

Correlation between microRNA-34a levels and lens opacity severity in age-related cataracts

Abstract

Purpose MicroRNA 34a (miR-34a) is involved in regulating tissue senescence. However, the role of miR-34a in age-related cataracts is unclear. In this study, we evaluated the correlations among the severity of lens opacity, patient age, and miR-34a expression level in the lens epithelium of age-related cataracts for clarifying the role of miR-34a in the lens senescence.

Methods This study was carried as a case control study in the Department of Ophthalmology, Taipei Veterans General Hospital, Taiwan. We recorded age of each patient at the time of their cataract surgery and information regarding lens opacity according to a modified version of the Lens Opacities Classification System III.

Correlations among age, lens opacity, and miR-34a expression levels were evaluated.
Results This study evaluated 110 patients with a mean age of 73.19 years (SD ± 10.2). Older patients had higher nuclear cataract (NC), cortical (C), and posterior subcapsular cataract (P) scores (one-way analysis of variance (ANOVA), $P < 0.05$). miR-34a expression levels were significantly different between each age group (ANOVA *post hoc* Bonferroni's test, $P < 0.001$), and there were moderate correlations between high NC, C, and P cataract scores and high miR-34a levels (Pearson correlation coefficient; $R = 0.606, 0.575,$ and 0.515 , respectively).

Conclusions The current study demonstrated positive correlations between high miR-34a levels and high lens opacity severity in NC, C, or P cataracts. These results suggest that miR-34a expression has a role in lens senescence.

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Introduction

Studies have provided many evidences of the participation of microRNAs in multiple cellular functions, such as cell proliferation,¹ cell apoptosis,² stress response,³ and senescence,⁴ mainly through post-transcriptional mRNA silencing and subsequent protein translation inhibition. MicroRNAs, which are groups of endogenous and small noncoding RNAs, have also been found to be biological modulators at the post-translational level through complementary or partial complementary binding with the 3' untranslated region (UTR) of target mRNA.^{5,6} MicroRNAs are also involved in DNA damage response-induced senescence, which is mainly governed by the p53 pathway.⁷ The microRNA-34a (miR-34a), the most apparent of the p53-induced microRNAs, has a role in senescence through the upregulation of acetylated p53 and activation of apoptosis in a positive feedback loop.² However, very less information is available regarding miR-34a in ocular aging processes, such as cataracts.

Cataracts, the most common cause of blindness worldwide,⁸ are significantly related to the aging process. Currently, many factors such as diabetes mellitus, ultraviolet exposure, systemic drugs, and other ocular diseases are known to be related to cataract formation. Among these disorders, oxidative stress with the subsequent formation of reactive oxygen species reactive oxygen species (ROS) is thought to be a major predisposing factor in age-related cataracts.⁹ Recently, a biological factor, silent information regulator T1 (SirT1), was proposed as an element involved in delaying the aging process and lifespan extension by suppressing NF- κ B signaling. We have found that decreased expression of the *SirT1* gene is associated with higher cataract severity and patient age in our prior study,¹⁰ but miR-34a expression in age-related cataracts has not been understood well.

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The aim of this study was to investigate the expression of miR-34a in the lens epithelium of patients with age-related cataracts. To further evaluate the correlations among patient age, cataract severity, and miR-34a expression, the Lens Opacities Classification System III (LOC III)¹¹ was conducted to stratify the patients according to cataract type and severity.

Materials and methods

Lens epithelium samples were obtained from 110 eyes between January 2008 and December 2010. This study followed the tenets of the Declaration of Helsinki. All patients underwent a complete preoperative ophthalmologic examination and only those who had no ocular diseases other than age-related cataracts were included. Patients who had previously undergone ocular surgery or those with diabetes were not included from this study. All samples were collected after obtaining informed consent from the patient. Lens epithelium samples were obtained by intact continuous curvilinear capsulorhexis, with care taken to avoid vascular contact or damage to the iris or other intraocular structures.

