

Curcumin Inhibits Retinoblastoma Cell Proliferation by miR-26a Targeting the Tumor Suppressor Gene *Rb1* in Y79 Cells

Jing Bai¹, Duo Guo² and Xiangyi Liu^{1,*}

¹Department of Clinical Laboratory, Beijing Tongren Hospital, Capital Medical University, Beijing, 100730, China

²Department of Histology and Embryology, School of Basic Medical Sciences, Capital Medical University, Beijing, 100069, China

Abstract: The retinoblastoma (*Rb1*) gene is one of the most important tumor suppressor genes. Dysfunction of Rb protein drives tumorigenesis by overcoming barriers to cellular proliferation. Consequently, factors modulating Rb function are of great clinical import. Here, we show that miR-26a was differentially expressed in human retinoblastoma cells, tissues and serums from retinoblastoma patients, compared with human retinal microvascular endothelial cells, non-tumor tissues and serums from healthy children, and that it tightly regulated the expression of *Rb1* by specifically targeting a conserved sequence motif in its 3' UTR, leading to low expression of *Rb1*. *In vitro* experiments determined that miR-26a directly participated in the regulation of cell proliferation of human Y79 RB cells. Our results also suggest that curcumin modulated the miR-26a expression profile, thereby exerting its anti-proliferation effects on Y79 RB cells via up-regulation of *Rb1*. To our knowledge, these data indicate for the first time that miR-26a directly regulates cell proliferation by targeting *Rb1* in retinoblastoma and that miR-26a could be a potential therapeutic approach for retinoblastoma.

Keywords: Retinoblastoma, miR-26a, *Rb1*, microRNA, Curcumin.

1. INTRODUCTION

Retinoblastoma is a primary intraocular malignancy arising from the retina. It is the most common intraocular tumor in children [1]. The known causes of retinoblastoma are either *Rb* gene mutation or epigenetic alterations of both alleles, and, yet, there are many unknown causes of RB that need to be deciphered [2]. The *Rb1* is one of the best characterized tumor suppressor genes, and its inactivation has been noted in a variety of human cancers including retinoblastoma, osteosarcoma, lung, breast, and prostate cancers [3]. Rb protein is one of the most important tumor suppressors and functions in multiple biological pathways that are deregulated during carcinogenesis and progression. Genomic alterations in the expression of several miRNAs are often present in human cancers, suggesting a potential role in carcinogenesis [4].

MicroRNAs are small (~22 nt) RNAs that influence gene expression networks by repressing target messenger RNAs (mRNAs) via specific base-pairing interactions in 3' untranslated regions (3'UTRs) [5]. miRNAs are differentially expressed in many tumors and often create a unique signature for each tumor type [6]. Aberrant miRNA expression has been observed in a wide range of human cancers, and in

several cases miRNAs have been shown to have oncogenic or tumor suppressor functions [7]. It was reported that a number of highly expressed miRNAs (miR-513, miR-518, miR-129, miR-198, miR-492, miR-498, miR-320, miR-373 and miR-503) in retinoblastoma play significant roles in regulating tumorigenesis [8]. Jong *et al.* reported that miR-132 and miR-212 are increased in pancreatic cancer and target the retinoblastoma tumor suppressor [9]. Researchers have also identified differentially regulated miRNAs from primary Rb tumors and Rb cell lines [10]. The tumor suppressing nature of miR-34a and the oncogenic nature of miR-17, miR-18a, miR-19b-1, miR-20a, and miR-92a-1 are reported in Rb [11-12]. It was reported that the *Rb1* gene can be suppressed by miR-26a in many tumors. For example, in glioma, *Rb1* could be regulated by has-miR-26a to participate in pathways in this cancer [13]. But, it is still unknown whether miR-26a can also regulate the expression of *Rb1* gene in retinoblastoma cells and tissues.

Herein, in this work, we investigated the regulation of *Rb1* gene by miR-26a in RB cell line based on reported literatures. Firstly, when using the TargetScan5.1 software, we found that miR-26a was predicted to target a conserved site within the *Rb1* 3' UTR. Subsequently, in this study, we report that miR-26a expression was highly upregulated in Y79 Rb cells. Our results demonstrated that an important tumor suppressor, *Rb1*, is a target of miR-26a in Y79 Rb cells. We also investigated the effect of phenolic

*Address correspondence to this author at the Department of Clinical Laboratory, Beijing Tongren Hospital, Capital Medical University, Beijing, 100730, China; Tel/Fax: +86 1058266273; E-mail: liuxiangyi2010@163.com

compound curcumin on miR-26a expression in Y79 Rb cells. As a result, treatment of Y79 Rb cell line with curcumin resulted in miR-26a down-regulation and Rb protein restoration. Finally, the effect of miR-26a and curcumin on Y79 Rb cell proliferation was also determined by cell proliferation assay.

2. MATERIALS AND METHODS

2.1. Patient Material

Ten retinoblastoma tissues were obtained directly from patients undergoing surgery and processed at Capital Medical University Tongren Hospital (Beijing, China). Also, ten non-tumor retinoblastoma tissues were obtained from patients undergoing ophthalmectomy surgery. Serum samples collected from twenty five patients diagnosed as Rb were used as test group. Twenty five control blood samples were collected from 12-age matched healthy children based on their physical examination. The parents/guardians of the children were informed about the purpose of the study, and signed consent forms had been received as means of acceptance. The samples were collected between Jan 2015 and June 2015 at the Hospital after informed consent and Ethics Committee's approval. Tissue samples were trimmed and cryopreserved in liquid nitrogen for miR-26a and *Rb1* quantitative study.

