, MA, USA). Cells were lysed in RIP buffer. The resulting cell extraction was incubated with ProteinA/G magnetic beads bounded to anti-Ago2 (Abcam,
Cambridge, UK) or anti-IgG (Abcam). Enrichment levels of RGMB-AS1, miR-4428 and PBX1 were individually measured with qRT-PCR.

**Statistical analysis**

Data were expressed as means ± SD. Statistical analysis was conducted using the SPSS (IBM, Armonk, NY, USA). Significance of the variance between two or several groups was evaluated by Student's t-test or one-way ANOVA. Correlation among RGMB-AS1, miR-4428 and PBX1 was assessed by Pearson's correlation analysis. P<0.05 was regarded as statistically significant in general. The experiments were conducted for at least thrice.

**Results**

**RGMB-AS1 is overexpressed in HCC and facilitates CC cell proliferation, invasion and restrained cell apoptosis**

To explore the expression pattern of RGMB-AS1 in CC tissues and cell lines (HeLa, C-33A, SiHa, CaSki), qRT-PCR analysis was conducted. The adjacent non-tumor tissues and cervical epithelial cell (H8) were utilized as negative controls, separately. As a result, RGMB-AS1 expression was dramatically elevated in CC tissues (Figure 1A). Consistently, the high RGMB-AS1 level was found in CC cell lines (Figure 1B). To estimate the role of RGMB-AS1 in CC cells, RGMB-AS1 was knocked down by sh-RGMB-AS1#1 and sh-RGMB-AS1#2 (Figure 1C). Then, CCK-8 data and EdU-labeling results indicated that SiHa and CaSki cell proliferation was remarkably reduced by RGMB-AS1 knockdown of (Figure 1D,E). Later, the impact of RGMB-AS1 depletion on cell apoptosis was evaluated. Shown by the result of TUNEL assay, RGMB-AS1 deficiency significantly encouraged apoptosis of SiHa and CaSki cells (Figure 1F). The reduced Bcl-2 and increased Bax protein levels upon RGMB-AS1 silence were observed with Western blot assay, further verifying that cell apoptosis was promoted by RGMB-AS1 knockdown (Figure 1G). Moreover, transwell assay depicted decreased invasion ability in SiHa and CaSki cells (Figure 1H). Lastly, the effect of RGMB-AS1 on the proteins that related to metastasis were assessed. The results showed that silenced RGMB-AS1 considerably restrained the levels of MMP2 and MMP9 proteins (Figure 1I).

Additionally, RGBM-ASI was overexpressed by pcDNA3.1/ RGMB-AS1 in HeLa and C-33A cells which expressed relatively low RGMB-AS1 level (Figure S1A). Then, we discovered that overexpressing RGMB-ASI drive proliferation and depressed apoptosis in 2 cell lines (Figure S1B,C,D). Accordingly, Bcl-2 level upregulated and Bax level de-regulated under RGMB-AS2 overexpression (Figure S1E). Invasion of HeLa and C-33A cells was weakened upon RGMB-ASI overexpression (Figure S1F), and MMP2 and MMP9 levels declined with RGMB-ASI overexpression as well (Figure S1G). Conclusively, RGMB-ASI is overexpressed in HCC and facilitates CC cell proliferation, invasion and restrained cell apoptosis.

