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MicroRNA-34a suppresses human lens epithelial cell proliferation and migration via downregulation of c-Met

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ABSTRACT

MicroRNAs (miRNAs) are endogenously expressed, non-coding, small RNAs which inhibit protein translation through binding to target mRNAs. Recent studies have demonstrated that miRNAs participate in the regulation of a variety of cell structures and functions including those for cell proliferation and migration. MicroRNA-34a (miR-34a), a potential effector of the p53 tumor suppressor gene, is extensively studied for its suppression of cell growth. In the present study, we investigated the function of miR-34a in human lens epithelial cells. Following confirming that miR-34a expression was increased in a P53 dependent manner in human lens epithelial cells after treatment with doxorubicin, we demonstrated that overexpression of miR-34a in the human lens epithelial cell line HLE B3 led to a significant decrease in cell proliferation and migration, with the use of MTS and transwell migration assays. Moreover, HGF enhanced the proliferation and migration of human lens epithelial cells. miR-34a was found to downregulate the expression of c-Met protein by Western blotting. Furthermore, overexpression of miR-34a downregulated the levels of phosphorylated Akt, phosphorylated ERK1/2 and other cell cycle regulators. miR-34a expression was significantly reduced in posterior capsule opacification (PCO) clinical samples. These results demonstrate that miR-34a may act as a suppressor in PCO by regulating human lens epithelial cell proliferation and migration through downregulation of c-Met.

1. Introduction

Cataract, defined as an opacity of the normally transparent lens or lens capsule, is the major cause of visual impairment and blindness in modern society. Posterior capsule opacification (PCO) is the most frequent complication of cataract surgery. Approximately 35% of patients who undergo cataract surgery experience a secondary loss of vision that requires further corrective surgery within 2 years [1]. PCO is mainly caused by the proliferation and migration of postoperative remnants of lens epithelial cells (LECs) in the posterior lens capsule [2]. Although findings from previous studies much increased our understanding of the pathogenesis of PCO, the molecular mechanisms underlying PCO development is elusive. Recent studies have revealed the cytokines involved in the development of PCO and suggested some strategies for its prevention [3]. While Doxorubicin (adramycin) is effective for PCO treatment, the molecular and cellular pathways remain to be characterized [4].

MicroRNAs (miRNAs) are an abundant class of endogenously expressed, non-protein-coding, short (20–25) nucleotide RNAs, which regulate gene expression [5]. These small RNA molecules negatively regulate the translation and stability of target mRNAs through direct binding of complementary sequences at their 3′ untranslated regions (3' UTRs) [5,6]. Computational analysis suggests that miRNAs may help regulate > 30% of the protein-coding genes in diverse processes such as development, metabolism, cell proliferation and differentiation [6]. Aberrant posttranscriptional regulation of mRNAs by miRNAs can lead to oncogenesis with increased cell proliferation, decreased apoptosis, and enhanced metastatic potential of affected cells [7,8]. Since its discovery in 1993 as a developmental modulator, miRNAs are increasingly found to be important regulators in cell proliferation and migration [4,9]. Recently, microRNA-34a (miR-34a) was found to be a proapoptotic transcriptional target of the *p53* tumor suppressor gene effector network [10–12]. These studies suggest that miRNAs are involved in cancer formation through regulation of cell growth and migration.

Dicer, which is an essential ribonuclease for miRNA maturation, was found to be abundantly expressed during corneal and lens development [13]. Conditional knockout of Dicer led to disruption of lens morphogenesis and corneal epithelium stratification during corneal and lens development [13]. This study reveals a critical role of Dicer and the importance of miRNAs in regulation of cell proliferation and apoptosis in the developing lens. Nevertheless, their exact roles in human lens

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epithelial cells remains largely unkown.

In this study, we specifically address the function of miR-34a in lens epithelial cells, by determining the miR-34a expression alteration following adramycin treatment of HLE cells, miR-34a expression in lens associated with PCO, regulation of miR-34a by P53. Moreover, specific effects of miR-34a on c-Met expression levels and Akt phosphorylation are also explored. The findings strongly suggest that miR-34a represents a potent effector of the p53 transcriptional network. A better understanding on miR-34a actions in lens epithelial cells may facilitate the design of new treatment modalities for PCO and other diseases.

