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Original Article

miR-215 controls proliferation, invasion, and apoptosis of human retinoblastoma cells by regulating RB1 expression

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Abstract: Purpose: The aim was to determine the effect of miR-215 on retinoblastoma cells proliferation, invasion, and apoptosis. Methods: ACBRI-181, HXO-Rb44 and SO-Rb50 cells were cultured. HXO-Rb44 cells were transfected. qRT-PCR, Western blot, MTT assay, transwell assay, flow cytometry analysis, and luciferase reporter assay were conducted. *In vivo* studies with nude mice were performed. Results: miR-215 in retinoblastoma cells was significantly up-regulated ($P < 0.01$). Compared with the Blank group and NC group, significantly higher OD_{495} value and invasive cell number and significantly lower apoptotic cells percentage was found in the miR-215 mimic group ($P < 0.05$), while the exact opposite result was found in the miR-215 inhibitor group. RB1 relative expression was significantly decreased ($P < 0.05$) in miR-215 mimic group and was significantly increased in miR-215 inhibitor group ($P < 0.05$). RB1 was the target gene of miR-215. siRNA-RB1 + mimic group was with much higher OD_{495} value and invasive cell number and much lower apoptotic cells percentage when compared with the siRNA-RB1 group ($P < 0.05$). Nude mice in the siRNA-RB1 + mimic group had a much higher tumor volume compared with that in the siRNA-RB1 group ($P < 0.05$). Conclusions: miR-215 affected HXO-Rb44 cells proliferation, invasion, and apoptosis through regulation of RB1 protein expression.

Keywords: miR-215, RB1, retinoblastoma, proliferation, invasion, apoptosis

Introduction

Retinoblastoma was an intraocular cancer that occurred frequently in infancy and childhood. Approximately 9,000 new cases of retinoblastoma were diagnosed every year in the world [1]. Retinoblastoma seriously affected patients' visual acuity and in most cases surgery for enucleation was necessary [2, 3]. More seriously, once a high-risk retinoblastoma was metastasized, the disease was very difficult to control and eventually led to an increased risk of death [4]. Therefore, early diagnosis was very important to improve the prognosis of patients with retinoblastoma.

With the further development of molecular biology techniques, the discovery of potential tumor biomarkers had realized the prevention and early diagnosis of tumors, which was of great significance to improve the prognosis of tumor patients. miR-215, a small molecule RNA, was associated with many tumors and influences their development and progression. Several studies have shown that, in some types of tumors, such as colon cancer and esophageal adenocarcinoma, miR-215 expression was significantly reduced, associated with increased tumor sizes and decreased disease-free survival rate after radical surgery [5-8]. However, in some other types of tumors, the expression of miR-215 was reported to be up-regulated. Deng et al. [9] concluded that up-regulation of miR-215 was found in gastric cancer tissues and they speculated that miR-215 might be a biomarker for the diagnosis of gastric cancer based on the results that inhibition of miR-215 suppressed the gastric cancer cell proliferation. Furthermore, they further studied the mechanism of miR-215 in gastric cancer cells and

Table 1. Primer sequences of miR-215 and internal reference

Name of primer	Sequences
miR-215-F	GGGTCCGAGGTATTCGCACT
miR-215-R	CGATGACCTATGAATTGACAGACG
U6-F	CTCGCTTCGGCAGCACA
U6-R	AACGCTTCACGAATTTGCGT

found that miR-215 might affect cell proliferation in gastric cancer by targeting RB1. This view was recognized by Wei et al. [10] and they also found that up-regulation of miR-215 promoted the migration and invasion of glioma cells by inhibiting the expression of the RB1. Similar research also revealed a significantly reduced proliferation rate of hepatoma cells in nude mice after miR-215 expression was inhibited [11]. Many other studies also confirmed the close relationship between miR-215 and tumors, and miR-215 was also considered as a potential biomarker for the early diagnosis and treatment of these tumors.

Unfortunately, as for the relationship between miR-215 and retinoblastoma, the related research was very rare. So in this study, we speculated that miR-215 might play a role in retinoblastoma based on previous studies, and related mechanism was further researched. This study would provide important clinical guidance for prevention and early diagnosis of retinoblastoma at molecular level.

Materials and methods

Cell culture, transfection and grouping

Human normal retinal vascular endothelial cell line (ACBRI-181) and retinoblastoma cell lines (HXO-Rb44 and SO-Rb50) were all purchased from American Type Culture Collection. These cells were cultured in DMEM containing 10% fetal bovine serum (FBS) in an incubator at 37°C, 5% CO_2, respectively. Then they were collected at logarithmic growth phase. After suspended with DMEM (10% FBS), these cells were seeded in 6-well plates at a density of $1 * 10^5$/mL with 1 mL cell suspension each well.

For HXO-Rb44 cells, they were transfected by miR-215 mimic and miR-215 inhibitor, and were named as the miR-215 mimic group and miR-215 inhibitor group, respectively. As NC group, the miR-215 negative mimic was also used to transfect HXO-Rb44 cells. miR-215 mimic, miR-215 inhibitor and miR-215 negative mimic were bought from Genepharma company (Shanghai, China). Furthermore, siRNA/RB1 transfection was performed on HXO-Rb44 cells and these cells were named as siRNA-RB1 group. We also co-transfected HXO-Rb44 cells using miR-215 mimic and siRNA/RB1, which was set at the siRNA-RB1 + mimic group. The siRNA/RB1 sequence was synthesized by Shanghai Bioengineering Company (China). In addition, HXO-Rb44 cells without any treatment were set as the Blank group. Transfection was performed using Lipofectamine 2000 (purchased from the American Company Invitrogen) according to the instructions. Cells that were successfully transfected were used for subsequent studies. All these cells were kept in the incubator under the same conditions after seeded in 6-well plates at a density of $1 * 10^5$/mL. A total of 1 mL cell suspension was added into each well, respectively.

