

LncRNA MT1JP acts as a tumor inhibitor via reciprocally regulating Wnt/ β -Catenin pathway in retinoblastoma

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Abstract. – **OBJECTIVE:** The aim of this study was to investigate the roles of MT1JP and β -catenin in retinoblastoma.

PATIENTS AND METHODS: We performed quantitative Reverse Transcriptase-Polymerase Chain Reaction (qRT-PCR) to quantify the expressions of MT1JP and β -catenin in 44 retinoblastoma tissues and matched non-tumor tissues. What's more, retinoblastoma cell lines were transfected with pcDNA3.1-MT1JP, after which proliferation, cell cycle, apoptosis, and expression of β -catenin as well as its downstream targets were assayed. We also conducted TOP-Flash reporter assay to explore the activity of Wnt/ β -catenin signaling pathway.

RESULTS: The results revealed that MT1JP was down-regulated, while β -catenin was highly expressed in retinoblastoma cells. Meanwhile, the forced expression of MT1JP impaired the expression of the β -catenin protein and its downstream targets such as cyclin D1, c-myc.

CONCLUSIONS: We demonstrated that MT1JP was a tumor suppressor by negatively modulating the activity of the Wnt/ β -catenin signaling pathway in the development of retinoblastoma and might function as a prognostic biomarker and therapeutic target.

Key Words:

Retinoblastoma, MT1JP, β -catenin signaling, EMT.

Introduction

Retinoblastoma (RB) is a relatively peculiar malignant tumor in childhood¹. As the commonest pediatric intraocular malignant cancer, the mortality rate of RB reached 50%-70% in under-developed countries^{2,3}. Children suffering from RB are facing various threats including RB cell

invasion, malignant transformation of intracranial neuroblastoma, heterochronous tumor, and so on⁴. In spite of the rapid development of RB treatment recently, the survival rate remains very low mainly because of the early diagnostic limitations of the disease⁵. The traditional therapies for RB, such as external beam radiotherapy, or chemotherapy, have side effects, including infections, blindness, etc^{6,7}. Therefore, in order to avoid the side effects above, it is urgent to study the biological functions and molecular mechanisms of RB.

LncRNAs are a class of RNA transcripts without protein-coding abilities and with the length of more than 200 nucleotides⁸. It is well-known that lncRNAs play crucial roles in a variety of biological function processes, such as cellular differentiation, growth, and apoptosis⁹⁻¹¹. Many lncRNAs are abnormally expressed in breast cancer¹², lung cancer¹³, and esophageal carcinoma¹⁴. LncRNAs are also ectopically expressed in different tumors and exert critical functions in cancer biology^{15,16}. MT1JP, a kind of lncRNA, seems to play a crucial role in RB progression. Quantitative Reverse Transcriptase-Polymerase Chain Reaction (qRT-PCR) analysis was used to detect the expression of MT1JP, finding that MT1JP had a lower expression level in RB tissues and cell lines than in the corresponding normal ones.

β -catenin is an important factor in the carcinogenesis of many cancers. For instance, the over-expression of Wnt/ β -catenin pathway member is a common CRC feature¹⁷; highly expressed long non-coding RNA NNT-AS1 promotes cell proliferation and invasion through Wnt/ β -catenin signaling pathway in cervical cancer¹⁸; lncRNA

PTCSC3/miR-574-5p governs cell proliferation and migration of papillary thyroid carcinoma via Wnt/ β -catenin signaling¹⁹; long noncoding RNA CRNDE activates Wnt/ β -catenin signaling pathway through acting as a molecular sponge of microRNA-136 in human breast cancer²⁰.

This study aimed to investigate the molecular mechanism of MT1JP, through which MT1JP modulated the progress of RB cells, and the possible reciprocal relationship between MT1JP and β -catenin signaling. We also explored the effects of MT1JP overexpression on the downstream targets of Wnt/ β -catenin pathway including cyclin D1 and c-Myc. Our data disclosed that the up-regulation of lncRNA MT1JP inhibited the malignant growth and invasion of RB cells through interdicting Wnt/ β -catenin signaling pathway.

Patients and Methods

Tissue Samples

Forty-four retinoblastoma tissues and matched non-tumor tissues were gathered from patients having suffered from enucleation in the Second

Affiliated Hospital, Mudanjiang College of Medicine, Mudanjiang, China. No patients had undergone chemotherapy or radiotherapy before the surgical resection. This study was approved by the Ethical Committee of the Second Affiliated Hospital, Mudanjiang College of Medicine. Each patient was provided with written informed consents. Whole surgical specimens were verified by pathologists and then stored in -80°C till the moment of being used. The traits of patients are shown in Table I.

Cell Culture

Human retinoblastoma cell lines Weri-Rb1 and Y79 were purchased from the Institute of Biochemistry and Cell Biology at the Chinese Academy of Sciences (Shanghai, China). Cell lines were cultured in Roswell Park Memorial Institute 1640 (RPMI-1640) medium (Gibco, Rockville, MD, USA) with 10% of fetal bovine serum (FBS, Gibco, Rockville, MD, USA) at 37°C in a humidified incubator with 5% of CO_2 .

Cell Transfection

Cells were transfected with a pcDNA3.1 vector for MT1JP and siRNA for MT1JP respectively

Table I. Correlation between MT1JP expression and clinical features (n = 44).

Variable	MT1JP expression		p-value
	Low	High	
Age			0.563
> 2.5	12	11	
≤ 2.5	9	12	
Gender			0.763
Male	11	10	
Female	10	13	
Laterality			0.547
Unilateral	12	10	
Bilateral	9	13	
Differentiation			0.563
Well and moderately	9	12	
Poor and undifferentiated	12	11	
IIRC stage			0.033*
Early stage (A, B, C)	14	7	
Advanced stage (D, E)	7	16	
Optic Nerve Invasion			0.019*
Negative	15	8	
Positive	6	15	
Nodal or distant metastasis			0.006*
Negative	15	6	
Positive	6	17	

Low/high by the sample median. Pearson χ^2 -test. $p < 0.05$ was considered statistically significant.

by using Lipofectamine 2000 (Invitrogen, CA, Carlsbad, USA) on the basis of the manufacturer's guidelines. At 48 h after transfection, cells were reaped for the following experiments including quantitative Reverse Transcriptase-Polymerase Chain Reaction (qRT-PCR), Western blot, proliferation assays, and Flow Cytometry. RNA oligonucleotides were purchased from GenePharma (Shanghai, China).

