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ORIGINAL ARTICLE

Dysregulation of miR-204-3p Driven by the Viability and Motility of Retinoblastoma via Wnt/β-catenin Pathway In Vitro and In Vivo

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Abstract

Retinoblastoma (RB) is a malignant intraocular tumor that frequently occurs in infants and toddlers. Although the most of RB patients in the developed countries could survival from this cancer, the patients in undeveloped areas are still suffering. The human retinal pigment epithelial cell line ARPE-19 and human retinoblastoma (RB) cell lines HXO-RB44, Y79, and WERI-Rb1 were cultured. The mRNA levels of BANCR and miR-204-3p in these cell lines were measured by qRT-PCR. After transfection with sh-BANCR or treatment with miR-204-3p inhibitor in Y79 cells, the cell proliferation rate, growth, invasion, migration, apoptosis and Wnt/β-catenin signaling pathway activity were measured. The regular Y79 and Y79 cells stably expressed sh-BANCR were injected subcutaneously into nude mice, respectively. The volumes and pathohistological futures of tumors were compared. The biochemical features similar to the cell culture were detected and compered. The mRNA measurements showed that BANCR negatively modulate miR-204-3p expression via directly integration with it. Besides, miR-204-3p and Wnt/β-catenin signalling pathway were found to participate in the oncogenic effects of BANCR on RB cell line by Hoechst staining, cell Counting Kit-8 (CCK-8) assay, wound healing assay, transwell assay, and Western blot analysis in vitro. In addition, an in vivo tumorigenesis experiment in nude mice injected with Y79 cells stably expressed sh-BANCR conformed in the effects of BANCR on RB. Taken together, the knockdown of BANCR inhibited cell proliferation, apoptosis, invasion, and migration in RB via targeting miR-204-3p, the mechanism may involve inhibiting Wnt/β-catenin signaling pathway.

Keywords Long non-coding RNA-BANCR · microRNA-204-3p · Retinoblastoma · WNT signaling pathway · Viability · Motility

Introduction

Retinoblastoma (RB) usually occurs in infants and toddlers, and is one of the most common intraocular tumors worldwide [1, 2]. Thousands of new RB cases are reported in the world per year, and some died prematurely in pain [3, 4]. Although RB is no longer an incurable cancer in the developed countries because of the improvement of the medical technologies during the past decades, [5–7], the mortality rate is over 60% due to the lack of effective therapies in the undeveloped countries [7, 8]. In addition, some survival RB patients are with abnormal eyeballs and vision loss [9]. Therefore, illustrating the underlying pathogenic mechanisms of RB might be helpful for the exploration of novel biomarkers and therapeutic strategies.

It is well documented that more than 70% of genome is transcribed into RNAs, but only a tiny fraction of them can encode proteins. Most of transcripts from genome lack the ability to encode protein, and are defined as non-coding RNAs (ncRNAs) [10, 11]. The molecular biology of ncRNAs has become the research focus in recent years. Accumulating evidences have demonstrated that microRNAs (miRNAs) and long non-coding RNAs (lncRNAs) may participate in the progression of various human tumors, such as breast, oral and lung cancers [12–15]. Recently, ncRNAs were also reported to be involved in the pathogenesis of RB. For example, microRNA655 (miR-655) reportedly suppresses the tumor progression of RB by directly interacting with paired box 6 (PAX6) and inhibiting the extracellular signal-regulated kinases (ERK) signaling pathway [16]. The decreased miR-204 in RB cells serves as tumor suppressor [17], while the increased BRAF-activated non-protein coding RNA (BANCR) in RB tissues and cell lines acts as an

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oncogenic agent [18]. Considering the opposite expression trend of lncRNA-BANCR and miR-204-3p in previous reports, this study aims to investigate the underlying mechanism in RB.

In the present study, we have investigated the expression of BANCR and miR-204-3p in RB cell lines compared with a human retinal pigment epithelial cell line. After knockdown of BANCR by shRNA transfection, the effects of the decreased BANCR on cell viability and motility were explored in vitro and in vivo. Our study indicates that BANCR may play as an oncogenic molecule by inhibiting Wnt/β-catenin pathway via targeting miR-204-3p.

Materials and Methods

Cell Culture

The human retinal pigment epithelial cell line ARPE-19, human retinoblastoma (RB) cell lines HXO-RB44, Y79, and WERI-Rb1 were purchased from ATCC (Rockville, MD, USA), and maintained in RPMI 1640 medium (Gibco, NY, USA) containing 100 U/L penicillin/streptomycin and 10% fetal bovine serum (FBS) under the conditions of 95% air and 5% CO_2 at 37 °C.

Cell Transfection

To establish Y79 cell line stably expressing BANCR, the cDNA of BANCR was amplified via PCR with specific primers and inserted into 1 μg lentiviral vector provided by Shanghai Genepharma Co., Ltd. (Shanghai, China). Recombinant lentiviruses containing BANCR (LV-BANCR) were produced, Y79 cells were infected with LV-BANCR and selected by puromycin. To obtain Y79 cell line stably expressing BANCR shRNA, the specific shRNA sequences were designed and synthesized by Shanghai Genepharma. The sequences of BANCR shRNAs were as follows: shRNA NC, 5'-GCA ACG GCC GGC AAA GGG AUU CAU-3′, BANCR shRNA1, 5'-GCU ACC GUU AAC CGC UAA CCG GGA CGC-3′; BANCR shRNA2, 5'-ACU AAC GCA AUA ACA GCU AGG CGA UA-3′. Cells were seeded into 6-well plates and grown to reach 70% confluence for transfection. Transfection was performed with these molecular productions using Invitroge™ Lipofectamine 2000 (Thermo Fisher Scientific, Inc.) according to the manufacturer's instructions. The transfection efficiency was validated by qRT-PCR assay. The BANCR shRNA2 transfection exhibited higher interference. Thus, the BANCR shRNA2 was sub-cloned to 1 μg lentiviral vector. Recombinant lentiviruses expressing BANCR shRNA (sh-BANCR) were produced. Y79 cells were infected with sh-BANCR lentiviruses and target cells were selected with puromycin. After the transfection for 48 h, cells were collected for the following experiments.

