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Original Article

Expression of the microRNAs hsa-miR-15a and hsa-miR-16-1 in lens epithelial cells of patients with age-related cataract

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Abstract: This study aimed to examine and analyze the expression levels of hsa-miR-15a and hsa-miR-16-1 in lens epithelial cells from patients with age-related cataract to understand better the roles of these microRNAs in the pathogenesis of this disease. Lens epithelial cells of 60 age-related cataract patients (including 20 with cortical cataracts, 20 with nuclear cataracts, and 20 with posterior subcapsular cataracts) and 20 normal patients were included in the study. Real-time PCR was used to detect the expression of hsa-miR-15a-5p, hsa-miR-15a-3p, hsa-miR-16-1-5p, and hsa-miR-16-1-3p. Expression of the target genes of these microRNAs, namely *bcl-2* and *mcl-1*, was also evaluated. hsa-miR-15a-5p, hsa-miR-15a-3p, and hsa-miR-16-1-5p were expressed at low levels in normal lens epithelial cells but at significantly higher levels in corresponding cells of patients with cortical, nuclear, or posterior subcapsular cataracts ($P < 0.01$). hsa-miR-16-1-3p was expressed at relatively high levels in normal lens epithelial cells but significantly decreased expression, or none at all, was detected in cells of patients from each cataract group ($P < 0.01$). Concerning their target genes *bcl-2* and *mcl-1*, expression was detectable in normal lens epithelial cells, but their levels were significantly decreased in cataract patients, irrespective of type ($P < 0.01$). Expression of hsa-miR-15a-5p, hsa-miR-15a-3p, and hsa-miR-16-1-5p rose in lens epithelial cells in the three types of age-related cataract, which may suppress the expression of the anti-apoptotic genes *bcl-2* and *mcl-1*, thereby contributing to the pathogenesis of age-related cataract through apoptosis.

Keywords: Age-related cataract, hsa-miR-15a, hsa-miR-16-1, *bcl-2*, *mcl-1*

Introduction

Owing to a rapidly aging population, vision impairment due to age-related cataract has become very common. Age-related cataract has also become one of the leading causes of blindness. It has been found that apoptosis of lens epithelial cells contributes to all types of cataracts, with the exception of congenital cataracts [1].

The microRNAs (miRNAs) hsa-miR-15a and hsa-miR-16-1 have been shown to be directly involved in the regulation of 14% of the genes in the human genome, which is predicted to contain around 25,000 genes [2]. These two miRNAs can negatively regulate the expression of anti-apoptotic genes such as *bcl-2* and *mcl-1*, therefore inducing apoptotic cell death. They also influence the expression of various genes required for growth, to inhibit cell growth and arrest the cell cycle, leading to apoptosis. Our current study aimed to compare the expression of miRNAs including hsa-miR-15a-5p, hsa-miR-15a-3p, hsa-miR-16-1-5p, and hsa-miR-16-1-3p, and their target genes *bcl-2* and *mcl-1*, between normal and diseased lens epithelial cells obtained from patients with age-related cataracts including cortical, nuclear, and posterior subcapsular forms. We also discuss the role of hsa-miR-15a and hsa-miR-16-1 in age-related cataracts, revealing possible mechanisms underlying their pathogenesis.

Materials and methods

Materials

Lens epithelial cells were derived from 60 patients (age: 61 ± 8.4 years) with age-related

cataracts, including 20 with cortical cataracts (group A), 20 with nuclear cataracts (group B), and 20 with posterior subcapsular cataracts (group C). Patients with eye injuries, glaucoma, diabetes, or long-term eye exposure to radiation were excluded. For the control group, the posterior capsules of 20 normal lenses from subjects with an average age of 59 ± 9.6 years were dissected by the same operator under a microscope. There was no significant age difference between the disease group and the normal group ($P > 0.05$). All samples were immediately frozen at -70°C after dissection.

This study was performed in accordance with the Declaration of Helsinki for Research Involving Human Tissue. The authors also received consent for research use with the approval of the Yantai Yuhuangding Hospital Human Ethics Committee.

