

Silent information regulator T1 in aqueous humor of patients with cataract

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Purpose: Silent information regulator T1 (SIRT1), a member of the sirtuin family, has a preventive role in various ocular diseases. We evaluated the relations between the aqueous humor level of SIRT1 and age, sex, systemic diseases, the severity of lens opacity, and other factors.

Setting: This study was conducted at a university teaching hospital in Tokyo, Japan.

Design: This study was designed based on the consecutive case series.

Methods: Aqueous humor samples were obtained from 29 eyes of the 21 consecutive patients undergoing surgery for age-related cataract (ARC). SIRT1 levels were determined by enzyme-linked immunosorbent assay.

Results: Aqueous humor levels of SIRT1 showed a positive correlation with visual acuity (logarithm of the minimum angle of resolution) and with the severity of nuclear cataract ($r=0.32$ and 0.30 , respectively, $P<0.05$). However, only visual acuity was correlated with SIRT1 according to the stepwise multiple regression analysis ($P<0.05$).

Conclusion: These findings suggest that SIRT1 may have an effect on the formation of ARC, acting as a defensive factor against ARC.

Keywords: SIRT1, sirtuin, cataract surgery, oxidative stress, resveratrol, ocular aging

Introduction

The sirtuins are a highly conserved family of nicotinamide adenine dinucleotide-dependent histone deacetylases that are involved in regulating the life span of diverse organisms. The human genome encodes seven different sirtuins (silent information regulator T1 [SIRT1]–7), which have a common catalytic core domain but distinct N- and C-terminal extensions. It has been suggested that dysfunction of sirtuins is associated with various age-related diseases, including cancer, type II diabetes, obesity-associated metabolic diseases, neurodegeneration, and cardiac aging, as well as the response to environmental stress. SIRT1 is one of the targets of resveratrol, a polyphenol SIRT1 activator that increases the lifespan and protects various organs from aging.¹ The role of sirtuins in ocular aging has been examined in a number of animal studies.¹ However, few authors have investigated the relation between SIRT1 and age-related cataract (ARC), even though it is the most common age-related ophthalmic disease leading to blindness, and (to our knowledge) there have been no studies on the relation between ARC and SIRT1 in aqueous humor. In persons with ARC, progressive cloudiness or opacity of the lens or capsule occurs with aging and leads to the impairment of vision. It is the leading cause of blindness worldwide, being responsible for binocular blindness in an estimated 17 million individuals. There is currently no effective method for preventing the onset/progression of cataract.^{2,3}

Against this background, we hypothesized that SIRT1 may have an effect on the formation of ARC. Accordingly, this study was performed to measure the aqueous

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humor level of SIRT1 in patients with ARC and to evaluate the correlations of SIRT1 with various demographic, clinical, and ocular factors.

Patients and methods

Aqueous humor samples were obtained from 29 eyes of the 21 patients with ARC who underwent cataract surgery between March and October 2013 at the Department of Ophthalmology, Tokyo Women's Medical University Medical Center East. Written informed consent was obtained from all patients, and the protocol of this study was approved by the Ethics Committee of Tokyo Women's Medical University. The mean age of the patients was 78.3 ± 3.7 years (range 65–92 years). Samples were obtained from 12 eyes of the nine males and 17 eyes of the 12 females. Prior to surgery, the skin around the eye was disinfected with popiyodon solution 10% (Yoshida Seiyaku Inc., Tokyo, Japan), and the conjunctival sac was washed with fourfold diluted PA·IODO Ophthalmic and Eye washing Solution (Nihon Tengan Kenkyujo, Aichi, Japan) under topical anesthesia with 4% lidocaine hydrochloride ophthalmic solution. The eyelids were draped with Tegaderm transparent film dressing (3M, Tokyo, Japan). After placing a lid retractor, paracentesis was performed at the limbus with a 27 G needle to obtain aqueous humor samples, which were stored in 1.5 mL microtubes in a freezer at -18°C . Information on the history of hypertension, diabetes, hypercholesterolemia, heart disease, cerebrovascular disease, and oral antiplatelet drug therapy was obtained from the patient, a family member, or the family doctor. Preoperative examination of visual acuity (logarithm of the minimum angle of resolution [logMAR]) was performed using a 5 m distance visual acuity chart (Inami Inc., Tokyo, Japan). The axial length, preoperative anterior chamber depth, and lens thickness were measured using an Ultrasonic A/B Scanner and Biometer (UD-6000; Tomey Inc., Nagoya, Japan), while the corneal endothelial cell density was determined with a Specular Microscope XIII (NSP-9900II; Konan Medical Inc., Hyogo, Japan). The type and severity of each cataract were graded and recorded according to a modified version of the Lens Opacities Classification System III (LOCS III).⁴ Six slit lamp images were used for grading the nuclear color, while five nuclear retroillumination images were used to grade cortical cataract and posterior subcapsular cataract. Each LOCS III scale is a decimal scale ranging from 0.1 (completely clear or colorless lens) to 5.9 (maximum value on the cataract scales indicating complete opacification of the cortex or posterior capsule) or 6.9 (maximum value on the nuclear color scale