Plasmid Construction and Dual Luciferase Activity Assay

The targets of BANCR were obtained from the following target prediction programs: PicTar (https://pictar.mdc-berlin.de/), miRanDB (http://mirdb.org/miRDB/custom.html) and TargetScan (http://www.targetscan.org/vert_72/), The fragment of miR-204 containing the target sequence of BANCR was amplified by RT-PCR and then sub-cloned into psi-CHECK vector (Promega, Madison, USA) to establish miR-204 wild type (miR-204 WT) or mutant (miR-204-3p Mut) recombinant plasmid. Next, the miR-204 WT or miR-204 Mut and BANCR were co-transfected into cells using Lipofectamine 2000 (Invitrogen) as the instructions described obtained from manufacturer. Luciferase activity was detected 36 h after transfection using the Dual-Luciferase Reporter Assay System (Promega) following the manufacturer's protocols. Renilla luciferase activity was normalized to Firefly luciferase activity.

Transwell Assay

In the invasion analysis, chambers (8 μm pores) coated with Matrigel matrix (BD Biosciences, Franklin Lakes, NJ, USA) were applied to the transwell assay. Treated Y79 cells were collected and suspended at a concentration of 1×10^4 cells/ml in 0.2 ml of culture medium, and then added into the upper chamber. The lower chamber was filled with 0.3 ml of culture medium containing 20% FBS. After cultured for 24 h, non-invaded Y79 cells were scraped and the invaded Y79 cells were stained with 0.5% crystal violet (Shanghai Chemical Reagent Company, China). The cell numbers were counted, and images were captured under an inverted microscope (magnification, ×400; Olympus Corporation, Tokyo, Japan) on 5 randomly selected fields in each well.

Cell Motility Assay

Wound-healing assay and transwell assay were used to evaluate the roles of miR-140-5p in Y79 cell migration and invasion, respectively. In the wound-healing assay, treated Y79 cells (2×10^3 cells/well) were seeded into glass dishes and cultured at 37 °C until cells reached 80% confluence. Then, a straight scratch was made in the Y79 cells via a plastic pipette tip. Cells were then cultured at 37 °C for an additional 24 h, and scratches were photographed at the time of 0 and 24 h after wounding.

In Vivo Tumor Assay

The work of animal of this study was approved by the Institutional Animal Care and Use Committee of The Second Clinical Medical College of Qingdao University. Six-week old

BALA/C athymic nude mice (male, Vitalriver, Beijing, China) were used for the in vivo tumor growth assay. Mouse were housed in a controlled environment at 25 ± 3 °C, humidity 60%, in a 12-h light/dark cycle with free access to food and water. Y79 cells (1×10^6 cells) transfected with sh-BANCR were injected subcutaneously in the right flank of nude mice. The volume of xenografts was assessed every 5 days via the equation Volume (mm^3) = a (largest diameter) × b (perpendicular diameter)2/2. Thirty days after injection, the mouse was euthanized by intraperitoneal injection of pentobarbital sodium (200 mg/kg body weight). The tumor samples were collected and processed for qRT-PCR, IHC, and western blot analysis. The maximum tumor burden observed in the study was 8.6% of body weight. The survival rates of nude mouse injected with control or BANCR blocked Y79 cells were estimated using Kaplan-Meier method.

Hoechst Staining

For Hoechst staining, Y79 cells transfected with sh BANCR or miR-204-3p inhibitor were cultured in 6-well plates, and were incubated with 30 ng/ml of Hoechst 33342 (Sigma-Aldrich, St Louis, MO, USA) at room temperature for 15 min. After washed twice with phosphate buffer saline (PBS), the nuclear morphology was detected by fluorescence microscopy at 365 nm, and the percentage of Hoechst -positive cells was counted in three independent experiments.

Cell Proliferation Analysis (Cell Counting Kit-8, CCK-8 Assay)

Cell proliferation rate of BANCR silenced Y79 cells transfected with miR-204-3p was determined via CCK-8 assay. Treated Y79 cells were seeded into 96-well plates at a density of 1000 cells/well and incubated for 12 h at 37 °C. Then, the proliferation rate was assessed through a CCK-8 detection kit (Dojindo Molecular Technologies, Inc., Kumamoto, Japan) at every 24 h for four consecutive days following the protocols of manufacturer.

RNA Extraction and Quantitative Real-Time PCR (qRT-PCR) Assay

Total RNA of RB cell lines or mouse tumor tissues was extracted and then performed qRT-PCR to measure the mRNA levels of BANCR and miR-204-3p according to the previous methods [18, 19].

