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LncRNA AB073614 promotes the proliferation and inhibits apoptosis of cervical cancer cells by repressing RBM5

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Abstract. – OBJECTIVE: Recent studies have determined the crucial role of long noncoding RNAs (lncRNAs) in cancer development. Cervical cancer (CC) is a common type of fatal gynecological cancer worldwide. This study aims to identify the role of lncRNA AB073614 in the progression of CC.

PATIENTS AND METHODS: Relative level of AB073614 in 3 CC cell lines and 48 paired CC samples was determined by the quantitative Real Time-Polymerase Chain Reaction (qRT-PCR). The potential regulatory effects of AB073614 on the cellular behaviors of CC cells were explored through apoptosis assay, colony formation assay, and proliferation assay *in vitro*. The underlying mechanism of AB073614 in mediating the progression of CC was also conducted.

RESULTS: The AB073614 expression was remarkably higher in CC tissues than that in [illegible]cent tissues. The knockdown of AB07361[illegible] [illegible]ited proliferation but induced apoptosis [illegible] CC cells. In addition, RBM5 (RNA binding mo[illegible]5) was upregulated in CC cells after [illegible]kdow[illegible] AB073614 *in vitro*. Furthermore[illegible] [illegible]tive c[illegible] relation was seen between t[illegible] expr[illegible]sions [illegible] RBM5 and AB073614 in CC ti[illegible]

CONCLUSIONS: AB07[illegible]14 [illegible] pro[illegible]ote proliferation and inhi[illegible] [illegible]optos[illegible] CC cells through downregula[illegible]M5, whic[illegible] [illegible]ay offer a new therapeutic in[illegible]erve[illegible] for CC patients.

Key Words:
Long non[illegible]g [illegible], AB073614, cervical cancer, RBM5.

[illegible]troduction

Cervi[illegible]ncer (CC) is the fourth most general gynecologi[illegible] malignancy in the world and is the most common cancer in Chinese women. Each year, there are about 530,000 n[illegible]set approximately 275,000 death cas[illegible]or CC. Moreover, the morbidity of CC acc[illegible]s for 85[illegible] developing countries, lea[illegible]ng t[illegible]re th[illegible] 250,000 deaths annually[2,3]. [illegible]onventi[illegible] [illegible]ments for CC mainly incl[illegible] sur[illegible]y, ch[illegible]therapy, and radiotherapy. De[illegible] advances in the molecular tumori[illegible]esis [illegible]ew [illegible]rapeutic strategies have be[illegible]de for th[illegible] decades, the prognosis of [illegible]eta[illegible] CC remains dismal. Therefore, it [illegible]gent to [illegible]ify the underlying molecular [illegible]chanism and [illegible]e out a new treatment strat[illegible]y for CC.

[illegible]s one [illegible]type of non-coding RNAs, long [illegible]NAs (lncRNAs) are widely studied in a [illegible]iety of biological behaviors and have [illegible]oved to be vital in the progression of ma[illegible]ant tumors. For example, lncRNA p23154 accelerates cell metastasis in oral squamous cell carcinoma by participating in the Glut1-mediated glycolysis[4]. LncRNA LUCAT1 inhibits the expressions of tumor suppressors and leads to the formation and invasion of esophageal squamous cell carcinoma by regulating the stability of DNMT1[5]. LncRNA AFAP1-AS1 down-regulation depresses cell proliferation and induces cell apoptosis in lung adenocarcinoma, which could offer a new therapeutic strategy for lung adenocarcinoma[6]. LncRNA PVT1 promotes glucose metabolism, cell motility, cell proliferation, and tumor progression in osteosarcoma by regulating the microRNA-497/human glandular kallikrein 2 (miR-497/HK2) axis[7]. Also, lncRNA LINC00052 depresses migration and invasion of hepatocellular carcinoma cells through upregu-

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lating EPB41L3, which is modulated by miR-452-5p[8]. However, the clinical role of AB073614 and its underlying biological mechanisms in CC remain unknown.

In our study, AB073614 expression was significantly upregulated in CC samples. AB073614 regulated apoptosis and proliferation of CC cells through mediating *RBM5* (RNA binding motif 5).