indicating advanced opacification and brunescence of the nucleus).⁴ Anterior subcapsular cataract was graded as 0 (clear) or 1 (present) on slit lamp images.

Enzyme-linked immunosorbent assay

SIRT1 levels in aqueous humor were determined by enzyme-linked immunosorbent assay (ELISA) with a Sirtuin 1 (human intracellular) ELISA Kit (BioVision Inc., Milpitas, CA, USA) according to the manufacturer's instructions. The assay was performed as follows.

The human SIRT1 quality control sample was reconstituted with 1 mL of distilled water, and the human SIRT1 standard was also reconstituted with 1 mL of distilled water to obtain a stock solution (20 ng/mL). Then, the standard curve was prepared by making twofold serial dilutions with $1 \times$ diluent. First, 300 μL of the SIRT1 standard (4 ng/mL) was added to 300 μL of $1 \times$ diluent to obtain a 2 ng/mL SIRT1 solution, and this 300 μL of the 2 ng/mL SIRT1 solution was added to 300 μL of $1 \times$ diluent to obtain a 1 ng/mL SIRT1 solution. This process was repeated further to obtain SIRT1 solutions of 0.5 ng/mL, 0.25 ng/mL, 0.125 ng/mL, 0.0625 ng/mL, and 0.03125 ng/mL.

Assay protocol

The required number of eight-well assay strips were inserted into the frame, followed by the addition of 100 μL of standards, samples, and quality control samples to the appropriate wells in duplicate. The plate was covered and incubated for 1 hour at 37°C , followed by aspiration and washing three times with 300 μL of $1 \times$ washing buffer. Then, 100 μL of detection antibody was added to each well, and the incubation and washing were repeated as earlier. Next, 100 μL of $1 \times$ detector was added to each well, and the plate was covered, incubated for 1 hour at 37°C , aspirated, and washed five times with 300 μL of $1 \times$ washing buffer. Then the plate was inverted and tapped on a stack of paper towels, 100 μL of tetramethylbenzidine substrate solution was added to each well, and the color was developed at room temperature in the dark for 10 minutes. The reaction was stopped by adding 100 μL of stop solution to each well, and the optical density was measured at 450 nm using an ELISA plate reader within 30 minutes.

Statistical analysis

Univariate analysis was performed to investigate the correlation between the SIRT1 level in aqueous humor and demographic factors (age and sex), clinical factors (hypertension, diabetes, hypercholesterolemia, heart disease,

cerebrovascular disease, and antiplatelet therapy), and ocular factors (preoperative visual acuity [logMAR], axial length, corneal endothelial cell density, anterior chamber depth, lens thickness, nuclear color, cortical cataract, posterior subcapsular cataract, and anterior subcapsular cataract). Stepwise multiple regression analysis was also performed.