RNA Extraction, and qRT-PCR

All RNAs were isolated by using the TRIzol reagent (TaKaRa, Otsu, Shiga, Japan), followed by the reverse transcription into cDNA by means of the One Step PrimeScript Kit (TaKaRa, Otsu, Shiga, Japan), in accordance with the manufacturer's instructions. Then, qRT-PCR was applied to quantify the expression of MT1JP with the SYBRP remix Ex Taq kit (TaKaRa, Otsu, Shiga, Japan) on CFX96 Real-Time PCR Detection System (Bio-Rad, Hercules, CA, USA). The relative expression was evaluated by using the comparative cycle threshold method ($2^{-\Delta\Delta CT}$), and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was served as an internal normalized reference. The primers for MT1JP and GAPDH were constructed by Generay Biotech (Shanghai, China), which were listed as the following: MT1JP-F: 5'-GGGACTCCTTTACTTCCTTGG-3' and MT1JP-R: 5'-CCTTGAGCCTCAGTATCCTTAAC-3'; GAPDH-F: 5'-TGCCATCAATGACCCCTTC-3' and GAPDH-R: 5'-CATCGC-CCCACTTGATTTTG-3';

Western Blot Analysis

Phosphate-buffered saline (PBS) was used to wash transfected cells for twice, followed by the lysis in radioimmunoprecipitation assay (RIPA) lysis buffer (Thermo Scientific, Waltham, MA, USA) with a protease inhibitor cocktail (Roche, Basel, Switzerland) for 30 min on ice. Then, an equal quantity of protein (50 μ g) was split by 10% of sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE), followed by the transfer onto a polyvinylidene difluoride (PVDF) membrane (Roche, Basel, Switzerland) by electro-blotting. Membranes were blocked for 1 h in Tris-buffered saline and Tween (TBS-T) buffer with 5% of skim milk in room temperature, followed by the cultivation with primary antibodies against β -catenin and β -actin (1:1000, Cell Signaling Technology, Danvers, MA, USA) overnight at 4°C. Subsequently, they were washed and cultivated for 2 h with HRP Goat-anti-Rab-

bit antibody (1:2000; Santa Cruz Biotechnology, Santa Cruz, CA, USA). Protein signals were detected with enhanced chemiluminescence reagent (ECL, Thermo Scientific, Waltham, MA, USA) based on the manufacturer's instructions.

Proliferation Assays

Cell viability was detected with Cell Proliferation Reagent Kit I (MTT) (Roche Applied Science). pcDNA-MT1JP-transfected Weri-Rb1 and Y79 cells (3,000/well) were allowed to grow in 96-well plates. Cell viability was documented every 24 h in accordance with the manufacturer's suggestions. All experiments were carried out in triplicate. For the colony formation assay, 500 cells were put in a fresh six-well plate and kept in media complemented with 10% of FBS, taking the place of the medium every 4 days. After 14 days, cells were fixed with methanol, followed by the staining of 0.1% of crystal violet (Sigma-Aldrich, St. Louis, MO, USA). Visible colonies were manually calculated. For each group cells were examined in triplicate.

Transwell Assays

For the transwell assays, after 48 h of post-transfection, 5×10^4 (migration) or 1×10^5 (invasion) cells in serum-free media were placed into the upper part of an insert (8- μ m pore size; Millipore, Billerica, MA, USA). Medium with 10% of FBS was added to the lower part. After incubation for 24 h, the cells remaining on the upper part were removed with cotton wool, while the cells having migrated or invaded through the membrane were stained with methanol and 0.1% of crystal violet, imaged, and counted using an IX71 inverted microscope (Olympus, Tokyo, Japan). Experiments were independently repeated three times.

Cell Cycle Assay

Transfected Weri-Rb1 and Y79 cells were cultivated in six-well plates. For cell cycle assay, the cells were reaped and fixed in 70% of ethanol at -20°C overnight. Fixed cells were washed with PBS and incubated in 400 μ L PBS, 50 μ L RNase (1 mg/mL) and 10 μ L propidium iodide (PI) (2 mg/mL, Keygen Biotechnology, Nanjing, China) for 30 min at room temperature, followed by flow cytometry analysis (FACScan). The experiments were carried out in triplicate.

Apoptosis Assay

Cells transfected with pcDNA-MT1JP for 48 h were resuspended at a concentration of $1 \times$

10^6 cells/mL. After double staining with 5 μ L of propidium iodide (PI) and 5 μ L of Annexin V-FITC (fluorescein isothiocyanate) from the FITC-AnnexinV Apoptosis Detection Kit (BD Biosciences, San Diego, CA, USA), cells were analyzed with a BD FACS Calibur™ flow cytometer within 1 h. Data were analyzed with FlowJo (Tree Star, San Carlos, CA, USA).

Wnt/ β -Actin Activity Luciferase Assay

Cells were seeded in 24-well plates and cotransfected with the TOP/FOP Flash expression plasmids (Biovector, Richmond, Canada) and Renilla TK-luciferase vector (Promega, Madison, WI, USA) as a control. 48 h after transfection, the cells were harvested and lysed for the luciferase assay.

Statistical Analysis

Data were statistically analyzed by using Statistical Product and Service Solutions (SPSS) 13.0 software (SPSS Inc., Chicago, IL, USA). All experiments were repeated in triplicate, and data were exhibited as mean $\pm$ SEM. Differences were assessed by two-tailed Student's *t*-test, and *p* < 0.05 was considered statistically significant.

Results

MT1JP is Down-Regulated in RB Tissues and Cell Lines

To determine the biological functions of MT1JP, we performed qRT-PCR to detect the ex-

pression of MT1JP in RB tissues. As shown in Figure 1A, the expression of MT1JP was lower in RB tissues than that in the paired adjacent normal tissues. Besides, the expression of MT1JP in RB cell lines (WERI-Rb1, Y79) was also much more down-regulated than that in healthy ones (ARPE-19, HRMEC), indicating that MT1JP might function as a tumor suppressor in RB development (Figure 1B).