Patients and methods

Patients and Cell Lines

The CC tissues were obtained from 48 CC patients who underwent surgery at Beijing Chaoyang Hospital. All tissues were stored at -80°C. The written informed consent was signed by CC patients before the surgery. This study was approved by the requirement of the Ethics Committee of Beijing Chaoyang Hospital. SiHa, HeLa and C4-1 human CC cell lines, and the normal cervical epithelium cell line (NC104) (Chinese Type Culture Collection, Chinese Academy of Sciences, Shanghai, China) were cultured in Dulbecco's modified Eagle's [illegible]um (DMEM, Hyclone, South Logan, UT[illegible]A) containing 1% penicillin and 10% fetal b[illegible] serum (FBS; Gibco, Rockville, MD, USA). C[illegible] were maintained in a humidified incubator wi[illegible] 5% CO_2 at 37°C.

Cell Transfection

Lentivirus expressing s[illegible] hairp[illegible] (shRNA) targeting AB0736[illegible]ounded and cloned to pGPH1/N[illegible] ve[illegible]ePharma, Shanghai, China). C[illegible]ransfecti[illegible]er-formed using Lipofec[illegible]e 2000 (Inv[illegible]gen, Carlsbad, CA, USA)[illegible]ion efficacy was determined at 48 h[illegible]qRT[illegible]

RNA Extracti[illegible] and qRT-PCR

TRIzol rea[illegible]t (In[illegible]trogen, Carlsbad, CA, USA) was [illegible]zed t[illegible]olate total RNA from tissues and[illegible]T[illegible], the t[illegible] RNA was reversely trans[illegible] com[illegible]entary deoxyribose nucleic acid[illegible] using the reverse Trans[illegible] Kit (Ta[illegible] Biotechnology Co., Ltd[illegible]hina). Following were the primers us[illegible]or R[illegible]073614 primers forward 5[illegible]TC[illegible]GGTCTTAC-3′, reverse 5′-A[illegible]TGTCTGTTAGAGTC-3′; Glyceraldehyd[illegible]phate dehydrogenase (GAPDH) primers for[illegible]5′-CCAAAATCAGATGGGGCAATGCTGG-3′ and reverse 5′-TGATGGCAT-GGACTGTGGTCATTCA-3′. [illegible] cycle was as follows: 30 sec at 95[illegible] sec a[illegible] and 35 sec at 60°C, for 40 cyc[illegible]

Colony Formation Ass[illegible]

HeLa cells were p[illegible]ced in [illegible] with 1×10^3 cells per well[illegible]er cell cultu[illegible]0 days, colonies were fix[illegible]with [illegible]% formal[illegible]hyde for 30 min and stai[illegible] with [illegible]% cry[illegible]tal violet for 5 min. Colonie[illegible]ed by [illegible]kon camera (Nikon, Tok[illegible] Jap[illegible]

Cell Pro[illegible]ation Assay

The [illegible]ration was monitored every 24 h by [illegible]cou[illegible] 8 (CCK-8) assay (Dojindo Mol[illegible]ar Techn[illegible]c., Kumamoto, Japan). T[illegible]sorbance at 4[illegible]m was monitored by a [illegible]trophotometer (Thermo Scientific, Rockford, [illegible] USA).

[illegible] Cytom[illegible] Analysis

[illegible]s was detected by Annexin-V[illegible]uorescein isothiocyanate) apoptosis detection kit (BD, Franklin Lakes, NJ, USA). [illegible] harvested cells were washed twice using [illegible]BS. Then, 100 µL of flow cytometry [illegible]uffer was added. After 5 µL of Annexin [illegible]FICC and 5 µL of Propidium Iodide (PI) were mixed at the room temperature, these cells were [illegible]tained in the mixture in the dark for 15 min. [illegible]ach tube was added with 400 microliters bind[illegible]ing buffer. The apoptotic cells were analyzed by FACSCalibur flow cytometer (BD Biosciences, Franklin Lakes, NJ, USA).

Statistical Analysis

Statistical analysis was performed by the Statistical Product and Service Solutions (SPSS) 18.0 (SPSS, Chicago, IL, USA). The significance was analyzed by two-tailed Student's *t*-test. $p < 0.05$ was considered as statistically significant.