Detection of the expression of hsa-miR-15a, hsa-miR-16-1, bcl-2, and mcl-1 by real-time PCR

Total RNA was prepared by TRIzol extraction (Takara, Dalian, China). A260/A280 values were between 1.8 and 2.0, demonstrating that the RNA samples were not contaminated by proteins or DNA. RNA was used for cDNA synthesis as follows. A solution containing 1 μL of miRNA-specific primers (25 μM) and 2 μg of total RNA was diluted with ddH_2O (RNase free) to a volume of 10 μL and then denatured at 70°C for 10 min before being immediately put on ice. This was then added to a reaction buffer containing 4 μL 5×RT buffer, 4 μL dNTPs mix, 1 μL ReverTra Ace (100 U/μL; TOYOBO, Osaka, Japan), and 11 μL ddH_2O (RNase free) and incubated at 42°C for 1 h, followed by 90°C for 10 min. cDNA was stored at -20°C for future use. Real-time PCR was conducted as follows. For each sample, three to six repeats were carried out and each repeat was tested in triplicate during real-time PCR. Amplification was carried out using Invitrogen Platinum SYBR Green qPCR SuperMix-UDG (Life Technologies Corporation, Carlsbad, CA, USA), according to the manufacturer's instructions. Briefly, each reaction contained 1 μL cDNA, 1.5 μL forward primer (10 μM), 1.5 μL reverse primer (10 μM), 25 μL SYBR mix, and 21 μL ddH_2O. A QIAGEN Rotor-Gene Q (QIAGEN, Hilden, Germany) was used for real-time PCR and fluorescence detection. Cycling conditions were as follows: 50°C for 10 min, 95°C for 10 min, 95°C for 15 s, 60°C for 45 s, for 40 cycles. Relative expression of target genes was calculated using the $2^{-\Delta\Delta CT}$ method: $^{\Delta\Delta}CT$ = (Ct (experimental target gene) –Ct (experimental internal control)) -(Ct (control target gene) – Ct (control internal control)). The following primers were synthesized by Takara:

Primers for retrotranscription of miR-15a and miR-16-1: hsa-miR-15a-5p RT: CTCAACTGGTGTCGTGGAGTCGGCAATTCAGTTGAGCACAAAC; hsa-miR-15a-3p RT: CTCAACTGGTGTCGTGGAGTCGGCAATTCAGTTGAGTGAGGCA; hsa-miR-16-1-5p RT: CTCAACTGGTGTCGTGGAGTCGGCAATTCAGTTGAGCGCCAAT; hsa-miR-16-1-3p RT: CTCAACTGGTGTCGTGGAGTCGGCAATTCAGTTGAGTCAGCA.

Forward primers for miR-15a and miR-16-1: hsa-miR-15a-5P-F: ACACTCCAGCTGGGTAGCAGCACATAATGGTTTGT; hsa-miR-15-3P-F: FACACTCCAGCTGGGCAGGCCATATTGTGCTGCCTC; hsa-miR-16-1-5P-F: ACACTCCAGCTGGGTAGCAGCACGTAAATATTGGC; hsa-miR-16-1-3P-F: ACACTCCAGCTGGGCCAGTATTAACTGTGCTGCTG; Universal primers: URP: TGGTGTCGTGGAGTCG.

Primers for U6 internal control: U6F: CTCGCTTCGGCAGCACA; U6R: AACGCTTCACGAATTTGCGT.

Primers for apoptotic genes: bcl-2F: GGAGGATTGTGGCCTTCTTT; bcl-2R: GGCCGTACAGTTCCACAAAT; mcl-1F: TGGTGCCTTTGTGGCTAAA; mcl-1R: CCACCTTCTAGGTCCTCTACAT.

Primers for GAPDH internal control: Gapdh F: ATCAAGTGGGGCGATGCTG; Gapdh R: ACCCATGACGAACATGGGG.