**RGMB-AS1 was associated with miR-4428 by acting as a sponge**

Mounting reports have suggested that lncRNAs and miRNAs were closely linked to regulate biological processes (18,19). To confirm whether RGMB-ASI played regulatory role in CC via functioning as a ceRNA, its cellular localization was detected. As a result, RGMB-ASI was more in cytoplasm than in nucleus (Figure 2A). Hence, we hypothesized that RGMB-ASI might serve as a ceRNA in cytoplasm. Thereafter, we applied StarBase tool (http://starbase.sysu.edu.cn/agoClipRNA.php?source=LncRNA&flag=target&clade=mammal&genome=human&assembly=hg19&miRNA=all&clipNum=1&deNum=0&panNum=0&target=RGMB-AS1) to search potential miRNAs for RGMB-AS1. The results manifested five miRNAs (miR-574-3p, miR-3614-5p, miR-670-3p, miR-4428 and miR-22-3p) that could interact with RGMB-ASI (Figure 2B). As illustrated in Figure 2C, only miR-4428 expression was notably increased in CC cells transfected with sh-RGMB-AS1#1. Therefore, miR-4428 was selected for the following investigation. To confirm the relationship between miR-4428 and CC, miR-4428 expression was measured with qRT-PCR analysis. The result displayed that miR-4428 expression was obviously under-expressed in CC tissues and cells versus the corresponding normal tissues and cervical epithelium (Figure 2D,E). Then, we verified the elevated miR-4428 expression after transfecting miR-4428 mimics in 2 CC cell lines (Figure 2F). Based on StarBase prediction, a miR-4428 binding site in RGMB-ASI was recognized, and we mutated this site for further detection (Figure 2G). Consequently, the luciferase activity for RGMB-AS1-WT declined evidently under miR-4428 overexpression, but that of RGMB-AS1-Mut was unchanged (Figure 2H). RNA pull-down assay uncovered that RGMB-ASI was pulled down by miR-4428-WT rather than miR-4428-Mut biotinylated probe (Figure 2I). At last, the negative relevance between RGMB-ASI and miR-4428...
Figure 1. Expression and function of RGMB-AS1 in CC. (A) RGMB-AS1 expression in CC tissues and corresponding normal tissues was detected by qRT-PCR; (B) qRT-PCR data of RGMB-AS1 expression in four CC cell lines, HeLa, C-33A, SiHa, CaSki, and cervical epithelium, H8 (control); (C) RGMB-AS1 expression was tested by qRT-PCR in CC cells with sh-RGMB-AS1#1/2/3 transfection; (D,E) CCK-8 test of viable SiHa and CaSki cells and EdU staining of proliferative SiHa and CaSki cells with RGMB-AS1 knockdown. Scale bar: 50 μm; (F) TUNEL-labeled CC cells was imaged and calculated under RGMB-AS1 silence. Scale bar: 100 μm; (G) Western blot of Bax and Bcl-2 in CC cells with RGMB-AS1 knockdown; (H) the invaded SiHa and CaSki cells in a transwell system was imaged and counted under RGMB-AS1 knockdown. Scale bar: 100 μm; (I) Western blots of MMP2 and MMP9 proteins in CC cells with RGMB-AS1 deficiency. **, P<0.01. RGMB-AS1, RGMB antisense RNA 1; CC, cervical cancer; qRT-PCR, quantitative real-time PCR; CCK-8, cell counting kit-8.
**Figure 2** **RGMB-AS1** sponged miR-4428. (A) The distribution of **RGMB-AS1** in SiHa and CaSki cells was conformed through subcellular localization; (B) StarBase predicted the potential miRNAs (miR-574-3p, miR-3614-5p, miR-670-3p, miR-4428 and miR-22-3p) interacting with **RGMB-AS1**; (C) qRT-PCR data of the levels of potential miRNAs (miR-574-3p, miR-3614-5p, miR-670-3p, miR-4428 and miR-22-3p) in CC cells with **RGMB-AS1** silence; (D) qRT-PCR was utilized to detect miR-4428 expression in CC tissues and adjacent normal tissues; (E) the expression of miR-4428 in CC cell lines (HeLa, C-33A, SiHa, CaSki) was determined by qRT-PCR. The control is cervical epithelium (H8); (F) the transfection efficiency of miR-4428 mimics was examined using qRT-PCR; (G) prediction of miR-4428 sites in **RGMB-AS1** was obtained from StarBase; (H) luciferase activity pmirGLO-**RGMB-AS1**-WT or pmirGLO-**RGMB-AS1**-Mut reporter was measured in two CC cells introduced with and NC or miR-4428 mimics; (I) SiHa and CasKi cells lysates were mixed with Bio-miR-4428-WT or Bio-miR-4428-Mut for RNA pull-down and **RGMB-AS1** level in the pulldown products were detected; (J) the correlation between the expression of **RGMB-AS1** and miR-4428 was analyzed with the employment of Pearson’s correlation analysis. **, P<0.01. **RGMB-AS1**, RGMB antisense RNA 1; miR-4428, microRNA-4428; qRT-PCR, quantitative real-time PCR; CC, cervical cancer.
RGMB-AS1 upregulated PBX1 by sponging miR-4428