Results

Expression Level of AB073614 in CC

The relative level of AB073614 in 3 CC cell lines and 48 paired CC samples was determined by quantitative Real Time-Polymerase Chain Reaction (qRT-PCR). As a result, AB073614 was significantly upregulated in CC tissue samples than that in adjacent tissues (Figure 1A). The AB073614 expression level of CC cells was higher than that of NC104 cells (Figure 1B).

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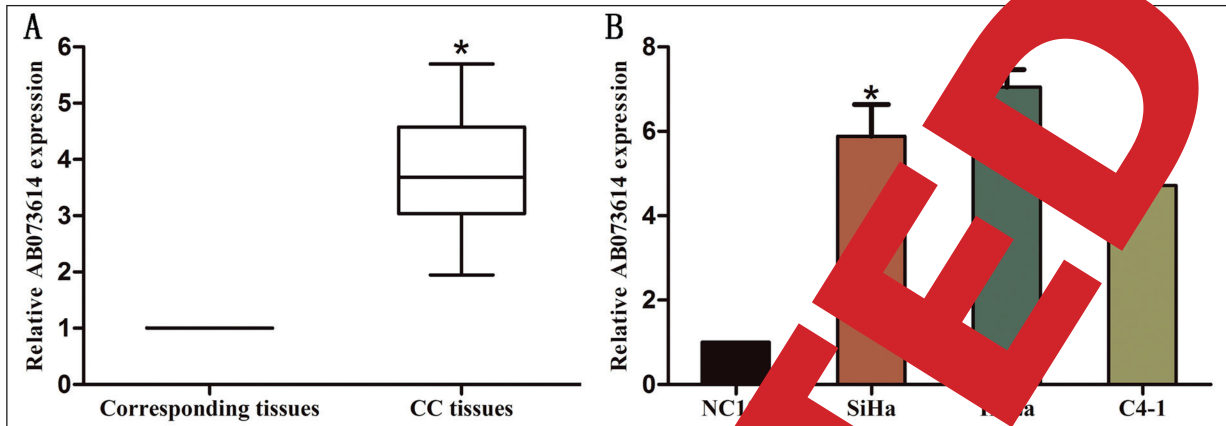

Figure 1. AB073614 expression in CC tissues and cell lines. ***A,*** The [illegible] was remarkably higher in CC tissues compared with that in corresponding tissues. ***B,*** The AB073614 expression was [illegible] SiHa, HeLa and C4-1 (human CC cell lines) than that in NC104 (normal cervical epithelium cell li[illegible] < 0.05.

Knockdown of AB073614 Inhibited the Proliferation of CC Cells

We chose HeLa cells for AB073614 knockdown. The transfection efficiency was detected by qRT-PCR (Figure 2A). Besides, CCK-8 assay revealed that the proliferation of CC cells [illegible] markably suppressed after the AB073614 [illegible]down (Figure 2B). In addition, the resu[illegible] colony formation assay revealed that AB07[illegible] the knockdown in CC cells decreased the numb[illegible] of colonies (Figure 2C).

Knockdown of AB073614 [illegible] Apoptosis of CC Cells

The flow cytometric analy[illegible]rmed to detect the apoptotic rate [illegible] CC c[illegible] results showed that the apoptot[illegible]te of He[illegible]as remarkably elevated a[illegible]B073614 kno[illegible]own (Figure 3).

AB073614 Promoted CC Tu[illegible]enesis via RBM5

RBM5 was [illegible]ewly [illegible]iscovered gene in CC. We then con[illegible]ed m[illegible]anism assays to identify the correla[illegible] AB0[illegible]14 and RBM5. The mRNA [illegible] RBM[illegible] was upregulated in CC cells trans[illegible] AB073614 shRNA (Figu[illegible] The RB[illegible]pression was signifi-cant[illegible] CC tissues compared with adja-ce[illegible]ssues [illegible] Identically, RBM5 was [illegible] cells than that of NC104 cel[illegible]4C). The linear correlation analysis showed [illegible]BM5 expression negatively cor-related to the [illegible]73614 expression in CC tissues (Figure 4D).