2.2. Cell Culture

The Y79 Rb cell lines (ATCC, Manassas, VA, USA) were cultured in Dulbecco's modified Eagle's medium (DMEM) (Gibco, CA, USA) and supplemented with 10% (v/v) fetal bovine serum (FBS) in a humidified environment (37 °C and 5% CO₂). Human retinal microvascular endothelial cells (HRECs) (ACBRI-181) were obtained from Cell Systems (Kirkland, WA, USA). The cells were grown on fibronectin-coated cell culture dishes in MCDB-131 (Invitrogen, Carlsbad, CA, USA) supplemented with 10% FBS, 10 ng/mL EGF, 1 µg/mL hydrocortisone, 0.2 mg/mL endothelial culture medium (Millipore, Manassas, VA, USA), and 0.09 mg/mL heparin (VEC Technologies, Rensselaer, NY, USA).

2.3. miRNA Expression Analysis

RNA was extracted from cultured cells, tissues and serum samples by using miRNA extraction kit (Qiagen, German) according to the manufacturer's instructions. As to the serum samples, a volume of 1.5 mL blood was collected from each child directly into serum collection tubes. The whole blood was allowed to stand for one hour before being centrifuged at 1800 g for 20

minutes. The resultant serum was aliquoted into sterile DEPC-treated 1.5 mL tubes and stored immediately in a freezer at -80°C while miRNAs from the cells samples were submitted to KangChen Bio-tech (Shanghai, China) for array hybridization on a miRCURY™ Array microarray kit (Exiqon, Denmark). Each microarray chip was hybridized with a single sample labeled with either Cy3 or Cy5. Background subtraction and normalization were performed. MiRNA expression in cells, tissues and serum samples was determined by miRNA TaqManVR qRT-PCR assays (Applied Biosystems, Foster City, CA, USA) on an ABI Prism 7500 Sequence Detection System (Applied Biosystems) according to supplier protocol. Expression was normalized to endogenous control U6 (Applied Biosystems).

2.4. Construction of Plasmids

For luciferase assay, the 3' UTR segments of *Rb1* mRNA containing the miR-26a binding sites were amplified by PCR from human genomic DNA and inserted into the pMIR-REPORT luciferase reporter vector (Ambion, CA, USA) and named pMIR-Rb1-3' UTR. The forward primer was 5' -ACTAGTCAGAGCAAGACTCGGCAGGGTGTCTG-3', and the reverse primer was 5' -GTCGACCACCAGGCTTCCAATGGATCAGTGGTC-3'. A mutant version shown in Figure 1 was also generated by Generate site-directed mutagenesis system (Invitrogen) and named pMIR-mut-Rb1-3' UTR. All of the plasmids were confirmed with DNA sequencing. The miR-26a mimics, a nonspecific negative control (miR-Ctrl) and anti-miR-26a were purchased from GenePharma (Shanghai, China).

2.5. miRNA Transfection and a Luciferase Assay

HRECs were transfected with 50 nM miR-26a mimics, anti-miR-26a, or control using Lipofectamine™ 2000 (Invitrogen), and then used for Real-time PCR and Western blot. For co-transfection experiments, Y79 Rb cells were plated at a density of 4×10^4 cells/well on 96-well culture plates and transfected with the miR-26a mimics or miR-Ctrl and the pMIR-Rb1-3' UTR or pMIR-mut-Rb1-3' UTR. Next, the cells were assayed for both firefly and Renilla luciferase activities using a dual-luciferase system and a GloMax™ 96-well microplate illuminometer (Promega, Madison, WI, USA), as described in the manufacturer's protocol. The Renilla luciferase expression vector, pRL-TK (Promega), was used as endogenous control.

2.6. Isolation of RNA and Real-Time PCR Analysis

Total RNA from HRECs and Y79 cell lines was purified using the standard procedure for TRIzol[®] RNA extraction (Invitrogen) and stored at -80°C. Reverse transcription was performed using the SuperScript[™]III first-strand synthesis system (Invitrogen). Real-time PCR was then performed with the ABI Prism 7500 Sequence Detection System (Applied Biosystems) using the Power SYBR Green master kit to quantitatively determine the mRNA level of *Rb1*, according to the manufacturer's protocol. The forward primer was 5' - GTGTGAGCATCTCTCCTGGAG -3', and the reverse primer was 5' - ACCACACCAGACCCTGAAAG-3'. The β -actin gene was used as an internal control for all of the reactions.

2.7. Western Blot Analysis

Western blot analysis was performed using previously described methods [14]. After treatment, HRECs and Y79 cells were harvested, and the nuclear protein extracts were prepared with NE-PER nuclear (Pierce, Rockford, IL, USA). For electrophoresis, 50 μ g of nuclear proteins was separated through SDS/PAGE, electrotransferred to a nitrocellulose membrane, and

subsequently incubated with an anti-Rb monoclonal antibody (1:500) (Santa Cruz Biotech, Dallas, TE, USA). The proteins were visualized using ECL (enhanced chemiluminescence) reagents (Pierce).

2.8. Curcumin Treatment

To analyze the effect of reagent on the miR-26 and *Rb1* expression, curcumin (Sigma, St. Louis, MO, USA) was dissolved in phosphate-buffered saline and used for the treatment of Y79 RB cells. Y79 RB cells were incubated in culture medium with or without 20 μ M curcumin for 48h and then used for real-time PCR and western blot [15].

2.9. Cell Proliferation Assay

Directly counting cells numbers from micromass pellets was used to detect the proliferation of Y79 RB cells. The micromass pellets which were treated with miR-26a mimics, anti-miR-26a or curcumin were induced to Y79 RB cells for 24 h. Subsequently, all micromass pellets were trypsinized by 0.25% trypsin and directly counted using a hemacytometer. All experiments were done in three independent experiments and repeated counting in triplicate.