Western Blot Assay

Treated Y79 cells or tumor samples were lysed in RIPA buffer and broken by ultrasonication on ice for the preparation of total proteins. After centrifuged at 20,817 xg in high speed refrigerated centrifuge (Eppendorf), supernatant was collected, and the concentration of proteins was determined via a BCA kit (Pierce, Rockford). A total 50 μg of proteins was separated by 10% SDS-PAGE followed by transferring to nitrocellulose membranes (Millipore, Billerica, MA, USA). Next, membranes were incubated with corresponding primary antibodies that against WNT4 (1:500, sc376279, Santa Cruz), total β-catenin (1:100, ab2982, Abcam), active β-catenin (1:500, ab27798, Abcam), transcription factor 4 (TCF4, 1:500, ab185736, Abcam), and GAPDH (1:10000, sc420485, Santa Cruz) at 4 °C overnight. Membranes were then incubated with horseradish peroxidase conjugated secondary antibodies (1:2000, #7056, Cell Signaling Technology, Danvers, MA, USA) at room temperature for 2 h. Proteins were visualized using enhanced chemiluminescence reagents (Pierce, USA). Analysis was performed using ImageJ software (version 1.48, National Institutes of Health, Bethesda, MD, USA).

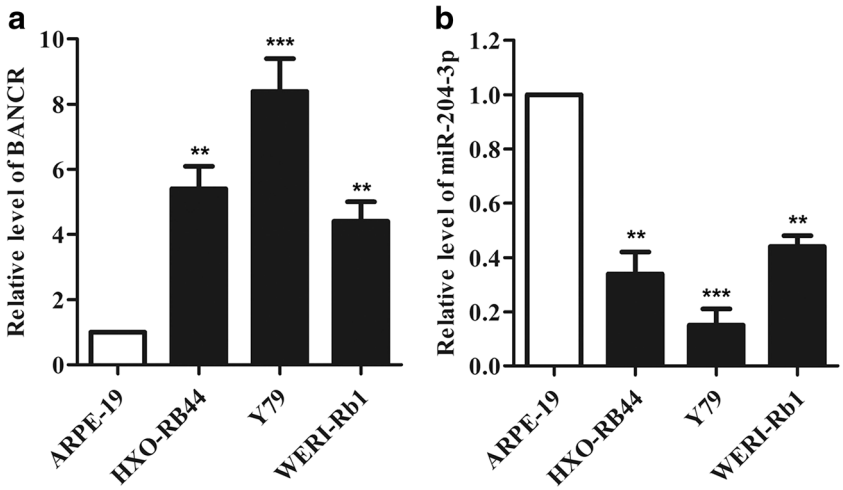

Fig. 1 The expression levels of BANCR and miR-204-3p in RB cells. The mRNA expression levels of (**a**) BANCR and (**b**) miR-204-3p were determined by qRT-PCR analysis in a human retinal pigment epithelial cell line (ARPE-19) and three RB cell lines (HXO-RB44, Y79, WERI-Rb1). Data was shown as means ± SD by at least three independent experiments (**$p < 0.01$, ***$p < 0.001$ versus ARPE-19 group)