[illegible]scussion

[illegible]vidence showed that lncRNAs fun[illegible]mportant factors in the regulation of various biological behaviors. For example, the [illegible]xpression of lncRNA NNT-AS1 facilitates [illegible]eration and invasion of cervical cancer [illegible] Wnt/beta-catenin signaling pathway[9]. The [illegible]pregulated lncRNA CCHE1 is associated with a poor prognosis of CC, which could serve as a [illegible]otential prognostic marker[10]. LncRNA CCAT2 [illegible]romotes cell proliferation and cell survival in CC[11]. LncRNA HOTAIR promotes the proliferation and invasion of CC cells through targeting the Notch pathway, which may be a potential therapeutic target[12]. In addition, low-expression of lncRNA ZNF667-AS1 represses the progression of CC, and its expression level is also related to the prognosis of CC[13].

LncRNA AB073614, as a novel lncRNA to promote tumorigenesis in ovarian cancer, is closely related to a poor prognosis for patients with ovarian cancer[14]. Through regulating epithelial-mesenchymal transition, the silence of AB073614 suppresses cell proliferation and migration in glioma, indicating a poor prognosis of glioma[15]. AB073614 plays an important role in regulating cell proliferation and migration in colorectal cancer by modulating PI3K/AKT signaling pathway[16]. In this work, we found that AB073614 was upregulated in CC samples and cells. Besides, the AB073614 knockdown inhibited proliferation and induced apoptosis of CC cells. The above data suggested that AB073614 promoted the tumorigenesis of CC and might act as an oncogene.

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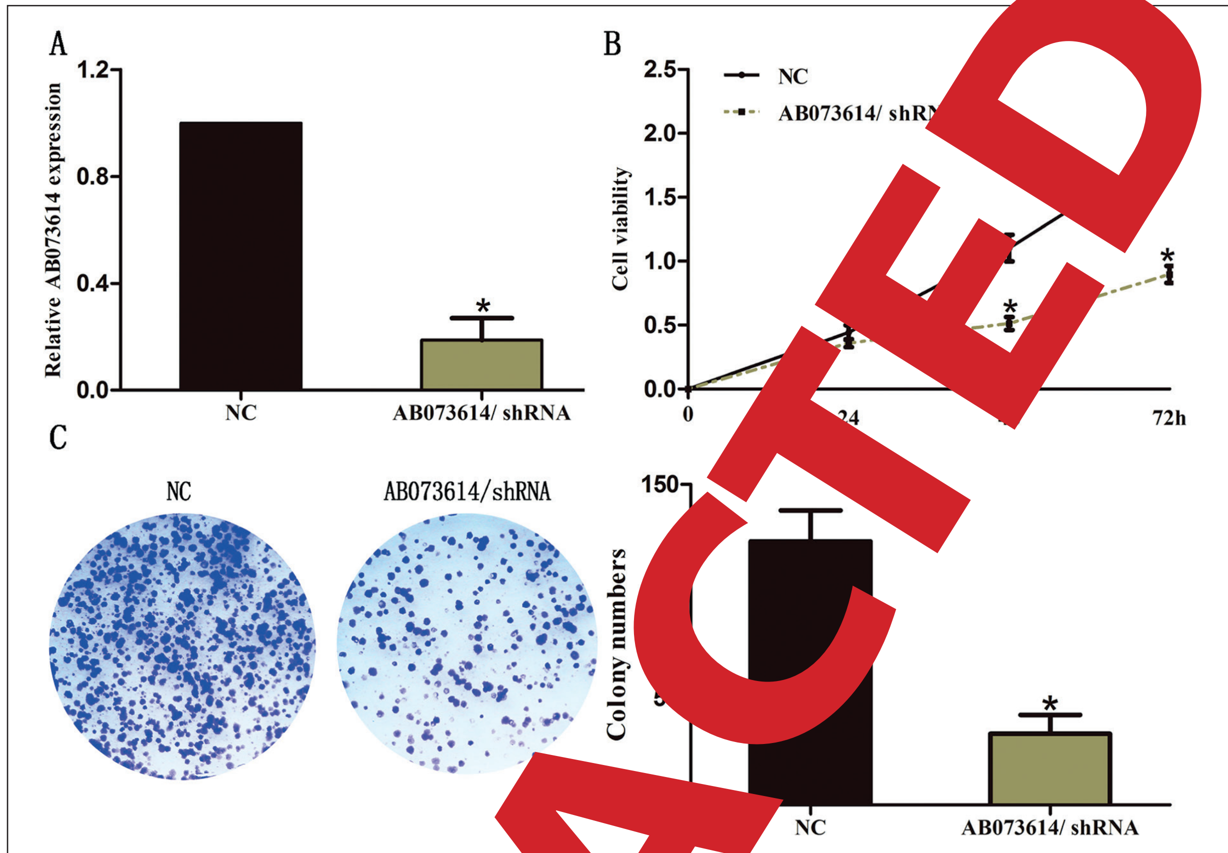