Cataract type and severity were graded and recorded on the basis of a modified version of the LOCSIII by using six slit-lamp images for grading nuclear color and nuclear retro-illumination images for grading posterior subcapsular (P) cataracts. Each scale on the LOCSIII is a decimalized scale ranging from 0.1 (a completely clear or colorless lens) to 5.9 (upper value on the C and P scales indicating complete opacification of the cortex or posterior capsule) and 6.9 on the NC scales (indicating advanced opacification and brunescence of the nucleus). All LOCSIII scorings among subjects was carried out and consisted up to at least three ophthalmologists. The control lens epithelium samples with a LOCSIII score of C1, NC1, and P1 were obtained from the vitrectomy operation for epiretinal membranes ($N = 13$).

MicroRNA isolation and microRNA quantitative real-time reverse transcriptase (RT-PCR)

The expression levels of miR-34a microRNA were determined by quantitative real-time RT-PCR (qRT-PCR) as previously described.¹² Briefly, a mirVana PARIS kit (Ambion, Grand Island, NY, USA) was used to isolate small RNAs from total RNA according to the manufacturer's instructions. The mature miR-34a sequence is (5'-3') UGGCAGUGUCUUAGCUGGUUGUU. For microRNA quantitation, qRT-PCR was performed using TaqMan miRNA assays (Applied Biosystems, Grand Island, NY, USA) with specific primer sets. All reagents and protocols were obtained from (Applied Biosystems)

and detection was performed using a 7900HT fast real-time PCR system (Applied Biosystems) with RNU6B as an internal control. The conditions for amplification were as follows: one cycle of 94 °C for 2 min, followed by 50 cycles of 94 °C for 20 s, 57 °C for 20 s, and 70 °C for 20 s.

MicroRNA-specific qRT-PCR was performed in triplicate and repeated three times, the expression ratio of miR-34a/RUN6B in control patients was defined as 1.

Statistical methods

For statistical analysis, patient age was expressed as mean \pm SD and miR-34a mRNA levels were expressed as mean \pm SE. The correlations among LOCSIII score, age, and levels of miR-34a mRNA were analyzed using one-way analysis of variance (ANOVA), followed by *post hoc* Bonferroni's test. The correlation between LOCSIII score and age, and LOCSIII score and levels of miR-34a mRNA expression were analyzed using the Pearson correlation coefficient and unpaired Student's *t*-test. A *P*-value of <0.05 was considered statistically significant. All analyses were performed using SPSS 12.0 (IBM, Armonk, NY, USA).

Results

At the end of enrollment, 110 patients were included in this study. Patient ages ranged from 55–92 years with an average of 73.19 years (SD 10.2). We further stratified patients according to age into four groups: between 55 and 64 years (25; 22.7%), between 65 and 74 years (35; 31.8%), between 75 and 84 years (28; 25.5%), and older than 85 years (22; 20%).

Considering the entire patient cohort, the age at the time of cataract surgery showed significant differences with respect to the grading of nuclear cataracts (NCs) (one-way ANOVA, $P < 0.05$) (Figure 1). In addition, the age at the time of cataract surgery showed significant differences with respect to the grading of cortical cataracts (C) (one-way ANOVA, $P < 0.05$) and the grading of P cataract (one-way ANOVA, $P < 0.05$) (Figure 1). There were correlations among older ages at the time of cataract surgery and higher NC, C, and P LOCSIII scores (Pearson correlation coefficient; $R = 0.399$; $P < 0.001$; $R = 0.479$; $P < 0.001$; $R = 0.410$; $P < 0.001$, respectively).

We compared miR-34a levels between each age group and found that the older the patient, the greater the miR-34a level. The mean miR-34a level of all patients was 1.18 (SE \pm 0.01) and the mean miR-34a levels in patients older than 85 years (mean \pm SE, 1.30 \pm 0.03) was significantly different from those in patients whose age was between 75 and 84 years (mean \pm SE, 1.24 \pm 0.03) (ANOVA *post hoc* Bonferroni's test, $P < 0.001$). In addition, the mean miR-34a levels of the patients

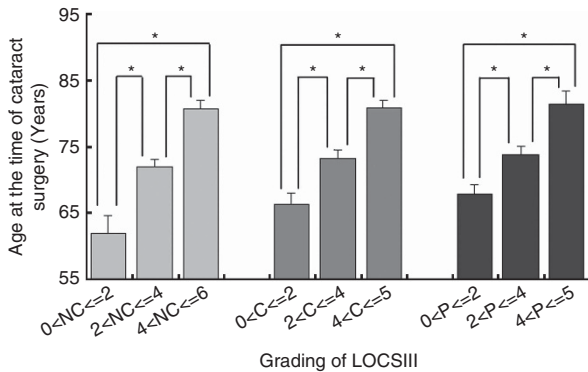


Figure 1 Differing severity of age-related cataract groups (NC, C, and P groups obtained from the Grading of Lens Opacities Classification System version III, LOCSIII), according to subtype and age of patients at the time of cataract surgery. Significantly higher grading of age-related cataracts correlated with older age at the time of surgery ($*P < 0.001$).