2. Materials and methods

2.1. Cell culture and clinical samples

The human lens epithelial cell line B-3 were purchased from ATCC (Manassas, VA), and grown in Dulbecco modified Eagle's media (DMEM; Invitrogen, Carlsbad, CA) supplemented with 10% fetal bovine serum (FBS; Hyclone, Logan, UT) and incubated at 37 °C in a humidified incubator containing 5% CO_2, as described.

Six human PCO specimens were obtained from patients treated at the First Affiliated Hospital, Zhejiang University (Hangzhou, China), with documented informed consent for each case. Three transparent lenses were from healthy donors in the Eye Bank of the First Affiliated Hospital. All studies and procedures involving human tissue were performed in compliance with the Helsinki Declaration, and approved by the Zhejiang University Ethics Committee.

2.2. Cell proliferation assay

Human lens epithelial cells were plated at 3×10^3 cells per well in 96-well plates (Costar, High Wycombe, UK) for each transfection. Cell transfection was performed using Lipofectamine 2000 (Invitrogen) and following the manufacturer's protocols. For each well, 50 nM of miR-34a precursor molecule (Ambion, Austin, TX) or a negative control precursor miRNA (Ambion) was transfected into cells. Pre-miR™ miRNA Precursor Molecules (Ambion) are small, chemically modified double-stranded RNA molecules designed to mimic endogenous mature miRNAs once properly transfected and expressed in recipient cells. For convenience, the miR-34a precursor is designated miR-34a throughout the manuscript. The negative control is scrambled oligonucleotides that have been validated to not produce identifiable effects on known miRNA function. After 24-hour culture, cell proliferation was assessed using the CellTiter 96 Aqueous MTS (3-[4,5-dimethylthiazol-2-yl]-5-[3-carboxymethoxyphenyl]-2-[4-sulfophenyl]-2H-tetrazolium, inner salt) assay (Promega, Madison, WI) according to the manufacturer's instructions. Briefly, the CellTiter 96 AQueous One Solution Reagent was added to each well and incubated at 37 °C for 3 h. Cell proliferation was assessed by measuring the absorbance at 490 nm using a microtiter plate reader (Molecular Devices, Sunnyvale, CA).

2.3. Transwell migration assays

Human lens epithelial cells were grown in DMEM containing 10% FBS to ~ 60% confluence and transfected with 50 nM miR-34a precursor molecule or a negative control. After 24 h, cells were harvested by trypsinization and washed once with D-Hanks solution (Invitrogen). To measure cell migration, 8-mm pore size-culture inserts (Transwell, Costar, Cambridge, MA) were placed into the wells of 24-well culture plates with separate upper and the lower chambers. In the lower chamber, 400 µl of DMEM containing recombinant human HGF (20 ng/ml) (R&D Systems, Minneapolis, MN) were added. 1×10^5 cells were seeded in the upper chamber. After 24 h of incubation at 37 °C with 5% CO_2, the number of cells that had migrated through the pores was quantified by counting ten independent visual fields under the microscope (Zeiss, Oberkochen, Germany) using a 20× objective, and cell morphology was observed by staining with hematoxylin and eosin.

2.4. Western blot analysis

HLE cells (1×10^5) were seeded and grown in DMEM with 10% FBS in 6-well plates for 24 h. After transfection, the cells were washed with cold phosphate-buffered saline (PBS) and subjected to lysis in a lysis buffer (50 mM/L Tris-Cl, 1 mM/L EDTA, 20 g/L sodium dodecyl sulfate [SDS], 5 mM/L dithiothreitol, 10 mM/L phenylmethylsulfonyl fluoride). Equal amounts of protein lysates (50 µg each) and rainbow molecular weight markers (Amersham Pharmacia Biotech, Amersham, UK) were separated in 10% SDS-polyacrylamide gel electrophoresis (PAGE), and proteins were electrotransferred onto nitrocellulose membranes. The membranes were blocked in PBS buffer containing 5% non-fat milk and 0.05% Tween 20 for 2 h. The membranes were incubated with primary antibody overnight at 4 °C. After extensive washing with PBS containing 0.05% Tween 20, the membranes were incubated with peroxidase conjugated secondary antibodies (Santa Cruz Biotechnology, Santa Cruz, CA) and developed with an electro-generated chemiluminescence (ECL) detection kit (Pierce, Rockford, IL). Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as a protein loading control. Antibodies for total ERK1/2, phosphorylated-ERK1/2 (threonine 202/tyrosine 204), total Akt, phosphorylated-Akt (serine 473, D9E), and phosphorylated-Rb (serine 795) were purchased from Cell Signaling Technology (Beverly, MA), and antibody against c-Met were purchased from Santa Cruz Biotechnology.