Results

Aqueous humor levels of SIRT1 are shown in Table 1. The correlation between the aqueous humor level of SIRT1 and age, sex, hypertension, diabetes, hypercholesterolemia, heart disease, cerebrovascular disease, oral antiplatelet therapy, preoperative visual acuity (logMAR), axial length, preoperative corneal endothelial cell density, preoperative anterior chamber depth, lens thickness, nuclear color, cortical cataract, posterior subcapsular cataract, and anterior subcapsular cataract was -0.03 , 0.03 , -0.14 , -0.13 , -0.01 , -0.06 , 0.01 , 0.19 , 0.32 , -0.02 , -0.15 , 0.06 , -0.31 , 0.30 , -0.14 , 0.02 , and 0.22 , respectively (one-tailed Pearson's correlation coefficients) (Table 2). Preoperative visual acuity (logMAR) and nuclear color showed a significant positive correlation (Figures 1 and 2). Thus, the aqueous humor level of SIRT1 became higher as preoperative visual acuity got worse and the nuclear cataract became harder.

However, only visual acuity (logMAR) was selected by stepwise multiple regression analysis, while the other factors did not show a significant correlation with the SIRT1 level in aqueous humor.

Discussion

In this study, we found that the aqueous humor level of SIRT1 increased as preoperative visual acuity declined and the nuclear cataract became more severe. To our knowledge, this is the first study that has shown SIRT1 in the aqueous humor may be associated with ARC and may regulate the senescence of lens cells in patients with cataract.

Jaliffa et al performed a detailed examination of the localization of SIRT1 in adult mouse eyes by immunohistochemistry and Western blotting, and they identified SIRT1 in the nucleus and/or cytoplasm of cells in all normal ocular structures, including the cornea, lens, iris, ciliary body, and retina.⁵ In the lens, SIRT1 was principally localized in the nuclei of epithelial cells and fiber cells, while it was not detected in the lens capsule.

In humans, expression of SIRT1 has also been detected in the lens epithelium of patients with ARC^{2,3} and in the retina.⁶ Lin et al reported that SIRT1 expression was lower in patients ≥ 51 years old with lens opacity than in

those < 51 years old and was negatively correlated with age,³ suggesting that decreased SIRT1 expression in the lens epithelium is associated with a higher grade of cataract and an increased age. Interestingly, Zheng and Lu reported that SIRT1 expression in the lens epithelium decreases with age but is higher in patients with ARC > 50 years old than in persons > 50 years old without cataract, and they suggested that SIRT1 may have a protective effect against ARC.² Moreover, an increase in SIRT1 expression in response to oxidative stress and its protective effect against such stress have been observed in retinal pigment epithelial cells.⁷

In this study, we showed that the aqueous humor level of SIRT1 was positively correlated with the progression of ARC. Our findings support the notion that SIRT1 expression may increase to compensate the insufficient defenses against factors, such as ultraviolet light and oxidative stress, thus playing a protective role against factors that promote ARC. Accordingly, it is possible that SIRT1 may have an effect on the development and/or progression of ARC. Zheng et al² and Lin et al³ reported the relationship of SIRT1 with lens epithelial cells, but we did not investigate lens epithelial cells in this study.

Resveratrol (3,5,4'-trihydroxy-*trans*-stilbene) is found in grapes and other plants,⁸ and it has been reported to activate SIRT1⁹ by inducing the deacetylation of peroxisome proliferator-activated receptor gamma coactivator-1 α .¹⁰ Doganay et al reported that resveratrol prevented cataract formation in a rat model,¹¹ and Pearson et al reported that resveratrol-fed elderly mice showed a marked reduction in ARC.¹² These findings suggest that the activation of SIRT1 by resveratrol may have a protective effect against cataract.

In human and mouse livers, Biel et al reported that the loss of SIRT1 led to defective autophagy, mitochondrial dysfunction, and death of hepatocytes after ischemia-reperfusion injury, which is a major cause of morbidity and mortality after liver surgery.¹³ In addition, Kaur et al reported that the neuroprotective effect of ischemic postconditioning, an important adaptive technique for limiting cerebral ischemia-reperfusion injury, probably involves the activation of SIRT1 in mice.¹⁴ Furthermore, Wu et al reported that SIRT1 is a potential new target for the treatment of hepatocellular carcinoma.¹⁵

A SIRT1 activator (SRT1720), which is much more potent than resveratrol, has been identified and has been suggested as a potential drug for the treatment of diabetes.¹⁶ It is possible that SIRT1 activators may also be useful for the prevention/treatment of ARC.