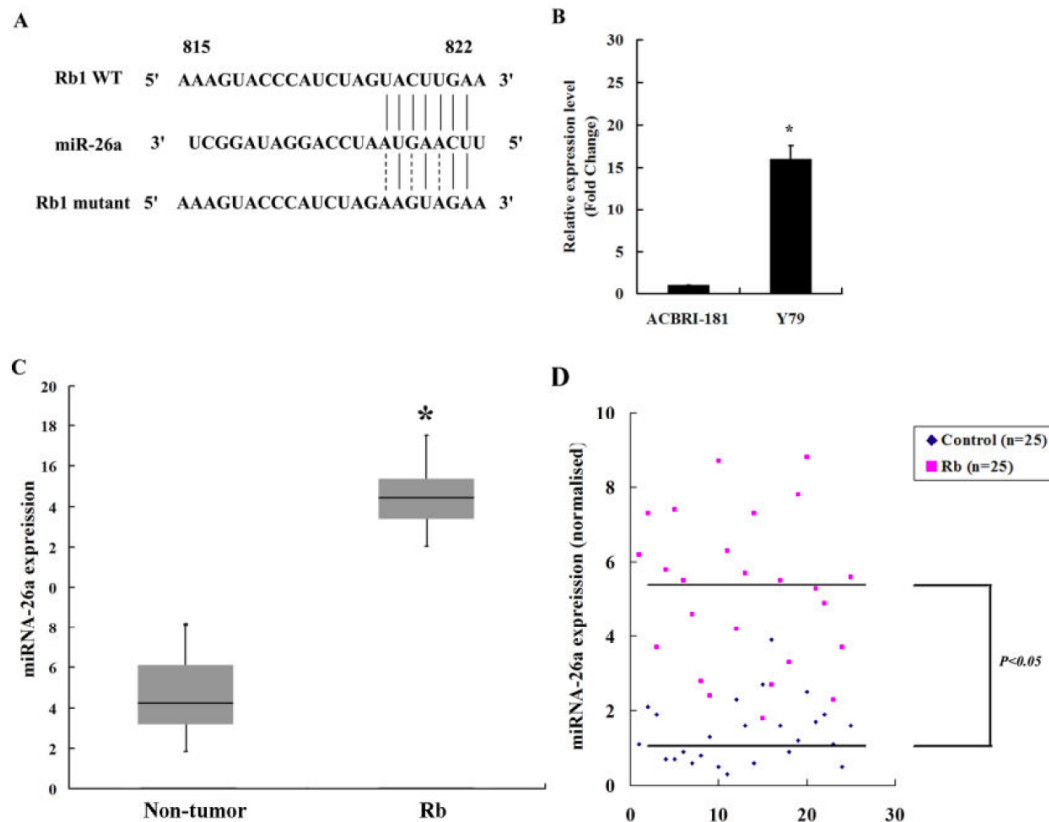


Figure 1: The expression profiling of miR-26a in Y79 cells, Rb tissues and serums. (A) Schematics of *Rb1* mRNA 3' UTR. Up-regulated expression of miR-26a in Y79 cells (B), Rb tissues (C) and serums (D) were assessed by qRT-PCR.

2.10. Data Analysis

The data are expressed as the means $\pm$ S.D. The results were evaluated with an un-paired Student's *t*-test. Statistical significance was determined at $p < 0.05$.

3. RESULTS

3.1. Identification of miR-26 as a Potential Regulator of Rb1 in Retinoblastoma Cells

We began our studies interested in probing possible regulation of the *Rb* expression by miR networks in retinoblastoma cells. Previously, *Rb* has been found down-regulated in multiple retinoblastoma cell lines and patient samples [16]. To determine if miRs might play some role in the dysregulation of the *Rb* expression, we first examined the expression profile of miRs in Y79 retinoblastoma cell line compared with that of the ACBRI-181 non-tumorigenic human retinal microvascular endothelial cell line. Table 1 shows that the results of our expression profiling revealed up-or down-regulation of 18 miRs by 4-fold or greater in Y79 RB cells. Among these 18 miRs was miR-26a found to be significantly up-regulated microRNA examined with this array. Importantly, when exploring the TargetScan5.1 miR target prediction algorithm, miR-26a was predicted to target a conserved site within the *Rb1* 3' UTR. In fact, only one conserved targeting site was identified within the *Rb1* 3' UTR, a site matching the seed sequences for miR-26a (Figure 1A). Based on the predicted targeting information and our array data, we further examined dysregulation of the miR-26a expression by qRT-PCR. We found that miR-26a levels were also significantly increased in Y79 cells, 10 retinoblastoma tissue samples and 25 retinoblastoma

serum samples compared with non-tumorigenic ACBRI-181 cells, 10 non-tumor tissues and 25 serum samples from noncancerous patients (Figure 1B, C and D, $p < 0.05$).

3.2. Rb mRNA 3'-UTR Contains a Validated miR-26-Targeted Site

Further, to ascertain whether miR-26a directly regulate *Rb1* expression through the target site in the 3' UTR of *Rb1* mRNA, we constructed a luciferase reporter vector with the putative *Rb1* 3' UTR target site for miR-26a downstream of the luciferase gene (pMIR-*Rb1*-3' UTR) and mutant version (pMIR-mut-*Rb1*-3' UTR). Luciferase reporter vector together with miR-26a mimics or mimics control were transfected into Y79 RB cells that weakly expressed miR-26a. A significant decrease in relative luciferase activity was noted when pMIR-*Rb1*-3' UTR was cotransfected with miR-26a mimics ($p < 0.05$) (Figure 2A). As expected, this suppression was abolished by deleting part of the perfectly complementary sequences in the *Rb1* 3' UTR (pMIR-mut-*Rb1*-3' UTR) which disrupts the interaction between miR-26a and *Rb1* (Figure 2A).