2.5. Quantitative RT-PCR

Total RNA was extracted from human PCO specimens and normal tissues with Trizol reagent (Invitrogen, Carlsbad, CA). RNA integrity was confirmed by spectrophotometry and agarose gel electrophoresis. The cDNA synthesis and evaluation of miR-34a expression level was respectively performed with the use of Taqman MicroRNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA) and Taqman MicroRNA Assay (Applied Biosystems, Foster City, CA), according to the manufacturer's instructions. The ViiA™ 7 Real-Time PCR System (Applied Biosystems, Foster City, CA) was used to perform the Quantitative PCR experiments. The expression levels of miR-34a are standardized with the levels of U6 small nuclear RNA (snRNA).

2.6. Statistical analysis

All data were presented as the mean ± SEM. Differences between transfected with miR-34a and negative control were analyzed using the Student's *t*-test. Statistical significance was set at $P < .05$.

3. Results

3.1. miR-34a expression was upregulated by P53 in human lens epithelial cells

Quantitative RT-PCR analysis was performed to determine the changes of miR-34a following Doxorubicin treatment of HLE B3 cells. It was found that miR-34a levels increased at both 24 h and 48 h (Fig. 1A). To determine if induction of miR-34a by Doxorubicin is p53 dependent, preceding the Doxorubicin treatment we transfected HLE B3 cells with a siRNA that specifically targets p53 or a scrambled control siRNA. As shown in Fig. 1B, while the control siRNA did not change the induction of miR-34a by Doxorubicin treatment, p53 knockdown by siRNA significantly reduced the expression of miR-34a, to a level comparable to that in cells with no Doxorubicin treatment. These results indicate that the induction of miR-34a by Doxorubicin treatment of HLE B3 cells is related to p53 function.