Low Expression of MT1JP is Related to Poor Prognosis in Patients With RB

Kaplan-Meier method analysis (log-rank test) was conducted to figure out the relationship between MT1JP expression and overall survival in patients with RB. Patients with low expression of MT1JP suffered from poorer overall survival than those with high expression of MT1JP (Figure 2). Additionally, the expression of MT1JP was found to be associated with IIRC stage, optic nerve invasion, and nodal or distant metastasis (Table I). Proportional hazards method analysis exposed that down-regulated MT1JP could be explored as an independent prognostic factor, in addition to laterality (Table II). These results demonstrated that MT1JP played a key role in RB growth and might be considered to be a specific biomarker for RB prognosis.

Up-Regulated MT1JP Suppresses the Proliferation of RB Cells Via Inducing Cell Cycle and Apoptosis Variations

To better comprehend the effect of MT1JP on RB development, the expression of MT1JP was

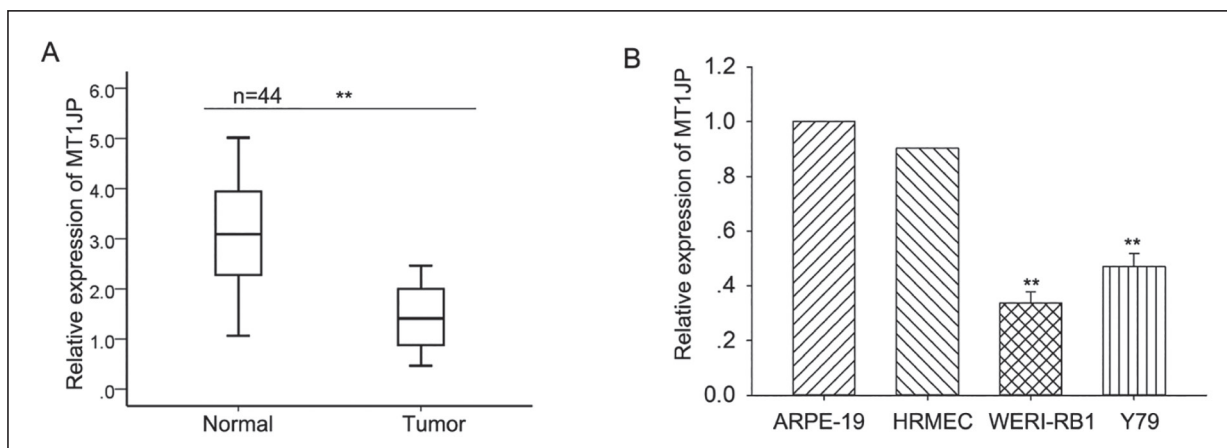


Figure 1. MT1JP is down-regulated in RB tissues and cell lines. **A**, The expression of MT1JP in RB tissues and in the adjacent normal tissues. **B**, The expression of MT1JP in two RB cell lines (WERI-Rb1, Y79) and in two normal RB cell lines (ARPE-19, HRMEC). Error bars represented the mean $\pm$ SD of at least three independent experiments. ***p* < 0.01 vs. control group.

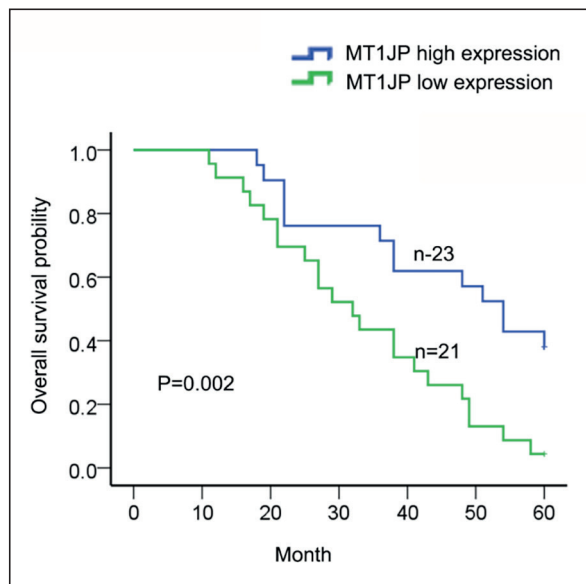


Figure 2. Kaplan-Meier method analysis (log-rank test) was carried out to detect the relationship between MT1JP expression level and overall survival in patients with RB ($p = 0.002$).

improved. As illustrated in Figure 3A, WERI-Rb1 and Y79 cells were transfected with pcDNA-MT1JP, followed by qRT-PCR analysis after 48h of transfection. To assess the biological effect of MT1JP on RB, we performed MTT assay to explore the effect of up-regulated MT1JP on cell proliferation. It was proved in Figure 3B that cell viability was distinctly decreased in pcDNA-MT1JP-transfected WERI-Rb1 and Y79 cells. Sim-

ilarly, the colony-formation assays showed that clonogenic survival rate was impaired after MT1JP was over-expressed in WERI-Rb1 and Y79 cells compared with that in the negative control (Figure 3C). To further confirm the repressive function of over-expressed MT1JP on RB progress, flow cytometry analysis of cell cycle was performed, suggesting that enhanced MT1JP increased cell proportion in G0/G1 phase and decreased that in S phase compared with that in the negative control (Figure 3D). Next, we examined the effect of MT1JP expression on the apoptosis changes. RB cells were transiently transfected with pcDNA-MT1JP for 48 h, and then were stained with Annexin V/PI. Compared to the negative control, the data presented an increased number of Annexin V+ (total apoptosis), Annexin V+/PI- (early apoptosis), and Annexin V+/PI+ (late apoptosis) cells after the enrichment of MT1JP (Figure 3E). The results revealed that MT1JP could inhibit the proliferation of RB cells through influencing the cell cycle and apoptosis.

Up-Regulated MT1JP Represses Cell Migration and Invasion Via Triggering EMT Alteration

Transwell assays were applied to define the effect of MT1JP expression on cell migration and invasion, exposing that up-regulated MT1JP reduced the migratory and invasive abilities of WERI-Rb1 and Y79 cells in comparison with the negative control (Figure 4A-B). To make sure the mechanism how MT1JP caused cell migration

Table II. Multivariate analysis of prognostic parameters in patients with MT1JP by Cox regression analysis.