We next examined the impact of miR-26a overexpression on endogenous *Rb1* expression in Rb cell lines. In ACBRI-181 cells, transfection of miR-26a expression vector significantly reduced *Rb1* mRNA levels (5-fold) (Figure 2B, $p < 0.05$) compared with transfection of empty vector controls. Contrast to the miR-26a expression vector, the transfection of anti-miR-26a can increase *Rb1* mRNA levels (Figure 2B, $p < 0.05$). Subsequently, we further assessed miR-26a regulation of Rb1 protein by performing western blot. As a result, we confirmed that miR-26a targeting of

Table 1: Differential Expression Profiles of miRNAs in Y79 and ACBRI-181 Assessed by miRNA Array. miRs Up-or Down-Regulated by 4-Fold or Greater are also Listed in the Table

Y79 vs. ACBRI-181			
miRs	Fold Change	miRs	Fold Change
hsa-miR-27b	23.87	hsa-miR-455	5.35
hsa-miR-26a	19.09	hsa-miR-200a	4.39
hsa-miR-4788	16.81	hsa-miR-21	4.99
hsa-miR-193b	7.56	hsa-miR-210	-4.55
hsa-miR-27a	7.45	hsa-miR-379	-5
hsa-miR-222	6.77	hsa-miR-380	-5.56
hsa-miR-374a	6.54	hsa-miR-5003	-7.14
hsa-miR-223	6.45	hsa-miR-23c	-7.69
hsa-miR-34c	5.88	hsa-miR-146a	-12.5

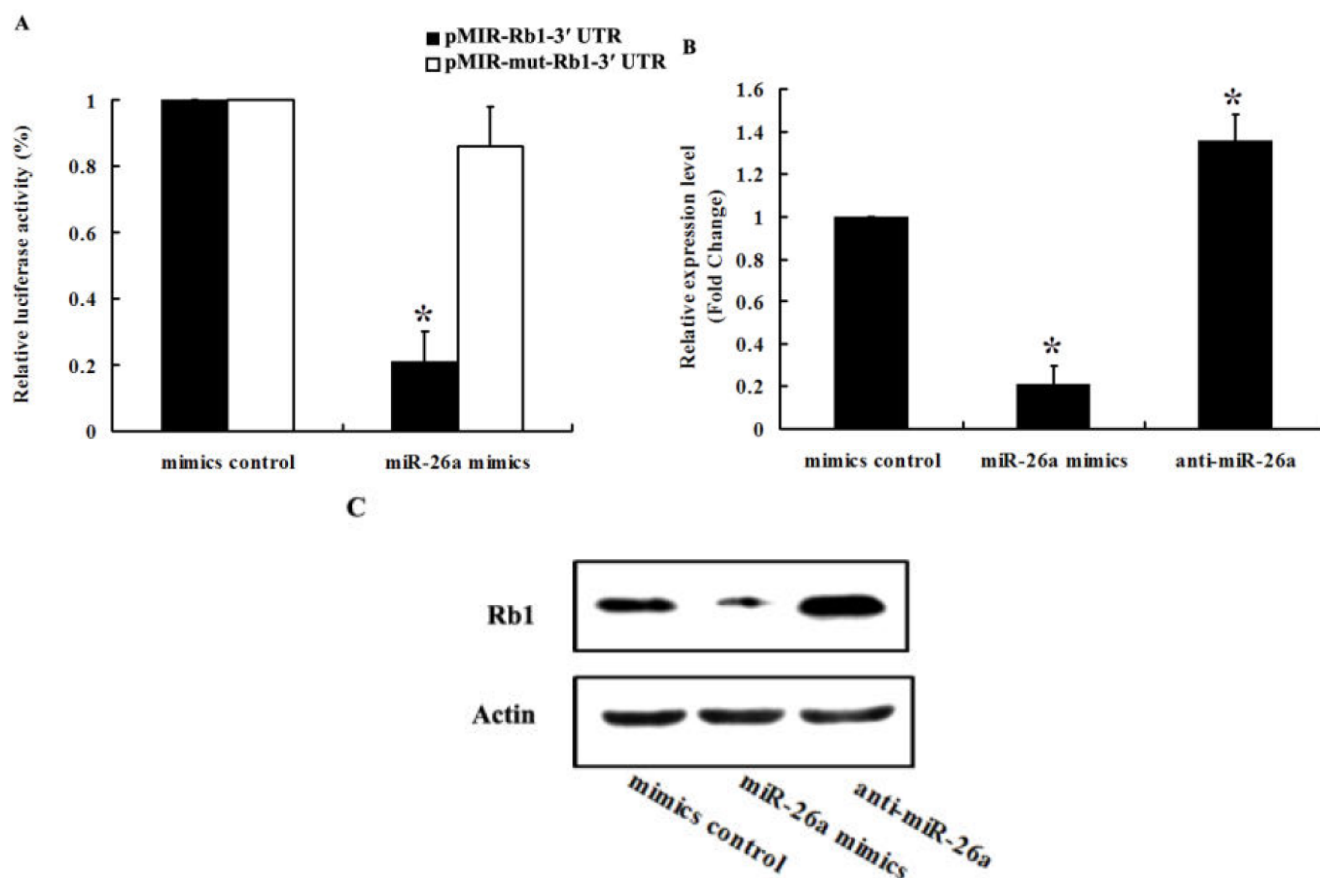


Figure 2: The miR-26a targets *Rb1* in retinoblastoma cell line. (A) Y79 Rb cells were co-transfected with wild type (WT) or mutant *Rb1* 3' UTR luciferase reporter plasmids along with miR-26a expression vector. (B) miR-26a targeting leads to *Rb1* mRNA down-regulation. (C) miR-26a targeting results in decreased *Rb1* protein levels.

Rb1 mRNA also resulted in decreased *Rb1* protein levels by Western blotting, while anti-miR-26a enhanced the *Rb1* protein levels (Figure 2C).

3.3. Treatment with Curcumin Results in the Down-Regulation of miR-26a and *Rb1* Re-Expression

It was reported that curcumin can modulate the miRNA expression profile, thereby exerting its anticancer effects on retinoblastoma cells. So qRT-PCR was performed to further determine the expression of miR-26a of Y79 RB cells induced by curcumin. The miR-26a which was treated by curcumin was reverse transcribed and amplified in the ABI 7500 sequence detection system. From the real-time PCR results, it was found that miR-26a were significantly down-regulated in Y79 cell lines treated by curcumin compared to those untreated (Figure 3A).