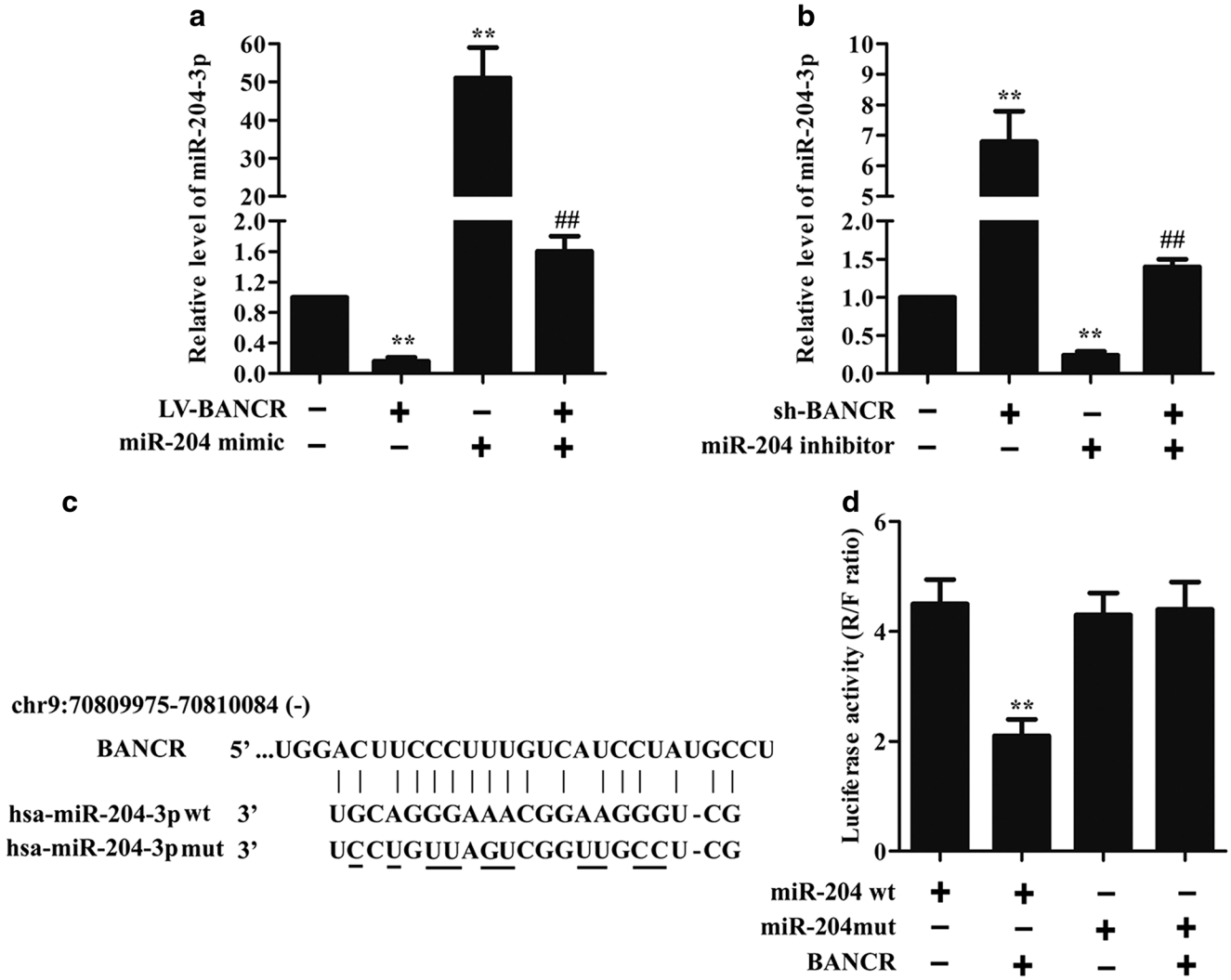

Fig. 2 Negative correlation between BANCR and miR-204-3p expression in RB cells. The mRNA expression level of miR-204-3p was quantified using qRT-PCR in (**a**) LV-BANCR or miR-204-3p mimic treated Y79 cells (**$p < 0.01$ versus LV-BANCR group) and (**b**) sh-BANCR or miR-204-3p inhibitor treated Y79 cells (**$p < 0.01$ versus sh-BANCR group, ##$p < 0.01$ versus miR-204 inhibitor group). (**c**) Bioinformatics analysis showed the predicted miR-204-3p interaction sites in BANCR. The mutated sequence in the seed regions of miR-204-3p was also exhibited in the lower panel. (**d**) The correlation between BANCR and miR-204-3p was determined by luciferase reporter assay. Data was shown as means ± SD by at least three independent experiments. (**$p < 0.01$ versus miR-204 wt group)

Immunohistochemistry (IHC)

For immunohistochemical, tumor tissue samples of RB were fixed in 4% formalin, embedded with paraffin, and cut into 4 μm thick slices. After dewaxed and rehydrated, all tumor slices were incubated with citrate buffer (10 mM) at 100 °C for around 5 mins. Then, slices were immersed into 10% FBS for 2 h at room temperature to block the non-specific binding sites. Slices were subjected to the incubation of primary antibodies against Ki67 (1: 500, rabbit polyclonal, ab833, Abcam), Capase-3 (1: 100, rabbit polyclonal, ab2302, Abcam), or VEGF (1: 50, rabbit polyclonal, ab2349, Abcam). After washed with PBS twice, slices were incubated with anti-rabbit secondary antibody at room temperature for 2 h, then the signals were detected by streptavidin horseradish peroxidase.

Statistical Analysis

Results of this study were repeated at least threee times and were all expressed as mean ± SEM. Comparisons were performed using one-way analysis of variance (ANOVA) followed by Bonferroni's post hoc test or Student's t-tests through GraphPad Prism Software v7.0. Values of $P < 0.05$ were considered statistically significant.