between the age of 75 and 84 years were significantly different from those of the patients aged between 65 and 74 years (mean \pm SE, 1.15 ± 0.02) (ANOVA *post hoc* Bonferroni's test, $P < 0.001$). There was also a significant difference between the mean miR-34a levels of patients aged 65–74 years and those of patients aged 55–64 years (mean \pm SE, 1.03 ± 0.02) (ANOVA *post hoc* Bonferroni's test, $P < 0.001$) (data not shown). A scatter plot showed a moderate correlation between age at the time of cataract surgery and miR-34a levels in the lens epithelium (Pearson correlation coefficient $R = 0.683$; $P < 0.001$, Figure 2).

In NC cataracts, there was a moderate correlation between miR-34a levels and lens opacity severity (Pearson correlation coefficient $R = 0.606$; $P < 0.001$, Figure 3). Moreover, there were moderate correlations between miR-34a levels and severity of C cataracts (Pearson correlation coefficient $R = 0.575$; $P < 0.001$) and between miR-34a levels and P cataract severity (Pearson correlation coefficient $R = 0.518$; $P < 0.001$).

To further examine relations among levels of miR-34a expression levels, age of patients, and different levels of cataracts, we stratified patients into nine subgroups according to their levels of cataracts. It was noted that in each subgroup, miR-34a expression levels increased as older age of patients (Figure 4a). Then, in each subgroup, we compared miR-34a expression levels between samples from male and female subjects (Figure 4b). There was no significant difference between different gender samples in any subgroup of our study.

Discussion

Cataract, the first leading cause of blindness in the world, accounts for 47.8% of blindness that equals 37 millions blind people in the world.⁸ Notably, 90% of

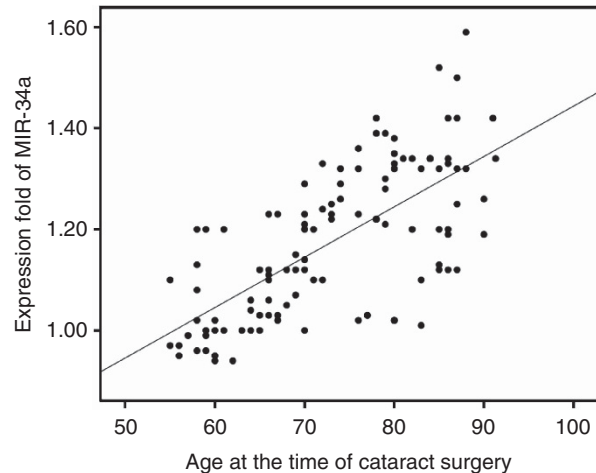


Figure 2 Relationship between age and miR-34a expression in cataract patients over 55 years of age. Each dot indicates miR-34a expression level in a single patient. Linear regression showed a significant positive correlation between miR-34a expression and age ($R = 0.683$, $P < 0.001$). Expression ratio of miR-34a/RUN6B in control patients was defined as 1.

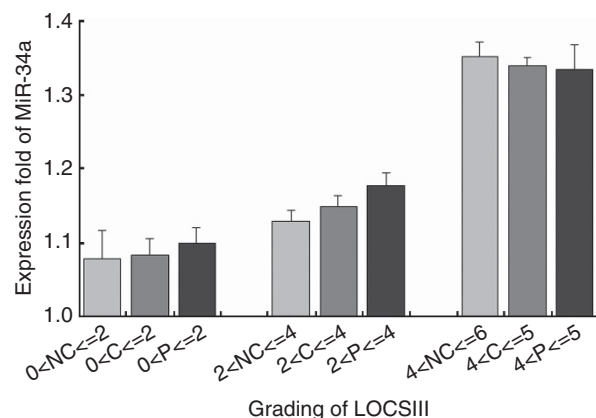


Figure 3 Relative miR-34a expression according to the severity of NC, C, and P. Real-time PCR revealed that there was a significant association between higher expression of miR-34a level and a higher severity score of NC, C, and P cataracts (Pearson correlation coefficient; $R = 0.606$, 0.575 , and 0.515 , respectively). Expression ratio of miR-34a/RUN6B in control patients was defined as 1.