To further examine that curcumin regulates *Rb1*, the expression of *Rb1* was assessed in Y79 RB cells treated by curcumin. According to the real-time PCR results, it was shown that *Rb1* mRNA was increased about 6-fold in Y79 RB cells treated by curcumin

(Figure 3B). Finally, we examined *Rb1* expression by western blotting and found that curcumin treatment also resulted in upregulation of *Rb1* protein levels in Y79 RB cells (Figure 3C).

3.4. miR-26a and Curcumin have an Influence on Proliferation of Y79 RB cells

To investigate whether miR-26a and curcumin inhibition that leads to a change in *Rb* protein expression affects cell proliferation, we assessed the proliferation of Y79 RB cells by direct cell counting. As a result, treatment of Y79 RB cells with either miR-26a mimics or anti-miR-26a significantly enhanced or inhibited the cell proliferation, respectively (Figure 4A). On the contrary, treatment of Y79 RB cells with curcumin resulted in a dramatic decrease of the Y79 RB cell proliferation (Figure 4B).

4. DISCUSSION

The miR-26a gene is now generally considered as a tumor suppressor gene. According to literatures, it was indicated that miR-26a expression in tumor cells could

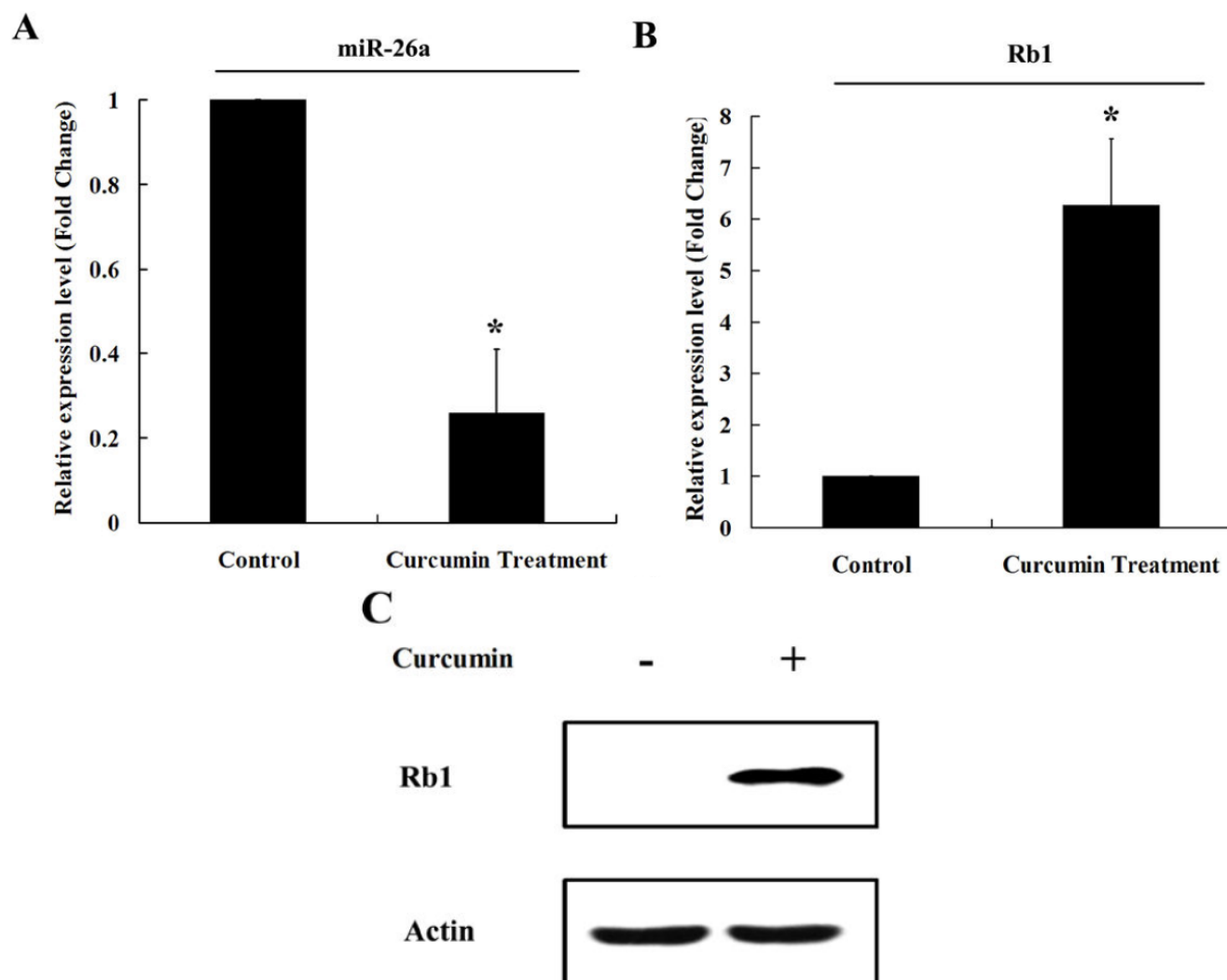


Figure 3: Curcumin alter in the expression of miR-26a and *Rb1* in Y79 Rb cells. (A) miR-26a was found to be regulated between curcumin treated and untreated Y79 RB cells. Curcumin can regulate the expression of *Rb1* mRNA (B) and protein (C) in Y79 Rb cells.

