3-month mortality after acute myocardial events.[13] Long pentraxin 3 is an acute-phase protein that is involved in innate immunity and inflammation. Pentraxins are a family of acute response proteins comprising 3 members—C-reactive protein, serum amyloid P, and PTX3—and these proteins are classic acute-phase reactants that closely reflect the level of inflammatory activity.[14,15] Long pentraxin 3 is induced by cytokines and is produced mainly by vascular endothelial cells, fibroblasts, and cells in some other extrahepatic tissues,[14,16-20] unlike the other 2 family members that are synthesized primarily in the liver.[21,22] In an animal model, there was a rapid increase in PTX3 expression after reperfusion of the ischemic superior mesenteric artery territory.[23] More important, overexpression of PTX3 was accompanied by an increase in death and tissue damage after intestinal ischemia and reperfusion.[23] In addition, PTX3 increases vascular permeability.[24] These findings suggest that PTX3 may play an important role in the pathogenesis of macular edema associated with BRVO. However, the level of PTX3 expression in patients with BRVO and its relationship to the pathogenesis of macular edema are unclear, just as the relative contribution of each of the molecules evaluated herein to the development of macular edema remains uncertain. Accordingly, we measured the vitreous fluid levels of 6 inflammatory factors (including PTX3) and 1 anti-inflammatory factor in patients with BRVO and macular edema, focusing on molecules that have been linked to the onset or exacerbation of this condition. The association between each of these molecules and the severity of macular edema was then assessed.

METHODS

PARTICIPANTS

Undiluted vitreous fluid samples were harvested at the start of pars plana vitrectomy (PPV) after written informed consent was obtained from each participant following an explanation of the purpose and potential adverse effects of the procedure. This study was performed in accordance with the Helsinki Declaration of 1975 (1983 revision). The institutional review boards of Tokyo Women's Medical University and Eguchi Eye Hospital approved the protocol for collection and testing of vitreous fluid and blood samples. This was a retrospective case-control study of 60 Japanese patients who underwent PPV in 1 eye (39 with BRVO and 21 with idiopathic macular hole [MH]) to treat macular edema. Seventy-two consecutive patients with BRVO who sought care at the hospitals associated with Tokyo Women's Medical University or Eguchi Eye Hospital between August 11, 2009, and November 15, 2011, were screened, using the criteria listed in the next sentence, and vitreous fluid samples were obtained from the 39 patients enrolled. The indications for PPV were (1) clinically detectable diffuse macular edema or cystoid macular edema persisting for more than 3 months and (2) best-corrected visual acuity worse than 20/40.

The Branch Vein Occlusion Study[25] demonstrated the effectiveness of argon laser photocoagulation for BRVO, but it was recommended that this should not be performed within 3 months of occurrence, during which time spontaneous improvement may occur. The absence of posterior vitreous detachment can contribute to persistent macular edema in patients with retinal vascular occlusion.[26] Saika et al[27] reported on the effectiveness of PPV combined with surgical posterior vitreous detachment for macular edema in patients with BRVO.

Table 1. Clinical and Laboratory Characteristics of the BRVO and MH Groups

Variable	BRVO Group	MH Group	*P* Value
No. of participants[a]	39	21	
Sex, No.			.66
Female	20	12	
Male	19	9	
Age, mean (SD), y	69.2 (9.6)	68.8 (8.4)	.88
Blood pressure, mean (SD), mm Hg			
Systolic	134 (14)	121 (11)	<.001
Diastolic	78 (8)	74 (8)	.07
Hypertension, No.	22	3	.002
Hyperlipidemia, No.	12	4	.33
Duration of BRVO, mean (SD), mo	5.1 (2.4)	...	...

Abbreviations: BRVO, branch retinal vein occlusion; ellipsis, not applicable; MH, macular hole.
[a] Number of participants with data.