cataract-related blindness are in the developing countries.⁸ Most cataracts are age-related; however, there are several risk factors about cataractogenesis.^{13,14} Previous studies focused on prevalence differences of three specific types of lens opacity: nuclear sclerosis, C cataract, and P cataract. Owing to variation of subject race and age, nuclear sclerosis is most commonly noted in Canadian, Chinese, and American whites,^{15–17} while African, Japanese, and Singaporean present higher prevalence of C cataract than nuclear sclerosis.¹⁶ However, there is scant information about certain factors among different types of lens opacity. Our study enrolled

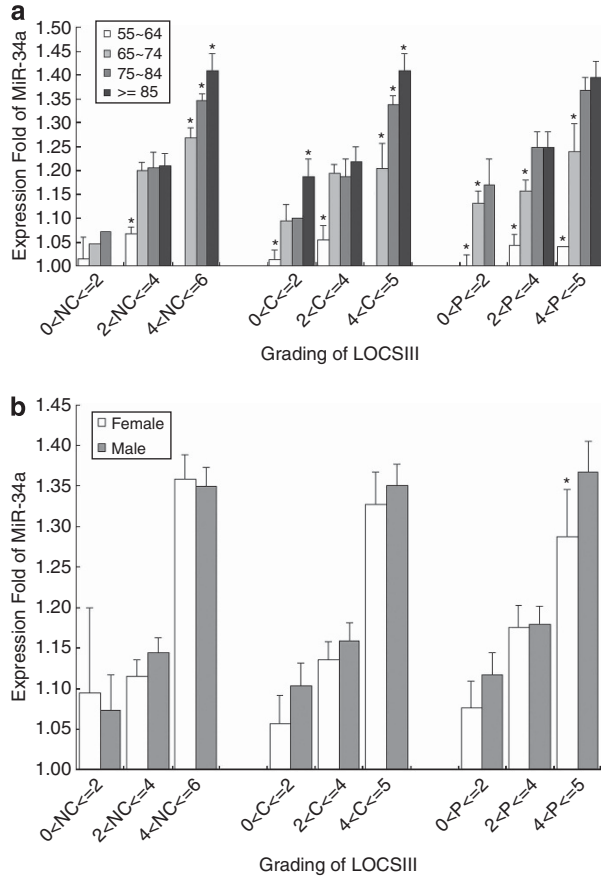


Figure 4 (a) MiR-34a expression levels in each age group stratified according to different levels of cataracts in NC, C, and P. Real-time PCR revealed that there was an association between higher expression of miR-34a level and an older age group. (b) Comparison of miR-34a expression levels between male and female subject samples stratified according to different levels of cataracts. There is no significant difference in any of subgroup. Expression ratio of miR-34a/RUN6B in control patients was defined as 1, * $P < 0.001$.

Chinese patients aged 55 and older with age-related cataract, and analyzed detailed information among individual grades of cataracts according to LOC III. We found that cataract in all subtypes of cataracts got more severe and higher miR-34a expression levels as subjects aged older. Gender is proposed as a risk factor to cataract formation;¹³ however, we could not find such results from our study (Figure 4b). We believed a larger scale of further study on miR-34a may be needed to answer the question.

Some confirmed risk factors to cataract formation are related to oxidative stress, such as chronic ultraviolet light exposure, smoking, and diabetes.^{14,18} Oxidative stress is proved to cause DNA damage and subsequent cataract formation.^{19,20} Blue Mountain Eye Study proved antioxidants intake, including beta-carotene, zinc, and

vitamins A, C, and E, reduced nuclear sclerosis cataract formation.²¹ Sorte *et al*²⁰ examined DNA damage amounts in lens epithelial cells right after cataract surgery and found that DNA damage products were noted maximally in C cataract subject samples. Those studies disclosed that oxidative stress prevention may be related to cataract prevention. Recently, one study on *Caenorhabditis elegans* with miR-34a loss-of-function mutation showed that subjects extend their lifespan, and increases resistance to oxidative stress.²² Taken together, we supposed that miR-34a may have their role in age-related cataracts and from our study, we proposed that miR-34a is correlated with age of patients (Figure 2) and severity of cataracts (Figure 4a).