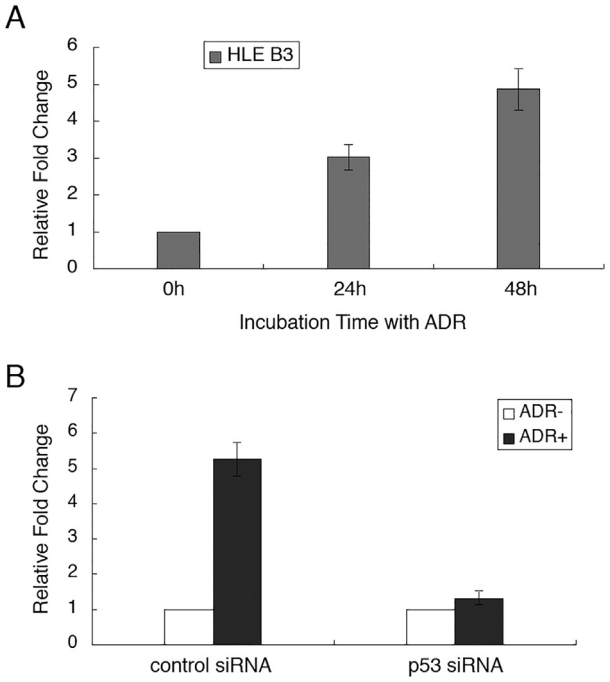

Fig. 1. Upregulation of miR-34a expression in human lens epithelial cell line B-3 after treated with ADR. (A) HLE B3 cells were incubated in the presence of doxorubicin at a concentration of 1 μg/mL. Quantitative RT-PCR analysis was performed to detect the expression of miR-34a in human lens epithelial cell line B-3 after treated with ADR. The value for miR-34a at time 0 was set as 1, and the relative amounts of miR-34a at other time points were plotted as folds of induction. miR-34a expression was upregulated when treated with doxorubicin. (B) HLE B3 cells were transfected with a siRNA targeting p53 or a scrambled negative control siRNA were treated with 1 μg/mL of doxorubicin for 48 h. miR-34a expression levels were indicated, as determined by Quantitative RT-PCR relative to the level of U6 snRNA expression. Representative data from three independent experiments are shown. The increase of miR-34a expression by doxorubicin was blocked when transfected with P53 siRNA.

3.2. miR-34a inhibited human lens epithelial cell proliferation

Since miR-34a was demonstrated to be regulated by P53 in HLE B3 cells, we next examine if increased miR-34a may exert biological effects. HLE B3 cells were transfected with either the miR-34a precursor or a negative control. After transfection, the MTS assay was carried out to assess growth inhibition at days 1, 2, 3, 4, and 5. While transfection with the negative control did not affect HLE B3 cell proliferation and viability, transfection with miR-34a caused a significant inhibition of cell proliferation at 5 day post-transfection (Fig. 2). A reduction in cell number was detected by day 2 following transfection, and the differences between cells transfected with the miR-34a precursor and cells transfected with a negative control were statistically significant starting from day 3. These results suggest that miR-34a participates in the regulation of cell proliferation, and increased miR-34a inhibits HLE B3 proliferation.

3.3. miR-34a induced human lens epithelial cell cycle G1 arrest

Since miR-34a inhibited HLE cell proliferation, we next investigate if cell cycle is regulated by miR-34a. Forty-eight hours after transfection of miR-34a, cells were stained with propidium iodide and analyzed by flow cytometry. HLE cells transfected with miR-34a showed 53.03% G1 arrest in comparison to 40.17% for negative control (Fig. 3). This result demonstrated that miR-34a overexpression leads to G1 cell cycle arrest in HLE cells.

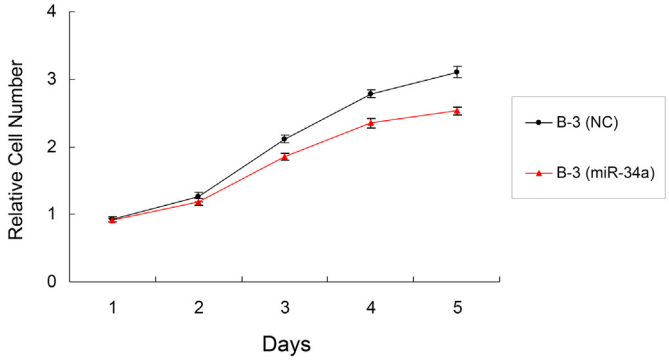

Fig. 2. miR-34a inhibited the proliferation of human lens epithelial cells. To evaluate cell proliferation, MTS assay was performed on days 1 to 5 as indicated after transfection of HLE B3 cells with either the miR-34a mimic or a negative control (NC). The negative control does not encode any known miRNAs. Cell populations transfected with miR-34a had a remarkable decrease than cells transfected with the negative control. Results represent those obtained in three experiments.

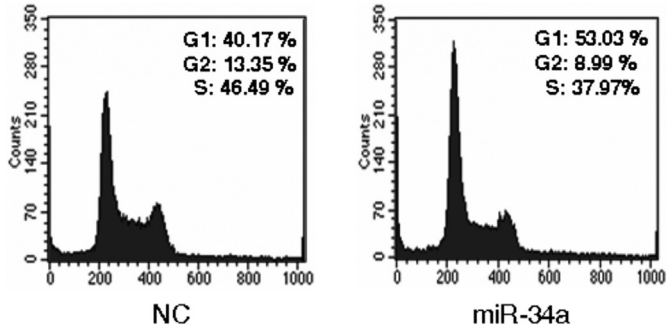

Fig. 3. miR-34a induces cell cycle G1 arrest. HLE cells were collected 48 h after transfection of HLE B3 cells with miR-34a or negative control (NC), stained with propidium iodide, and analyzed by flow cytometry. Ten thousand cells were evaluated in each sample. Three independent experiments were carried out and a representative result is shown.