Table 1 Aqueous humor level of SIRT1, clinical profile, and the severity and type of cataract

Case number	SIRT1 (μg/mL)	Age	Sex	Hypertension	Diabetes mellitus	Hypercholesterolemia	Heart disease	Cerebrovascular disease	Oral antiplatelet therapy	Preoperative logMAR visual acuity	Nuclear color	Cortical cataract	Posterior subcapsular cataract	Anterior subcapsular cataract
1	0.778	73	M	0	1	0	0	0	0	0.097	2.0	2.0	0.1	0
2	0.450	86	M	1	0	1	1	0	1	2.000	5.0	0.1	0.1	1
3	0.535	86	F	1	0	0	1	0	1	1.699	5.0	2.0	2.0	0
4	0.613	68	F	0	1	0	0	0	1	0.398	2.0	4.0	0.1	0
5	0.323	86	M	0	0	0	0	0	1	0.523	3.0	4.0	0.1	0
6	0.950	86	M	0	0	0	0	0	1	0.523	3.0	4.0	0.1	0
7	2.149	72	M	0	0	0	0	0	1	2.000	6.0	0.1	0.1	1
8	0.827	70	M	1	1	0	0	1	1	-0.079	2.0	4.0	0.1	0
9	0.609	70	M	1	1	0	0	1	1	-0.079	2.0	4.0	0.1	0
10	0.644	74	M	0	0	0	1	0	1	0.398	3.0	0.1	2.0	0
11	0.496	65	F	0	1	0	0	0	0	0.046	3.0	0.1	0.1	1
12	0.912	74	F	0	0	0	0	0	0	1.097	4.0	0.1	0.1	0
13	0.457	72	M	0	0	0	0	0	1	0.222	3.0	4.0	0.1	0
14	0.541	65	F	0	1	0	0	0	0	0.097	3.0	0.1	0.1	0
15	0.597	86	F	1	0	0	0	0	0	-0.079	2.0	2.0	0.1	1
16	1.051	70	F	0	0	0	0	0	0	0.155	3.0	4.0	0.1	0
17	0.726	86	F	1	0	0	0	0	0	-0.079	2.0	2.0	0.1	0
18	0.735	92	F	0	0	1	0	0	0	0.699	3.0	4.0	0.1	0
19	0.388	77	M	1	1	1	0	0	0	1.046	6.0	0.1	0.1	0
20	0.653	82	F	0	0	0	0	0	0	1.398	4.0	4.0	4.0	0
21	0.805	75	F	1	0	1	0	0	0	0.222	4.0	0.1	0.1	0
22	1.419	79	F	1	0	0	1	0	1	1.398	3.0	0.1	5.9	0
23	0.710	85	F	1	0	0	1	0	0	0.097	3.0	0.1	0.1	0
26	1.047	77	F	1	1	1	0	0	0	0.523	4.0	4.0	0.1	0
25	0.431	77	F	1	1	1	0	0	0	0.155	3.0	4.0	0.1	0
26	0.454	85	F	1	0	0	1	0	0	0.000	3.0	0.1	0.1	0
27	0.500	79	F	1	0	0	1	0	1	0.155	2.0	2.0	0.1	0
28	0.342	75	M	0	0	0	0	0	0	0.398	3.0	4.0	4.0	0
29	0.423	76	M	1	0	0	0	0	0	1.699	3.0	0.1	5.9	0

Abbreviations: logMAR, logarithm of the minimum angle of resolution; M, male; SIRT1, silent information regulator T1; F, female.

Table 2 The correlations (one-tailed Pearson's correlation coefficients) between aqueous humor levels of SIRT1 and patients' factors