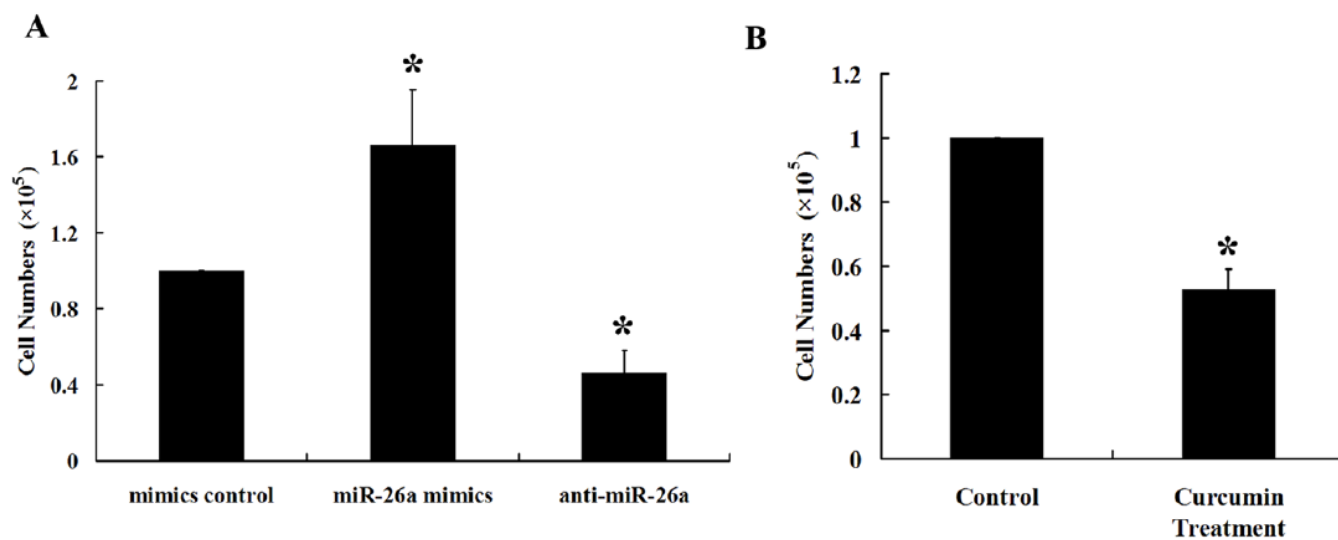


Figure 4: The miR-26a and curcumin have a reverse influence on proliferation of Y79 RB cells.

induce cell cycle arrest at G1 phase through inhibiting the expression of EZH2 at post-transcriptional level [17]. It was also reported that in HepG2 cells miR-26a directly downregulates cyclins D2 and E2 and induces a G1 arrest [18].

In this study, we investigated the effects of miR-26a on the expression of *Rb1* in Y79 RB cells. As shown in our results, miR-26a expression was found to directly participate in the regulation of *Rb1* in Y79 RB cells. However, differently, it was observed that upregulation but not downregulation of miR-26a expression can be examined by the miRNA microarray and qRT-PCR in Y79 RB cells and retinoblastoma tissues. Next, we found that miR-26a upregulation can decrease *Rb1* expression by binding to the *Rb1* mRNA 3' UTR in Y79 RB cells. According to our results, the miR-26a, which downregulated tumor suppressor gene *Rb1*, could belong to the oncogenic miRNA in Y79 RB cells. Our observation differs from previous reports, suggesting that in different cell contexts, miR-26a may exhibit markedly different characteristics, many of which are unique or even opposite from the pattern found in different cells.

Rb pathways represent the major tumor suppressor pathways in mammals and are crucial for the control of cell proliferation [19]. Rb is frequently mutated or expressed at low levels in many tumors such as retinoblastoma as well as small lung, bladder, and breast carcinomas [20-21]. Down-regulation of tumor suppressors by miRNAs could contribute to the malignant progression of carcinomas. Thus, miRNAs controlling Rb protein levels are of high clinical interest. Here, we show that miR-26a, which was significantly increased in retinoblastoma, efficiently controls Rb1 protein levels by directly targeting a conserved region in the 3' UTR of *Rb1* in Y79 RB cells. While it is possible for *Rb1* to be regulated by other miRNAs, our data suggest that reduced Rb protein in Y79 RB cells results from increased miR-26a expression. According to literatures, it was reported that miR-26a can also decrease the RB1 protein expression by targeting the 3' UTR of *Rb1* gene in other tumor cells. Kim *et al.* demonstrate that miR-26a alone can transform cells and it promotes glioblastoma cell growth *in vitro* and in the mouse brain by decreasing PTEN, RB1, and MAP3K2/MEKK2 protein expression, thereby increasing AKT activation, promoting proliferation, and decreasing c-JUN N-terminal kinase-dependent apoptosis [22]. Zhang *et al.* also identified that *Rb1* is the direct functional target of miR-26a, and revealed

that the reduction of miR-26a expression leads to increased Rb1 protein level and thus inhibits the function of E2F1, by which it influences the phenotypes of cell cycle and anoikis human esophageal adenocarcinoma cells [23].

Curcumin, a natural polyphenolic compound, is considered one of the most powerful anticancer agents. It is a major ingredient in the rhizome of the herb *curcuma longa*. It exhibits various properties like antioxidant, antiinflammatory, antiviral, antibacterial and anticancer activities [24]. Extensive studies have shown that many biological effects of curcumin are through regulation of cellular signaling pathways, including nuclear factor kappa-light-chain-enhancer of activated B cells (NF- κ B), protein kinase B (PKB/Akt), mitogen-activated protein kinase (MAPK) and other pathways [25]. However, there is no report on the effect of curcumin on miRNA regulation in RB cells. In this study, we have used Real-time PCR technology to investigate the effect of curcumin on the expression profile of miRNAs in Y79 RB cells. Our results showed that curcumin did alter miR-26a expression in Y79 RB cells, and thereby increase the RB1 protein expression. Furthermore, the effects of miR-26a and curcumin on proliferation of Y79 RB cells were also clarified in our study. It was demonstrated that miR-26a can enhance the proliferation of Y79 RB cells while curcumin inhibit it in our results. Its mechanism could be related to the regulation of Rb1/E2F1 pathway. E2F1 plays an important role in regulating cell proliferation, and its function is mostly controlled by Rb1 protein [26].