3.4. miR-34a inhibited human lens epithelial cell migration

We next assessed the effect of miR-34a on HLE B3 cell migration, using a transwell migration assay. Following transfection of HLE B3 cells with either a miR-34a precursor or control oligonucleotides, vertical migration studies was then performed. Cells were seeded on culture inserts and the ability of cells to migrate to the underside of the inserts was determined. As shown in Fig. 4, the HGF-induced migration was significantly decreased when comparing miR-34a transfected cells to negative control. Therefore, miR-34a overexpression results in a reduced cell motility.

3.5. HGF enhanced human lens epithelial cell proliferation and migration

It has been reported that HGF can enhance HLE cell proliferation, which was also confirmed by our experiment. MTS assay showed that cell growth increased after 72 h treatment with HGF (Fig. 5A). Furthermore, HLE B3 cell migration was enhanced by HGF (Fig. 5B). These results indicated that HGF/c-Met axis may play an important role in HLE cell proliferation and migration.

3.6. Ectopic miR-34a downregulated the expression of c-Met pathway and cell cycle related proteins in HLE cells

c-Met has been shown to activate diverse intracellular signaling pathways. To examine intracellular proteins affected by miR-34a in

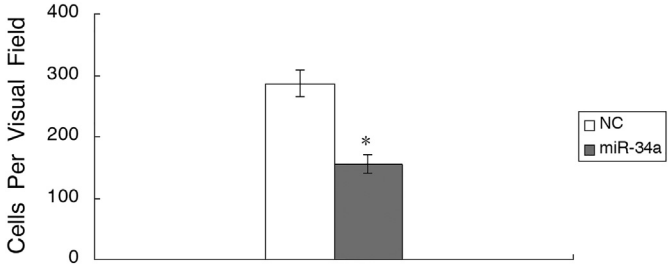

Fig. 4. Overexpression of miR-34a leads to a reduced migration of human lens epithelial cells. HLE B3 cells were transfected with miR-34a or a negative control (NC) for 24 h and plated on matrigel inserts in DMEM medium containing 20 ng/ml of HGF to assess the number of migrating cells. The number of cells that had migrated through the pores was counted under five independent visual fields using a 20× magnitude. Results are expressed as mean ± SEM. *: Differences in cell migration between miR-34a and negative control transfected cells were significant, $P < .01$.

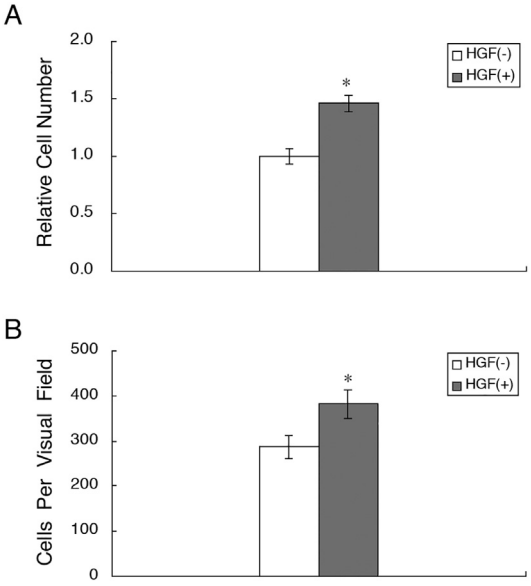

Fig. 5. HGF enhanced human lens epithelial cell proliferation and migration. (A) MTS proliferation assay was performed after treatment of HLE B3 cells with HGF (20 ng/ml) for 72 h. The number of cells showed a significant increase after treatment with HGF as compared with control. (B) Transwell migration assay of human lens epithelial cell line was performed. HLE B3 cells were treated with HGF (20 ng/ml) or without HGF as a control for 24 h and plated on cultured inserts in DMEM medium to assess the number of migrating cells.

