Inflammation Research (2021) 70:217–227
https://doi.org/10.1007/s00011-020-01432-z

Inflammation Research

ORIGINAL RESEARCH PAPER

LncRNA MBLN1-AS1 inhibits the progression of retinoblastoma through targeting miR-338-5p-Wnt/β-catenin signaling pathway

Lei Xu[1] · Shenyu Zhu[2] · Aidong Tang[1] · Wanrong Liu[1]

Received: 19 November 2020 / Revised: 7 December 2020 / Accepted: 13 December 2020 / Published online: 16 January 2021

Abstract

Objective and design Retinoblastoma is the most common primary intraocular malignancy of childhood, which brings a heavy burden to the countries across the world, especially the developing countries. It has been shown that lncRNA muscleblind-like 1 antisense RNA 1 (MBNL1-AS1) exerts anti-tumor effects in various cancers, including bladder cancer, papillary thyroid cancer, and retinoblastoma. In the present study, we hypothesized that MBNL1-AS1 might play a protective role against retinoblastoma.

Methods The expression of MBNL1-AS1 and its potential target miR-338-5p were evaluated in retinoblastoma cell line by real-time quantitative PCR and western blot. The involvement of MBNL1-AS1-miR-338-5p in the cell proliferation was evaluated by cell counting kit-8 (CCK8), and colony formation assay. The cell migration was evaluated by Transwell assay in Y79 cells, a retinoblastoma cell line. The involvement of MBNL1-AS1-miR-338-5p in tumor formation was also evaluated in mice.

Results It was found that MBNL1-AS1 overexpression inhibited proliferation and migration in Y79 cells. In addition, the inhibitory effects of MBNL1-AS1 on Y79 cells were significantly reversed in the presence of miR-338-5p mimics, and MBNL1-AS1 overexpression significantly decreased miR-338-5p level in Y79 cells. Furthermore, MBNL1-AS1 overexpression significantly inhibited Wnt/β-catenin signaling pathway, and this inhibitory effect was almost lost in the presence of miR-338-5p mimics. Finally, our in vivo study showed that MBNL1-AS1 overexpression significantly inhibited Y79-induced retinoblastoma in mice, and this inhibitory effect was lost in the presence of miR-338-5p mimics.

Conclusion Our study shows that MBNL1-AS1 exerts its anti-tumor effect by targeting miR-338-5p, thereby inactivating wnt/β-catenin signaling pathway in retinoblastoma.

Keywords MBNL1-AS1 · miR-338-5p · Retinoblastoma · Proliferation · Migration

Introduction

Retinoblastoma is the most common intraocular malignant neoplasm in children [1]. The incidence of retinoblastoma is about 1 in 14,000–20,000 live births worldwide, and it accounts for 3% of all childhood cancers [2]. In clinical practice, the major treatment for retinoblastoma was eye removal, and most retinoblastoma patients have poor prognosis due to lack of early diagnosis and effective therapies, especially in developing countries [2, 3]. Therefore, it is necessary for us to investigate the molecular mechanisms of retinoblastoma to develop new targets for the prevention and treatment of retinoblastoma.

It has been demonstrated that long noncoding RNAs (lncRNAs) play a crucial role in biological regulation and the development of various cancers, including bladder cancer [4], papillary thyroid cancer [5], and retinoblastoma [6]. Among them, it has been reported that lncRNA muscleblind-like 1 antisense RNA 1 (MBNL1-AS1) works as an anti-tumor lncRNA in various cancers. For example, Wei et al., reported that MBNL1-AS1 was significantly downregulated

Communicated by John Di Battista.

✉ Wanrong Liu
WanrongLiu2020@163.com

[1] Department of Ophthalmology, The First Affiliated Hospital of Gannan Medical University, No. 23 Qingnian Road, Zhanggong District, Ganzhou 341000, Jiangxi Province, China

[2] The First Affiliated Hospital of Gannan Medical University, Ganzhou 341000, Jiangxi Province, China

in bladder tumor tissues, and that MBNL1-AS1 worked as a tumor suppressor since its knockdown increased the number of living cells and bromodeoxyuridine positive cells in bladder cancer cells, accelerated cell cycle, and inhibited proliferation regulators (Ki67, p21, p27 and cyclin D1) [7]. In addition, Zhu et al., reported that the expression of MBNL1-AS1 and MYL9 was decreased in colon cancer cells, and that the enhancement of MBNL1-AS1 reduced the proliferation, migration and invasion of cancer stem cells (CSC), but promoted apoptosis through inhibition of miR-412-3p and stimulation of myosin light chain 9 (MYL9) [8]. Studies have showed that MYL9 is essential for the maintenance of cytoskeletal dynamics, experimental metastasis, and tumor cell migration. Moreover, its downregulation is a hallmark for the decreased median survival rate in patients with colon cancer [9]. Similarly, it was reported that low expression of MBNL1-AS1 was observed in non-small cell lung cancer tissues, and that overexpression of MBNL1-AS1 significantly reduced the proliferation, migration and invasion of CSC in non-small-cell lung carcinoma (NSCLC) [10]. However, the role of MBNL1-AS1 in the development of retinoblastoma has not been studied. We performed Starbase analysis and found that miR-338-5p is a potential target of MBNL1-AS1. Importantly, it has been shown that serum miR-338-5p may be a potential tumor marker of retinoblastoma, and that the combination of miR-338-5p and neuron-specific enolase (NSE) can improve the early diagnosis rate of retinoblastoma [11].

Increasing evidence has showed that Wnt/β-Catenin signal transduction is abnormally activated, which can promote cell proliferation and tumor progression in various types of human cancer including retinoblastoma [12–14]. For example, Wu et al., reported that sclerostin siRNA significantly increased the proliferation, invasion and migration of retinoblastoma cells through increased expression of Wnt/β-catenin signaling pathway [13].

Materials and methods

Cell culture and transfection

The human retinal pigment epithelium cell line ARPE-19, and the human retinoblastoma cell lines Y79, WER1-RB1, and SO-RB were from the American Type Culture Collection (Manassas, VA). The culture was performed as previously described [15–17]. In brief, ARPE-19 cells were cultured in a 1:1 mixture of Dulbecco's modified Eagle's medium and Ham's F-12 medium (Invitrogen, Carlsbad, CA) containing 10% fetal bovine serum (FBS). Y79 cells were cultured in RPMI 1640 medium (Invitrogen) containing 20% FBS. WER1-RB1 cells were cultured in RPMI 1640 containing 10% FBS. SO-RB cells were cultured in DMEM containing 10% FBS. All of these four kinds of cells were cultured at 37 °C in a humidified atmosphere of 5% CO_2 and 95% O_2. miR-338-5p mimics, inhibitor, the adenovirus that overexpressed MBNL1-AS1 (Oe-MBNL1-AS1), and the control (Oe-NC) were constructed by Hanbio company (Shanghai, China). Cell transfection was performed according to manufacturer's instructions.

Real-time quantitative PCR

Total RNA was extracted with Trizol reagent (Invitrogen, USA) following the manufacturer's protocol, and quantitative real-time PCR (RT-PCR) was performed using Premix Ex Taq DNA polymerase for real-time PCR (RR039B, Takara). U6 and GAPDH were used as internal controls.