Real-time fluorescence quantitative RT-PCR

Total RNA of cells was extracted by using miRNeasy Mini Kit (obtained from Germany Qiagen company). Reverse transcriptase kit (bought from Germany Qiagen company) was used to perform reverse transcription reaction. PCR amplification reactions were conducted in PCR instrument with a 20 μL reaction system, including 1 μL template cDNA, 1 μL forward primer and 1 μL reverse primer. The reaction conditions were as follows: degeneration at 95°C for 10 seconds, reannealing at 58°C for 20 seconds, extension at 72°C for 34 seconds. The reaction was cycled 40 times. U6 was used as an internal reference. The forward and reverse primer sequences of miR-215 and U6 were shown in **Table 1**. Data were analyzed using the 2-ΔΔCt method.

MTT assay

Cells were incubated for 24, 48 and 72 hours and then 20 μL of MTT solution (5 mg/mL, Sigma) was added into each well for additional 4 hours of incubation at 37°C. Total 150 μL dimethyl sulfoxide (DMSO) solutions was added into each well after the upper liquid was discarded, and the plate was shaken for 10 minutes to promote formazan crystals dissolution. Finally, the absorbance value of each well was measured at 495 nm by enzyme-linked immunosorbent assay.

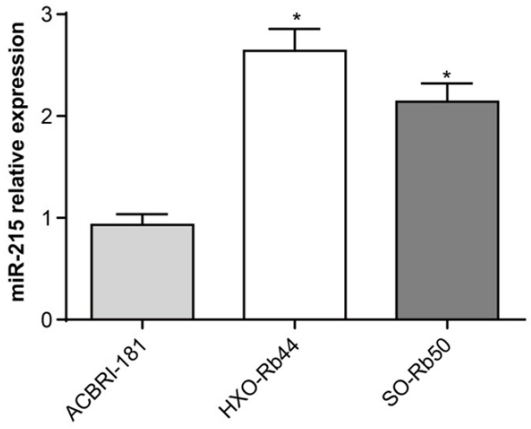


Figure 1. Up-regulation of miR-215 expression in retinoblastoma cells. *P < 0.01 when compared to the expression of miR-215 in ACBRI-181 cells.

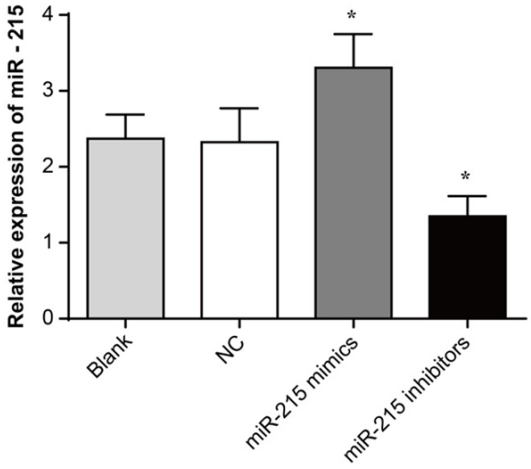


Figure 2. Expression of miR-215 in HXO-Rb44 cells after transfection. *P < 0.05 when compared with the Blank and NC groups.

Transwell experiment

Cells were resuspended in serum-free medium and then were seeded in the upper chamber of a 6-well Matrigel™ Invasion Chamber (BD Biosciences, San Diego, CA, USA) coated with Matrigel. Medium containing 10% FBS was added into the bottom chamber. After incubation for 48 hours, cells that passed through the membrane were fixed with 10% formaldehyde and stained by 0.05% crystal violet. The number of cells that passed through the membrane was counted under microscope. The greater the number of cells, the greater the invasive ability.

Flow cytometry analysis

After 48 hours of incubation, cells were harvested and washed with PBS and fixed by ice-cold 70% ethanol. Then these cells were centrifuged after incubation for 12 hours at 4°C, and were resuspended with PBS. Ethidium bromide (50 μg/mL) and RNaseA (100 μg/mL) was added before incubated in the dark at 4°C for 30 minutes. Cell cycle analysis was conducted by flow cytometry.

Western blot

Cells to be tested were collected after 48 hours of incubation and total protein was extracted and electrophoresed. After electrophoresis, the protein was transferred to the PVDF membrane using wet transfer method. Skimmed milk powder (5%) was used to block the membrane for 2 hours at room temperature. Then the membrane was placed in incubations with primary antibodies (rabbit anti-rat primary antibodies, 1:1000, Invitrogen) overnight at 4°C. HRP-labeled secondary antibody (1:5000) was added to incubate for 1 hour at room temperature after the membrane washed by TBST buffer for three times. After the same TBST cleaning procedure, chemiluminescence and data analysis were performed. U6 was used as an internal reference.

Luciferase reporter assay

Target Scan determined that the RB1 3'UTR was the targeting binding sites of RB1 and miR-215 (**Figure 6A**). The RB1 3'UTR was inserted into psiCHECK-2 vector after amplified by PCR. The mutated- and wild-type sequences of the miR-215 binding sites were designed and inserted into psiCHECK-2 report vectors, respectively. Co-transfection of HXO-Rb44 cells was performed by using miR-215 mimic and mutated-type psiCHECK-2 Report vectors or wild-type psiCHECK-2 Report vectors, and was set as MT + mimic group or WT + mimic group, respectively. In addition, miR-215 negative mimic and mutated-type psiCHECK-2 Report vectors or wild-type psiCHECK-2 report vectors were also used to co-transfect HXO-Rb44 cells, and were named as MT + NC group or WT + NC group, respectively. Cells were collected after 48 hour incubation and assayed by using Dual Luciferase Assay (Promega).