HLE cells, we next determined the expression patterns of ERK1/2 and Akt after downregulation of c-Met by miR-34a. HLE cells were transfected with either the miR-34a precursor or a negative control. Cells were solubilized and subjected to Western blot analysis with phosphorylation-specific antibodies to Akt and ERK1/2, molecules involved in the two major signaling pathways previously shown to be stimulated by HGF. As shown in Fig. 6B, the dramatic downregulation of c-Met by miR-34a was accompanied by significant reduction of phosphorylated-Akt and phosphorylated-ERK1/2 in HLE cells. Neither total Akt nor total ERK1/2 was affected following transfection with the negative control (Fig. 6). In addition to its effects on phosphorylated-Akt, ectopic miR-34a also downregulated phosphorylation of cell cycle regulatory proteins such as CDK4, CDK6 and retinoblastoma protein (Rb) in HLE cells (Fig. 6).

3.7. Downregulation of miR-34a expression in PCO clinical samples

To evaluate if miR-34a expression levels are abnormal in PCO pathogenesis, we performed real-time RT-PCR to compare miR-34a levels in PCO clinical samples with those in normal transparent lenses from three healthy donors. In six PCO clinical specimens, miR-34a levels were significantly reduced compared to those in the normal controls (Fig. 7).

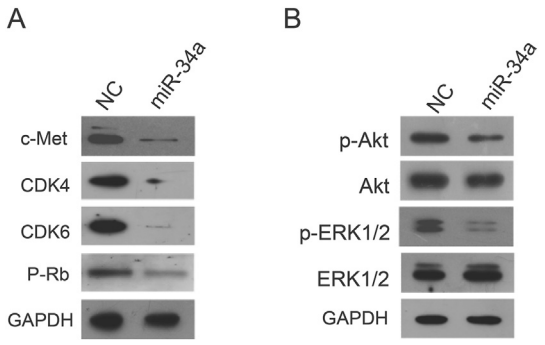

Fig. 6. Transfection of miR-34a downregulated c-Met signal pathway and cell cycle regulators in HLE B3 cells. (A) Results of Western blotting showing that overexpression of miR-34a downregulated c-Met, CDK4, CDK6, and p-Rb protein expression. (B) Overexpression of miR-34a downregulated phosphorylated Akt and phosphorylated ERK1/2, and cell cycle regulators p-Rb, CDK4, and CDK6.

4. Discussion

Emerging evidence suggests that miRNA may have a regulatory role in the pathogenesis of cataract development through suppression of genes involved in cell proliferation, differentiation, and migration [14–18]. Analysis of the p53 gene products identified miR-34a as a direct transcriptional target and an important component of the tumor suppressor network [10–12]. miR-34a helps to regulate cell cycle progression, DNA repair and apoptosis following its elaboration within the cellular machinery. But so far, little is known about the role of miR-34a in HLE cells during PCO.

Although p53 has been identified as a potential regulator of miR-34a, downstream effectors of miR-34a remains a mystery. The MET oncogene is a cell surface receptor tyrosine kinase that is upregulated in a variety of tumor cells similar in scope to p53 mutants [19,20]. c-Met activation, through aberrant HGF paracrine stimulation, can contribute to cell growth, invasiveness, and metastasis [19,20]. Overall, c-Met mutations occur at a far lower frequency than expected given the types of aggressive tumors seen with p53 mutants. However, the expression

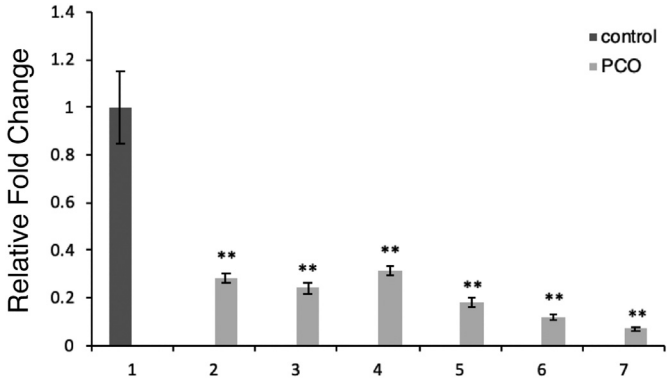

Fig. 7. MiR-34a expression is significantly reduced in PCO clinical samples. Quantitative RT-PCR was carried out to determine the expression of miR-34a in six PCO specimens. Human transparent lenses were used as normal controls. The average value for miR-34a in lenses from three donors was set as 1, and the relative amount of miR-34a in the PCO specimens was plotted as folds of change. 1: Normal control; 2–7: PCO specimens. **: $P < .01$.

pattern of MET mutations is seemingly similar to the ones with miR-34a deficiency. Therefore, we examined the c-Met pathway of HLE cells following transfection with miR-34a.