To support the hypothesis of ceRNA mechanism, we subsequently searched the downstream mRNAs for miR-4428 using StarBase with the setting of 3 prediction programs (RNA22, mirmap and microT). The Venn diagram showed that there are 16 potential mRNAs for miR-4428 (Figure 3A). qRT-PCR analysis showed that among 16 candidate genes, PBX1 exhibited the most significant upregulation in 3 CC specimens versus corresponding para-tumor normal ones (Figure S2). PBX1 was commonly reported as oncogene (20-22), so we speculated PBX1 as the target for miR-4428 in CC. qRT-PCR analysis confirmed the upregulated PBX1 in 32 CC tissues and 4 cell lines (Figure 3B,C). Then, effects of RGMB-AS1 and miR-4428 on PBX1 were measured. As presented in Figure 3D,E, the levels of PBX1 mRNA and protein were diminished with the transfection of sh-RGMB-AS1#1/2. Besides, miR-4428 mimics observably attenuated PBX1 mRNA and protein levels (Figure 3F,G). Then, the overexpression of RGMB-AS1 by pcDNA3.1/RGMB-AS1 in CC cells was confirmed by qRT-PCR (Figure 3H). Moreover, as shown in Figure 3I, RIP assay demonstrated that RGMB-AS1, miR-4428 and PBX1 could be detected in Ago2 immunoprecipitates rather than IgG (Figure 3I). In addition, luciferase reporter assay confirmed that the decrease of PBX1-WT luciferase activity caused by miR-4428 mimics was restored by RGMB-AS1 overexpression, but no evident difference was discovered in PBX1-Mut (Figure 3J). RNA pull-down assay further validated that both RGMB-AS1 and PBX1 were pulled down by miR-4428-WT, rather than miR-4428-Mut (Figure 3K). Furthermore, PBX1 expression was positively associate with RGMB-AS1 and negatively related to miR-4428 in CC specimens (Figure 3L). Jointly, RGMB-AS1 regulates PBX1 by sponging miR-4428.

RGMB-AS1 enhances CC progression through regulating PBX1

Finally, we tested whether PBX1 was a target for RGMB-AS1 to regulate CC cellular activities. We first used pcDNA3.1/PBX1 to increase the expression of PBX1 so as to unfold rescue experiments (Figure 4A). Later, we showed that the proliferative ability of CC cells was restrained by the silence of RGMB-AS1 and was recovered by PBX1 overexpression (Figure 4B,C). Besides, TUNEL staining suggested that the promoting effect of RGMB-AS1 silence on CC cell apoptosis was offset by transfecting pcDNA3.1/PBX1 through TUNEL (Figure 4D). Decreased Bcl-2 and increased Bax in CC cells with RGMB-AS1 silence were reversed by PBX1 overexpression (Figure 4E). According to transwell assay, overexpressing PBX1 counterbalanced the repressive impact of RGMB-AS1 depletion on CC cell migration (Figure 4F). Additionally, silenced RGMB-AS1 lessened the levels of MMP2 and MMP9, while the transfection of pcDNA3.1/PBX1 reversed the effect of silenced RGMB-AS1 (Figure 4G). In brief, RGMB-AS1 enhances CC progression through regulating PBX1.