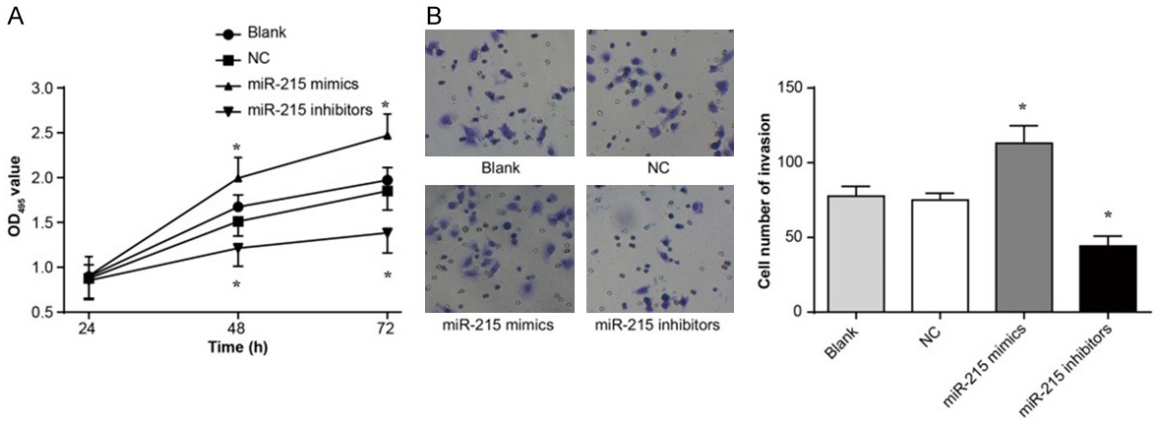


Figure 3. Effect of up-regulation or down-regulation of miR-215 expression on proliferation and invasion of HXO-Rb44 cells. A. The effect of miR-215 expression on the proliferation of HXO-Rb44 cells was detected by MTT assay; B. The effect of miR-215 expression on the invasion of HXO-Rb44 cells by transwell assay. *$P < 0.05$ when compared with the Blank and NC groups.

In vivo transplantation experiment

Fifteen nude mice were housed in a sterile isolator at room temperature. Feed, water and other items in contact with the mice were also autoclaved. After 2 days of feeding, cells of the Blank group, siRNA-RB1 group, and siRNA-RB1 + mimic group were collected in logarithmic growth phase and were made into cell suspension at a density of $1 * 10^7$/mL. A total volume of 200 μL cell suspension was subcutaneously injected into the mice. Tumor volume was measured weekly and was continuously observed for 5 weeks. All operations were in line with the ethical standards of animal experiments.

Statistical analysis

All data were processed using SPSS 18.0 statistical software, and expressed as mean ± SD. Data were analyzed using t-test and $P < 0.05$ was considered statistically significant.

Results

Upregulation of miR-215 in retinoblastoma cells

The results of qRT-PCR showed that the relative expression of miR-215 in HXO-Rb44 cells and SO-Rb50 cells was significantly higher than that in ACBRI-181 cells ($P < 0.01$), which indicated that expression of miR-215 in retinoblastoma cells was significantly up-regulated (**Figure 1**).

Expression of miR-215 in HXO-Rb44 cells after transfection

Compared with the Blank and NC groups, the relative expression of miR-215 in the miR-215 mimic group was significantly increased ($P < 0.05$) and significantly decreased miR-215 relative expression was found in miR-215 inhibitor group ($P < 0.05$). These results demonstrated that miR-215 expression was successfully regulated after transfection (**Figure 2**).

Effect of miR-215 expression on proliferation and invasion of HXO-Rb44 cells

Of the Blank group, NC group, miR-215 mimic group, and miR-215 inhibitor group, there was no significant difference in OD_{495} value among all groups at 24 hours. However, at 48 and 72 hours, compared to the Blank group and the NC group, the OD_{495} value of miR-215 mimic group was significantly increased ($P < 0.05$) and the OD_{495} value of the miR-215 inhibitor group was significantly decreased ($P < 0.05$) (**Figure 3A**). This result suggested that up-regulation of miR-215 promoted proliferation of HXO-Rb44 cells, and inhibition of miR-215 expression could attenuate HXO-Rb44 cell proliferation.

In addition, at 48 hours, the number of cells passing through the membrane of the miR-215 mimic group was significantly higher than that of the Blank and NC groups ($P < 0.05$), and at the same time, significantly lower cell number passing through the membrane of miR-215