Variable	Category	p-value
Age	> 2.5	0.510
	≤ 2.5	
Gender	Male	0.286
	Female	
Laterality	Unilateral	0.039*
	Bilateral	
Differentiation	Well and moderately	0.182
	Poor and undifferentiated	
IIRC stage	Early stage (A, B, C)	0.632
	Advanced stage (D, E)	
Optic nerve invasion	Negative	0.854
	Positive	
Nodal or distant metastasis	Negative	0.346
	Positive	
MT1JP	Low	0.008*
	High	

Proportional hazards method analysis showed a positive, independent prognostic importance of MT1JP expression ($p = 0.006$).

* $p < 0.05$ was considered statistically significant.

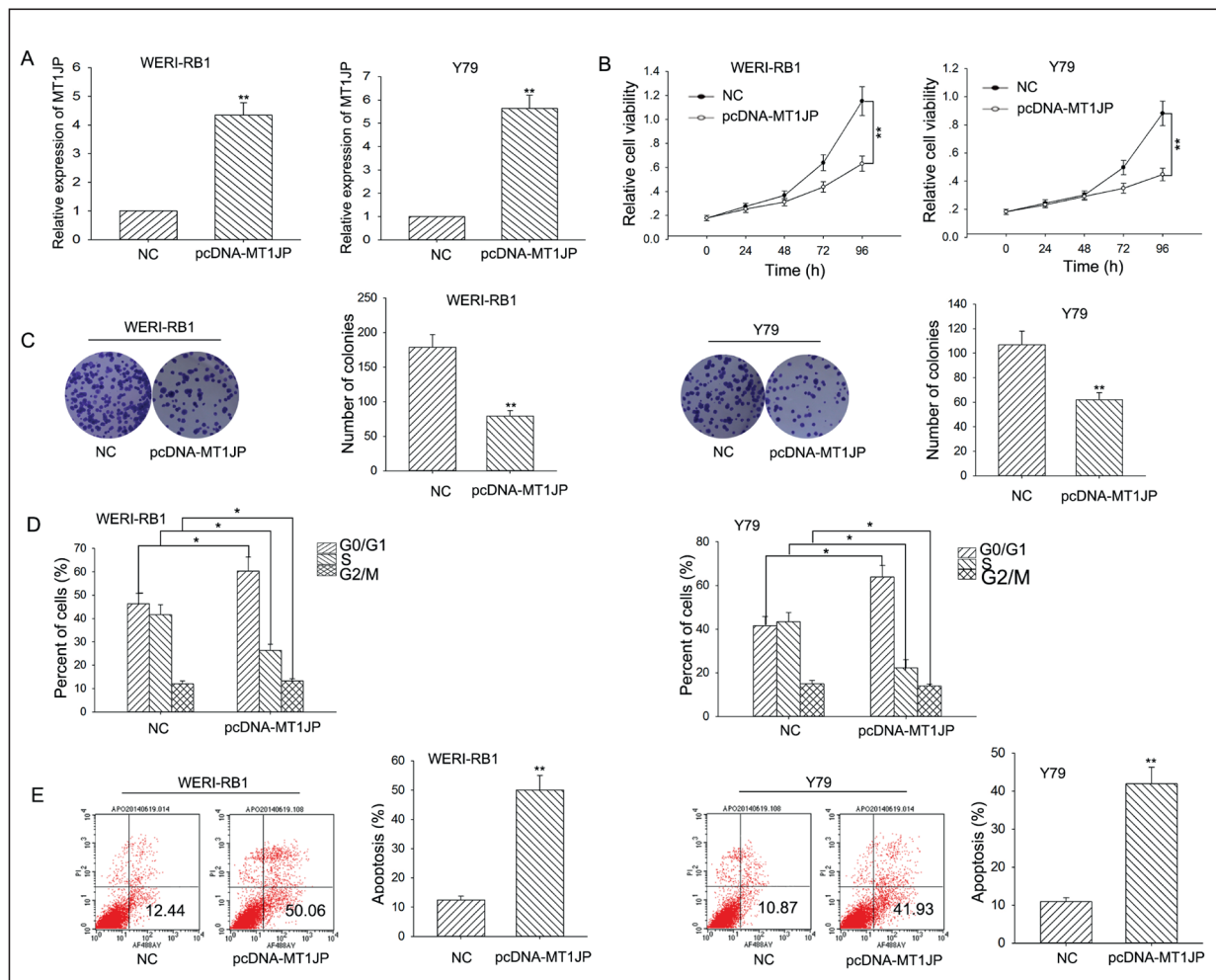


Figure 3. Up-regulation of MT1JP suppresses the proliferation of RB cells *via* variations in cell cycle and apoptosis. **A**, qRT-PCR analyzed the expression of MT1JP transfected with pcDNA-MT1JP in two RB cell lines (WERI-Rb1, Y79). **B**, MTT assay investigated the effect of the overexpression of MT1JP on RB cells proliferation. **C**, The colony-formation assay detected the clonogenic survival in RB cells transfected with pcDNA-MT1JP. **D-E**, Flow cytometry analysis of cell cycle and apoptosis demonstrated the effect of overexpression of MT1JP on RB cells cycle and apoptosis. Error bars represented the mean $\pm$ SD of at least three independent experiments. * $p < 0.05$, ** $p < 0.01$ vs. control group.

and invasion changes, Western blot assays were carried out to determine the expression changes of EMT-related proteins after the transfection of pcDNA-MT1JP. As exhibited in Figure 4C, E-cadherin was markedly up-regulated while N-cadherin was greatly down-regulated after the improvement of MT1JP, compared to the negative control. These findings explained that MT1JP accelerated cell migration and invasion *via* causing EMT phenotype changes.

