



MicroRNA-454 functions as an oncogene by regulating PTEN in uveal melanoma



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ABSTRACT

MicroRNAs (miRNAs) regulate gene expression by targeted repression of transcription and translation, and are involved in carcinogenesis. In this study, we demonstrated that the expression of miR-454 was up-regulated in uveal melanoma tissues compared to normal tissues. Ectopic expression of miR-454 resulted in significant promotion of cell proliferation, colony formation, invasion and induction of cell cycle in uveal melanoma cells. Furthermore, we identified PTEN as a direct target of miR-454. Our data revealed that ectopic expression of PTEN restored the effects of miR-454 on cell proliferation and invasion in uveal melanoma cells. These findings support an oncogene role of miR-454 in development of uveal melanoma.

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1. Introduction

Uveal melanoma is the most common malignancy of the eye. It is arising out of pigmented cells including the ciliary body, choroid, or iris [1–4]. The annual incidence of uveal melanoma is five to seven cases per million populations [5–7]. Metastatic disease of the liver accounts for the high death rate of uveal melanoma, with up to 50% of patients having liver metastases [8–11]. Currently, the etiology and pathogenic mechanism of uveal melanoma are still not elucidated. Therefore, it is important to identify the new crucial biomarkers for early prognosis and therapeutic targets [12–14].

MicroRNAs (miRNAs) are a class 18–22 nucleotide-length non-coding RNAs that are involved in posttranslational regulation of gene expression by repressing translation or cleaving RNA transcripts in a sequence-specific manner [15–18]. A growing body of evidence suggests that miRNAs play crucial roles in various

physiological and pathological processes, including development, cell differentiation, proliferation, apoptosis, migration and signal transduction [19–22]. Recent evidence also identifies muted or aberrantly expressed miRNAs in various human cancers such as gastric cancer, bladder cancer, breast cancer, osteosarcoma, hepatocellular carcinoma and Ewing's sarcoma, indicating that miRNAs can function as tumor suppressors or oncogenes [23–29].

The expression of miR-454 was reported to be upregulated in human colorectal cancer tissues and cell lines, functioning as a novel oncogenic miRNA contributing to colon tumorigenesis by regulating TGF- β /Smad signaling by targeting Smad4 [30]. Moreover, another study showed that the expression of miR-454 was downregulated in the TGF- β 1-treated LX-2 cells and miR-454 could inhibit the activation of hepatic stellate cells (HSCs) by directly targeting Smad4 [31]. However, the expression and role of the miR-454 in the uveal melanoma development and progression remain unknown. In our study, we demonstrated that miR-454 acted as an oncogene in uveal melanoma. The expression of miR-454 was upregulated in uveal melanoma tissues compared to normal tissues and this was also observed in uveal melanoma cells and human melanocyte cell line. Ectopic expression of miR-454 resulted in significant promotion of cell proliferation, colony formation, invasion and induction of cell cycle in uveal melanoma cells. Furthermore, we identified PTEN as a direct target of miR-454.

Author contributions: Lei Sun, Qiaoling Wang, Xiangchun Gao, Dejing Shi, Shuyong Mi, Qing Han conceived and supervised the study; Lei Sun, Qiaoling Wang, Xiangchun Gao, Dejing Shi, Shuyong Mi, Qing Han designed experiments; Lei Sun, Qiaoling Wang performed experiments; Qing Han provided new tools and reagents; Lei Sun, Qiaoling Wang developed new software and performed simulation studies; Lei Sun, Qiaoling Wang analyzed data; Lei Sun, Qiaoling Wang wrote the manuscript; Lei Sun, Qiaoling Wang made manuscript revisions.

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2. Materials and methods

2.1. Ethics statement

All patients provided written informed consent for this study and this study was also approved by the Medical Ethics Committee of The Fourth Hospital of Harbin Medical University.

2.2. Tissue specimens and cell lines

Tumor samples and their morphologically normal tissues (located > 0.3 cm from the tumor) were obtained from 25 uveal melanoma patients and were immediately frozen in liquid nitrogen. Human uveal melanoma cell lines (OCM-1A, MUM-2C, C918 and MUM-2B) and human melanocyte cell line (D78) were obtained from the Cell Bank of the Chinese Academy of Sciences (Beijing, People's Republic of China). The OCM-1A, MUM-2C and D78 cells were maintained in Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal bovine serum (FBS), and MUM-2B, C918 in RPMI 1640.