Because of the relative nature of quantification using the $2^{-\Delta\Delta CT}$ method, adjustment is required for each sample. A more detailed account is described by Livak and Schmittgen [3]. Briefly, cDNA was diluted 10-, 10^2-, 10^3-, 10^4-, 10^5-, and 10^6-fold prior to amplification by real-time PCR and a standard curve was derived in order to obtain optimal amplification conditions.

Statistical analysis

SPSS 16.0 software was used for *t*-tests of independent samples. *p*-values < 0.05 were considered to have statistical relevance, and *p*-values < 0.01 were of statistical significance. Our aim was to analyze whether the expression levels of hsa-miR-15a and hsa-miR-16-1 differ

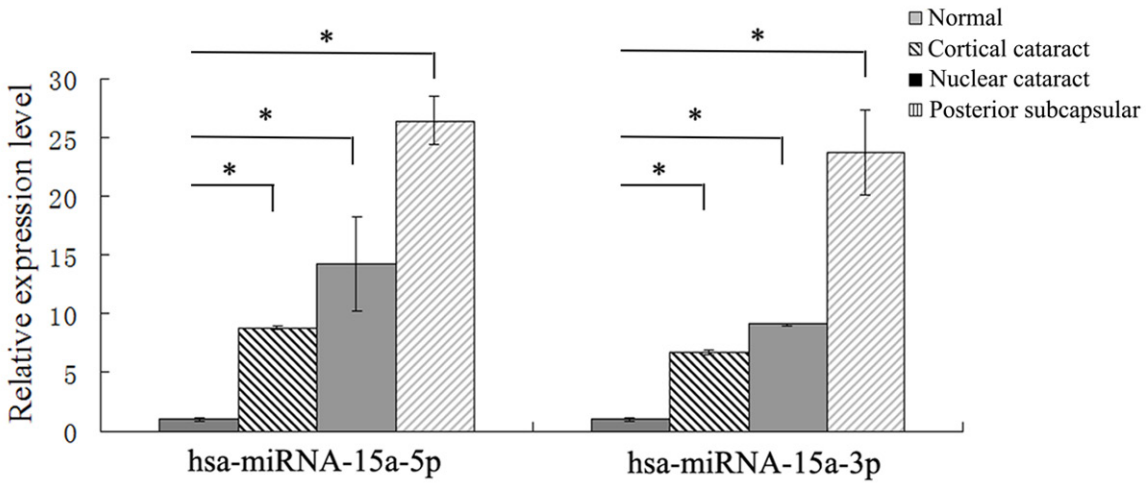

Figure 1. Relative expression of hsa-miR-15a-5p and hsa-miR-15a-3p in normal lens epithelial cells and in those from patients with age-related cataracts, including cortical cataracts (group A), nuclear cataracts (group B), and posterior subcapsular cataracts (group C). Data are expressed as the mean ± standard deviation; n = 20 per group. *$P < 0.01$ vs. the control group.