Discussion

It was proposed that lncRNAs could function as regulators in the biological processes, including cancers. Recent studies have revealed that lncRNAs might act as sponges for miRNAs in cancer progression (23). Several lncRNA-miRNA axes are revealed in cancers, such as MALAT1-miR-218 axis in choriocarcinoma (24), NEAT1-miR-181a-5p in lung cancer (25), and PVT1-miR-128-3p axis in thyroid carcinoma cell proliferation and invasion (27). RGMB-AS1 is also associated with clinical stage in hepatocellular carcinoma (28). RGMB-AS1 indicates an unfavorable prognosis in lung adenocarcinoma and regulates its progression (17). Hence, we assumed that RGMB-AS1 might participate in CC. Expectedly, our study first suggested that RGMB-AS1 was significant overexpressed in CC tissues and cells. RGMB-AS1 knockdown lessened proliferation and invasion, and increased apoptosis, whereas its overexpression had opposite effects. These data consistently implied the oncogenic property of RGMB-AS1 in CC.

MiRNAs are a kind of short RNAs with 21–25 nucleotides (29). MiRNA serves as tumor promoters or tumor suppressors in tumorigenesis and development of cancers (30,31). For instance, miRNA-518 represses cell growth and induces apoptosis in gastric cancer via targeting MDM2. MiR-183 is overexpressed in glioblastoma and downregulates LRIG1 expression to promote glioblastoma radioresistance (32). MiRNA-423 promotes hepatocellular carcinoma cell invasion via regulating BRMS1 (33). A variety of miRNAs was reported to relate to CC progression. For example, miRNA-433 represses CC tumor size and distant metastasis...
Figure 3 MiR-4428 targets to PBX1. (A) The Venn diagram showed the potential mRNAs binding with miR-4428 from StarBase; (B) PBX1 expression in CC tissues and matched normal tissues was detected by qRT-PCR; (C) qRT-PCR and Western blot data of PBX1 expression in four CC cell lines and one normal cell line; (D,E) PBX1 mRNA and protein levels were examined by qRT-PCR and Western blot in CC cells transfected with sh-RGMB-AS1#1/2; (F,G) qRT-PCR and Western blot data of PBX1 mRNA and protein under miR-4428 mimics transfection in CC cells; (H) qRT-PCR of RGMB-AS1 level in CC cells transfected with pcDNA3.1/RGMB-AS1 versus pcDNA3.1; (I) enrichments of RGMB-AS1, miR-4428 and PBX1 bound in Ago2 RIP was estimated by qRT-PCR. IgG was negative reference; (J) luciferase activity of PBX1-WT and PBX1-Mut was measured in CC cells transfected with miR-4428 mimics and miR-4428 mimics + pcDNA3.1/RGMB-AS1; (K) RGMB-AS1 and PBX1 enrichment was evaluated by qRT-PCR in the pulldown of bio-miR-4428-WT/Mut; (L) expression correlation of PBX1 with RGMB-AS1 and miR-4428 was tested by Pearson's correlation analysis in CC tissues. **, P<0.01. miR-4428, microRNA-4428; PBX1, PBX homeobox 1; CC, cervical cancer; qRT-PCR, quantitative real-time PCR; RGMB-AS1, RGMB antisense RNA 1; RIP, RNA immunoprecipitation.
Figure 4  

RGMB-AS1 enhanced CC progression through regulating PBX1. (A) The transfection efficiency of pcDNA3.1/PBX1 was tested by qRT-PCR; (B,C) cell proliferation was tested by CCK-8 and EdU in CC cells transfected with sh-NC, sh-RGMB-AS1#1 or sh-RGMB-AS1#1 + pcDNA3.1/PBX1. Scale bar: 50 μm; (D) images of TUNEL-labeled apoptotic CC cells in each group. Scale bar: 100 μm; (E) Western blot of Bax and Bcl-2 in CC cells of each group; (F) cell invasion in cells transfected with sh-NC, sh-RGMB-AS1#1 or sh-RGMB-AS1#1 + pcDNA3.1/PBX1 was determined by transwell assay. Scale bar: 100 μm; (G) MMP2 and MMP9 protein levels in CC cells of each group was estimated by Western blot assay. **, P<0.01. RGMB-AS1, RGMB antisense RNA 1; CC, cervical cancer; PBX1, PBX homeobox 1; qRT-PCR, quantitative real-time PCR; CCK-8, cell counting kit-8.