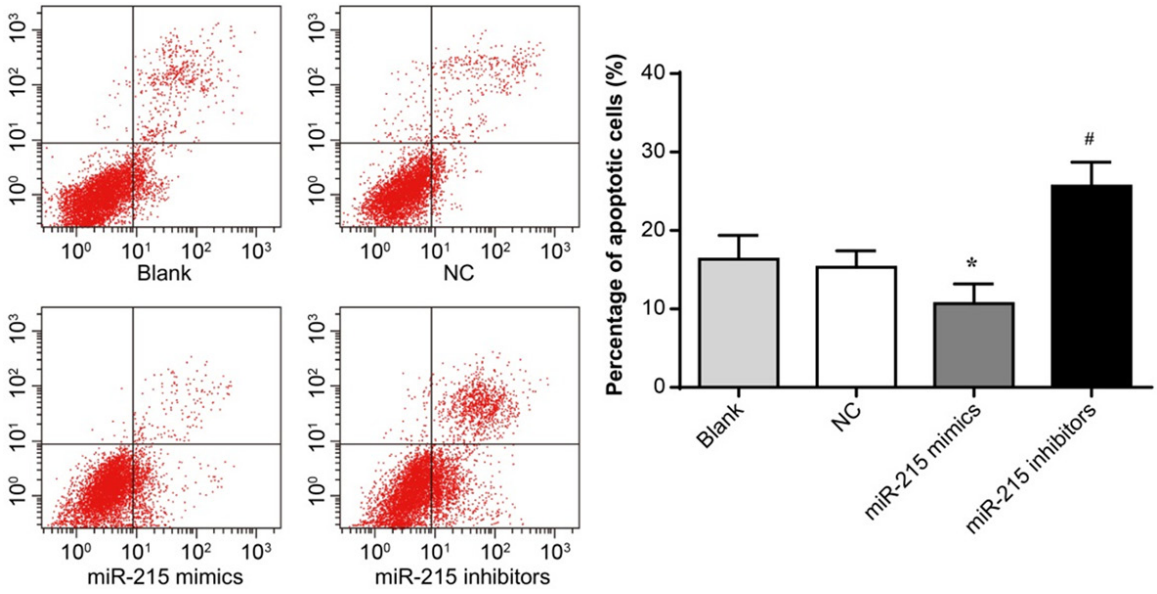


Figure 4. Effect of up-regulation or down-regulation of miR-215 expression on apoptosis of HXO-Rb44 cells. *P < 0.05 when compared with the Blank and NC groups; #P < 0.01 when compared with the Blank and NC groups.

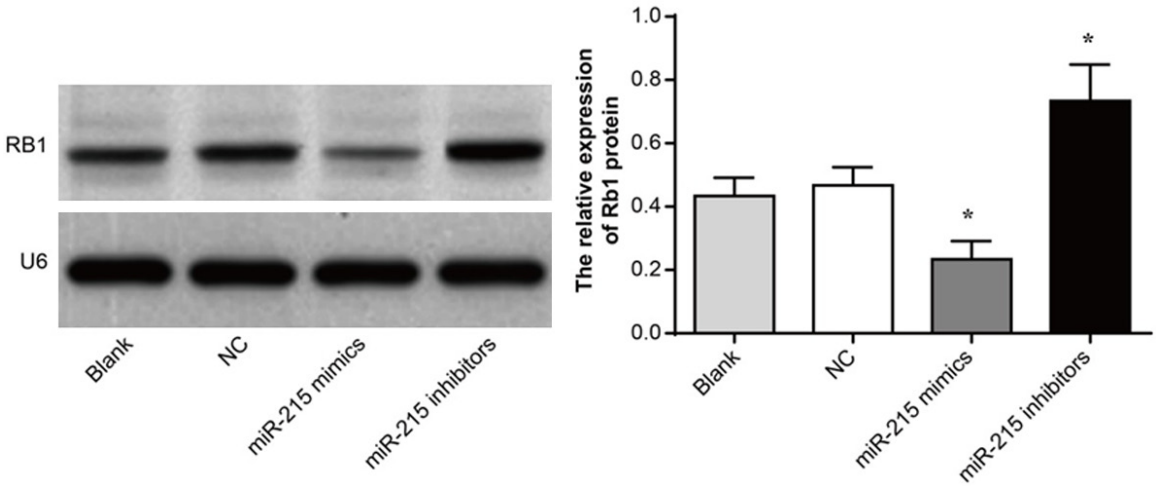


Figure 5. miR-215 regulated RB1 protein expression *P < 0.05 when compared with the Blank and NC groups.

inhibitor was found (P < 0.05) (**Figure 3B**), which indicated that up-regulation of miR-215 could enhance the invasion ability of HXO-Rb44 cells, while down-regulation of miR-215 could weaken HXO-Rb44 cells invasion ability.

Effect of miR-215 expression on apoptosis of HXO-Rb44 cells

At 48 hours, compared with the Blank group (16.32 ± 2.49%) and the NC group (15.43 ± 1.70%), the percentage of apoptotic cells in the miR-215 mimic group was significantly decreased (10.67 ± 2.05%) (P < 0.05) while that in the miR-215 inhibitor group (25.67 ± 2.49%) was significantly increased (P < 0.05) (**Figure 4**). miR-215 expression had a negative regulatory effect on the apoptosis of HXO-Rb44 cells.

miR-215 regulated RB1 protein expression

Compared with the Blank group and the NC group, the relative expression of RB1 protein in miR-215 mimic group was significantly decreased (P < 0.05), while that in miR-215 inhibitor group was significantly increased (P < 0.05) (**Figure 5**). miR-215 negatively regulated RB1 protein expression.

A

	Predicted consequential pairing of target region (top) and miRNA (bottom)	Site type	Context++ score	Context++ score percentile	Weighted context++ score	Conserved branch length	PCT
Position 1582-1589 of RB1 3'UTR	5' ...AAAUCAGUUAGUUUUUAGGUCAA...	8mer	-0.30	98	-0.30	2.004	<0.1
hsa-miR-215-5p	3' CAGACAGUUAAGUAUCCAGUA						

B

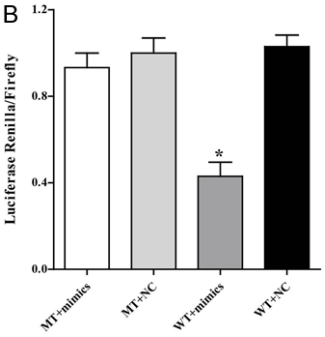


Figure 6. miR-215 directly targeted RB1. A. Target Scan predicted the target site for RB1 and miR-215; B. Dual luciferase reporter gene activity assay. *P < 0.05 when compared with the WT + NC group.