Cell proliferation assay

The viability of Y79 cells was evaluated with cell counting kit-8 (CCK8) assay (CCK8 kit, Beyotime, Shanghai, China) according to manufacturer's instructions. In brief, cells were seeded into 96-well plates (2×10^3cells/well), and incubated at 37 °C and 5% CO_2 for 24 h. Then, the CCK8 solution was added to fresh medium for 2 h at 37 °C. The absorbance optical density was measured at 450 nm using the UV–Vis SpectraMax 190 Microplate Reader and SoftMax Pro software (Molecular Devices, Sunnyvale, CA).

Colony formation

Colony formation was performed as previously described [18]. Y79 cells were seeded in 6-well plates at a density of 500 cells per well. After 10 days of incubation, cells were immobilized with 4% paraformaldehyde for 20 min, and stained with crystal violet for 30 min. The stained cell colonies were counted.

Cell wound healing assay

Cell wound healing assays were performed as previously described [19]. Briefly, the cell layers were wounded by a sterile pipette tip at 75% confluence. After being washed with PBS for several times to remove cell debris, the cells were incubated in serum-free medium for 48 h. The cell that had migrated into wound surface was considered as wound healing. The wound healing was photographed, and the rate of wound healing was calculated as [(the wound width of 0 h–48 h)/0 h wound width] × 100%.

Transwell migration and invasion experiments

Transwell assay was performed to evaluate cell migration as previously described [19]. In brief, 1×10^5 cells were

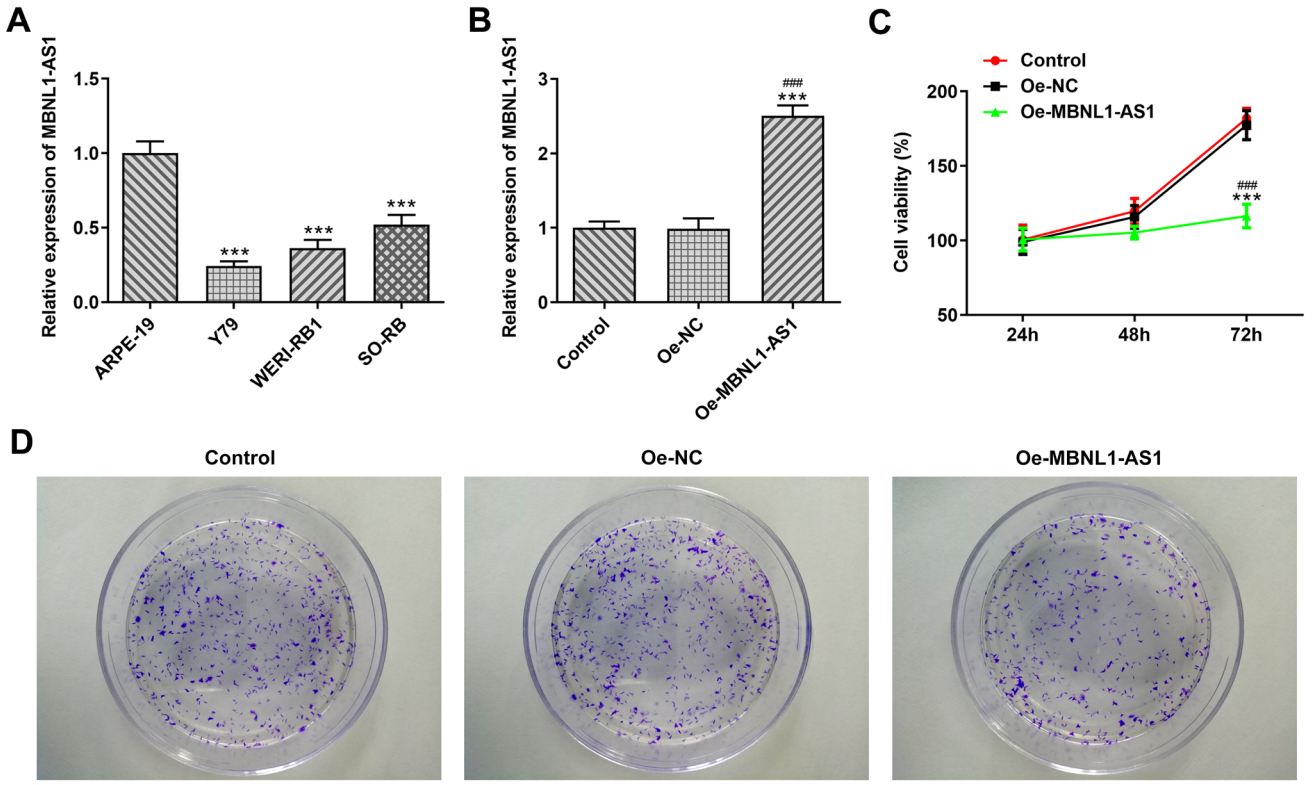


Fig. 1 MBNL1-AS1 overexpression inhibited the proliferation of Y79 cells. **a** Summarized data showing the level of MBNL1-AS1 in different retinoblastoma cell lines including ARPE-19, Y79, WER1-RB1, and SO-RB cells in real-time qPCR assay. **b** Summarized data showing the efficiency of MBNL1-AS1 overexpression in Y79 cells. **c** Summarized data showing the effect of MBNL1-AS1 overexpression on the proliferation of Y79 cells in CCK8 assay. **d** Summarized data showing the effect of MBNL1-AS1 overexpression on clone formation in Y79 cells. $^{***}P < 0.001$ vs. control group or ARPE-19 group; $^{\#\#\#}P < 0.001$ vs. Oe-NC group

seeded in the upper chamber in each well (24-well, 8.0-μm pore membranes, Corning USA) in 100 μL of serum-free medium, and 600 μL of complete medium was added to the lower chamber as a chemoattractant at the same time. After incubation for 24 h at 37 °C, the cells at the upper surface of the membrane were removed with cotton swabs, and the cells on the lower surface of the membrane are indicated as the migrated cells. After fixing with 4% paraformaldehyde and stained with 0.1% crystal violet solution, the cells on the lower surface of the membrane were counted.

Subcutaneous tumor formation

Subcutaneous tumor formation assay was performed as previously described [20, 21]. All the experimental operations performed on all mice were approved by the Animal Experiment Ethics Committee of The First Affiliated Hospital of Gannan Medical University. Oe-MBNL1-AS1 and Oe-NC were transfected into Y79 cells, and then the transfected Y79 cells were injected into nude mice to form subcutaneous tumors. The body weight, tumor weight, and tumor volume were evaluated each week. After 5 weeks treatment, tumors were collected for taking pictures.