It has also been reported[28,29] that PPV contributes to an increase in oxygen tension in the inner retina. If retinal oxygen tension increases after PPV, macular edema would be lessened for several reasons. First, an increase in oxygen tension would reduce VEGF production and thus decrease vascular permeability. Second, an increase in oxygen tension would alleviate autoregulatory arteriolar vasoconstriction and thus reduce the hydrostatic pressure in the retinal capillaries and venules. This would decrease water flux from the vascular compartment to the tissue compartment and reduce edema according to the Starling law. Finally, PPV reduces the intraocular levels of various other inflammatory factors in addition to VEGF,[30] and this may be another mechanism by which it alleviates macular edema in patients with BRVO. In fact, it has been reported[31,32] that PPV improves both functional and tomographic outcomes in patients with BRVO and macular edema. Accordingly, we performed PPV in patients with clinically detectable diffuse macular edema or cystoid macular edema more than 3 months after the onset of BRVO.

Thirty-three of the 72 patients were excluded because of previous ocular surgery or intravitreous injection of anti-VEGF agents or triamcinolone acetonide in 23 patients, diabetic retinopathy in 2 patients, previous retinal photocoagulation in 7 patients, and a history of ocular inflammation or vitreoretinal disease in 1 patient. Patients with intravitreous injection of anti-VEGF agents or triamcinolone acetonide were excluded because such treatment could influence vitreous fluid levels of inflammatory factors. Vitreous fluid samples were also obtained from 21 patients with nonischemic ocular diseases as a control group (MH group). None of the patients in the MH group had proliferative vitreoretinopathy. The mean (SD) age of the BRVO group (19 men and 20 women) was 69.2 (9.6) years, and the control group (9 men and 12 women) was aged 68.8 (8.4) years. The mean duration of BRVO was 5.1 (2.4) months (range, 3-11 months). Clinical and laboratory characteristics of the BRVO and MH groups are shown in **Table 1**.

FUNDUS FINDINGS

Both preoperative and operative fundus findings were recorded for each participant. A masked grader (H.N.) independently assessed ischemic retinal vascular occlusion by examining fluorescein angiograms. The ischemic region of the retina was measured with the public domain Scion Image program (Scion Corp), as reported previously.[8-10] On digital fundus pho-

Table 2. Vitreous Fluid Levels of Factors in the Groups

Variable	Median (Interquartile Range)		P Value
	BRVO Group	MH Group	
sVEGFR-2, pg/mL	1500 (1083-2035)	1020 (721-1343)	.002
VEGF, pg/mL	229 (33.9-1353)	15.6 (15.6-31.2)	<.001
sICAM-1, ng/mL	8.20 (5.33-15.6)	4.50 (3.60-5.65)	<.001
IL-6, pg/mL	10.7 (5.53-29.0)	1.00 (0.50-1.18)	<.001
MCP-1, pg/L	1190 (747-1993)	458 (375-636)	<.001
PTX3, ng/mL	0.86 (0.50-1.62)	0.50 (0.50-0.81)	.01
PEDF, ng/mL	25.6 (8.14-40.7)	59.9 (25.0-101)	.005

Abbreviations: BRVO, branch retinal vein occlusion; IL-6, interleukin 6; MCP-1, monocyte chemotactic protein 1; MH, macular hole; PEDF, pigment epithelium-derived factor; PTX3, pentraxin 3; sICAM-1, soluble intercellular adhesion molecule 1; sVEGFR-2, soluble vascular endothelial growth factor (VEGF) receptor 2.

Table 3. Correlation of Vitreous Factors With the Nonperfused Area and Retinal Thickness

Variable	Nonperfused Area		Retinal Thickness	
	r	P Value	r	P Value
sVEGFR-2	0.19	.25	0.36	.02
VEGF	0.77	<.001	0.47	.003
sICAM-1	0.36	.02	0.56	<.001
IL-6	0.46	.004	0.41	.01
MCP-1	0.52	.001	0.63	<.001
PTX3	0.37	.02	0.39	.02
PEDF	−0.39	.02	−0.36	.02

Abbreviations: See Table 2; r, correlation coefficient.

tographs, the disc area was outlined with a cursor and then measured, and the same was done for the nonperfused area. The severity of retinal ischemia was assessed as the nonperfused area divided by the disc area.