A

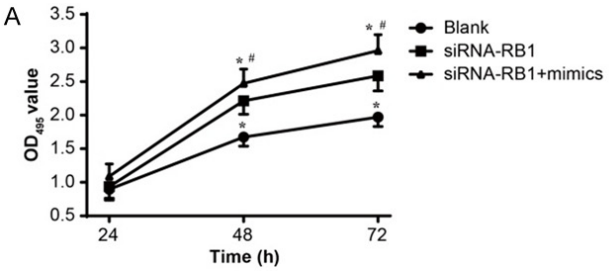
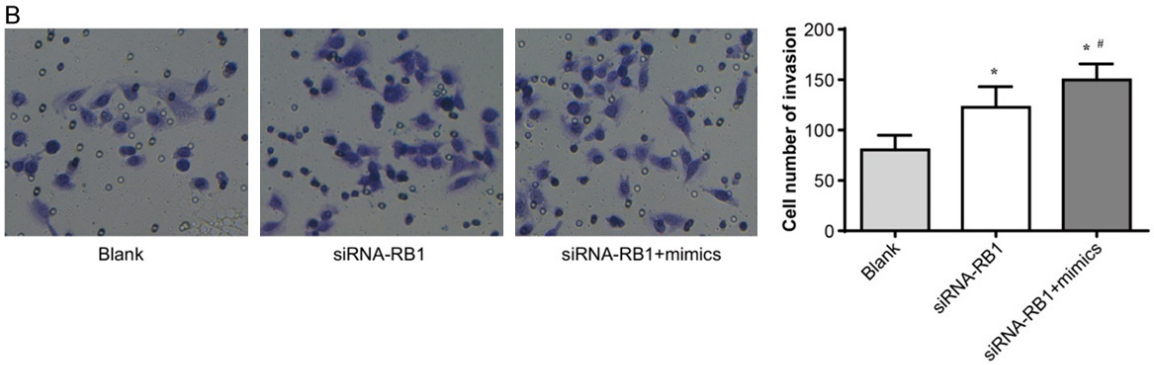
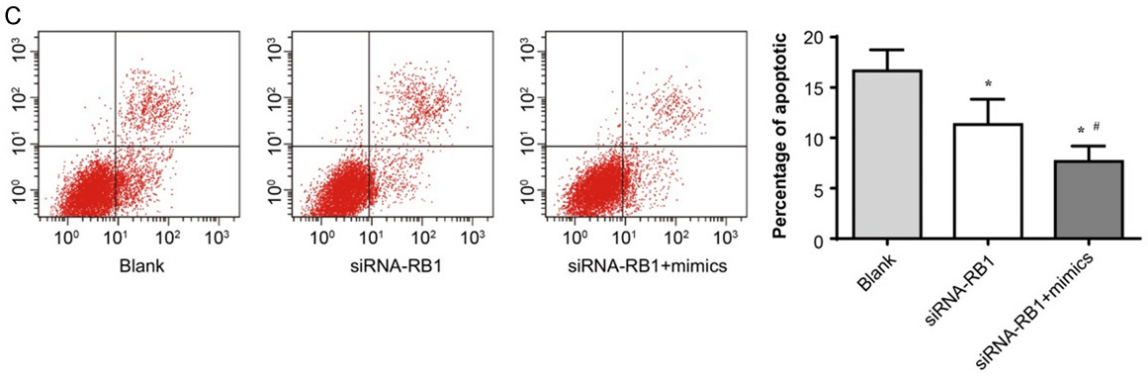


Figure 7. miR-215 affected the proliferation, invasion, and apoptosis of HXO-Rb44 cells through the regulation of RB1 protein expression. A. Detection of cell proliferation by MTT assay; B. Detection of cell invasion by transwell assay; C. Detection of cell apoptosis by flow cytometry. *P < 0.05 when compared with the Blank group; #P < 0.05 when compared with the Blank or siRNA-RB1 group.

B



C



RB1 was the target gene of miR-215

Target Scan, an online prediction software, identified the 3'-UTR region as the target site for the binding of RB1 and miR-215 (**Figure 6A**). Luciferase reporter assay was performed to further confirm the targeting between RB1 and miR-215 and the results showed no significant

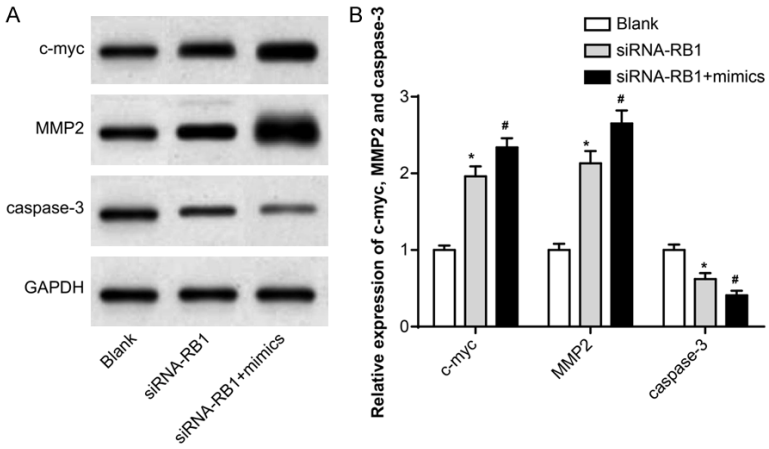


Figure 8. miR-215 affected proliferation, invasion, and apoptosis related proteins expression by regulation of RB1. A. Western blot analysis of c-myc, MMP2, and caspase-3 proteins; B. Statistical histogram of c-myc, MMP2, and caspase-3 proteins relative expression. $^{**}P < 0.01$ when compared with the Blank group; $\#P < 0.05$ when compared with the Blank or siRNA-RB1 group.