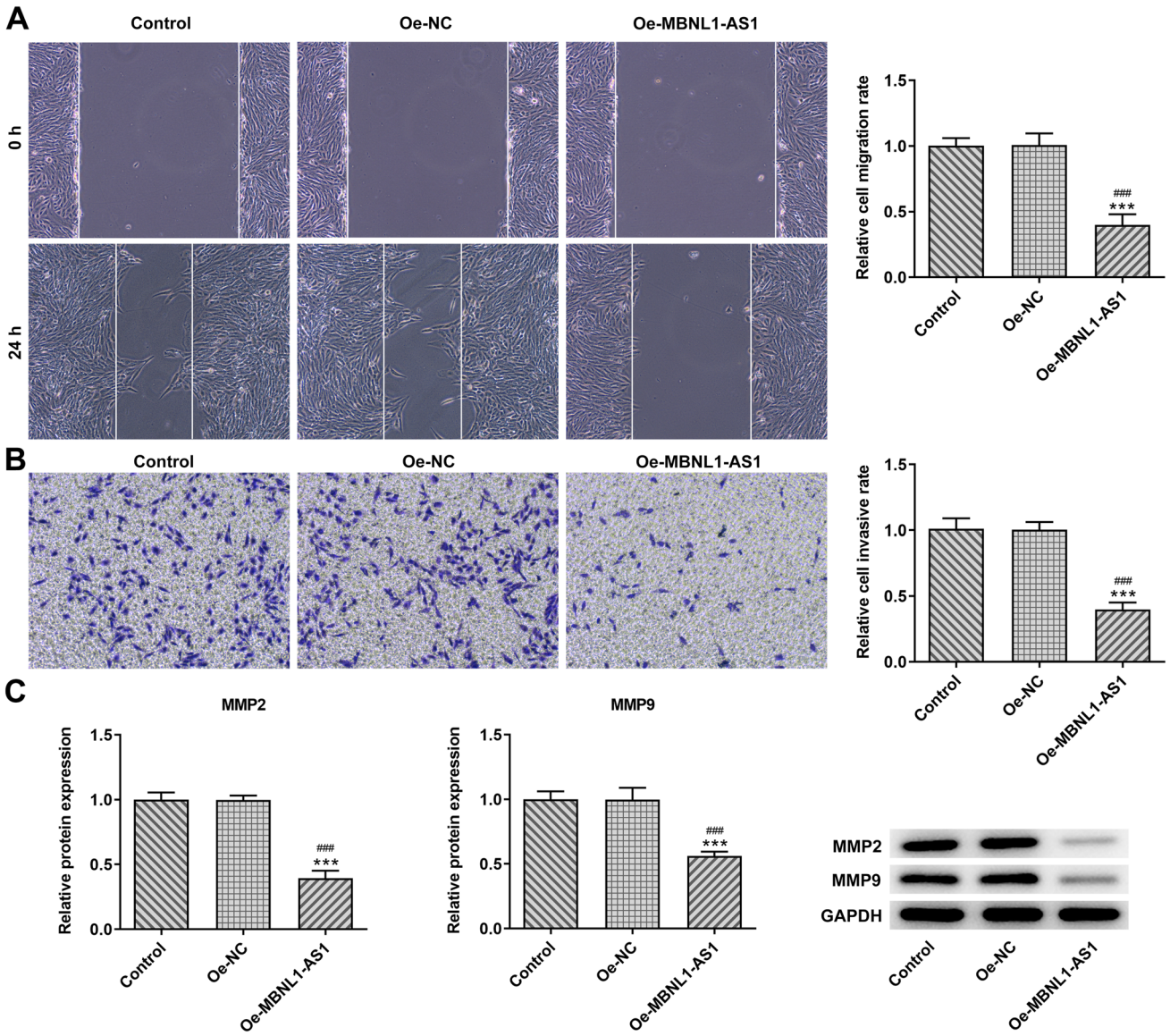


Fig. 2 MBNL1-AS1 overexpression inhibited the migration of Y79 cells. **a**–**b** Summarized data showing the effect of MBNL1-AS1 overexpression on migration of Y79 cells in wound healing and transwell assay. **c** Representative western blot images and summarized data showing the effect of MBNL1-AS1 overexpression on MMP2 and MMP9 protein level in Y79 cells. $^{***}P < 0.001$ vs. control group; $^{\#\#\#}P < 0.001$ vs. Oe-NC group

Western blot

Western blotting was performed as described previously. Extracted protein samples were boiled for 5 min in gel-loading buffer. Each sample was separated via SDS–polyacrylamide gel electrophoresis (PAGE) and transferred to PVDF membranes at 15 V for 1 h. After being blocked with 1% non-fat milk, the blots were allowed to block at room temperature for 1 h and then incubated with specific primary antibodies overnight at 4 °C overnight, including MMP2, MMP9, Ki67, PCNA, Wnt4, TCF4, active β-catenin, and-total β-catenin. Then, membranes were incubated with HRP-conjugated secondary antibody at room temperature for 1 h. Signals were detected using enhanced chemiluminescence according to the manufacturer's instructions.

Luciferase assay

Luciferase reporter assay was performed as previously described [22]. Briefly, a 3′UTR segment of miR-338-5p was amplified and validated prior to transfection in Y79 cells. Then, cells were transfected with pSI-Check2-MBNL1-AS1 wild type or pSI-Check2-MBNL1-AS1 mutant. After 48 h