Optical coherence tomography was performed in each participant within 1 week before PPV (Zeiss-Humphrey Ophthalmic Systems). The thickness of the central fovea was defined as the distance between the inner limiting membrane and the retinal pigment epithelium (including any serous retinal detachment) and was automatically measured by computer software (Zeiss-Humphrey Ophthalmic System). The thickness of the neurosensory retina was defined as the distance between the inner and outer neurosensory retinal surfaces,[26] and the severity of macular edema was graded from the measured retinal thickness.

SAMPLE COLLECTION

Samples of undiluted vitreous fluid (0.5-1.0 mL) were collected at the start of PPV by aspiration into a 1-mL syringe attached to the vitreous cutter before the intravitreal infusion of balanced salt solution was begun. The vitreous samples were immediately transferred into sterile tubes and were rapidly frozen at −80°C. Blood samples were collected simultaneously and were centrifuged at 3000g for 5 minutes to obtain plasma, after which aliquots were stored at −80°C until assays were performed.

MEASUREMENT OF INFLAMMATORY AND ANTI-INFLAMMATORY FACTORS

The levels of VEGF, sVEGFR-2, sICAM-1, IL-6, MCP-1, and PTX3 were measured in vitreous samples from the same eye and in plasma samples by enzyme-linked immunosorbent assay, using kits for human VEGF, sVEGFR-2, IL-6, MCP-1, and PTX3 (R&D Systems); sICAM-1 (Bender Med Systems); and PTX3 (Perseus Proteomics Inc).[8,10,33] Similarly, levels of anti-inflammatory PEDF were measured in vitreous samples with a human PEDF sandwich enzyme-linked immunosorbent assay kit (Chemicon International).[9] The VEGF kit was able to detect 2 of the 4 VEGF isoforms ($VEGF_{121}$ and $VEGF_{165}$), probably because these 2 shorter isoforms are secreted and the 2 longer isoforms are cell associated. Each assay was performed according to the manufacturer's instructions.

STATISTICAL ANALYSIS

Analyses were performed with commercial software (SAS, version 9.1; SAS Institute Inc). A *t* test was used to compare normally distributed unpaired continuous variables between the 2 groups, and the Mann-Whitney test was used for variables with a skewed distribution. The χ^2 test or Fisher exact test was used to compare discrete variables. Differences between the median plasma and vitreous levels were assessed with the Wilcoxon single rank test. To examine relationships among the variables, Spearman rank order correlation coefficients or Pearson correlation coefficients were calculated. Statistical significance was set at $P < .05$, with 2-tailed values.

RESULTS

The vitreous fluid concentration of sVEGFR-2 (median [interquartile range]) was significantly higher in the BRVO group (1500 pg/mL [1083-2035]) than in the MH group (1020 pg/mL [721-1343]; P = .002) (**Table 2**). The vitreous fluid concentration of VEGF was significantly higher in the BRVO group (229 pg/mL [33.9-1353]) compared with the MH group (15.6 pg/mL [15.6-31.2]; $P < .001$) (Table 2). Likewise, vitreous sICAM-1 levels were significantly higher in the BRVO group (8.20 ng/mL [5.33-15.6]) than in the MH group (4.50 ng/mL [3.60-5.65]; $P < .001$) (Table 2). Furthermore, the vitreous level of IL-6 was significantly higher in the BRVO group (10.7 pg/mL [5.53-29.0]) than in the MH group (1.00 pg/mL [0.50-1.18]; $P < .001$), as was the vitreous level of MCP-1 (1190 pg/mL [747-1993] vs 458 pg/mL [375-636]; $P < .001$) and the vitreous level of PTX3 (0.86 ng/mL [0.50-1.62] vs 0.50 ng/mL [0.50-0.81]; P = .01) (Table 2). In contrast, the vitreous fluid level of PEDF was significantly lower in the BRVO group (25.6 ng/mL [8.14-40.7]) than in the MH group (59.9 ng/mL [25.0-101]; P = .005) (Table 2).