Variable	Correlation coefficients		
	R	(95% CI)	P-value
Age	-0.03	(-0.39 to 0.34)	0.5605
Sex	0.03	(-0.34 to 0.39)	0.4335
Hypertension	-0.14	(-0.48 to 0.24)	0.7580
Diabetes mellitus	-0.13	(-0.48 to 0.24)	0.7551
Hypercholesterolemia	-0.01	(-0.38 to 0.36)	0.5258
Heart disease	-0.06	(-0.41 to 0.32)	0.6127
Cerebrovascular disease	0.01	(-0.36 to 0.37)	0.4862
Oral administration of antiplatelet drug	0.19	(-0.19 to 0.52)	0.1678
Preoperative logMAR visual acuity	0.32	(-0.05 to 0.62)	0.0447
Axial length	-0.02	(-0.39 to 0.35)	0.5460
Preoperative corneal endothelium cells	-0.15	(-0.49 to 0.23)	0.7748
Preoperative anterior chamber depth	0.06	(-0.32 to 0.41)	0.3879
Lens thickness	-0.31	(-0.61 to 0.06)	0.9490
Nuclear color	0.30	(-0.08 to 0.60)	0.0583
Cortical cataract	-0.14	(-0.48 to 0.24)	0.7663
Posterior subcapsular cataract	0.02	(-0.35 to 0.38)	0.4597
Anterior subcapsular cataract	0.22	(-0.16 to 0.54)	0.1269

Abbreviations: CI, confidence interval; logMAR, logarithm of the minimum angle of resolution; SIRT1, silent information regulator T1.

This study had the following limitations. The number of patients was small, and there were many explanatory variables, which might explain why nuclear color was not selected by stepwise multiple regression analysis despite showing a significant positive correlation. In addition, there were nine patients with diabetes, one patient with age-related macular degeneration, and one patient with pigment degeneration, and there is a possibility that these conditions may have had an effect on the SIRT1 level.

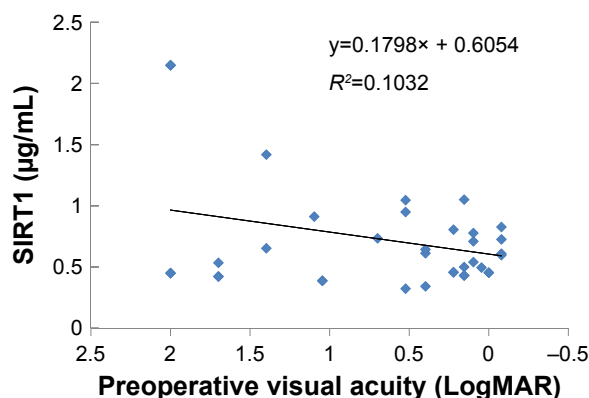
Conclusion

Although animal studies have shown that SIRT1 regulates ocular aging and protects ocular tissues from oxidative stress, suggesting that it is an attractive target for the

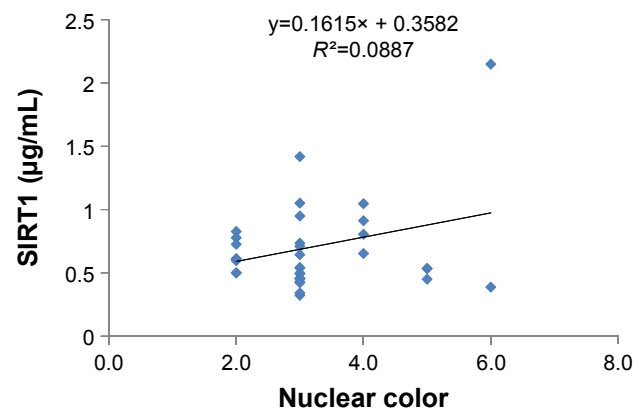
prevention of ocular aging, it is uncertain how closely animal data apply to humans.¹ The SIRT1 activator is currently being investigated for its use in treating cardiovascular disease, malignancy, diabetes, and Alzheimer's disease.¹ The role of SIRT1 in ocular aging should be further clarified by more clinical studies, since there is a possibility that the activation of SIRT1 could be effective for the treatment of cataract.

SIRT1 may be involved in the regulation of cellular senescence and may have a protective effect against oxidative stress. Expression of SIRT1 has been detected in the retina and in the lens epithelium of patients with ARC.

In patients with ARC, the aqueous humor level of SIRT1 was positively correlated with the progression of cataract.

**Figure 1** Correlation of SIRT1 with preoperative visual acuity (logMAR).

Abbreviations: logMAR, logarithm of the minimum angle of resolution; SIRT1, silent information regulator T1.

**Figure 2** Correlation of SIRT1 with nuclear color.

Abbreviation: SIRT1, silent information regulator T1.

Ocular expression of SIRT1 may increase as a defense against factors that promote ARC.

Disclosure

The authors report no conflicts of interest in this work.

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