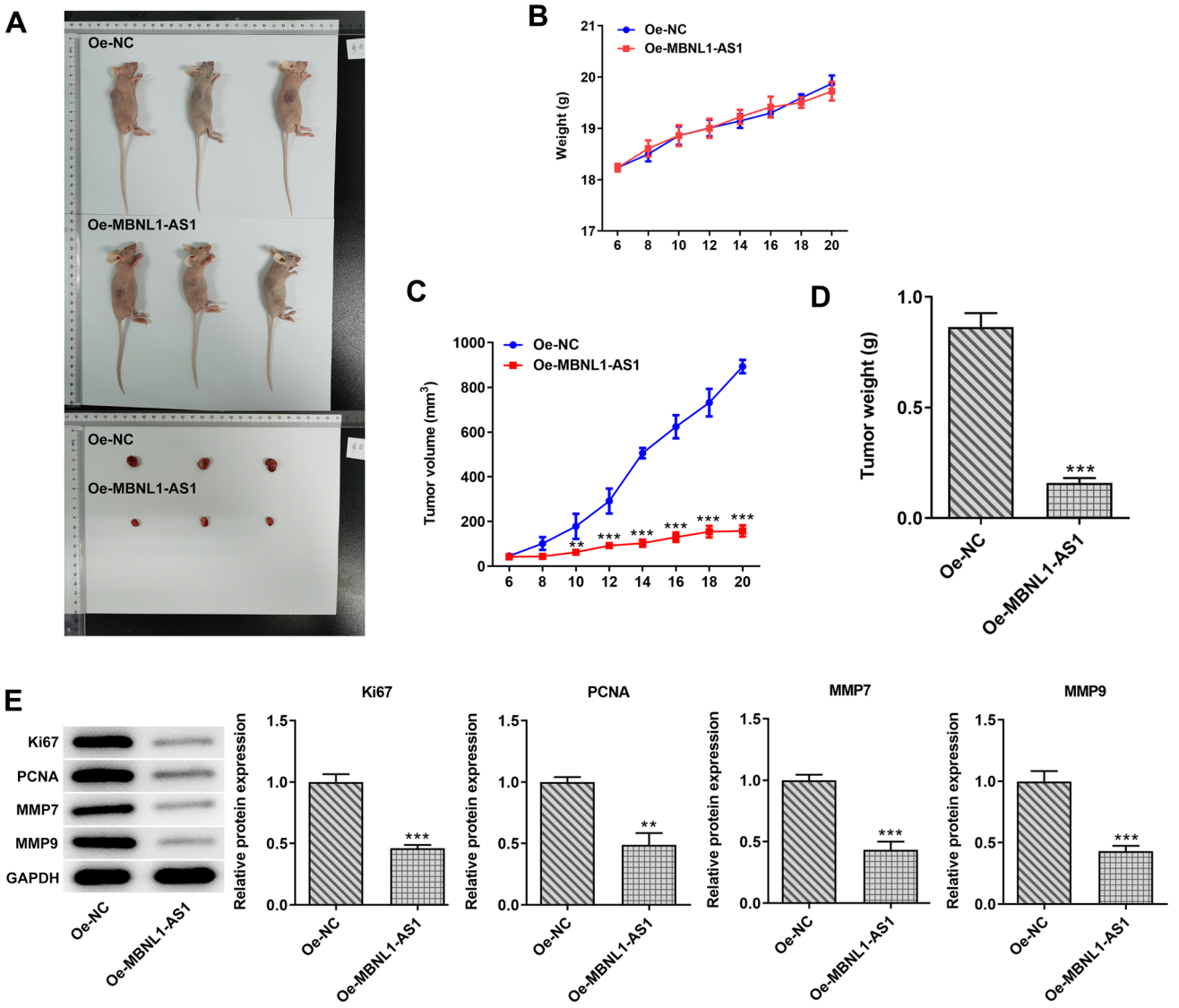


Fig. 3 MBNL1-AS1 overexpression in Y79 cells inhibited the development of Y79-induced retinoblastoma in vivo. **a–d** Summarized data showing the effect of MBNL1-AS1 overexpression on Y79-induced retinoblastoma. **e** Representative western blot images and summarized data showing the effect of MBNL1-AS1 overexpression on Ki-67, PCAN, MMP2 and MMP9 protein level in Y79-induced retinoblastoma. $^{**}P<0.01$, $^{***}P<0.001$ vs. Oe-NC group

treatment, cell lysates were prepared for luciferase activity measurement.

Statistical analysis

The data in the study were presented as the mean ± standard deviation (SD). The analysis was performed by one-way analysis of variance (ANOVA) with Tukey's multiple comparison test or unpaired two-tailed Student's *t* test. The differences were considered significant when $P<0.05$.

Results

Expression of MBNL1-AS1 in different retinoblastoma cell lines

First, we compared the expression of lncRNA MBNL1-AS1 in different retinoblastoma cell lines. As shown in Fig. 1a, the levels of MBNL1-AS1 in the four kinds of retinoblastoma cell lines were significantly lower than that in normal cells. Notably, the level of MBNL1-AS1 in Y79 cell line was significantly lower compared with that in ARPE-19,

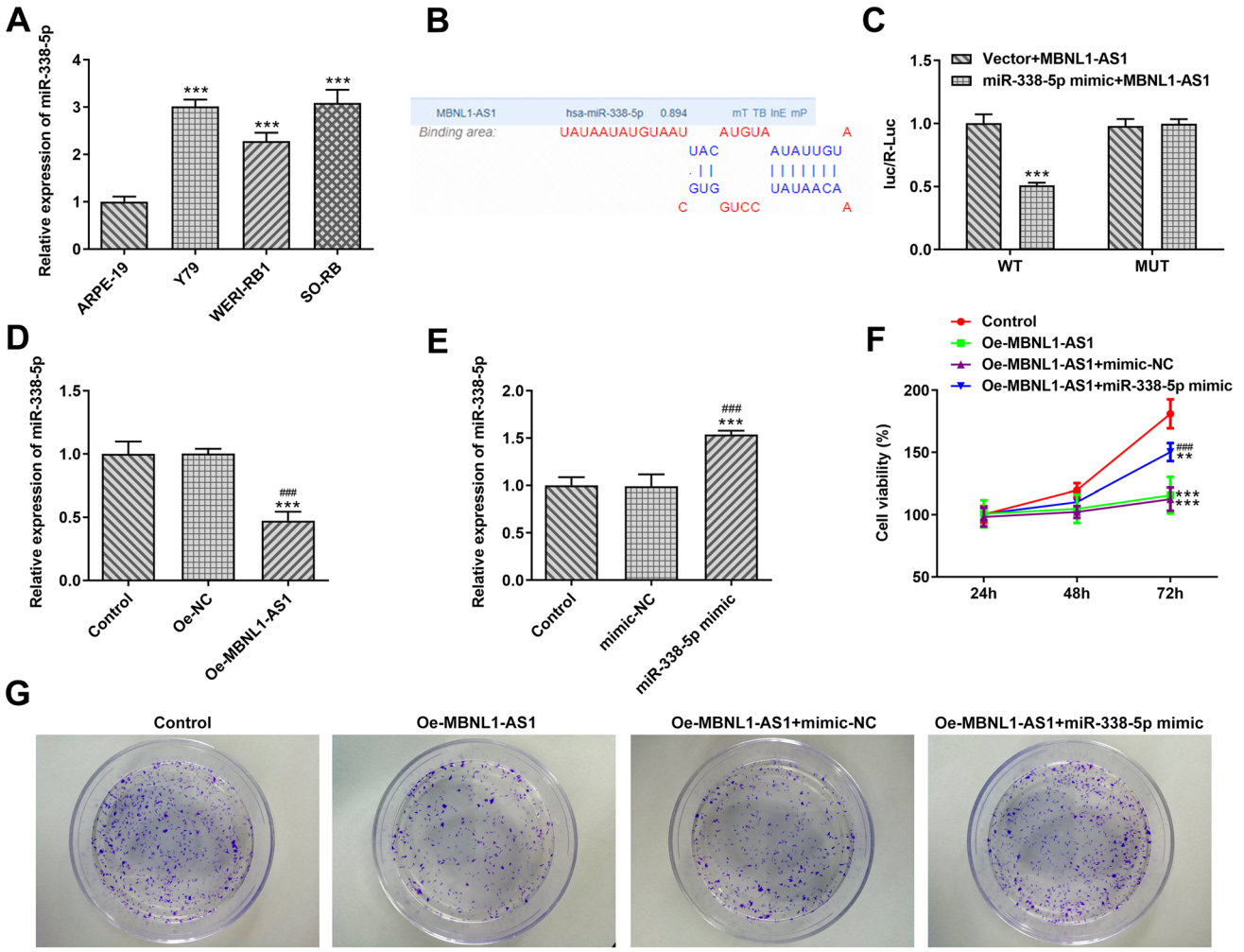


Fig. 4 MBNL1-AS1 inhibited the development of Y79-induced retinoblastoma through downregulation of miR-338-5p. **a** miR-338-5p was significantly increased in human retinoblastoma cells. **b–c** Summarized data showing that MBNL1-AS1 overexpression decreased luciferase activity in luciferase activity assay, and the inhibitory effect was lost in mutant MBNL1-AS1. **d** Summarized data showing that MBNL1-AS1 overexpression decreased miR-338-5p level. **e** Summarized data showing the efficiency of miR-338-5p overexpression in real-time PCR assay. **f** Summarized data showing that miR-338-5p overexpression prevented the inhibitory effect of MBNL1-AS1 on the proliferation of Y79 cells in CCK8 assay. **g** Summarized data showing that miR-338-5p overexpression prevented the inhibitory effect of MBNL1-AS1 overexpression on the clone formation in Y79 cells. $^{***}P<0.001$ vs. control group or ARPE-19 group; $^{\#\#\#}P<0.001$ vs. Oe-NC group or mimic-NC or Oe-MBNL1-AS1 + mimic-NC

WER1-RB1, and SO-RB cells. Therefore, Y79 cells were chosen in the following experiments.