Figure 2. Silence of AB073614 inhibited proliferati[illegible] cells. ***A,*** QRT-PCR detected the AB073614 expression in CC cells transfected with AB073614 shRNA (AB073[illegible]RNA) or negative control (NC). GAPDH was used as an internal control. ***B,*** The CCK8 assay sh[illegible]t silence [illegible]73614 significantly inhibited proliferation of CC cells. ***C,*** The colony formation assay showe[illegible]ber of colo[illegible] was significantly reduced *via* silence of AB073614 in CC cells. The results represent the a[illegible]ge o[illegible] independent experiments (mean ± standard error of the mean). $*p < 0.05$.

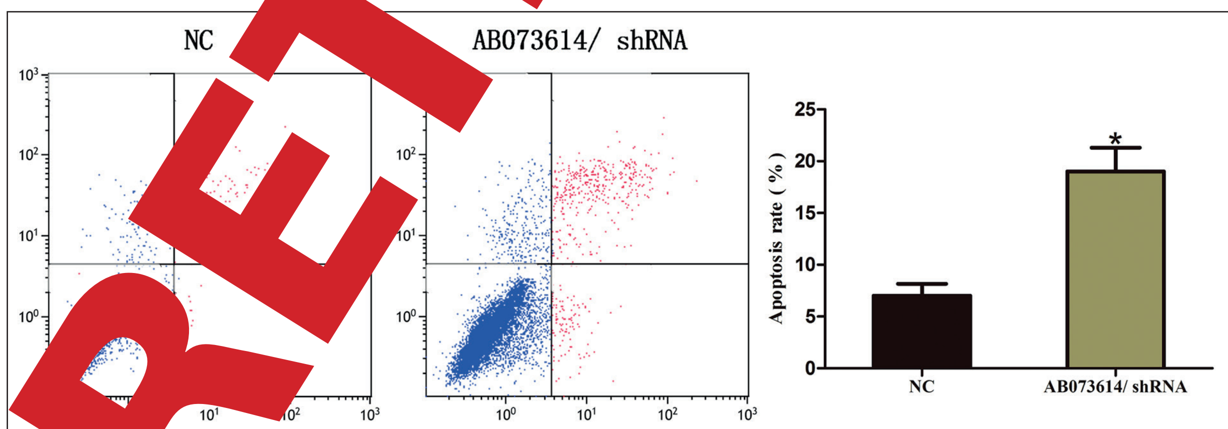

Figure 3. [illegible]AB073614 promoted apoptosis of CC cells. The flow cytometric analysis assay showed that apoptotic rate in CC cel[illegible]ficantly increased *via* silence of AB073614. The results represent the average of three independent experiments (mean ± standard error of the mean). $*p < 0.05$.