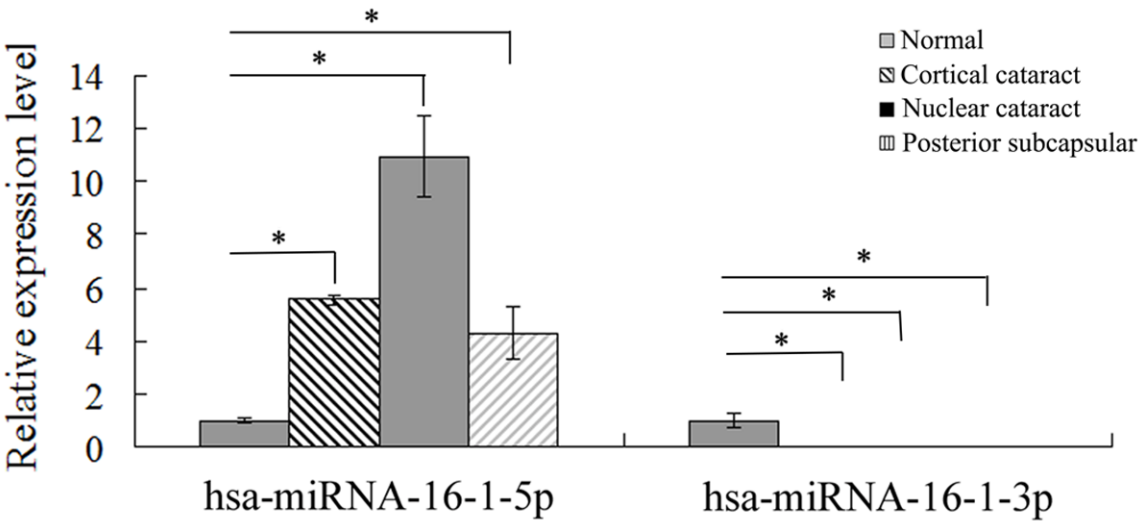

Figure 2. Relative expression of hsa-miR-16-1-5p and hsa-miR-16-1-3p in normal lens epithelial cells and in those from patients with age-related cataracts, including cortical cataracts (group A), nuclear cataracts (group B), and posterior subcapsular cataracts (group C). Data are expressed as the mean ± standard deviation; n = 20 per group. *$p < 0.01$ vs. the control group.

between the cataract groups and the normal group, therefore, in order to express statistical differences clearly, we used Student's *t*-test.

Results

Expression of hsa-miR-15a and hsa-miR-16-1

Lens epithelial expression of hsa-miR-15a-5p was 8.76 ± 0.09 compared to the control group (1.00 ± 0.15) for cortical cataract patients (group A), 14.26 ± 4.00 for nuclear cataract patients (group B), and 26.39 ± 2.07 for posterior subcapsular cataract patients (group C; **Figure 1**). Compared to the control group without age-related cataract, groups A, B, and C showed a significant increase in hsa-miR-15a-5p expression ($P < 0.01$).

Similar to hsa-miR-15a-5p, relative expression of hsa-miR-15a-3p in lens epithelial cells for group A with cortical cataracts was 6.68 ±

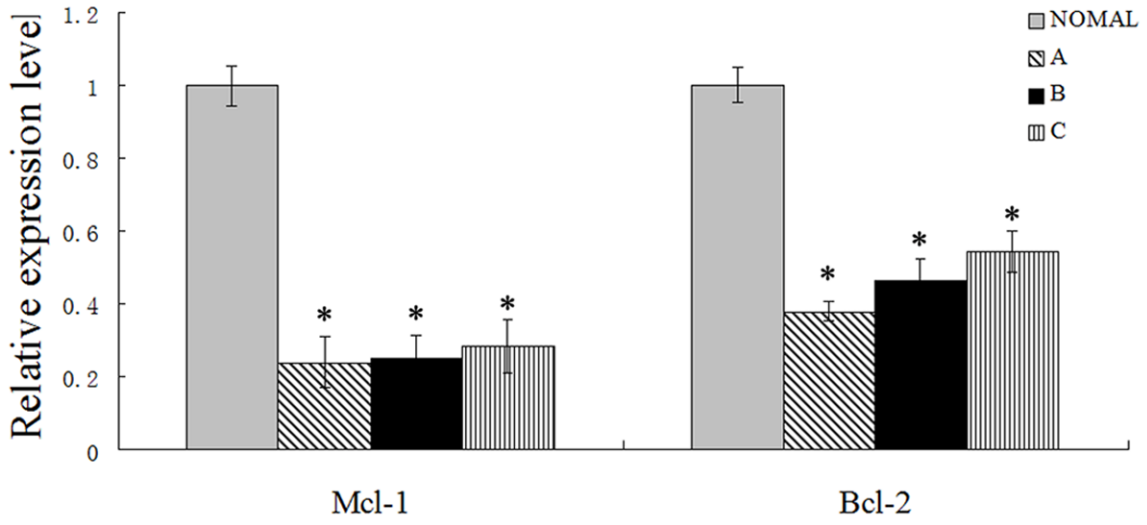

Figure 3. Relative expression of *bcl-2* and *mcl-1* in normal lens epithelial cells and in those from patients with age-related cataracts, including cortical cataracts (group A), nuclear cataracts (group B), and posterior subcapsular cataracts (group C). Data are expressed as the mean ± standard deviation; n = 20 per group. *$P < 0.01$ vs. the control group.