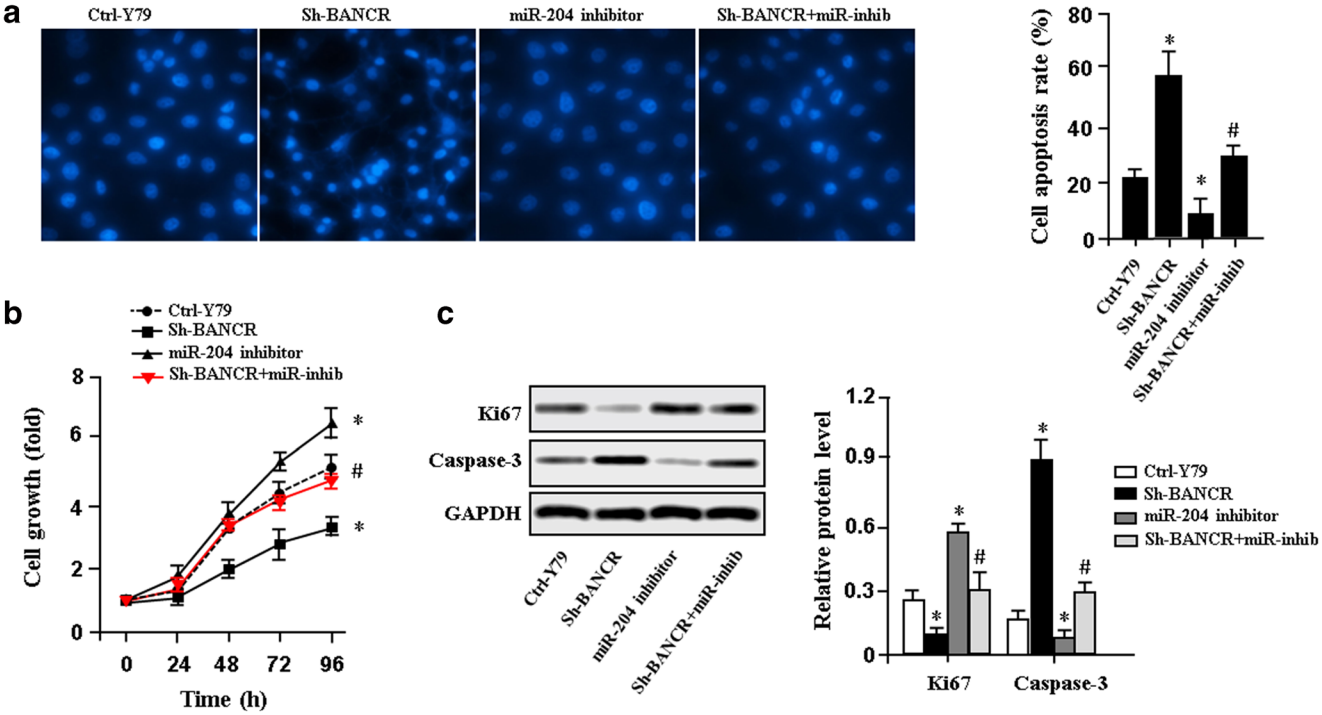

Fig. 3 The inhibition of the growth and promotion of apoptosis by knockdown of BANCR in RB cells. **a** Cell apoptosis rate was determined in Y79 cells transfected with sh-BANCR and treated with miR-204-3p inhibitor, respectively or together, by Hoechst staining. **b** CCK-8 assay was performed to measure the effects of BANCR and miR-204-3p on cell proliferation. **c** The protein expression levels of ki67 and cleaved Caspase-3 were measured by Western blot in Y79 cells transfected with sh-BANCR and treated with miR-204-3p inhibitor, respectively or together. Data was shown as means ± SD by at least three independent experiments (*$p < 0.05$, versus control Y79 cells; #$p < 0.05$, versus miR-204 inhibitor group)

Results

RB Cells Express the Higher Level of BANCR and Lower Level of miR-204-3p

To explore whether BANCR and miR-204-3p play roles in the pathogenesis of RB, we firstly examined their mRNA expression levels by qRT-PCR in a human retinal pigment epithelial cell line, ARPE-19, and three RB cell lines, HXO-RB44, Y79, and WERI-Rb1. The expression level of BANCR was significantly higher ($p < 0.01$) (Fig. 1a), while miR-204-3p expression was remarkably lower in the RB cell lines compared to ARPE-19 cell line ($p < 0.01$) (Fig. 1b). The results suggested that BANCR and miR-204-3p may be involved in the tumorigenesis of RB.

BANCR Inhibits miR-204-3p Expression by Interaction with miR-204-3p in RB Cells

Accumulating evidences have demonstrated that lncRNAs may interact with miRNAs as competing RNAs (ceRNAs) in various tumors. Therefore, the opposite alteration of BANCR and miR-204-3p expression remind us to explore whether their expression was negatively correlated in RB cell lines. As expected, miR-204-3p mRNA expression was significantly lower in the BANCR overexpressed Y79 cells than blank control cells, and miR-204-3p significantly reversed the downregulation of miR-204-3p induced by BANCR overexpression in Y79 cells (Fig. 2a). In addition, miR-204-3p expression was significantly upregulated in BANCR knockdown Y79 cells, and miR-204-3p inhibitor could reverse this upregulation of miR-204-3p (Fig. 2b). Bioinformatics analysis predicted a putative miR-204-3p binding site in BANCR (Fig. 2c). Next, luciferase reporter assay was used to verify the interaction between BANCR and miR-204-3p. The luciferase intensity was only obviously attenuated in the cells that co-transfected BANCR and miR-204-3p WT, but not in the miR-204-3p Mut lacking the putative miR-204-3p binding site (Fig. 2d). These results indicated that BANCR might regulate miR-204-3p expression by directly binding to it.

BANCR Promotes but miR-204-3p Inhibits the Growth, Invasion, and Migration of RB Cells

We next to explore whether miR-204-3p is involved in the biological functions of lncRNA-BANCR in RB cells. The knockdown of BANCR in Y79 cells significantly promoted cell apoptosis, inhibited cell proliferation, whereas knockdown of miR-204-3p exhibited opposed effects, which could partly reverse the effects of BANCR on cell proliferation and apoptosis (Fig. 3a and b). In Y79 cells, the knockdown of BANCR decreased the Ki67 expression and increased the Caspase-3 expression, whereas a reverse trend was observed when miR-204-3p was inhibited