In the present study, we demonstrated that miR-34a is an important modulator of HLE proliferation and migration with its effects linked to the c-Met signaling pathway. Doxorubicin-stimulated miR-34a expression is upregulated by P53 in HLE cells. Transfection of miR-34a into HLE cells dis0played an inhibitory activity on cell growth. Cell migration, a corner stone capability necessary for PCO process, has been shown to be affected by the presence of miR-204 [17]. Here, we were able to reveal for the first time that miR-34a can regulate HLE cell proliferation and migration through its target gene *c-Met*. Transwell experiment illustrated that HLE cell migration can be inhibited with the restoration of miR-34a activity. miR-34a inhibited HLE cell migration dramatically by targeting c-Met in an HGF-dependent fashion. Our findings also supported by previous studies that show the critical role of miRNA/c-Met axis in multiple tumors [21–23].

In addition to regulation of c-Met activity, introduction of miR-34a downregulated phosphorylated Akt and cell cycle-related proteins, including Rb, CDK4, and CDK6. It has been reported previously that PI3K/Akt and ERK1/2 signaling pathway was involved in HGF-induced migration of HLE cells through activation of the HGF transmembrane receptor tyrosine kinase c-Met [24,25]. As c-Met levels are directly affected by miR-34a, its downstream effects could be similarly altered in melanoma cells. The retinoblastoma (Rb) gene was also affected by miR-34a, which was the first tumor suppressor gene identified in humans and one of the most important genes in cell cycle regulation. The major action of Rb in cell growth is to control the G_1-S transition in proliferating cells [26]. miR-34a, which downregulates Rb, in turn, downregulates the transcriptional activity of its downstream targets.

In summary, we demonstrated that miR-34a modulates several signaling pathways involved in HLE cell proliferation and migration. miR-34a downregulates c-Met, which in turn inhibits cell proliferation and migration through the Akt and ERK1/2 signaling pathway in an HGF-dependent fashion. miR-34a also affects cell proliferation via cell cycle proteins Rb, CDK4, and CDK6. Specifically, we were able to show upregulation of the cellular expression of miR-34a by P53 in HLE cells. A decreased level of miR-34a was found in human lens tissues associated with PCO. Importantly, restoration of this miRNA leads to inhibition of HLE cell growth and migration. These findings suggest that miR-34a may play an important role in regulating the development of HLE, and miR-34a, with its deficiency in lens, could be a promising therapeutic target for the treatment of PCO and other eye diseases.