MT1JP is Negatively Associated With β -Catenin in RB

β -catenin had been widely reported to involve in tumorigenesis with different genes. To

determine whether MT1JP could also interact with β -catenin in RB, we firstly detect the expression of β -catenin in RB tissues. It turned out that β -catenin was more highly expressed in RB tissues than in healthy ones (Figure 5A). Considering the expression of MT1JP in RB, we hypothesized that MT1JP was negatively correlated with β -catenin, further proved by Spearman's Correlation analysis (Figure 5B). qRT-PCR was designed to definitely determine the reverse relationship between such two factors. Obviously, transfection with pcDNA-MT1JP enhanced the expression of MT1JP, but decreased the expression of β -catenin (Figure 5C). On the contrary, the over-expression of MT1JP yielded

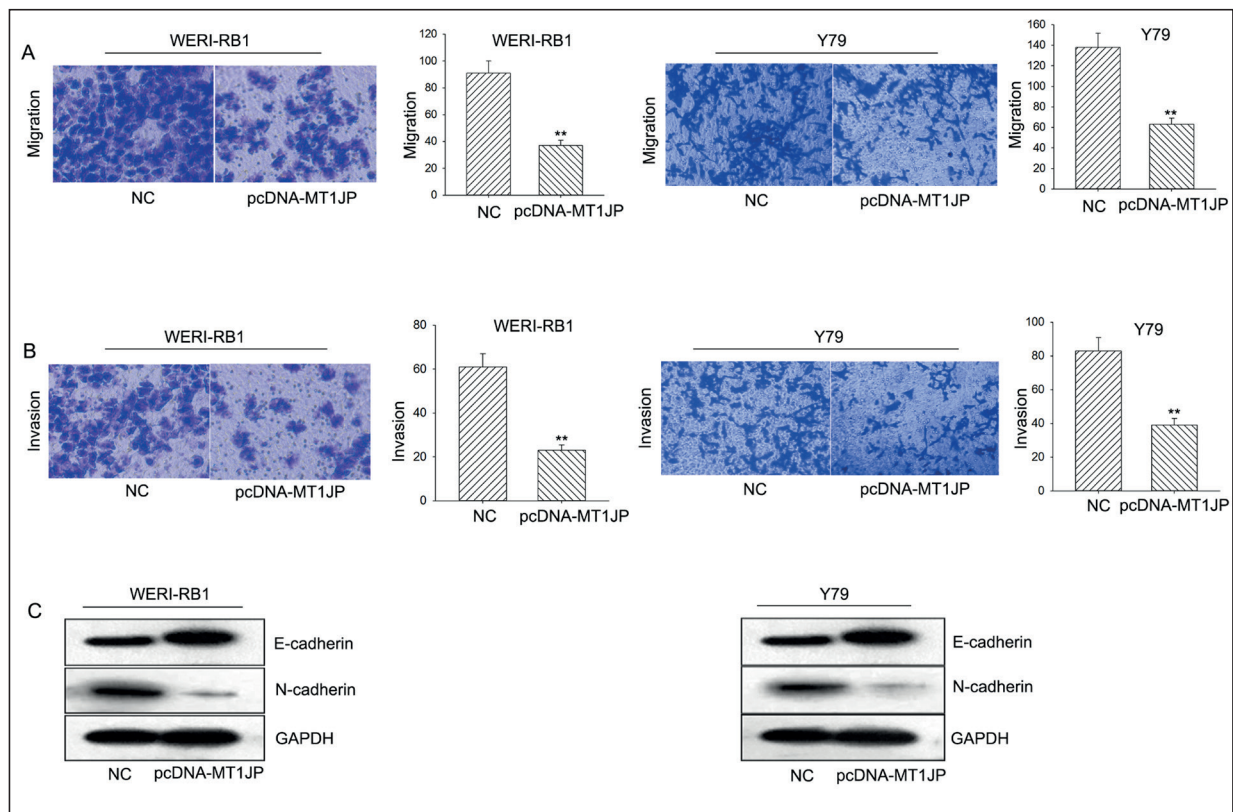


Figure 4. Up-regulation of MT1JP blocks the metastasis and EMT progress. *A-B*, Transwell assays confirmed the repressive functions of up-regulation of MT1JP on the migration and invasion of RB cells. *C*, Western blot assays indicated the effect of overexpression of MT1JP on EMT transformation. Error bars represented the mean $\pm$ SD of at least three independent experiments. ** $p < 0.01$ vs. control group.

the opposite results (Figure 5D). Such data indicated the negative correlation between MT1JP and β -catenin.

MT1JP Suppresses the Malignancy of Retinoblastoma Through Regulating Wnt/ β -Catenin Pathway

To explore the molecular mechanisms by which MT1JP attenuated the growth of RB cell lines, we firstly examined the effect of MT1JP on the luciferase activity of β -catenin. It was disclosed that the up-regulation of MT1JP decreased the luciferase activity of β -catenin, implying the interaction of MT1JP with β -catenin (Figure 6A). Subsequently, Western blot was applied to further demonstrate the interaction between MT1JP and β -catenin pathway. It was shown that enriched MT1JP attenuated the protein levels of the downstream targets of β -catenin including Cyclin D1 and c-Myc, while impaired MT1JP exerted the opposite results (Figure 6B-6C). These findings

suggested that MT1JP suppressed the tumorigenesis of RB *via* regulating Wnt/ β -catenin signaling pathway.

Discussion

An accumulating number of long non-coding RNAs have been discovered with the advances in the next-generation sequencing technology²¹. The expression of MALAT1, a type of lncRNA, is deregulated in a variety of cancers²¹. For example, MALAT1 was first found to be highly expressed in hepatoblastoma compared to hepatocellular carcinomas²². Soon after, it was reported²³⁻²⁶ that MALAT1 was also up-regulated in colon, breast, prostate, and non-small cell lung cancers. In this study, we discovered that lncRNA MT1JP seemed to play a critical role in repressing tumors. Our results verified that MT1JP was