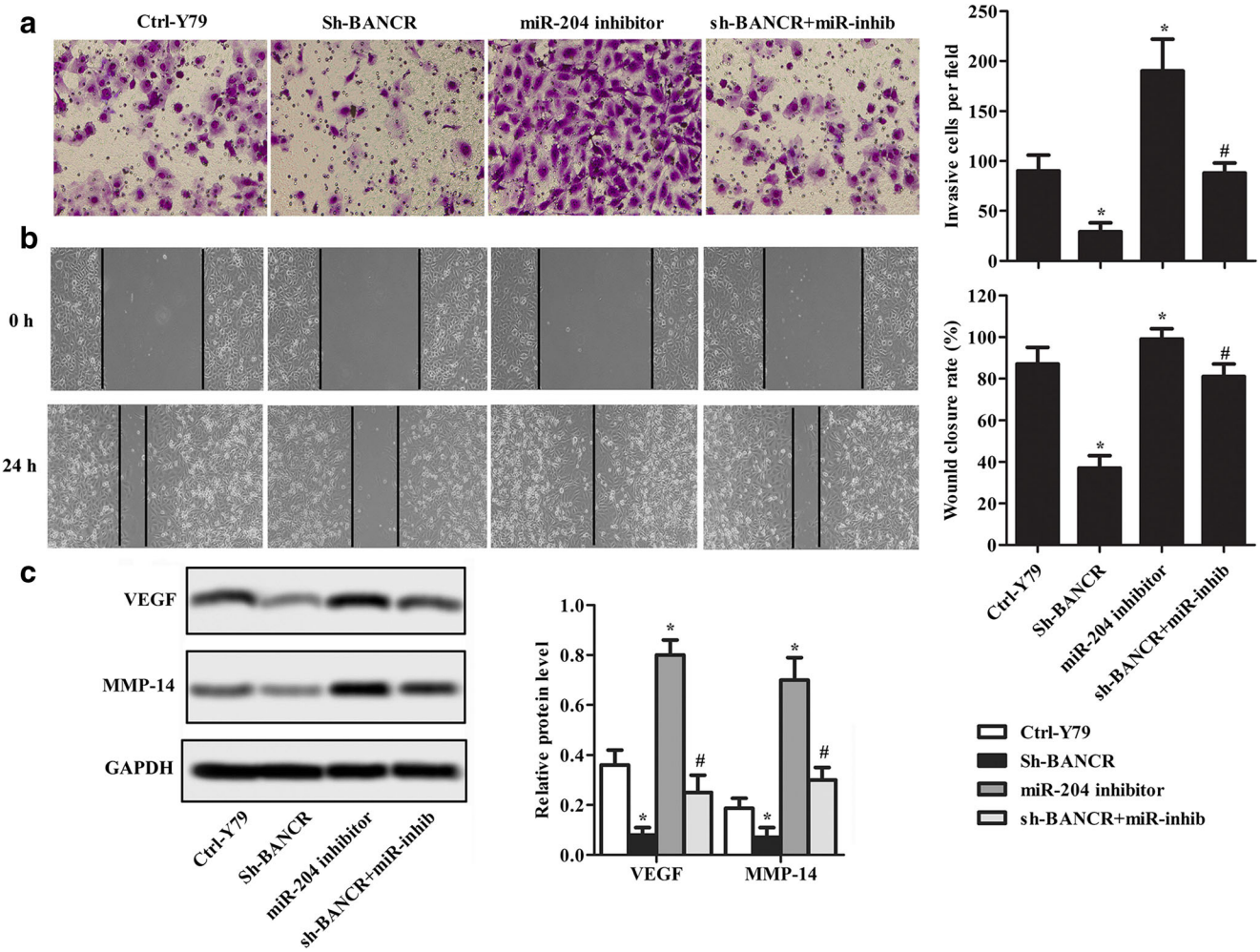

Fig. 4 The inhibition of the cell invasion and migration by the knockdown of BANCR in RB cell line. **a** Cell invasion and (**b**) cell migration of Y79 cells transfected with sh-BANCR and treated with miR-204-3p inhibitor, respectively or together, was assessed by transwell assay and wound-healing assay, respectively. **c** The protein expression levels of VEGF and MMP-14 were measured by Western blots in Y79 cells transfected with sh-BANCR and treated with miR-204-3p inhibitor, respectively or together. Data was shown as means ± SD by at least three independent experiments (*$p < 0.05$, versus control Y79 cells; #$p < 0.05$, versus miR-204 inhibitor group)

(Fig. 3c). Moreover, the knockdown of BANCR significantly suppressed cell invasion and migration, whereas the knockdown of miR-204-3p exhibited opposed effects and reversed the effects of BANCR (Fig. 4a and b). The knockdown of BANCR remarkably decreased the expression of vascular endothelial growth factor (VEGF) and matrix metalloproteinase 14 (MMP-14), whereas knockdown of miR-204-3p not only exhibited opposed effects but also abolished the effects of BANCR on VEGF and MMP-14 expression (Fig. 4c). These data showed that miR-204-3p is involved in the oncogenic functions of BANCR in RB cells.

Wnt/β-catenin Signaling Pathway Was Involved in the Oncogenic Effects of BANCR in RB Cells

Dyregulation of Wnt/β-catenin signaling pathway was frequently resulted in development disorder, and considered to be related with the formation of many human tumors. Besides, accumulating researches have suggested that miRNAs may interact with Wnt/β-catenin signaling pathway by regulating the protein expression of Wnt4. Our results from bioinformatics analysis (TargetScan Human) also predicted binding sites in Wnt4 for miR-204-3p. To determine whether Wnt/β-catenin signaling pathway is involved in the BANCR/miR-204-3p effects on RB cells, we evaluated the protein expression of Wnt4, total β-catenin, active β-catenin and TCF4 by western blot. The expression levels of Wnt4, active β-catenin, and TCF4 were significantly downregulated in the BANCR knockdown Y79 cells, and this downregulation was blocked by miR-204-3p inhibitor (Fig. 5). These results suggest that Wnt/β-catenin signaling pathway may participate in the effects of BANCR/miR-204-3p axis in RB cells.