0.04, for group B with nuclear cataracts was 9.07 ± 0.06, and for group C with posterior subcapsular cataracts was 23.72 ± 3.59 (**Figure 1**). Expression in normal lens epithelial cells was 1.00 ± 0.15. The increase in expression observed for each group compared to the normal control was statistically significant ($P < 0.01$) in all cases.

For hsa-miR-16-1-5p (**Figure 2**), expression in lens epithelial cells was higher for patients with cortical cataracts (group A, 5.56 ± 0.17), nuclear cataracts (group B, 10.94 ± 1.49), and posterior subcapsular cataracts (group C, 4.29 ± 0.98). Differences were statistically significant for each group compared to the control group ($P < 0.01$).

For hsa-miR-16-1-3p, we did not detect any expression in lens epithelial cells for groups A and B, and only a trace expression for group C (0.01 ± 0.00). However, relative expression in the control group was 1.02 ± 0.28 (**Figure 2**). The difference between each disease group and the control group was statistically significant ($P < 0.01$).

Expression of bcl-2 and mcl-1

For group A with cortical cataracts, *bcl-2* and *mcl-1* relative expression levels were 0.38 ± 0.02 and 0.24 ± 0.05, respectively, compared to the control group (**Figure 3**). For group B with nuclear cataracts, these values were 0.54 ± 0.05 and 0.25 ± 0.07, respectively. For group C with posterior subcapsular cataracts, they were 0.54 ± 0.05 and 0.28 ± 0.07, respectively. Finally, the expression level of both *bcl-2* and *mcl-1* was 1.00 ± 0.05 for the control group. The difference in expression of *bcl-2* and *mcl-1* between each disease group and the control group was statistically significant ($P < 0.01$).

Discussion

Age-related cataracts include three major types: cortical cataracts, nuclear cataracts and posterior subcapsular cataracts. Regardless of the differences between these types, all are associated with abnormal growth or apoptotic cell death in the lens. In recent years, studies into the mechanisms behind the pathogenesis of cataracts have found that apoptosis of lens epithelial cells is significantly involved and this process has therefore attracted substantial interest. In cultured lens epithelial cells, exposure to UV light, calcium iontophoresis, or H_2O_2 treatment increases the abundance of the apoptotic protein caspase-3 and expression of the oncogenes *c-Myc* and *c-Fos*, thereby inducing cell death [4-6]. Further analysis revealed that *bcl-2* is expressed at decreased levels, or not at all, in lens epithelial cells from cataract patients, which may have resulted in cell death

and hence cataracts. Wang et al. [7] found that *bcl-2* can inhibit superoxide-induced cell death and that its expression is negatively correlated with lens epithelial cell death: *bcl-2* is expressed in normal lenses but at distinctly lower levels in those of cataract patients. *mcl-1* is a member of the *bcl-2* protein family and also plays a role in antagonizing apoptosis. In addition, Geatrell et al. [8] found that Mcl-1 is important for the development and maturation of the lens.

miRNAs are a family of small, single-stranded RNAs, 21 to 25 nucleotides in length. They bind to the 3'-UTR of mRNA to regulate the expression of target genes at the post-transcriptional or translational level [9]. Recent studies have found that miRNAs are specifically expressed in the cornea, lens, and retina, suggesting their important role in regulating eye growth, development, and functioning. It has also been inferred that changes in miRNA expression are closely correlated with the development, progression, and prognosis of various eye diseases [10]. Studies in recent years have also found that many genes that are post-transcriptionally regulated by miRNA play important roles in lens regeneration, epithelial differentiation, and lens-associated pathologies [11, 12]. In a study on human epithelial cells, Peng et al. [13] used quantitative fluorescent PCR to detect the expression of the miRNAs let-7a, let-7b, and let-7c in cataract patients and found that let-7b expression levels are positively correlated with age, while expression of let-7a and let-7c is not correlated with age or lens opacity. This suggests that let-7b expression is an important factor in age-related cataracts. This study also found that let-7b promotes cell death by suppressing *bcl-2* expression. Furthermore, Wu et al. [14] determined by microarray analysis that let-7b and miR-923 are not expressed in normal human lens epithelial cells, but their expression is elevated in corresponding cells from cataract patients. With respect to animal studies, many miRNAs, including miR-184, miR-125b, miR-31, miR-204, miR-26a, and let-7b, were found to be expressed in the mouse lens [15, 16]. However, studies aimed at understanding the role of miR-15a and miR-16-1 in human age-related cataracts have not been reported.