MBNL1-AS1 overexpression inhibited proliferation of Y79 cells

Next, we evaluated the involvement of MBNL1-AS1 in the proliferation of Y79 cells. As shown in Fig. 1b, MBNL1-AS1 overexpression significantly increased MBNL1-AS1 level, indicating the high efficiency of MBNL1-AS1 overexpression. Figure 1c showed that MBNL1-AS1 overexpression significantly inhibited proliferation in CCK8 assay. Similarly, MBNL1-AS1 overexpression significantly decreased clone formation (Fig. 1d). These results suggested that MBNL1-AS1 could regulate the proliferation of Y79 cells.

MBNL1-AS1 overexpression inhibited migration and invasion of Y79 cells

Next, we evaluated the involvement of MBNL1-AS1 in migration of Y79 cells. As shown in Fig. 2a, b, MBNL1-AS1 overexpression significantly inhibited migration and invasion of Y79 cells in wound healing and Transwell assay.

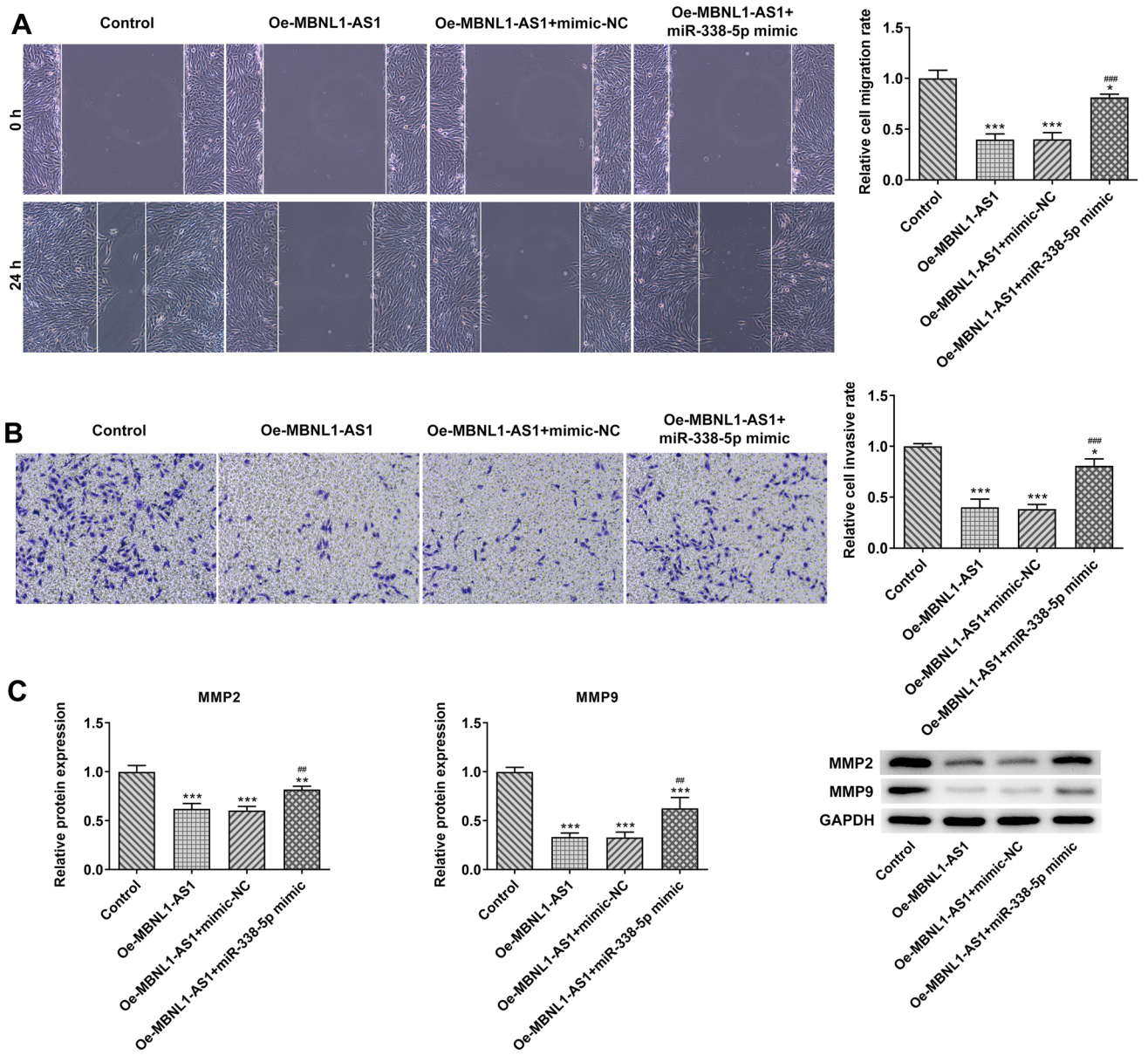
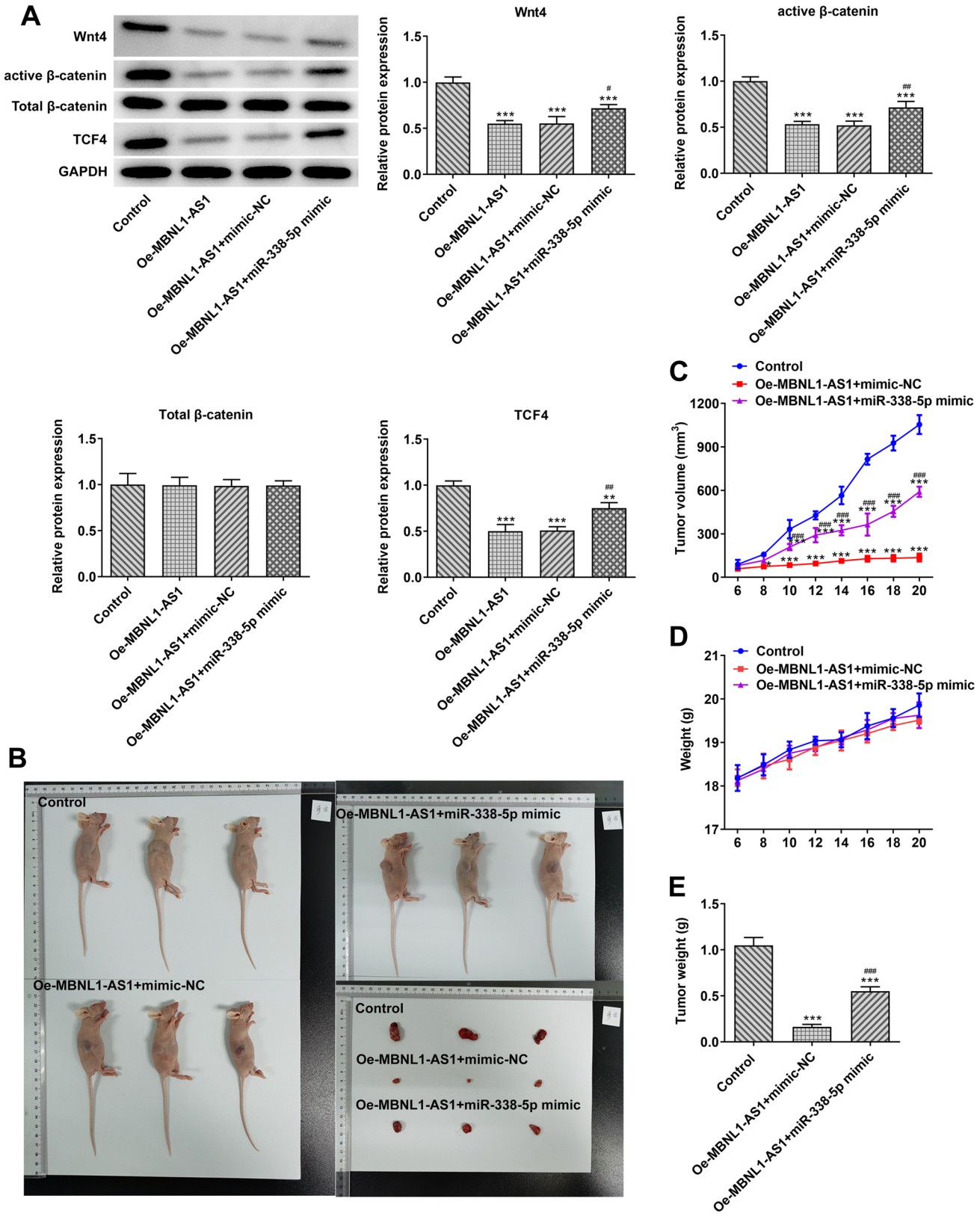