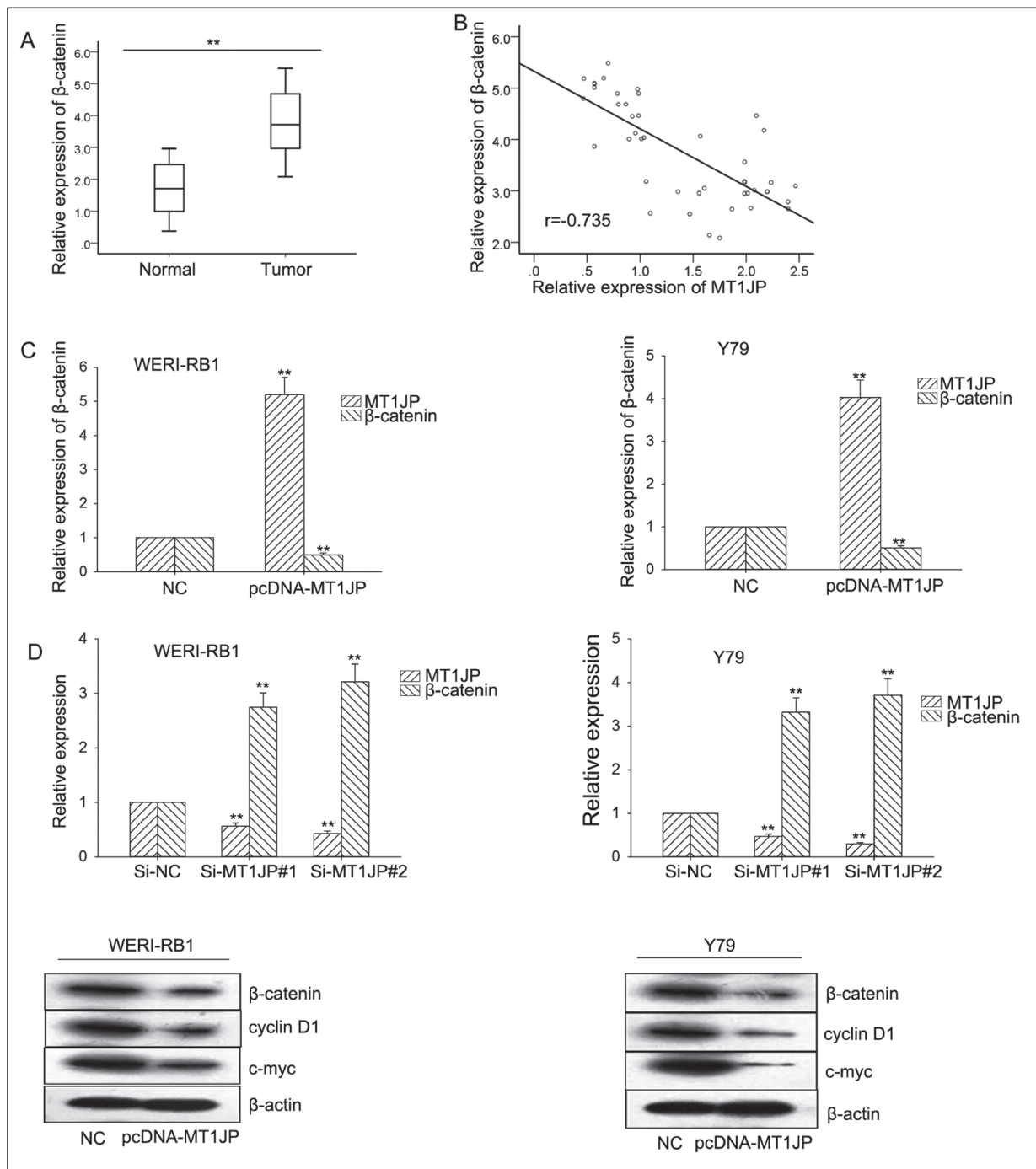


Figure 5. MT1JP is negatively associated with β -catenin in RB. **A**, The expression of β -catenin in RB tissues and normal tissues based on qRT-PCR. **B**, Spearman's correlation analysis determined the association of MT1JP with β -catenin. **C-D**, qRT-PCR determined the effect of MT1JP transfection on the expression of β -catenin. Error bars represented the mean $\pm$ SD of at least three independent experiments. ** $p < 0.01$ vs. control group.

quite low-expressed in tumor tissues and cell lines than in adjacent normal ones. In addition, the over-expression of MT1JP markedly hindered the proliferation, metastasis, inva-

sion, as well as the EMT progression of RB cells. Furthermore, the up-regulation of MT1JP induced cell cycle arrest in G0/G1 phase and increased cell apoptosis.