miR-15a and miR-16-1 are clustered within a 30-kb region of chromosome 13 (13q14) in humans, a region known to be deleted or down-regulated in more than half of B-cell chronic lymphocytic leukemia cases. miR-15a and miR-16-1 share nine nucleotides at their 5' ends, which anneal to *bcl-2* nucleotides 3287-3279. This functions to down-regulate *bcl-2* expression and promote apoptotic cell death [17]. miR-15a and miR-16-1 act to endogenously interfere with *bcl-2* gene activity. Recent research on miR-15a and miR-16-1 has been focused on various types of malignancy and leukemia. For example, Cimmino et al. [17] found that in chronic lymphocytic leukemia, expression of miR-15a and miR-16-l is negatively correlated with that of *bcl-2* and that both miRNAs can down-regulate *bcl-2* expression at the post-transcriptional level. Mcl-1 is a member of the Bcl-2 protein family and the *mcl-1* transcript is also a target of miR-15a and miR-16-1; studies by Calin et al. showed that expression of *mcl-1* is inhibited by these miRNAs [18].

Our study examined gene expression in lens epithelial cells from normal subjects and patients with three types of age-related cataracts. We found that hsa-miR-15a-5p, hsa-miR-15a-3p, and hsa-miR-16-1-5p are expressed at low levels in normal lens epithelial cells, while being highly expressed in corresponding cells taken from patients with cortical, nuclear, and posterior subcapsular cataracts. Compared to the control group, the expression of these miRNAs were five to eight times higher in the cortical cataract group, more than 10 times higher in the nuclear cataract group, and up to 20 times higher in the subcapsular cataract group.

The target genes of these miRNAs, *bcl-2* and *mcl-1*, are expressed in normal lens epithelial cells. However, their expression levels are apparently down-regulated in all types of cataracts. It is conceivable that hsa-miR-15a-5p, hsa-miR-15a-3p, and hsa-miR-16-1-5p promote cell death in the lens epithelium by suppressing *bcl-2* and *mcl-1* expression. The expression of these three miRNAs may therefore be associated with the pathogenesis of age-related cataracts. hsa-miR-16-1-3p is expressed in normal lens epithelial cells, but not in corresponding cells from cortical or nuclear cataract patients and is only slightly expressed in cells from patients with subcapsular cataracts. These observations suggest that hsa-miR-16-1-3p may be important in maintaining the normal physiology of the lens. However, the details of this mechanism remain unclear.

Age-related cataract is, nowadays, a very common disease, and a major cause of blindness. Elucidation of its pathogenesis remains a high-

ly active area of research. The rapid development of molecular biosciences should further illuminate the mechanisms of how miRNA expression influences age-related cataract and provide new insights into its prevention and treatment.

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Disclosure of conflict of interest

None.

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References

[1] Li WH, Kang ZF and Li L. Research of controlling genes of lens epithelial cell apoptosis and cataract. Int J Ophthalmol 2010; 10: 88-89.

[2] Stein LD. Human genome: End of the beginning. Nature 2004; 431: 915-916.

[3] Livak KJ, Schmittgen TD. Schmittgen Analysis of Relative Gene Expression Data Using Real-Time Quantitative PCR and the 2-ΔΔCT Method. Methods 2001; 25: 402-408.

[4] Li WC and Spector A. Lens epithelial cell apoptosis is an early event in the development of UVB-induced cataract. Free Radic Biol Med 1996; 20: 301-311.