Fig. 5 miR-338-5p prevented the inhibitory effect of MBNL1-AS1 overexpression on migration of Y79 cells. **a**–**b** Summarized data showing that miR-338-5p overexpression prevented the inhibitory effect of MBNL1-AS1 overexpression on the migration of Y79 cells in wound healing and transwell assay. **c** Western blot showing that miR-339-5p prevented the inhibitory effect of MBNL1-AS1 overexpression on MMP2 and MMP9 protein level in Y79 cells. $^{*}P<0.05$, $^{**}P<0.01$ and $^{***}P<0.001$ vs. control group; $^{\#\#}P<0.01$ and $^{\#\#\#}P<0.001$ vs. Oe-MBNL1-AS1 + mimic-NC group

The involvement of migration-related proteinase including MMP2 and MMP9 was evaluated. As shown in Fig. 2d, MBNL1-AS1 overexpression significantly decreased the mRNA and protein levels of MMP2 and MMP9 in Y79 cells. These results suggested that MBNL1-AS1 could regulate migration and invasion in Y79 cells.

MBNL1-AS1 overexpression in Y79 cells inhibited the development of Y79-induced retinoblastoma in vivo

We then evaluated whether MBNL1-AS1 overexpression could suppress the progression of retinoblastoma in vivo. As shown in Fig. 3a–d, MBNL1-AS1 overexpression significantly decreased body weight, and the size and weight of


A
Wnt4
active β-catenin
Total β-catenin
TCF4
GAPDH
Control
Oe-MBNL1-AS1
Oe-MBNL1-AS1+mimic-NC
Oe-MBNL1-AS1+miR-338-5p mimic
Relative protein expression
B
C
Tumor volume (mm3)
D
Weight (g)
E
Tumor weight (g)

◂**Fig. 6** MBNL1-AS1 overexpression inhibited wnt/β-catenin signaling pathway through downregulation of miR-338-5p. **a** Representative western blot images and summarized data showing that miR-338-5p prevented the inhibitory effect of MBNL1-AS1 on wnt/β-catenin signaling pathway. **b–e** Summarized data showing that miR-338-5p overexpression prevented the inhibitory effect of the effect of MBNL1-AS1 overexpression on Y79-induced retinoblastoma in vivo. $^{*}P<0.05$, $^{**}P<0.01$ and $^{***}P<0.001$ vs. control group; $^{\#}P<0.05$, $^{\#\#}P<0.01$ and $^{\#\#\#}P<0.001$ vs. Oe-MBNL1-AS1 + mimic-NC group

retinoblastoma. Figure 3e showed that MBNL1-AS1 overexpression significantly decreased Ki-67 and PCAN protein levels in Y79-induced retinoblastoma. Similarly, MBNL1-AS1 overexpression significantly decreased MMP2 and MMP9 protein levels in Y79-induced retinoblastoma (Fig. 3e). These results suggested that LncRNA MBNL1-AS1 overexpression in Y79 cells inhibited the development of Y79-induced retinoblastoma in vivo.

MBNL1-AS1 inhibited the development of Y79-induced retinoblastoma through downregulation of miR-338-5p

We then evaluated the potential target of MBNL1-AS1. As shown in Fig. 4a, miR-338-5p was significantly increased in human retinoblastoma cells. The relationship between MBNL1-AS1 and miR-338-5p was also evaluated using luciferase assay. As shown in Fig. 4b–d, MBNL1-AS1 significantly decreased miR-338-5p. These results suggested that LncRNA MBNL1-AS1 inhibited the development of Y79-induced retinoblastoma through downregulation of miR-338-5p.

miR-338-5p prevented the inhibitory effect of MBNL1-AS1 overexpression on proliferation and migration of Y79 cells

We then evaluated whether MBNL1-AS1 exerted its inhibitory effects through inhibition of miR-338-5p. As shown in Fig. 4e, miR-338-5p overexpression significantly increased miR-338-5p level. Functionally, miR-338-5p overexpression effectively prevented the inhibitory effect of MBNL1-AS1 on proliferation in CCK8 assay and clone formation (Fig. 4f, g). As expected, miR-338-5p overexpression prevented the inhibitory effect of MBNL1-AS1 overexpression on migration of Y79 cells in wound healing and Transwell assay (Fig. 5a, b). Furthermore, miR-339-5p mimics prevented the inhibitory effect of MBNL1-AS1 overexpression on MMP2 and MMP9 mRNA and protein levels in Y79 cells (Fig. 5d). These results suggested that miR-338-5p prevented the inhibitory effect of MBNL1-AS1 overexpression on proliferation and migration of Y79 cells.