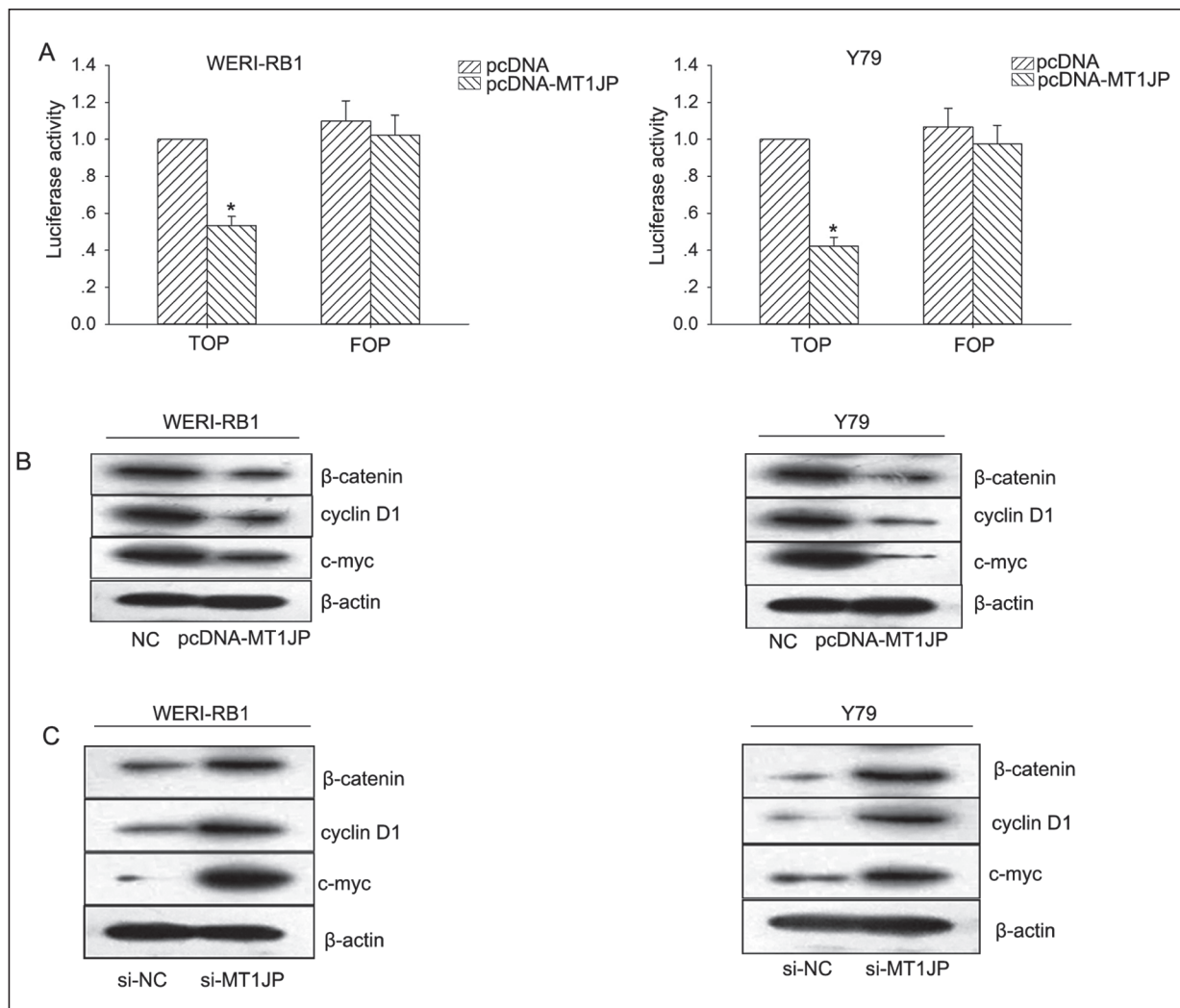


Figure 6. MT1JP inhibits RB growth *via* regulating β -catenin. **A**, Wnt/ β -actin activity luciferase assay was used to prove the interaction between MT1JP and β -catenin. **B-C**, Western blot was applied to detect the effect of MT1JP transfection on the protein levels of the downstream targets of β -catenin. Error bars represented the mean $\pm$ SD of at least three independent experiments. * $p < 0.05$ vs. control group.

It is well-known that Wnt/ β -catenin signaling can regulate a series of cellular processes, including proliferation, invasion, and metastasis¹. β -catenin can regulate the ability of multifunctional β -catenin protein, which is a pivotal signaling molecule in the Wnt/ β -catenin signaling pathway²⁷. Moreover, Wnt/ β -catenin signaling activity has been exhibited to be referred to multiple human cancers including the regulation of cancer stem cells in retinoblastoma²⁸⁻³¹. It has been related that the repression of the Wnt/ β -catenin signaling contributes to inhibit retinoblastoma cell proliferation³². And Wnt/ β -catenin is also a molecular pathway which transfers inhibitive signals to Y79 cells from indomethacin³³. All the

data above elucidated that Wnt/ β -catenin signaling is quite significant in RB cell proliferation and apoptosis.

Although both MT1JP and β -catenin could affect the progression of RB, the molecular mechanism underlying the changes of RB cells still remained unclear. In this study, we realized that both the forced and silenced expressions of MT1JP could influence the expression of β -catenin as well as its downstream targets such as cyclin D1 and c-myc. And we also found a reciprocal relationship between MT1JP and β -catenin. Thus, we determined that the ectopic expression of lncRNA-MT1JP inhibited RB cell growth and metastasis *via* blocking Wnt/ β -catenin signaling pathway.

Acknowledgements

The authors thanked all the laboratory members.

Conflict of Interest

The Authors declare that they have no conflict of interests.