Additionally, the dysregulation of miRNAs in body fluids such as plasma, serum, urine, and saliva has given insights to biomarkers for a variety of cancers [27-29]. Recently, identification of serum miRNA biomarkers and potential gene targets are gaining importance as biomarkers [27-28]. In our study, real-time PCR analysis shown that the miRNA-26a levels in retinoblastoma serum samples were significantly increased contrasted to that in serum samples from healthy children. Although the possible mechanism of miR-26a upregulation expression in retinoblastoma serum samples remains unclear, our results suggest that miR-26a could be used as biomarker for retinoblastoma diagnosis.

5. CONCLUSION

In summary, we report here that miR-26a is over-expressed in Y79 Rb cells, retinoblastoma tissues or serums from Rb patients and targets the tumor

suppressor Rb1. The present report also illustrates the regulation of miR-26a expression profile induced by curcumin on Y79 Rb cells and its effect on the expression of *Rb1* and Y79 Rb cell proliferation. Our results suggest that miR-26a might also be useful as a serum biomarker and potential therapeutic target for retinoblastoma and more in depth analysis is required.

ACKNOWLEDGEMENTS

This work was supported by grant Nos.81001044 from the National Science Foundation of China (NSFC).

DISCLAIMERS

Conflicts of Interest and Source of Funding: We confirm that there is no potential conflict of interest regarding this paper.

REFERENCES

- [1] Vogel F. Genetics of retinoblastoma. *Hum Genet* 1979; 52(1): 1-54. <https://doi.org/10.1007/BF00284597>
- [2] Zhang J, Benavente CA, McEvoy J, Flores-Otero J, Ding L, Chen X, Ulyanov A, Wu G, Wilson M, Wang J, Brennan R, Rusch M, Manning AL, Ma J, Easton J, Shurtleff S, Mullighan C, Pounds S, Mukatira S, Gupta P, Neale G, Zhao D, Lu C, Fulton RS, Fulton LL, Hong X, Dooling DJ, Ochoa K, Naeve C, Dyson NJ, Mardis ER, Bahrami A, Ellison D, Wilson RK, Downing JR, Dyer MA. A novel retinoblastoma therapy from genomic and epigenetic analyses. *Nature* 2012; 481(7381): 329-34. <https://doi.org/10.1038/nature10733>
- [3] Sakai T1, Toguchida J, Ohtani N, Yandell DW, Rapaport JM, Dryja TP. Allele-specific hypermethylation of the retinoblastoma tumor-suppressor gene. *Am J Hum Genet* 1991; 48(5): 880-8.
- [4] Dalgard CL, Gonzalez M, deNiro JE, O'Brien JM. Differential microRNA-34a expression and tumor suppressor function in retinoblastoma cells. *Invest Ophthalmol Vis Sci* 2009; 50(10): 4542-51. <https://doi.org/10.1167/iops.09-3520>
- [5] Filipowicz W, Bhattacharyya SN, Sonenberg N. Mechanisms of post-transcriptional regulation by microRNAs: are the answers in sight? *Nat Rev Genet* 2008; 9(2): 102-14. <https://doi.org/10.1038/nrg2290>
- [6] Volinia S, Calin GA, Liu CG, Ambs S, Cimmino A, Petrocca F, Visone R, Iorio M, Roldo C, Ferracin M, Prueitt RL, Yanaihara N, Lanza G, Scarpa A, Vecchione A, Negrini M, Harris CC, Croce CM. A microRNA expression signature of human solid tumors defines cancer gene targets. *Proc Natl Acad Sci U S A* 2006; 103(7): 2257-61. <https://doi.org/10.1073/pnas.0510565103>
- [7] Esquela-Kerscher A, Slack FJ. Oncomirs-microRNAs with a role in cancer. *Nat Rev Cancer* 2006; 6: 259-269. <https://doi.org/10.1038/nrc1840>
- [8] Zhao JJ, Yang J, Lin J, et al. Identification of miRNAs associated with tumorigenesis of retinoblastoma by miRNA microarray analysis. *Nat Rev Cancer* 2006; 6(4): 259-69.
- [9] Park JK, Henry JC, Jiang J, Esau C, Gusev Y, Lerner MR, Postier RG, Brackett DJ, Schmittgen TD. miR-132 and miR-212 are increased in pancreatic cancer and target the retinoblastoma tumor suppressor. *Biochem Biophys Res Commun* 2011; 406(4): 518-23. <https://doi.org/10.1016/j.bbrc.2011.02.065>
- [10] Jo DH, Kim JH, Park WY, Kim KW, Yu YS, Kim JH. Differential profiles of microRNAs in retinoblastoma cell lines of different proliferation and adherence patterns. *J Pediatr Hematol Oncol* 2011; 33(7): 529-33. <https://doi.org/10.1097/MPH.0b013e318228280a>
- [11] Dalgard CL, Gonzalez M, deNiro JE, O'Brien JM. Differential microRNA-34a expression and tumor suppressor function in retinoblastoma cells. *Invest Ophthalmol Vis Sci* 2009; 50(10): 4542-51. <https://doi.org/10.1167/iops.09-3520>
- [12] Kandamam MM, Beta M, Maheswari UK, Swaminathan S, Krishnakumar S. Oncogenic microRNA 17-92 cluster is regulated by epithelial cell adhesion molecule and could be a potential therapeutic target in retinoblastoma. *Mol Vis* 2012; 18: 2279-87.
- [13] Yu J, Cai X, He J, Zhao W, Wang Q, Liu B. Microarray-based analysis of gene regulation by transcription factors and microRNAs in glioma. *Neurol Sci* 2013; 34(8): 1283-9. <https://doi.org/10.1007/s10072-012-1228-1>
- [14] Guo D, Dong LY, Wu Y, Yang L, An W. Down-regulation of hepatic nuclear factor 4α on expression of human hepatic stimulator substance via its action on the proximal promoter in HepG2 cells. *Biochem J* 2008; 415(1): 111-21. <https://doi.org/10.1042/BJ20080221>
- [15] Sreenivasan S, Thirumalai K, Danda R, Krishnakumar S. Effect of curcumin on miRNA expression in human Y79 retinoblastoma cells. *Curr Eye Res* 2012; 37(5): 421-8. <https://doi.org/10.3109/02713683.2011.647224>
- [16] Lee EY, Bookstein R, Young LJ, Lin CJ, Rosenfeld MG, Lee WH. Molecular mechanism of retinoblastoma gene inactivation in retinoblastoma cell line Y79. *Proc Natl Acad Sci USA* 1988; 85(16): 6017-21. <https://doi.org/10.1073/pnas.85.16.6017>
- [17] Ciarapica R, Russo G, Verginelli F, Raimondi L, Donfrancesco A, Rota R, Giordano A. Deregulated expression of miR-26a and Ezh2 in rhabdomyosarcoma. *Cell Cycle* 2009; 8(1): 172-5. <https://doi.org/10.4161/cc.8.1.7292>
- [18] Kota J, Chivukula RR, O'Donnell KA, Wentzel EA, Montgomery CL, Hwang HW, Chang TC, Vivekanandan P, Torbenson M, Clark KR, Mendell JR, Mendell JT. Therapeutic microRNA delivery suppresses tumorigenesis in a murine liver cancer model. *Cell* 2009; 137(6): 1005-17. <https://doi.org/10.1016/j.cell.2009.04.021>
- [19] Chau BN, Wang JY. Coordinated regulation of life and death by RB. *Nat Rev Cancer* 2003; 3(2): 130-8. <https://doi.org/10.1038/nrc993>
- [20] Kanoe H, Nakayama T, Murakami H, Hosaka T, Yamamoto H, Nakashima Y, Tsuboyama T, Nakamura T, Sasaki MS, Toguchida J. Amplification of the CDK4 gene in sarcomas: tumor specificity and relationship with the RB gene mutation. *Anticancer Res* 1998; 18(4A): 2317-21.
- [21] Horowitz JM, Park SH, Bogenmann E, Cheng JC, Yandell DW, Kaye FJ, Minna JD, Dryja TP, Weinberg RA. Frequent inactivation of the retinoblastoma anti-oncogene is restricted to a subset of human tumor cells. *Proc Natl Acad Sci USA* 1990; 87(7): 2775-9. <https://doi.org/10.1073/pnas.87.7.2775>
- [22] Kim H, Huang W, Jiang X, Pennicooke B, Park PJ, Johnson MD. Integrative genome analysis reveals an oncomir/ oncogene cluster regulating lioblastoma Survivorship. *Proc Natl Acad Sci USA* 2010; 107(5): 2183-8. <https://doi.org/10.1073/pnas.0909896107>
- [23] Zhang YF, Zhang AR, Zhang BC, Rao ZG, Gao JF, Lv MH, Wu YY, Wang SM, Wang RQ, Fang DC. MiR-26a regulates cell cycle and anoikis of human esophageal adenocarcinoma