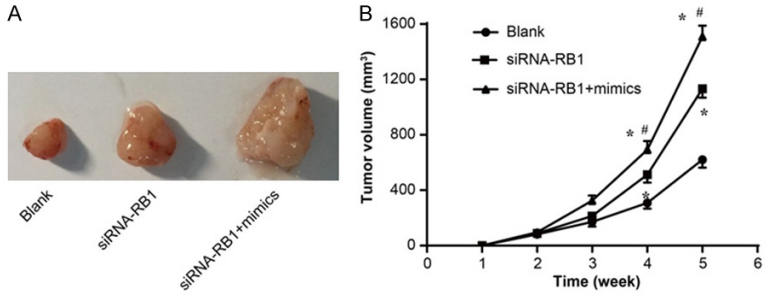


Figure 9. Up-regulation or down-regulation of miR-215 regulated the growth of HXO-Rb44 tumors in nude mice. A. Tumor tissues of each group at 5 weeks; B. Tumor volume of each group at 1, 2, 3, 4 and 5 weeks. $^{*}P < 0.05$ when compared with the Blank group; $\#P < 0.05$ when compared with the Blank or siRNA-RB1 group.

difference in luciferase activity between the MT + mimic group and the MT + NC group. However, significantly decreased luciferase activity occurred in the WT + mimic when compared with the WT + NC group ($P < 0.05$), and luciferase activity was significantly reduced by about 58% (**Figure 6B**).

miR-215 affected proliferation, invasion, and apoptosis of HXO-Rb44 cells through regulation of RB1 protein expression

MTT assay showed that no significant difference was found in OD_{495} value among the Blank group, the siRNA-RB1 group, and the siRNA-RB1 + mimic group at 24 hours ($P > 0.05$). However, at 48 and 72 hours, compared with the Blank group, the OD_{495} value of the siRNA-RB1 group and the siRNA-RB1 + mimic group was significantly higher ($P < 0.05$). At these two time points, the siRNA-RB1 + mimic group had a much higher OD_{495} value when compared with the siRNA-RB1 group ($P < 0.05$) (**Figure 7A**).

Transwell assay illustrated that the number of cells passing through the membrane of the siRNA-RB1 and siRNA-RB1 + mimic groups was significantly higher than that of the Blank group ($P < 0.05$) at 48 hours. In contrast, the number of cells across the membrane of the siRNA-RB1 + mimic group was significantly higher than that of the siRNA-RB1 group ($P < 0.05$) (**Figure 7B**).

Flow cytometry results revealed that the percentage of apoptotic cells in the siRNA-RB1 group and the siRNA-RB1 + mimic group was significantly lower than that in the Blank group ($P < 0.05$) at 48 hours. At the same time, the siRNA-RB1 + mimic group had a much lower percentage of apoptotic cells when compared to the siRNA-RB1 group ($P < 0.05$) (**Figure 7C**). The above results showed that miR-215 affected proliferation, invasion, and apoptosis of HXO-Rb44 cells through regulation of RB1 protein expression.

miR-215 affected proliferation, invasion, and apoptosis related proteins expression by regulation of RB1

In this research, we further studied miR-215 effects on proliferation, invasion, and apoptosis related proteins expression. We observed that compared with the Blank group, cells of the siRNA-RB1 and siRNA-RB1 + mimic groups were both with much higher c-myc and MMP2 relative expression, as well as obviously lower caspase-3 relative expression ($P < 0.05$). Fur-

thermore, we also noted that dramatically increased c-myc and MMP2 relative expression and decreased caspase-3 relative expression was found in the siRNA-RB1 + mimic group when compared with the siRNA-RB1 group ($P < 0.05$) (**Figure 8A**, **8B**). This indicated that miR-215 affected proliferation, invasion, and apoptosis related proteins expression by regulation of RB1.

Nude mice in vivo transplantation experiments

There was no significant difference in tumor size among the Blank group, the siRNA-RB1 group, and the siRNA-RB1 + mimic group at 1, 2, and 3 weeks. However, at 4 and 5 weeks, the tumor volume of the siRNA-RB1 group and the siRNA-RB1 + mimic group was significantly higher than that of the Blank group ($P < 0.05$). Furthermore, nude mice in the siRNA-RB1 + mimic group had a much higher tumor volume compared with nude mice in the siRNA-RB1 group ($P < 0.05$) (**Figure 9A** and **9B**). These results further confirmed that miR-215 affected proliferation, invasion, and apoptosis of HXO-Rb44 cells through regulation of RB1 protein expression.

Discussion

Much evidence suggested that miRNAs played an important role in tumor development [2, 12, 13]. They could play a biological role in cancer promotion or tumor suppression, and could be used as an effective biomarker for early diagnosis and prognosis of tumors [14-16]. In this paper, the function of miR-215 in retinoblastoma was researched through *in vitro* as well as *in vivo* studies, and the results revealed that miR-215 was up-regulated in retinoblastoma cells. Further study demonstrated that miR-215 affected the proliferation, invasion, and apoptosis of retinoblastoma as well as c-myc, MMP2 and caspase-3 proteins relative expression by negatively regulating expression of RB1. It regulated RB1 expression at mRNA and protein levels through binding to the 3'-UTR region.