[5] Kronschlager M, Lofgren S, Yu Z, Talebizadeh N, Varma SD and Soderberg P. Caffeine eye drops protect against UV-B cataract. Exp Eye Res 2013; 113: 26-31.

[6] Wang D, Guo D, Bi H, Wu Q, Tian Q and Du Y. Zinc oxide nanoparticles inhibit Ca2+-ATPase expression in human lens epithelial cells under UVB irradiation. Toxicol In Vitro 2013; 27: 2117-2126.

[7] Wang Z and Wang Z. Effects of rapamycin on expression of Bcl-2 and Bax in human lens epithelial cells and cell cycle in rats. J Huazhong Univ Sci Technolog Med Sci 2011; 31: 555-559.

[8] Geatrell JC, Gan PM, Mansergh FC, Kisiswa L, Jarrin M, Williams LA, Evans MJ, Boulton ME and Wride MA. Apoptosis gene profiling reveals spatio-temporal regulated expression of the p53/Mdm2 pathway during lens development. Exp Eye Res 2009; 88: 1137-1151.

[9] Roy S, Khanna S, Hussain SR, Biswas S, Azad A, Rink C, Gnyawali S, Shilo S, Nuovo GJ and Sen CK. MicroRNA expression in response to murine myocardial infarction: MiR-21 regulates fibroblast metalloprotease-2 via phosphatase and tensin homologue. Cardiovasc Res 2009; 82: 21-29.

[10] Karali M, Peluso I, Gennarino VA, Bilio M, Verde R, Lago G, Dolle P and Banfi S. MiRNeye: A microRNA expression atlas of the mouse eye. BMC Genomics 2010; 11: 715.

[11] Nakamura K, Maki N, Trinh A, Trask HW, Gui J, Tomlinson CR and Tsonis PA. MiRNAs in newt lens regeneration: Specific control of proliferation and evidence for miRNA networking. PLoS One 2010; 5: e12058.

[12] Hoffmann A, Huang Y, Suetsugu-Maki R, Ringelberg CS, Tomlinson CR, Del RK and Tsonis PA. Implication of the miR-184 and miR-204 competitive RNA network in control of mouse secondary cataract. Mol Med 2012; 18: 528-538.

[13] Peng CH, Liu JH, Woung LC, Lin TJ, Chiou SH, Tseng PC, Du WY, Cheng CK, Hu CC, Chien KH and Chen SJ. MicroRNAs and cataracts: Correlation among let-7 expression, age and the severity of lens opacity. Br J Ophthalmol 2012; 96: 747-751.

[14] Wu C, Lin H, Wang Q, Chen W, Luo H, Chen W and Zhang H. Discrepant expression of microRNAs in transparent and cataractous human lenses. Invest Ophthalmol Vis Sci 2012; 53: 3906-3912.

[15] Karali M, Peluso I, Marigo V and Banfi S. Identification and characterization of microRNAs expressed in the mouse eye. Invest Ophthalmol Vis Sci 2007; 48: 509-515.

[16] Ryan DG, Oliveira-Fernandes M and Lavker RM. MicroRNAs of the mammalian eye display distinct and overlapping tissue specificity. Mol Vis 2006; 12: 1175-1184.

[17] Cimmino A, Calin GA, Fabbri M, Iorio MV, Ferracin M, Shimizu M, Wojcik SE, Aqeilan RI, Zupo S, Dono M, Rassenti L, Alder H, Volinia S, Liu CG, Kipps TJ, Negrini M and Croce CM. MiR-15 and miR-16 induce apoptosis by targeting BCL2. Proc Natl Acad Sci U S A 2005; 102: 13944-13949.

[18] Calin GA, Cimmino A, Fabbri M, Ferracin M, Wojcik SE, Shimizu M, Taccioli C, Zanesi N, Garzon R, Aqeilan RI, Alder H, Volinia S, Rassenti L, Liu X, Liu CG, Kipps TJ, Negrini M and Croce CM. MiR-15a and miR-16-1 cluster functions in human leukemia. Proc Natl Acad Sci U S A 2008; 105: 5166-5171.