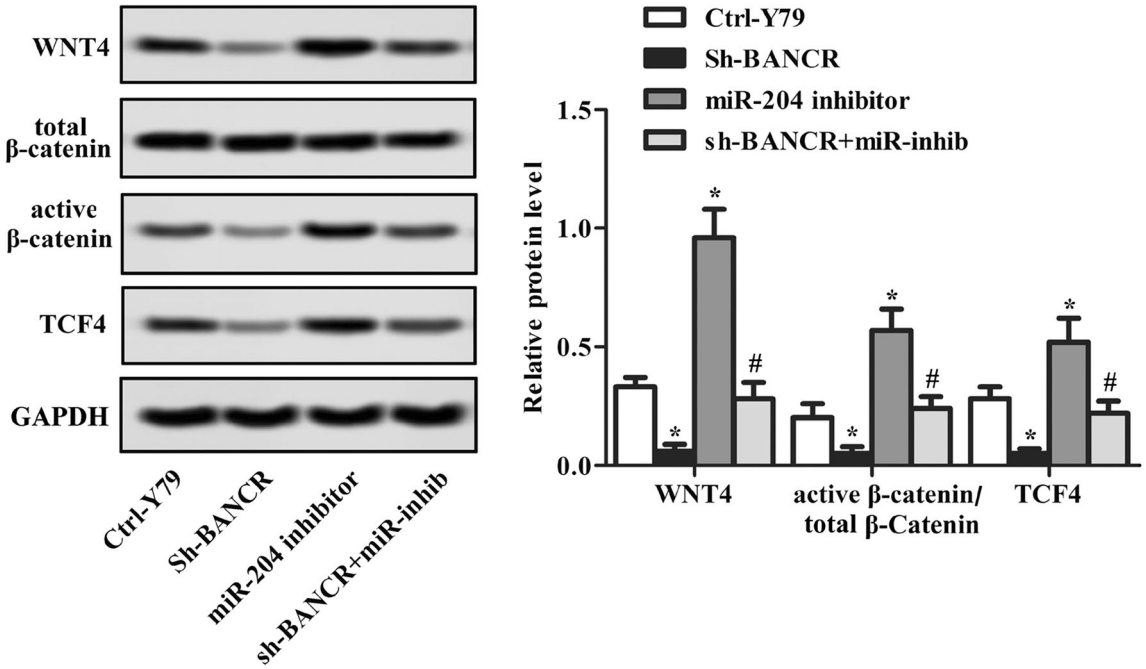

Fig. 5 The inhibition of Wnt/β-catenin signaling pathway by the knockdown of BANCR. The protein expression levels of WNT4, total β-catenin, active β-catenin and TCF4 were measured by Western blots in Y79 cells transfected with sh-BANCR and treated with miR-204-3p inhibitor, respectively or together (*$p < 0.05$, #$P < 0.05$). Data was shown as means ± SD by at least three independent experiments (*$p < 0.05$, versus control Y79 cells; #$P < 0.05$, versus miR-204 inhibitor group)

BANCR Knockdown Results in Tumor Inhibition Via miR-204-3p Upregulation and Wnt/β-catenin Pathway Inactivation In Vivo

To further verify our in vitro results, we applied an in vivo xenograft model. Y79 cells stably expressed sh-BANCR were injected subcutaneously into nude mice. Compared to the control Y79 cell group, the sh-BANCR group exhibited an obvious reduction in tumor volume (Fig. 6a). The diameter of the largest subcutaneous tumor was 14.5 mm and no multiple tumor was detected in any mouse. The mice with stable sh-BANCR expression displayed a significantly longer survival time than mice in the control group (Fig. 6b). Results from in vivo assay also indicated a similar suppressed effect of BANCR on miR-204-3p expression (Fig. 6c), and IHC performed in the tumor samples with BANCR knockdown revealed a similar downregulation of Ki-67 and VEGF and an upregulation of Caspase-3. The results suggest the suppressed effects of BANCR on cell apoptosis and the promoted effects of BANCR on cell apoptosis (Fig. 6d). Finally, we evaluated the activation of Wnt/β-catenin signaling pathway by measuring the protein expression of WNT4, total β-catenin, active β-catenin, and TCF4 in vivo, and obtained similar results with in vitro (Fig. 6e).

Discussion

Due to the lack of ability in protein coding, ncRNAs were once considered as a transcripts noise in the genome [20]. However, with more and more research data available, the knowledge of ncRNAs on biological effects has changed from meaningless to significant [21–23]. Accumulating studies revealed the oncogenic or suppressive roles of ncRNAs in various of human tumors, suggesting that ncRNAs may server as the potential effective biomarkers or therapeutic targets for many human cancers [24–26].

BANCR is identified in a massively parallel complementary DNA (cDNA) sequencing screen [27], and associated with the initiation and progression of many different human tumors, including melanoma, colorectal cancer, and thyroid cancer [28–30]. The overexpressed BANCR has been found in RB tissues and cell lines, which may promote tumor progression of RB [18]. In addition, miR-204 is reportedly involved in the pathogenesis of various of human cancers, including RB [17]. However, the exact role of BANCR and miR-204-3p in RB remains need to be explored. In our study, we revealed that the lncRNA-BANCR expression is increased and miR-204-3p is decreased in RB cell lines, and BANCR may negatively regulate miR-204-3p expression by directly interaction with it.