- cells through Rb1-E2F1 signaling pathway. *Mol Biol Rep* 2013; 40(2): 1711-20.
<https://doi.org/10.1007/s11033-012-2222-7>
- [24] Sharma RA, Gescher AJ, Steward WP. Curcumin: the story so far. *Eur J Cancer* 2005; 41(13): 1955-68.
<https://doi.org/10.1016/j.ejca.2005.05.009>
- [25] Mukhopadhyay A, Bueso-Ramos C, Chatterjee D, Pantazis P, Aggarwal BB. Curcumin downregulates cell survival mechanisms in human prostate cancer cell lines. *Oncogene* 2001; 20(52): 7597-609.
<https://doi.org/10.1038/sj.onc.1204997>
- [26] Rogoff HA, Kowalik TF. Life, death and E2F: linking proliferation control and DNA damage signaling via E2F1. *Cell Cycle* 2004; 3(7): 845-6.
<https://doi.org/10.4161/cc.3.7.975>
- [27] Liu R, Zhang C, Hu Z, Li G, Wang C, Yang C, Huang D, Chen X, Zhang H, Zhuang R, Deng T, Liu H, Yin J, Wang S, Zen K, Ba Y, Zhang CY. A five-microRNA signature identified from genome-wide serum microRNA expression profiling serves as a fingerprint for gastric cancer diagnosis. *Eur J Cancer* 2011; 47(5): 784-91.
<https://doi.org/10.1016/j.ejca.2010.10.025>
- [28] Tsujiura M, Ichikawa D, Komatsu S, Shiozaki A, Takeshita H, Kosuga T, Konishi H, Morimura R, Deguchi K, Fujiwara H, Okamoto K, Otsuji E. Circulating microRNAs in plasma of patients with gastric cancers. *Br J Cancer* 2010; 102(7): 1174-9.
<https://doi.org/10.1038/sj.bjc.6605608>
- [29] Yamada Y, Enokida H, Kojima S, Kawakami K, Chiyomaru T, Tatarano S, Yoshino H, Kawahara K, Nishiyama K, Seki N, Nakagawa M. MiR-96 and miR-183 detection in urine serve as potential tumor markers of urothelial carcinoma: correlation with stage and grade, and comparison with urinary cytology. *Cancer Sci* 2011; 102(3): 522-9.
<https://doi.org/10.1111/j.1349-7006.2010.01816.x>

Received on 25-11-2020

Accepted on 28-12-2020

Published on 31-12-2020

<https://doi.org/10.30683/1927-7229.2020.09.08>© 2020 Bai *et al.*; Licensee Neoplasia Research.

This is an open access article licensed under the terms of the Creative Commons Attribution Non-Commercial License (<http://creativecommons.org/licenses/by-nc/3.0/>) which permits unrestricted, non-commercial use, distribution and reproduction in any medium, provided the work is properly cited.