MBNL1-AS1 overexpression inhibited wnt/β-catenin signaling pathway through downregulation of miR-338-5p

Next, we aimed to investigate the mechanisms in the MBNL1-AS1-miR-338-5p signaling pathway. As shown in Fig. 6a, MBNL1-AS1 overexpression inhibited Wnt/β-catenin signaling pathway. In the presence of miR-338-5p mimics, however, the inhibitory effect of MBNL1-AS1 was reduced. These results suggested that MBNL1-AS1 overexpression inhibited Wnt/β-catenin signaling pathway through downregulation of miR-338-5p.

miR-338-5p overexpression prevented the inhibitory effect of MBNL1-AS1 on the development of Y79-induced retinoblastoma in vivo

Finally, we investigated whether miR-338-5p overexpression could prevent the inhibitory effect of MBNL1-AS1 overexpression on Y79-induced retinoblastoma in vivo. As shown in Fig. 6b–e, miR-338-5p overexpression prevented the inhibitory effect of MBNL1-AS1 overexpression on Y79-induced retinoblastoma. Consistently, Fig. 7a, b show that miR-338-5p overexpression prevented the inhibitory effects of MBNL1-AS1 overexpression on Ki-67, PCAN and Wnt/β-catenin signaling pathway protein levels in Y79-induced retinoblastoma, indicating that MBNL1-AS1 inhibited the development of Y79-induced retinoblastoma through targeting miR-338-5p in vivo.

Discussion

First, we evaluated the expression of MBNL1-AS1 in four retinoblastoma cell lines, including ARPE-19, WER1-RB1, SO-RB and Y79 cells. We found that the expression of MBNL1-AS1 was low in all of these four cell lines. Among them, Y79 cells showed the lowest expression of MBNL1-AS1. Then Y79 cells were chosen for further experiments. This finding was consistent with a previous study, which showed that MBNL1-AS1 was significantly downregulated in bladder tumor tissues, and that MBNL1-AS1 worked as a tumor suppressor [7]. Therefore, MBNL1-AS1 may have a protective role against retinoblastoma.

Functionally, we found that MBNL1-AS1 overexpression significantly inhibited the proliferation and migration of Y79 cells. Furthermore, our in vivo study showed that MBNL1-AS1 overexpression significantly inhibited Y79 cells-induced retinoblastoma. This finding was consistent with previous studies, which showed that MBNL1-AS1 exerted anti-tumor effects in various tumor cells, such as bladder cancer [7], colon cancer [8], and lung cancer [10].

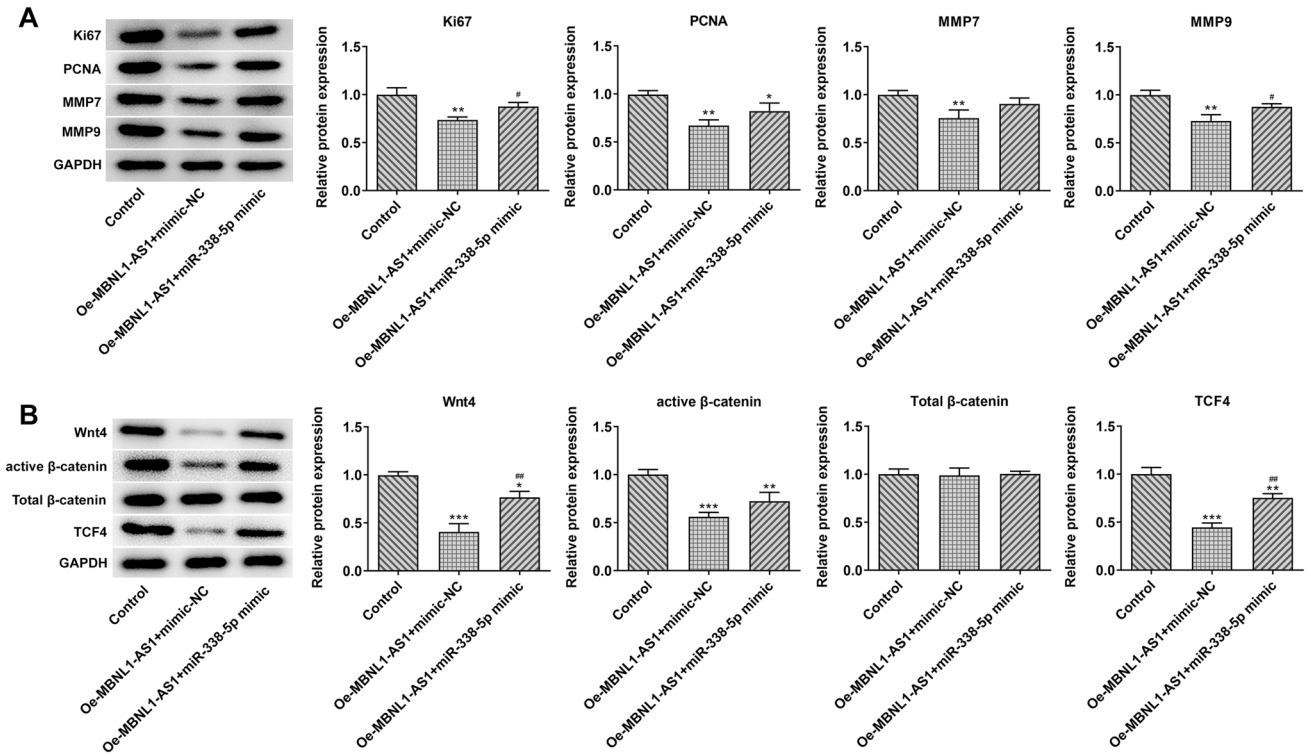


Fig. 7 miR-338-5p overexpression prevented the inhibitory effect of the effect of MBNL1-AS1 overexpression on wnt/β-catenin signaling pathway. **a** Representative western blot images (upper panel) and summarized data (lower panel) showing that miR-338-5p overexpression prevented the inhibitory effect of MBNL1-AS1 overexpression on Ki-67 and PCAN protein levels in Y79-induced retinoblastoma. **b** Representative western blot images (upper panel) and summarized data (lower panel) showing that miR-338-5p overexpression prevented the inhibitory effect of the effect of MBNL1-AS1 overexpression on wnt/β-catenin signaling pathway in Y79-induced retinoblastoma. $^{*}P<0.05$, $^{**}P<0.01$ and $^{***}P<0.001$ vs. control group; $^{\#}P<0.05$ and $^{\#\#}P<0.01$ vs. Oe-MBNL1-AS1 + mimic-NC group

Therefore, these studies suggested that MBNL1-AS1 has an anti-tumor effect in various tumor types.

Notably, we found that MBNL1-AS1 showed strong anti-tumor effects through binding to and degrading miR-338-5p. For example, Zhu et al., reported that MBNL1-AS1 could target miR-412-3p, thereby promoting the expression of MYL9 [8]. Similarly, it was reported that low expression of MBNL1-AS1 was observed in non-small cell lung cancer tissues, and that overexpression of MBNL1-AS1 significantly reduced the proliferation, migration and invasion of CSC in non-small-cell lung carcinoma (NSCLC) [10]. Therefore, MBNL1-AS1 may exert its anti-tumor effect through distinct miRNAs in different tumors. The present finding that MBNL1-AS1 could target miR-338-5p and exert its anti-tumor effects may provide an important molecular mechanism in retinoblastoma.