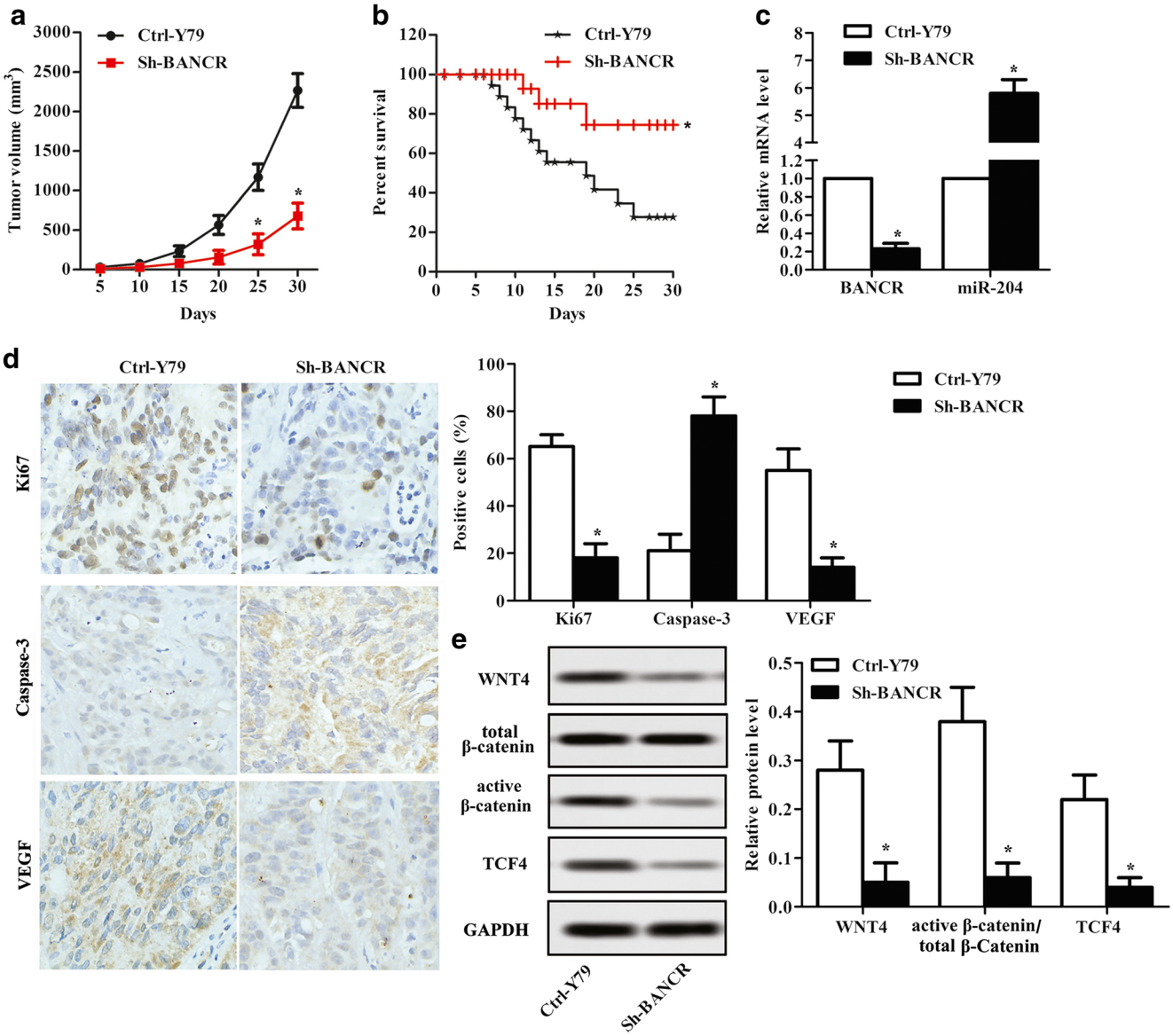

Fig. 6 The in vivo oncogenic roles of BANCR in RB. Y79 cells transfected sh-BANCR were subcutaneously injected into nude mouse, then (**a**) tumor size and (**b**) survival time was examined. (**c**) The relative mRNA expression levels of BANCR and miR-204-3p were detected by qRT-PCR in tumors from nude mouse injected with lncRNA-BACNCR silenced Y79 cells. (**d**) Representative images and statistical graphs for Ki67, Caspase-3, and VEGF immunostaining of tumor samples from nude mouse injected with lncRNA-BACNCR knockdown Y79 cells. (**e**) Proteins expression levels of WNT4, total β-catenin, active β-catenin and TCF4 in tumor samples from nude mouse were examined by western blot analysis. Data was shown as means ± SD by at least three independent experiments (*$p < 0.05$ versus control Y79 cells)

The ncRNAs may influence the physiological activities of cells, such as apoptosis, proliferation, invasion, and migration [31, 32]. The lncRNA-BANCR and miR-204-3p are related with these physiological activities of various human tumors, including RB [17, 18]. Our data reveal that BANCR and miR-204-3p influence the cell proliferation, apoptosis, invasion, and migration of RB cell line, which is consistent with the previous studies.

Wnt/β-catenin pathway, characterized by regulating cell development and tissue homeostasis, is a critical signaling pathway, which participates in the formation of various tumors by interacting with miRNAs [33–35]. β-catenin might be captured by a compound protein that consisted by adenomatous polyposis coli (APC), glycogen synthase kinase 3 β (GSK3 β) and Axin in the normal situation. However, once activated by Wnt protein, β-catenin is released to cytoplasm and eventually enters nucleus [36, 37]. Finally, β-catenin could regulate the corresponding gene expression by interacting with transcription factor 4 (TCF4) [38–40]. Recently, miR-204 is reported to promote adipogenic differentiation by suppressing Wnt signaling pathway [41, 42]. In addition, according to the bioinformatics analysis, we have

found that WNT4 exists predict binding sites for miR-204. Subsequently, WNT signaling pathway is necessary for the effects of BANCR and miR-204-3p on the RB cell line.

Although the in vitro roles of BANCR in RB cells were examind in our and other studies [18, 28, 43], the oncogenic effects of BANCR still need to be confirmed in vivo. Therefore, we established a xenograft model by injecting sh-BANCR silenced Y79 cells into the right flank of nude mice. The in vivo results were consistent with the in vitro results, which suggested that BANCR functions as a tumor promoter in RB by inhibiting miR-204-3p and WNT signaling pathway.

In conclusion, in RB cell lines the mRNA expression level of BANCR is higher and of miR-204-3p is lower when compared with that in a human retinal pigment epithelial cell line. The knockdown of BANCR inhibits the cell growth, invasion and migration with enhanced cell apoptosis in vitro via targeting miR-204-3p. Wnt/β-catenin signaling pathway is inhibited in the BANCR silenced Y79 cells, which could be reversed by the application of miR-204-3p inhibitor. In addition, we confirm the oncogenic roles of BANCR in vivo. BANCR may server as an oncogenic agent in RB by negatively regulating miR-204-3p expression, and inhibiting Wnt/β-catenin signaling pathway.

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Compliance with Ethical Standards

Competing Interests The authors declare that they have no competing interests.

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