Research findings involving miR-215 expression level were not consistent. Several existing studies showed that miR-215 expression in some types of cancers was down-regulated [8, 17]. For example, researchers have found that miR-215 was acted as a tumor suppressor in colorectal cancer, human non-small cell lung cancer, and epithelial ovarian cancer [18-20]. However, other studies have revealed that up-regulation of miR-215 expression was occurred in some other tumors, including glioma and gastric cancer [9, 21-25]. Zang et al. [24] observed that miR-215 was frequently overexpressed in gastric cancer and it promoted gastric cancer cells migration and invasion through regulating FOXO1. Another study also illustrated the similar role of miR-215 in gastric cancer [26]. Our results suggested that miR-215 was up-regulated in retinoblastoma cells. Overexpression of miR-215 was associated with significantly increased HXO-Rb44 cells proliferation ability as well as invasion ability, while dramatically decreased proliferation ability and invasion ability were found when the miR-215 was down-regulated by transfection with miR-215 inhibitors. Furthermore, the data also illustrated an exact opposite relationship between the HXO-Rb44 cells apoptotic capacity and miR-215 expression level. Dramatically decreased percentage of apoptotic HXO-Rb44 cells was occurred when expression of miR-215 was up-regulated while down-regulation of miR-215 expression resulted in an increase in the percentage of apoptotic HXO-Rb44 cells. Our results demonstrated that miR-215 was an oncogene in retinoblastoma, which could be used as a potential biomarker of retinoblastoma development.

More importantly, our data found that RB1 expression was affected by miR-215 expression level. RB1 was a gene with tumor suppressor effect, which was found to inhibit proliferation and invasion in many types of cancer cells [27, 28]. There was study indicating that the mechanism of RB1 inhibiting tumor development was through the cell cycle by preventing progression from G1 to S phase [10]. Another research study found that RB1 achieved tumor suppressor effect by regulating the expression of p53 [29]. It was well known that p53 was involved in a variety of cancers [30-32]. In this article, we confirmed that RB1 was the direct target gene of miR-215 and their binding sites was 3'-UTR region. The impact of miR-215 on HXO-Rb44 cells proliferation, invasion, and apoptosis was achieved through the negative regulation of RB1. Consensus findings were found between our results and previous studies. In a previous research of gastric cancer, researchers also revealed in their study that

miR-215 could enhance the proliferation of gastric cancer cells via inhibiting the expression of RB1 [9]. Our work further proved this mechanism in retinoblastoma that miR-215 was through the negative regulation of RB1 to achieve its effect on retinoblastoma cells proliferation, invasion, and apoptosis as well as their related protein expression, such as c-myc, MMP2, and caspase-3. C-myc, MMP2, and caspase-3 could directly affected proliferation, invasion, and migration of tumor cells, respectively.

In conclusion, this study explored the role and mechanisms of miR-215 in affecting the development of retinoblastoma through *in vitro* study and *in vivo* study with nude mice. The results showed that miR-215 was significantly up-regulated in retinoblastoma cells and it affected retinoblastoma cell proliferation, invasion, and apoptosis as well as c-myc, MMP2, and caspase-3 protein expression by negatively regulating the expression of RB1. miR-215 could be used as a potential biomarker for the clinical treatment retinoblastoma. This paper had important guiding significance for the clinical treatment of retinoblastoma. Clinicians could use some drugs that had specific inhibitory effects on miR-215 to treat patients with retinoblastoma.

Disclosure of conflict of interest

None.

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References

[1] Dimaras H, Kimani K, Dimba EA, Gronsdahl P, White A, Chan HS and Gallie BL. Retinoblastoma. Lancet 2012; 379: 1436-1446.

[2] Fabian ID, Naeem Z, Stacey AW, Chowdhury T, Duncan C, Reddy MA and Sagoo MS. Long-term visual acuity, strabismus, and nystagmus outcomes following multimodality treatment in group d retinoblastoma eyes. Am J Ophthalmol 2017; 179: 137.

[3] Abramson DH, Fabius AW, Issa R, Francis JH, Marr BP, Dunkel IJ and Gobin YP. Advanced unilateral retinoblastoma: the impact of ophthalmic artery chemosurgery on enucleation rate and patient survival at MSKCC. PLoS One 2015; 10: e0145436.

[4] Fabian ID, Stacey AW, Chowdhury T, Duncan C, Karaa EK, Scheimberg I, Reddy MA and Sagoo MS. High-risk histopathology features in primary and secondary enucleated international intraocular retinoblastoma classification group D eyes. Ophthalmology 2017; 124: 851.

[5] Wijnhoven BP, Hussey DJ, Watson DI, Tsykin A, Smith CM and Michael MZ. MicroRNA profiling of Barrett's oesophagus and oesophageal adenocarcinoma. Br J Surg 2010; 97: 853.

[6] Karaayvaz M, Pal T, Song B, Zhang C, Georgakopoulos P, Mehmood S, Burke S, Shroyer K and Ju J. Abstract 2263: prognostic significance of miR-215 in colon cancer. Cancer Research 2011; 71: 340-347.

[7] Chiang Y, Song Y, Wang Z, Liu Z, Gao P, Liang J, Zhu J, Xing C and Huimian XU. microRNA-192, -194 and -215 are frequently downregulated in colorectal cancer. Exp Ther Med 2012; 3: 560.

[8] Li S, Gao J, Gu J, Yuan J, Hua D and Shen L. MicroRNA-215 inhibits relapse of colorectal cancer patients following radical surgery. Med Oncol 2013; 30: 549.

[9] Deng Y, Huang Z, Xu Y, Jin J, Zhuo W, Zhang C, Zhang X, Shen M, Yan X and Wang L. MiR-215 modulates gastric cancer cell proliferation by targeting RB1. Cancer Lett 2014; 342: 27-35.

[10] Wei Y, Sun J and Li X. MicroRNA-215 enhances invasion and migration by targeting retinoblastoma tumor suppressor gene 1 in high-grade glioma. Biotechnol Lett 2017; 39: 197-205.