by regulating the AKT/β-catenin signaling pathway and targeting to metadherin (34). MicroRNA-150 targets to PDCD4 in CC and boosts cell proliferation, migration and invasion (35). MiR-214 enhances the drug sensitivity of CC via regulating FOXM1 (36). MiR-4428 is first identified as a downregulated miRNA in CC tissues and cells. Further, we first predicted and demonstrated that RGMB-AS1 directly combine with miR-4428 via putative MRE. Moreover, we
first exhibited that RGMB-AS1 expression was negatively correlated with miR-4428 expression in CC. Thus, we provided first data to link RGMB-AS1 to miR-4428 in CC.

Further, we found that PBX1 was a target for miR-4428 and upregulated overtly in CC. As formerly reported, PBX1 promotes the proliferation of clear cell renal carcinoma by JAK2/STAT3 signaling (20). PBX1 is upregulated in breast cancer and plays essential role in the regulation of breast cancer development (21). PBX1 serves as a stem cell reprogramming factor and modulates the chemoresistance of ovarian cancer (22). However, the role and mechanism of PBX1 in CC is first explained by our study. We showed that both RGMB-AS1 knockdown and miR-4428 overexpression could decline the expression of PBX1. Importantly, we found that RGMB-AS1 regulates PBX1 expression by sponging miR-4428. Moreover, we delineated the positive relevance between RGMB-AS1 and PBX1 and the negative relevance between miR-4428 and PBX1. According to rescue experiments, PBX1 overexpression restored RGMB-AS1 depletion-mediated repressing effect on CC progression, indicating that PBX1 was a target for RGMB-AS1 to regulate CC.

Conclusively, RGMB-AS1 contributes to CC progression through acting as a ceRNA, and RGMB-AS1/miR-4428/PBX1 axis could offer an inspiring thought for the therapy in CC patients.

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Footnote

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at http://dx.doi.org/10.21037/tcr.2020.04.19). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. Written informed consent was signed by every patient and this experiment gained permission from ethics committee of the First Affiliated Hospital of Hebei North University (approval number: 1809036). The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013).

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Supplementary

Figure S1 Effect of RGMB-AS1 overexpression on CC cells. (A) RGMB-AS1 expression in CC cells transfected with pcDNA3.1/RGMB-AS1 was evaluated by qRT-PCR; (B,C) proliferation of CC cells with RGMB-AS1 overexpression was estimated by CCK-8 and EdU. Scale bar: 50 μm; (D) TUNEL-labeled apoptotic CC cells with RGMB-AS1 overexpression was imaged and evaluated. Scale bar: 100 μm; (E) Western blot tested Bcl-2 and Bax level in CC cells under RGMB-AS1 overexpression; (F) invasion of CC cells under RGMB-AS1 overexpression was tested by transwell assay. Scale bar: 100 μm; (G) Western blot of MMP2 and MMP9 in CC cells under RGMB-AS1 overexpression. **, P<0.01. RGMB-AS1, RGMB antisense RNA 1; CC, cervical cancer; qRT-PCR, quantitative real-time PCR; CCK-8, cell counting kit-8.
Figure S2 Expressions of candidate genes in CC specimens. Heat-map showed the qRT-PCR levels of 16 candidate miR-4428 target genes in 3 CC tissues versus para-tumor ones. CC, cervical cancer; qRT-PCR, quantitative real-time PCR; miR-4428, microRNA-4428.