MiR-34a is a microRNA that regulates biological processes through complementary binding to its target at the 3' UTR site.²³ MiR-34a also interferes with the cell cycle and proptosis via the p53 pathway in cancer cells.²⁴⁻²⁶ MiR-34a was recently reported to be involved in senescence. Upregulation of miR-34a was observed in a premature senescence model induced by hydrogen peroxide²⁷ and, alternatively, antisense inhibition of miR-34a hindered the onset of replicative senescence.²⁸ Zhao *et al*¹² studied bone-marrow-derived endothelial progenitor cells (EPCs) from rats and found that overexpression of miR-34a led to the inhibition of EPC-mediated angiogenesis and induction of senescence by suppressing SirT1. In addition, Ito *et al*²⁹ found that miR-34a increased with age in endothelial cells in senescent human umbilical cord vein endothelial cells and in the hearts and spleens of older mice. However, there is no study yet about correlation between miR-34a expression and ocular senescent tissues. Our current study focused on miR-34a expression in the lens epithelium of age-related cataracts for investigating their role in the ocular aging process. There were significant correlations among NC, C, and P cataracts and age at the time of surgery (Figure 1). We also investigated the association between miR-34a expression in the lens epithelium and cataract subtypes and severity, which was classified according to LOCSIII. Our results show that the level of miR-34a expression in the lens epithelium increased with aging (Figure 2) and with the increase in the severity of NC, C, and P cataracts. (Figure 3) We believe that this is the first report describing a role for miR-34a in the senescence of aging lenses.

Age-related cataract formation involves several mechanisms, including ROS accumulation.⁹ UV exposure and cigarette smoking are two examples of ROS-generating stress resources. Lou *et al*³⁰ used hydrogen peroxide exposure to generate ROS in rat lenses and found that most of the ROS accumulated in the lens epithelium and this resulted in a cataract. Grape

seed proanthocyanidin extract is known to protect lens epithelial cells from ROS damage³¹ in a dose-dependent manner and it exerts its effects via nuclear SirT1 expression.³² Recently, microRNAs, such as let-7 members, have been shown to have a role in newt lens regeneration.^{33,34} MiR-34a participates in the aging process by suppression of SirT1 expression.^{2,35} Ito *et al*²⁹ demonstrated miR-34a regulates endothelial senescence directly through SirT1 that overexpression of miR-34a can reduce SirT1 expression and increase senescence; conversely, SirT1 expression will increase if miR-34a is knocked down. From our prior study,¹⁰ we found that decreased expression of SirT1 correlated with the severity of age-related cataracts, age at the time of surgery, and with NC, C, and P subtypes. In this study, we found that miR-34a expression positively correlated with the cataract severity according to LOCSIII classification (Figure 1). In our current study, our results support the hypothesis that miR-34a expression increases with aging ($R = 0.683$; Figure 2). This result implies that miR-34a expression in the ocular aging process occurs in parallel with the aging process of the entire body through miR-34a–SirT1 pathway. We also stratified the age-related cataracts into different subtypes according to LOCSIII and found that miR-34a positively correlated with the severity of all subtypes (Figure 3). Ito *et al*²⁹ used population doubling time as a time course to follow miR-34a expression, whereas our study showed that the LOCSIII system correlated with miR-34a expression in aging human lenses.

In conclusion, in order to understand miR-34a expression specifically in human age-related cataracts, we evaluated miR-34a expression in lens epithelial cells obtained via cataract surgeries. We discovered that miR-34a expression not only correlated with age at the time of surgery but also correlated with the different severity of aging cataracts in nuclear, C, or P subtypes. Given these results, it is apparent that miR-34a expression has a role in lens senescence and may be a target for scavengers of oxidative stresses in the human ocular aging process.

Summary

What was known before

- Senile cataracts were known to be age-related and microRNA-34a recently is studied to be related to tissue senescence.

What this study adds

- This study disclosed the role of microRNA-34a in ocular aging tissue. MicroRNA-34a expression levels were shown correlations among different lens opacity severities in nuclear cataract, cortical, and posterior subcapsular cataracts.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgements

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