Vitreous fluid levels of VEGF, sICAM-1, IL-6, MCP-1, and PTX3 were significantly correlated with the nonperfused area of the retina in the BRVO group ($r = 0.77$, $P < .001$; $r = 0.36$, $P = .02$; $r = 0.46$, $P = .004$; $r = 0.52$, $P = .001$; and $r = 0.37$, $P = .02$, respectively) (**Table 3**). Conversely, the vitreous fluid level of PEDF showed a significant negative correlation with the nonperfused area in the BRVO group ($r = -0.39$, $P = .02$) (Table 3). However, the vitreous fluid level of sVEGFR-2 was not significantly correlated with the nonperfused area in this group ($r = 0.19$, $P = .25$) (Table 3).

Table 4. Correlation Matrix for Vitreous Factors

	sVEGFR-2		VEGF		sICAM-1		IL-6		MCP-1		PTX3		PEDF	
Variable	*r*	*P* Value	*r*	*P* Value	*r*	*P* Value	*r*	*P* Value	*r*	*P* Value	*r*	*P* Value	*r*	*P* Value
sVEGFR-2			0.14	.38	0.76	<.001	0.63	<.001	0.69	<.001	0.66	<.001	−0.12	.44
VEGF					0.34	.03	0.41	.01	0.46	.004	0.23	.19	−0.33	.04
sICAM-1							0.63	<.001	0.66	<.001	0.64	<.001	0.03	.87
IL-6									0.70	<.001	0.65	<.001	−0.10	.52
MCP-1											0.53	<.001	−0.39	.02
PTX3													0.04	.82

Abbreviations: See Table 2; *r*, correlation coefficient.

Vitreous fluid levels of sVEGFR-2, VEGF, sICAM-1, IL-6, MCP-1, PTX3, and PEDF were significantly correlated with the retinal thickness at the central fovea according to simple linear regression analysis ($r = 0.36$, $P = .02$; $r = 0.47$, $P = .003$; $r = 0.56$, $P < .001$; $r = 0.41$, $P = .01$; $r = 0.63$, $P < .001$; $r = 0.39$, $P = .02$; and $r = -0.36$, $P = .02$, respectively) (Table 3).

In the BRVO group, there were significant correlations between the vitreous fluid level of sVEGFR-2 and the levels of sICAM-1, IL-6, MCP-1, and PTX3 ($r = 0.76$, $P < .001$; $r = 0.63$, $P < .001$; $r = 0.69$, $P < .001$; and $r = 0.66$, $P < .001$; respectively) (**Table 4**). There were also significant correlations between the vitreous fluid level of VEGF and the levels of sICAM-1, IL-6, MCP-1, and PEDF in the BRVO group ($r = 0.34$, $P = .03$; $r = 0.41$, $P = .01$; $r = 0.46$, $P = .004$; and $r = -0.33$, $P = .04$, respectively) (Table 4). Furthermore, there was a significant correlation between the vitreous fluid level of sICAM-1 and the levels of IL-6, MCP-1, and PTX3 ($r = 0.63$, $P < .001$; $r = 0.66$, $P < .001$; and $r = 0.64$, $P < .001$, respectively) (Table 4). Moreover, there was a significant correlation between the vitreous fluid level of IL-6 and the levels of MCP-1 and PTX3 ($r = 0.70$, $P < .001$; and $r = 0.65$, $P < .001$, respectively) (Table 4), as well as a significant correlation between MCP-1 and PTX3 or PEDF ($r = 0.53$, $P < .001$; and $r = -0.39$, $P = .02$, respectively) (Table 4). In contrast, there was no significant correlation between the vitreous levels of sVEGFR-2 and VEGF ($r = 0.14$, $P = .38$) or between the vitreous levels of VEGF and PTX3 in the BRVO group ($r = 0.23$, $P = .19$) (Table 4). There were also no significant correlations between the vitreous level of PEDF and the levels of sVEGFR-2, sICAM-1, IL-6, and PTX3 in the BRVO group ($r = -0.12$, $P = .44$; $r = 0.03$, $P = .87$; $r = -0.10$, $P = .52$; and $r = 0.04$, $P = .82$, respectively) (Table 4).