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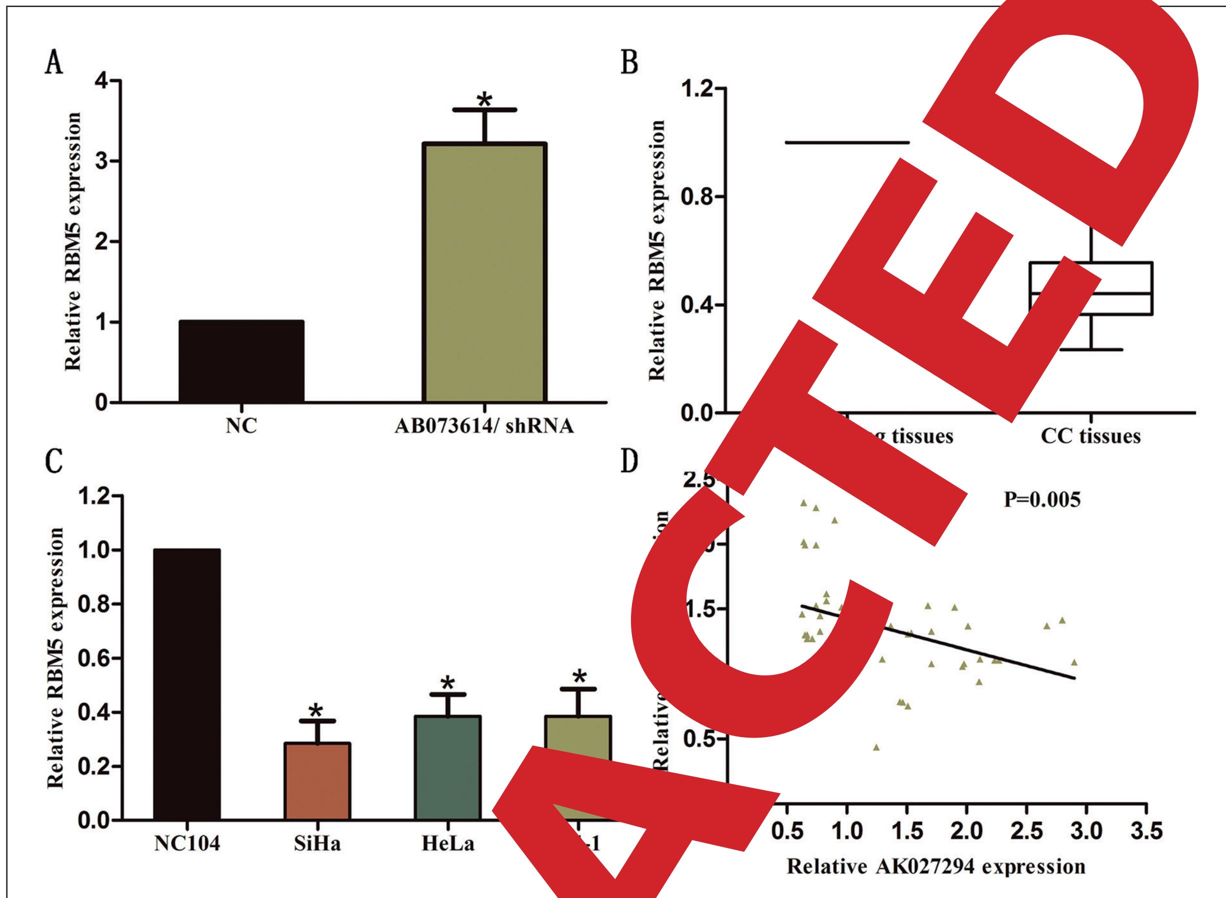

Figure 4. RBM5 expression was prom[illegible] silence of [illegible]614 in CC. ***A,*** The RNA expression level of RBM5 in AB073614/ shRNA group significantly [illegible] compared w[illegible] NC group. ***B,*** RBM5 was significantly downregulated in CC tissues compared with correspon[illegible] tissu[illegible] The RBM[illegible] expression was lower in SiHa, HeLa and C4-1(human CC cell lines) than that in NC104 (norm[illegible] vical e[illegible]ne). ***D,*** The linear correlation between the expression levels of RBM5 and AB073614 in CC tissu[illegible] 05.

RBM5 (RNA bind[illegible] motif 5) is [illegible]ted on the cancer inhi[illegible] 3p21.3, which is significantly d[illegible]regu[illegible] tumor progressions of many cancers [illegible]xample, RBM5 depress[illegible] umorigenesis o[illegible]liomas by inhibiting the[illegible]nt/be[illegible] catenin signaling and inducing ce[illegible] popt[illegible]. RBM5 is lowly expressed i[illegible] ducta[illegible] enocarcinoma and related t[illegible] clini[illegible] thological characteristic of these [illegible] RBM5 functions as a t[illegible] ppresso[illegible] in the progression of [illegible] cer by enhancing the transcripti[illegible] acti[illegible] Also, overexpression [illegible] 5 [illegible] growth and invasion of pro[illegible] cer cells by downregulating miR-483-5p [illegible] udy indicated that RBM5 was upregulated [illegible] AB073614 knockdown *in vitro*. Moreover, a negative correlation between RBM5 and AB073614 expression was discovered in CC tissues. The results above showed that RBM5 was a target of AB073614 in CC development.

Conclusions

We observed that AB073614 is a new biomarker in the carcinogenesis of CC and could be served as a promising mark for clinical treatment of CC.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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