2.3. RNA preparation and quantitative real-time PCR (qPCR) assay

Total RNA was extracted from tissues or cells using TRIzol reagent (Invitrogen, Carlsbad, CA, USA) following to the manufacturer's instruction. Quantitative real-time PCR was performed using SYBR Premix Ex Taq II (TaKaRa). TaqMan microRNA assays (Applied Biosystems, Foster City, California, USA) were used to quantify the expression levels of miR-454. Forward and reverse primers were used as follows: PTEN, CACCTATTCTCAGCCCTTAT and AACCTCATTCAGACCTTCAC; GAPDH, AATGGGCAGCCGTTAGG AAA and TGAAGGGGTCATTGATGGCA; miR-454, ACCCTATCAA TATTGTCTCTGC and GCGAGCACAGAATTAATACGAC; U6, CGCTTCG GCAGCACATATACTA and CGCTTCACGAATTTGCGTGTC.

2.4. Oligonucleotide transfection

miR-454 mimics and scramble were obtained by Genepharma (Genepharma, Shanghai, China). PTEN vector were synthesized by RiboBio (RiboBio, Guangzhou, China). Cells were transfected with oligonucleotides using Lipofectamine 2000 Reagent (Invitrogen Life Technologies) following the manufacturer's instructions.

2.5. Cell proliferation, colony formation and cell cycle assay

Cell proliferation was performed using a Cell Counting Kit-8 (CCK-8) (Dojindo, Kumamoto, Japan) according to the manufacturer's instructions and cell numbers were counted by the optical density at 450 nm. For colony formation assay, cells were cultured in a 6-well plate and seeded for 10 days. Colonies were fixed with paraformaldehyde and stained with 1% crystal violet (Sigma–Aldrich) and the numbers of colonies were counted. The fixed cells were incubated with propidium iodide (PI) and ribonuclease A for 30 min, and then detected by flow cytometric analysis using FL2 histogram of a flow cytometer (FACSort; Becton–Dickinson, San Jose, CA, USA).

2.6. Cell invasion assay

Invasion assay was performed using Matrigel invasion chamber system (BD Biosciences, Bedford, MA). Cells were cultured on the top of matrigel-coated chambers in a serum-free medium and DMEM containing 10% FBS was used as a chemoattractant. Invasion of cells to the lower side were fixed and stained with % crystal violet (Sigma–Aldrich) and were counted.

2.7. Western blot analysis

Proteins were extracted from cells using Protein Extraction Kit (KeyGen, Nanjing, China) and were separated on 10% sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) and transferred to nitrocellulose membrane (Bio-Rad, Hercules, California, USA). The membranes were blocked for 1 h with 5% non-fat milk and then detected with primary antibodies recognizing PTEN (Abcam, Cambridge, MA, USA), mouse anti-GAPDH antibody (1:3000; Sigma–Aldrich), respectively. Proteins were detected with enhanced chemiluminescence reagents (Thermo Scientific, Waltham, Massachusetts, USA) after incubation with appropriate horseradish peroxidase (HRP)-conjugated secondary antibodies (Jackson ImmunoResearch).

2.8. Luciferase reporter assay

The mutated (MUT) and wild-type (WT) putative miR-454 target on PTEN 3'UTR were cloned into the pGL3 Dual-Luciferase miRNA Target Expression Vector (Invitrogen Corporation). Luciferase activity was performed using the Dual-Luciferase Reporter Assay System (Promega) according to manuscript's instruction.

2.9. Statistical analysis

SPSS16.0 (Chicago, IL, USA) was performed for statistical analysis. Data were presented as the mean $\pm$ standard deviation (S.D.) from at least three independent experiments. Student's *t*-test or a one-way analysis of variance (ANOVA) was used to evaluate the statistical significance of the results. Differences were considered statistically significant at $P < 0.05$.

3. Result

3.1. The expression of miR-454 was upregulated in uveal melanoma tissues and cell

The expression of miR-454 was upregulated in uveal melanoma samples compared to their normal samples (Fig. 1A). Further results confirmed that miR-454 was upregulated in uveal melanoma (OCM-1A, MUM-2C, C918 and MUM-2B) compared to human melanocyte cell line (D78) (Fig. 1B).

3.2. MiR-454 promoted the uveal melanoma cell proliferation and invasion

To measure the functional role of miR-454 overexpression in uveal melanoma, we transfected uveal melanoma cell line MUM-2B with miR-454 mimic (Fig. 2A). Ectopic expression of miR-454 promoted cell proliferation as compared to cells expressing scramble (Fig. 2B). Cell cycle analysis revealed that overexpression of miR-454 decreased the number of cells in the G0/G1 phase of the cell cycle while the S-phase population increased in MUM-2B cells (Fig. 2C). Colony formation assay also showed that ectopic expression of miR-454 increased the MUM-2B cell colony formation (Fig. 2D). Invasion assay analysis demonstrated that miR-454 overexpression promoted the MUM-2B cell invasion (Fig. 2E).

3.3. PTEN was a direct target of miR-454 in uveal melanoma

We used TargetScan to find that 3'UTR of PTEN contained the highly conserved putative miR-454 binding sites (Fig. 3A). Luciferase reporter analysis demonstrated that overexpression of miR-454 reduced luciferase activity in the PTEN wild-type reporter

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