In the BRVO group, the vitreous fluid levels of VEGF, IL-6, and MCP-1 were significantly higher (all $P < .001$) than the plasma levels of these molecules (18.1 pg/mL [15.6-44.1], 0.59 pg/mL [0.35-0.98], and 142 pg/mL [117-167], respectively), whereas the vitreous levels of sVEGFR-2, sICAM-1, and PTX3 were significantly lower (all $P < .001$) than their plasma levels (6750 pg/mL [5895-8245], 423 ng/mL [332-508], and 3.66 ng/mL [2.66-5.11], respectively).

COMMENT

There were 3 main findings in this study. First, vitreous fluid levels of sVEGFR-2, VEGF, sICAM-1, IL-6, MCP-1, and PTX3 were significantly higher in patients with BRVO and macular edema than in controls with MH. Second, vitreous fluid levels of sVEGFR-2, VEGF, sICAM-1, IL-6, MCP-1, PTX3, and PEDF were also correlated with the retinal thickness at the central fovea. Finally, there were significant correlations among the vitreous fluid levels of sICAM-1, IL-6, MCP-1, PTX3, and sVEGFR-2 in the BRVO group, as well as among the vitreous levels of sICAM-1, IL-6, MCP-1, PEDF, and VEGF.

These findings suggest that not only VEGF but also VEGFR-2, ICAM-1, IL-6, MCP-1, and PTX3 may play important roles in the occurrence of macular edema associated with BRVO. Vascular endothelial growth factor has a potent influence on vascular permeability, and its production is upregulated by retinal hypoxia in patients with BRVO and macular edema.[8] Breakdown of the BRB and retinal vascular hyperpermeability are important pathophysiologic features of macular edema associated with BRVO, and there is evidence that inflammation is a key mediator of both endothelial cell damage and BRB breakdown.[5-7] Upregulation of inflammatory factors, including VEGF, VEGFR-2, ICAM-1, IL-6, and MCP-1, as well as increased rolling and adhesion of leukocytes, is observed before and during the increase in retinal permeability.[5-7] Leukocyte recruitment is modulated by PTX3 in inflammation,[34] so its upregulation could also lead to an increase in vascular permeability.[24] This possibility is supported by the report[24] that the response of vascular permeability is less marked in PTX3-deficient mice. Thus, interactions among the network of inflammatory factors evaluated here may enhance vascular permeability. Activation of ICAM-1 and the subsequent increase in leukocyte-endothelial adhesion may be essential for VEGF to induce vascular hyperpermeability[5] because blocking ICAM-1 activity almost completely prevents VEGF-induced leukostasis and BRB breakdown.[35] However, blocking VEGF activity in the diabetic retina markedly reduces the upregulation of ICAM-1 as well as the increase in leukocyte adhesion and BRB breakdown.[36] These findings suggest that VEGF is the key factor mediating the response to hypoxia in the retina.

Interestingly, we found a significant correlation between the vitreous fluid level of sVEGFR-2 and the levels of various inflammatory factors (sICAM-1, IL-6, MCP-1, and PTX3) in patients with BRVO and macular edema, but there was no significant correlation between the vitreous fluid levels of sVEGFR-2 and VEGF. Binding of VEGF to VEGFR-2 triggers a signaling cascade that results in tyro-