References

- GAO Y, LU X. Decreased expression of MEG3 contributes to retinoblastoma progression and affects retinoblastoma cell growth by regulating the activity of Wnt/ β -catenin pathway. *Tumour Biol* 2016; 37: 1461-1469.
- SHIELDS CL, SHIELDS JA. Retinoblastoma management: Advances in enucleation, intravenous chemoreduction, and intra-arterial chemotherapy. *Curr Opin Ophthalmol* 2010; 21: 203-212.
- SHIELDS CL, SHIELDS JA. Basic understanding of current classification and management of retinoblastoma. *Curr Opin Ophthalmol* 2006; 17: 228-234.
- JABBOUR P, CHALOUHI N, TJOU MAKARIS S, GONZALEZ LF, DUMONT AS, CHITALE R, ROSENWASSER R, BIANCIOTTO CG, SHIELDS C. Pearls and pitfalls of intraarterial chemotherapy for retinoblastoma. *J Neurosurg Pediatr* 2012; 10: 175-181.
- ZHANG H, ZHONG J, BIAN Z, FANG X, PENG Y, HU Y. Long non-coding RNA CCAT1 promotes human retinoblastoma SO-RB50 and Y79 cells through negative regulation of miR-218-5p. *Biomed Pharmacother* 2017; 87: 683-691.
- LIN P, O'BRIEN JM. Frontiers in the management of retinoblastoma. *Am J Ophthalmol* 2009; 148: 192-198.
- GOMBOS DS, CHEVEZ-BARRIOS AP. Current treatment and management of retinoblastoma. *Curr Oncol Rep* 2007; 9: 453-458.
- PONTING CP, BELGARD TG. Transcribed dark matter: Meaning or myth? *Hum Mol Genet* 2010; 19: R162-R168.
- BATISTA PJ, CHANG HY. Long noncoding RNAs: Cellular address codes in development and disease. *Cell* 2013; 152: 1298-1307.
- LI N, GAO WJ, LIU NS. LncRNA BCAR4 promotes proliferation, invasion and metastasis of non-small cell lung cancer cells by affecting epithelial-mesenchymal transition. *Eur Rev Med Pharmacol Sci* 2017; 21: 2075-2086.
- PONTING CP, OLIVER PL, REIK W. Evolution and functions of long noncoding RNAs. *Cell* 2009; 136: 629-641.
- LIU H, LI J, KOIRALA P, DING X, CHEN B, WANG Y, WANG Z, WANG C, ZHANG X, MO YY. Long non-coding RNAs as prognostic markers in human breast cancer. *Oncotarget* 2016; 7: 20584-20596.
- WU Y, LIU H, SHI X, YAO Y, YANG W, SONG Y. The long non-coding RNA HNF1A-AS1 regulates proliferation and metastasis in lung adenocarcinoma. *Oncotarget* 2015; 6: 9160-9172.
- ZHANG H, LUO H, HU Z, PENG J, JIANG Z, SONG T, WU B, YUE J, ZHOU R, XIE R, CHEN T, WU S. Targeting WISP1 to sensitize esophageal squamous cell carcinoma to irradiation. *Oncotarget* 2015; 6: 6218-6234.
- TAFT RJ, PANG KC, MERCER TR, DINGER M, MATTICK JS. Non-coding RNAs: Regulators of disease. *J Pathol* 2010; 220: 126-139.
- GIBB EA, BROWN CJ, LAM WL. The functional role of long non-coding RNA in human carcinomas. *Mol Cancer* 2011; 10: 38.
- SAMUELS Y, VELCULESCU VE. Oncogenic mutations of PIK3CA in human cancers. *Cell Cycle* 2004; 3: 1221-1224.
- HUA F, LIU S, ZHU L, MA N, JIANG S, YANG J. Highly expressed long non-coding RNA NNT-AS1 promotes cell proliferation and invasion through Wnt/ β -catenin signaling pathway in cervical cancer. *Biomed Pharmacother* 2017; 92: 1128-1134.
- WANG X, LU X, GENG Z, YANG G, SHI Y. LncRNA PTCSC3/miR-574-5p governs cell proliferation and migration of papillary thyroid carcinoma via Wnt/ β -Catenin signaling. *J Cell Biochem* 2017; 118: 4745-4752.
- HUAN J, XING L, LIN Q, XUI H, QIN X. Long noncoding RNA CRNDE activates Wnt/ β -catenin signaling pathway through acting as a molecular sponge of microRNA-136 in human breast cancer. *Am J Transl Res* 2017; 9: 1977-1989.
- LIU L, YUE H, LIU Q, YUAN J, LI J, WEI G, CHEN X, LU Y, GUO M, LUO J, CHEN R. LncRNA MT1JP functions as a tumor suppressor by interacting with TIAR to modulate the p53 pathway. *Oncotarget* 2016; 7: 15787-15800.
- LUO JH, REN B, KERYANOV S, TSENG GC, RAO UN, MONGA SP, STROM S, DEMETRIS AJ, NALESNIK M, YU YP, RANGANATHAN S, MICHALOPOULOS GK. Transcriptomic and genomic analysis of human hepatocellular carcinomas and hepatoblastomas. *Hepatology* 2006; 44: 1012-1024.
- LIN R, MAEDA S, LIU C, KARIN M, EDGINGTON TS. A large noncoding RNA is a marker for murine hepatocellular carcinomas and a spectrum of human carcinomas. *Oncogene* 2007; 26: 851-858.
- Ji P, DIEDERICH S, WANG W, BOING S, METZGER R, SCHNEIDER PM, TIDOW N, BRANDT B, BUERGER H, BULK E, THOMAS M, BERDEL WE, SERVE H, MULLER-TIDOW C. MALAT-1, a novel noncoding RNA, and thymosin β 4 predict metastasis and survival in early-stage non-small cell lung cancer. *Oncogene* 2003; 22: 8031-8041.
- GUFFANTI A, IACONO M, PELUCCHI P, KIM N, SOLDA G, CROFT LJ, TAFT RJ, RIZZI E, ASKARIAN-AMIRI M, BONNAL RJ, CALLARI M, MIGNONE F, PESOLE G, BERTALOT G, BERNARDI LR, ALBERTINI A, LEE C, MATTICK JS, ZUCCHI I, DE BELLIS G. A transcriptional sketch of a primary human breast cancer by 454 deep sequencing. *Bmc Genomics* 2009; 10: 163.
- REN S, WANG F, SHEN J, SUN Y, XU W, LU J, WEI M, XU C, WU C, ZHANG Z, GAO X, LIU Z, HOU J, HUANG J,

- SUN Y. Long non-coding RNA metastasis associated in lung adenocarcinoma transcript 1 derived miniRNA as a novel plasma-based biomarker for diagnosing prostate cancer. *Eur J Cancer* 2013; 49: 2949-2959.
- 27) MACDONALD BT, TAMAI K, HE X. Wnt/beta-catenin signaling: Components, mechanisms, and diseases. *Dev Cell* 2009; 17: 9-26.
- 28) SHI Z, QIAN X, LI L, ZHANG J, ZHU S, ZHU J, CHEN L, ZHANG K, HAN L, YU S, PU P, JIANG T, KANG C. Nuclear translocation of beta-catenin is essential for glioma cell survival. *J Neuroimmune Pharmacol* 2012; 7: 892-903.
- 29) KHRAMTSOV AI, KHRAMTSOVA GF, TRETIAKOVA M, HUO D, OLOPADE OI, GOSS KH. Wnt/beta-catenin pathway activation is enriched in basal-like breast cancers and predicts poor outcome. *Am J Pathol* 2010; 176: 2911-2920.
- 30) CHUNG MT, LAI HC, SYTWU HK, YAN MD, SHIH YL, CHANG CC, YU MH, LIU HS, CHU DW, LIN YW. SFRP1 and SFRP2 suppress the transformation and invasion abilities of cervical cancer cells through Wnt signal pathway. *Gynecol Oncol* 2009; 112: 646-653.
- 31) SILVA AK, YI H, HAYES SH, SEIGEL GM, HACKAM AS. Lithium chloride regulates the proliferation of stem-like cells in retinoblastoma cell lines: A potential role for the canonical Wnt signaling pathway. *Mol Vis* 2010; 16: 36-45.
- 32) XIAO W, CHEN X, HE M. Inhibition of the Jagged/Notch pathway inhibits retinoblastoma cell proliferation via suppressing the PI3K/Akt, Src, p38MAPK and Wnt/betacatenin signaling pathways. *Mol Med Rep* 2014; 10: 453-458.
- 33) ZHENG Q, ZHANG Y, REN Y, WU Y, YANG S, ZHANG Y, CHEN H, LI W, ZHU Y. Antiproliferative and apoptotic effects of indomethacin on human retinoblastoma cell line Y79 and the involvement of beta-catenin, nuclear factor-kappaB and Akt signaling pathways. *Ophthalmic Res* 2014; 51: 109-115.