[11] Liu F, You X, Chi X, Wang T, Ye L, Niu J and Zhang X. Hepatitis B virus X protein mutant HBxΔ127 promotes proliferation of hepatoma cells through up-regulating miR-215 targeting PTPRT. Biochem Biophys Res Commun 2014; 444: 128-134.

[12] Zhu L, Zhao J, Wang J, Hu C, Peng J, Luo R, Zhou C, Liu J, Lin J and Jin Y. MicroRNAs are involved in the regulation of ovary development in the pathogenic blood fluke schistosoma japonicum. PLoS Pathog 2016; 12: e1005423.

[13] Chandra S, Vimal D, Sharma D, Rai V, Gupta SC and Chowdhuri DK. Role of miRNAs in development and disease: lessons learnt from small organisms. Life Sci 2017; 185: 8-14.

[14] Zhao Y, Liu X, Zhong L, He M, Chen S, Wang T and Ma S. The combined use of miRNAs and mRNAs as biomarkers for the diagnosis of papillary thyroid carcinoma. Int J Mol Med 2015; 36: 1097-1103.

[15] Liu R, Chen X, Du Y, Yao W, Shen L, Wang C, Hu Z, Zhuang R, Ning G, Zhang C, Yuan Y, Li Z, Zen K, Ba Y, Zhang CY. Serum microRNA expression profile as a biomarker in the diagnosis

and prognosis of pancreatic cancer. Clin Chem 2012; 58: 610.
[16] Huang J, Liu J, Chen-Xiao K, Zhang X, Lee WN, Go VL and Xiao GG. Advance in microRNA as a potential biomarker for early detection of pancreatic cancer. Biomark Res 2016; 4: 20.
[17] Wijnhoven BP, Hussey DJ, Watson DI, Tsykin A, Smith CM, Michael MZ; South Australian Oesophageal Research Group. MicroRNA profiling of Barrett's oesophagus and oesophageal adenocarcinoma. Br J Surg 2010; 97: 853-861.
[18] Hou Y, Zhen J, Xiaodong XU, Zhen K, Zhu B, Pan R and Zhao C. miR-215 functions as a tumor suppressor and directly targets ZEB2 in human non-small cell lung cancer. Oncol Lett 2015; 10: 1985-1992.
[19] Vychytilova-Faltejskova P, Merhautova J, Machackova T, Gutierrez-Garcia I, Garcia-Solano J, Radova L, Brchnelova D, Slaba K, Svoboda M, Halamkova J, Demlova R, Kiss I, Vyzula R, Conesa-Zamora P and Slaby O. MiR-215-5p is a tumor suppressor in colorectal cancer targeting EGFR ligand epiregulin and its transcriptional inducer HOXB9. Oncogenesis 2017; 6: 399.
[20] Ge G, Zhang W, Niu L, Yan Y, Ren Y and Zou Y. miR-215 functions as a tumor suppressor in epithelial ovarian cancer through regulation of the X-chromosome-linked inhibitor of apoptosis. Oncol Rep 2016; 35: 1816.
[21] Wang C, Chen Q, Li S, Zhao Z, Gao H, Wang X, Li B, Zhang W and Yuan Y. Dual inhibition of PCDH9 expression by miR-215-5p up-regulation in gliomas. Oncotarget 2017; 8: 10287.
[22] Meng X and Shi B. miR-215 functions as an oncogene in high-grade glioma by regulating retinoblastoma 1. Biotechnol Lett 2017; 39: 1351-8.
[23] Xu YJ and Fan Y. MiR-215/192 participates in gastric cancer progression. Clin Transl Oncol 2015; 17: 34.
[24] Zang Y, Wang T, Pan J, Wu R, Ge H, Qu B and Zuo X. miR-215 promotes cell migration and invasion of gastric cancer cell lines by targeting FOXO1. Neoplasma 2017; 64: 579-587.
[25] Na L, Zhang QY, Zou JL, Li ZW, Tian TT, Dong B, Liu XJ, Ge S, Yan Z and Jing G. miR-215 promotes malignant progression of gastric cancer by targeting RUNX1. Oncotarget 2016; 7: 4817.
[26] Li N, Zhang QY, Zou JL, Li ZW, Tian TT, Dong B, Liu XJ, Ge S, Zhu Y and Gao J. miR-215 promotes malignant progression of gastric cancer by targeting RUNX1. Oncotarget 2016; 7: 4817-4828.
[27] Dyson NJ. RB1: a prototype tumor suppressor and an enigma. Genes Dev 2016; 30: 1492.
[28] Indovina P, Pentimalli F, Casini N, Vocca I and Giordano A. RB1 dual role in proliferation and apoptosis: cell fate control and implications for cancer therapy. Oncotarget 2015; 6: 17873.
[29] Hu W, Feng Z, Teresky AK and Levine AJ. p53 regulates maternal reproduction through LIF. Nature 2007; 450: 721-724.
[30] Shrestha M and Park PH. p53 signaling is involved in leptin-induced growth of hepatic and breast cancer cells. Korean J Physiol Pharmacol 2016; 20: 487-498.
[31] Charni M, Molchadsky A, Goldstein I, Solomon H, Tal P, Goldfinger N, Yang P, Porat Z, Lozano G and Rotter V. Novel p53 target genes secreted by the liver are involved in non-cell-autonomous regulation. Cell Death Differ 2016; 23: 509-520.
[32] Mahfoudhi E, Lordier L, Marty C, Pan J, Roy A, Roy L, Rameau P, Abbes S, Debili N and Raslova H. P53 activation inhibits all types of hematopoietic progenitors and all stages of megakaryopoiesis. Oncotarget 2016; 7: 31980-31992.