Finally, we evaluated the downstream effector of miR-338-5p and found that miR-338-5p promoted the development of retinoblastoma through activating Wnt/β-catenin signaling pathway. This was consistent with a previous study, which showed that serum miR-338-5p may be a potential tumor marker of retinoblastoma, and that the combination of miR-338-5p and NSE can improve the early diagnosis rate of retinoblastoma [11]. In contrast, Song et al. reported that miR-338 inhibited epithelial-mesenchymal transition (EMT) of gastric cancer cells through deactivation of Wnt/β-catenin signaling pathway [23]. Another study showed that miR-338-3p could inhibit angiogenesis by targeting metastasis associated in colon cancer 1 (MACC1), β-catenin and vascular endothelial growth factor (VEGF) in hepatocellular carcinoma [24]. These results suggested miR-338 may have a protective or detrimental role in different cancers. Of note, we provided the detailed mechanism that MBNL1-AS1 exerted anti-tumor effects by regulation of miR-338-5p/Wnt/β-catenin signaling pathway. Considering the decreased expression of MBNL1-AS in retinoblastoma and increased expression of miR-338-5p, this signaling pathway may play a critical role in the initiation and progression of retinoblastoma.

However, due to the limitation of time and funds, we have only observed that MBNL1-AS1 regulates cell proliferation, migration and invasion in Y79 cells and mice via inactivating the wnt/β-catenin signaling. Further research on the

clinical samples and other signaling pathways is needed to confirm and support the findings in our study.

Conclusion

In summary, the present study showed that MBNL1-AS1 exerts anti-tumor activity by targeting miR-338-5p, thereby decreasing Wnt/β-catenin signaling pathway. This novel finding may provide a new pharmacological target for the prevention and treatment of retinoblastoma.

Author contributions LX and WRL contributed to the conception and design of the study; LX, SYZ and ADT performed the experiments and collected the data; SYZ and ADT performed the statistical analysis; LX and SYZ completed data interpretation; LX and WRL wrote the manuscript and revised manuscript; All authors contributed to reading and revising the manuscript and approved the submitted version.

Funding Not applicable.

Availability of data and materials The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Compliance with ethical standards

Conflict of interest There are no conflict of interest in this investigation.

References

1. Hao F, Mou Y, Zhang L, Wang S, Yang Y. LncRNA AFAP1-AS1 is a prognostic biomarker and serves as oncogenic role in retinoblastoma. Biosci Rep. 2018;38(3):BSR20180384.
2. Fabian ID, Onadim Z, Karaa E, et al. The management of retinoblastoma. Oncogene. 2018;37(12):1551–60.
3. Rao R, Honavar SG. Retinoblastoma. Indian J Pediatr. 2017;84(12):937–44.
4. Wei X, Wang B, Wang Q, et al. MiR-362-5p, which is regulated by long non-coding RNA MBNL1-AS1, promotes the cell proliferation and tumor growth of bladder cancer by targeting QKI. Front Pharmacol. 2020;11:164.
5. Fu XM, Guo W, Li N, et al. The expression and function of long noncoding RNA lncRNA-ATB in papillary thyroid cancer. Eur Rev Med Pharmacol Sci. 2017;21(14):3239–46.
6. Yang G, Fu Y, Lu X, Wang M, Dong H, Li Q. LncRNA HOTAIR/miR-613/c-met axis modulated epithelial-mesenchymal transition of retinoblastoma cells. J Cell Mol Med. 2018;22(10):5083–96.
7. Wei X, Yang X, Wang B, et al. LncRNA MBNL1-AS1 represses cell proliferation and enhances cell apoptosis via targeting miR-135a-5p/PHLPP2/FOXO1 axis in bladder cancer. Cancer Med. 2020;9(2):724–36.
8. Zhu K, Wang Y, Liu L, Li S, Yu W. Long non-coding RNA MBNL1-AS1 regulates proliferation, migration, and invasion of cancer stem cells in colon cancer by interacting with MYL9 via sponging microRNA-412-3p. Clin Res Hepatol Gastroenterol. 2020;44(1):101–14.
9. Wang JH, Zhang L, Huang ST, et al. Expression and prognostic significance of MYL9 in esophageal squamous cell carcinoma. PLoS ONE. 2017;12(4):e0175280.
10. Li P, Xing W, Xu J, et al. microRNA-301b-3p downregulation underlies a novel inhibitory role of long non-coding RNA MBNL1-AS1 in non-small cell lung cancer. Stem Cell Res Ther. 2019;10(1):144.
11. Zhou P, Li X. Serum miR-338-5p has potential for use as a tumor marker for retinoblastoma. Oncol Lett. 2019;18(1):307–13.
12. Gao Y, Lu X. Decreased expression of MEG3 contributes to retinoblastoma progression and affects retinoblastoma cell growth by regulating the activity of Wnt/beta-catenin pathway. Tumour Biol. 2016;37(2):1461–9.
13. Wu T, Wang LN, Tang DR, Sun FY. SOST silencing promotes proliferation and invasion and reduces apoptosis of retinoblastoma cells by activating Wnt/beta-catenin signaling pathway. Gene Ther. 2017;24(7):399–407.
14. Yang M, Li Y, Wei W. MicroRNA-188-5p promotes epithelial-mesenchymal transition by targeting ID4 through Wnt/betacatenin signaling in retinoblastoma. Onco Targets Ther. 2019;12:10251–62.
15. Oshikawa M, Tsutsui C, Ikegami T, et al. Full-length transcriptome analysis of human retina-derived cell lines ARPE-19 and Y79 using the vector-capping method. Invest Ophthalmol Vis Sci. 2011;52(9):6662–70.
16. Liu Y, Hu H, Liang M, et al. Regulated differentiation of WERI-Rb-1 cells into retinal neuron-like cells. Int J Mol Med. 2017;40(4):1172–84.
17. Xu L, Li W, Shi Q, et al. MicroRNA936 inhibits the malignant phenotype of retinoblastoma by directly targeting HDAC9 and deactivating the PI3K/AKT pathway. Oncol Rep. 2020;43(2):635–45.
18. Ju C, Zhou R, Sun J, et al. LncRNA SNHG5 promotes the progression of osteosarcoma by sponging the miR-212-3p/SGK3 axis. Cancer Cell Int. 2018;18:141.
19. Li J, Guo Y, Duan L, et al. AKR1B10 promotes breast cancer cell migration and invasion via activation of ERK signaling. Oncotarget. 2017;8(20):33694–703.
20. Wu XZ, Cui HP, Lv HJ, Feng L. Knockdown of lncRNA PVT1 inhibits retinoblastoma progression by sponging miR-488–3p. Biomed Pharmacother. 2019;112:108627.
21. Huo Y, Wang Q, Liu Y, et al. A temperature-sensitive phase-change hydrogel of topotecan achieves a long-term sustained antitumor effect on retinoblastoma cells. Onco Targets Ther. 2019;12:6069–82.
22. Aldred SF, Collins P, Trinklein N. Identifying targets of human micrornas with the LightSwitch Luciferase Assay System using 3'UTR-reporter constructs and a microRNA mimic in adherent cells. J Vis Exp. 2011; (55).
23. Song B, Lin HX, Dong LL, Ma JJ, Jiang ZG. MicroRNA-338 inhibits proliferation, migration, and invasion of gastric cancer cells by the Wnt/beta-catenin signaling pathway. Eur Rev Med Pharmacol Sci. 2018;22(5):1290–6.
24. Zhang T, Liu W, Zeng XC, et al. Down-regulation of microRNA-338–3p promoted angiogenesis in hepatocellular carcinoma. Biomed Pharmacother. 2016;84:583–91.