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References

[1] D.S. Clark, Posterior capsule opacification, Curr. Opin. Ophthalmol. 11 (2000) 56–64.
[2] D.J. Apple, K.D. Solomon, M.R. Tetz, et al., Posterior capsule opacification, Surv. Ophthalmol. 37 (1992) 73–116.
[3] Suresh K. Pandey, David J. Apple, L. Werner, et al., Posterior capsule opacification: a review of the aetiopathogenesis, experimental and clinical studies and factors for prevention, Current Ophthalmology 52 (2004) 99–112.
[4] R. Guha, S. Chowdhury, H. Palui, et al., Doxorubicin-loaded MePEG-PCL nanoparticles for prevention of posterior capsular opacification, Nanomedicine (Lond) 8 (2013) 1415–1428.
[5] L. He, G.J. Hannon, MicroRNAs: small RNAs with a big role in gene regulation, Nat. Rev. Genet. 5 (2004) 522–531.
[6] D.P. Bartel, MicroRNAs: genomics, biogenesis, mechanism, and function, Cell 116 (2004) 281–297.
[7] Y. Wang, C.G. Lee, MicroRNA and cancer—focus on apoptosis, J. Cell. Mol. Med. 13 (2009) 12–23.
[8] J. Hayes, P.P. Peruzzi, S. Lawler, MicroRNAs in cancer: biomarkers, functions and therapy, Trends Mol. Med. 20 (2014) 460–469.
[9] R.C. Lee, R.L. Feinbaum, V. Ambros, The C. elegans heterochronic gene lin-4 encodes small RNAs with antisense complementarity to lin-14, Cell 75 (1993) 843–854.
[10] L. He, X. He, L.P. Lim, et al., A microRNA component of the p53 tumour suppressor network, Nature 447 (2007) 1130–1134.
[11] H. Hermeking, p53 enters the microRNA world, Cancer Cell 12 (2007) 414–418.
[12] T.C. Chang, E.A. Wentzel, O.A. Kent, et al., Transactivation of miR-34a by p53 broadly influences gene expression and promotes apoptosis, Mol. Cell 26 (2007) 745–752.
[13] Y. Li, J. Piatigorsky, Targeted deletion of dicer disrupts lens morphogenesis, corneal epithelium stratification, and whole eye development, Dev. Dyn. 238 (2009) 2388–2400.
[14] C. Wu, H. Lin, Q. Wang, et al., Discrepant expression of microRNAs in transparent and cataractous human lenses, Invest. Ophthalmol. Vis. Sci. 53 (2012) 3906–3912.
[15] K.H. Chien, S.J. Chen, J.H. Liu, et al., Correlation between microRNA-34a levels and lens opacity severity in age-related cataracts, Eye (Lond) 27 (2013) 883–888.
[16] N. Dong, X. Tang, B. Xu, et al., miRNA-181a inhibits the proliferation, migration, and epithelial-mesenchymal transition of lens epithelial cells, Invest. Ophthalmol. Vis. Sci. 56 (2015) 993–1001.
[17] Y. Wang, W. Li, X. Zang, et al., MicroRNA-204-5p regulates epithelial-to-mesenchymal transition during human posterior capsule opacification by targeting SMAD4, Invest. Ophthalmol. Vis. Sci. 54 (2013) 323–332.
[18] X. Yu, H1. Zheng, M.T. Chan, et al., MicroRNAs: new players in cataract, Am. J. Transl. Res. 9 (2017) 3896–3903.
[19] C. Birchmeier, W. Birchmeier, E. Gherardi, G.F. Vande Woude, Met, metastasis, motility and more, Nat. Rev. Mol. Cell Biol. 4 (2003) 915–925.
[20] M. Rosario, W. Birchmeier, How to make tubes: signaling by the met receptor tyrosine kinase, Trends Cell Biol. 13 (2003) 328–335.
[21] D. Yan, X. Zhou, X. Chen, et al., MicroRNA-34a inhibits uveal melanoma cell proliferation and migration through downregulation of c-met, Invest. Ophthalmol. Vis. Sci. 50 (2009) 1559–1565.
[22] D. Yan, E. Dong Xda, X. Chen, et al., MicroRNA-1/206 targets c-Met and inhibits rhabdomyosarcoma development, J. Biol. Chem. 284 (2009) 29596–29604.
[23] W. Qu, X. Chen, J. Wang, et al., MicroRNA-1 inhibits ovarian cancer cell proliferation and migration through c-Met pathway, Clin. Chim. Acta 473 (2017) 237–244.
[24] I.M. Wormstone, S. Tamiya, J.M. Marcantonio, et al., Hepatocyte growth factor function and c-Met expression in human lens epithelial cells, Invest. Ophthalmol. Vis. Sci. 41 (2000) 4216–4622.
[25] J. Choi, S.Y. Park, C.K. Joo, Hepatocyte growth factor induces proliferation of lens epithelial cells through activation of ERK1/2 and JNK/SAPK, Invest. Ophthalmol. Vis. Sci. 45 (2004) 2696–2704.
[26] R.A. Weinberg, The retinoblastoma protein and cell cycle control, Cell 81 (1995) 323–330.