sine phosphorylation of phospholipase Cγ,[37-39] which in turn increases the intracellular levels of inositol 1,4,5-triphosphate and diacylglycerol. Inositol 1,4,5-triphosphate increases the intracellular calcium level by promoting efflux of calcium from the endoplasmic reticulum. This increase in intracellular calcium stimulates sphingosine kinase to produce sphingosine 1-phosphate,[40] which then activates protein kinase C (PKC). Activated phospholipase Cγ also activates PKC by increasing the level of diacylglycerol, and activated PKC is a strong activator of nuclear factor κ B (NF-κB).[41] There is ample evidence that NF-κB promotes the transcription of inflammatory factors (including ICAM-1, IL-6, and MCP-1).[42-47] Nuclear factor–κB is found in almost all cell types and is involved in cellular responses to stimuli such as stress, proinflammatory gene expression (including cytokines, adhesion molecules, and chemokines), free radicals, UV irradiation, and bacterial or viral antigens in addition to its central role in the immune response.[48-50] It has also been reported[51-54] that VEGF, via the VEGFR-2–PKC axis, induces the production of proinflammatory cytokines (including IL-6 and MCP-1) in endothelial cells. Thus, VEGF promotes the expression of inflammatory factor messenger RNAs (including ICAM-1, IL-6, and MCP-1), mainly through the activation of PKC and NF-κB, indicating that VEGF induces the expression of inflammatory proteins by vascular endothelial cells through binding to VEGFR-2. This is supported by reports[53,55,56] that a specific VEGFR-2 antagonist blocks VEGF-induced expression of inflammatory factors (including ICAM-1, IL-6, and MCP-1) and also blocks activation of NF-κB by VEGF. Expression of the PTX3 gene also requires the activation of NF-κB.[57] In addition, Souza et al[24] reported that NF-κB activation was significantly suppressed in PTX3-deficient mice. Taken together with our results, these reports suggest that the vitreous level of sVEGFR-2 influences various inflammatory factors (including ICAM-1, IL-6, MCP-1, and PTX3) in patients with BRVO and macular edema. On the other hand, the vitreous level of sVEGFR-2 may be regulated independently of VEGF, although the VEGF–VEGFR-2 signaling pathway is considered essential for controlling vascular permeability.[58,59] The VEGF is upregulated by hypoxia through hypoxia-inducible factor 1α,[60] which is another transcription factor that regulates genes responding to hypoxia.[61] Vascular endothelial growth factor may act via an independent pathway to promote the retinal changes that occur in BRVO; therefore, additional studies are required to identify the mechanism. Differences in the activation of various transcription factors may determine the severity of ocular ischemic and inflammatory changes.

Considering our results, as well as the balance between VEGF and inflammatory cytokines, we should select treatment with anti-VEGF agents (to reduce the level of free VEGF) or triamcinolone acetonide (with a broad spectrum of action, as appropriate). Because the aqueous level of VEGF is significantly correlated with the vitreous level of VEGF,[62] measuring the concentrations of various molecules in aqueous humor by enzyme-linked immunosorbent assay or multiplex bead analysis could help with the selection of treatment between anti-VEGF agents, triamcinolone acetonide, or combined therapy. In addition, upregulation of inflammatory factors may be dependent on VEGFR-2 because there were significant correlations between the vitreous fluid level of sVEGFR-2 and the vitreous levels of 4 inflammatory factors (sICAM-1, IL-6, MCP-1, and PTX3) in our patients with BRVO and macular edema. Accordingly, multiple inflammatory factors could be inhibited by an antibody targeting VEGFR-2, so it may be worth also considering anti–VEGFR-2 therapy to treat macular edema in this population. However, a prospective clinical trial would be required to investigate the efficacy of such therapy.

This study also had some other limitations. For example, it is unclear from our data whether elevated vitreous levels of cytokines and chemokines were related to increased retinal vascular permeability or local production in the retina, but the mechanism involved may be revealed by animal studies.

In the present study, the vitreous fluid levels of sVEGFR-2, VEGF, sICAM-1, IL-6, MCP-1, PTX3, and PEDF were strongly correlated with retinal vascular permeability and the severity of macular edema. The sVEGFR-2 level was significantly correlated with the levels of sICAM-1, IL-6, MCP-1, and PTX3 but not with the level of VEGF. These findings suggest the importance of investigating relationships among VEGF and the cytokine network and may contribute to understanding the mechanism of macular edema in patients with BRVO and developing new treatments.

Submitted for Publication: May 9, 2012; final revision received July 20, 2012; accepted July 24, 2012.
Correspondence: Hidetaka Noma, MD, Department of Ophthalmology, Yachiyo Medical Center, Tokyo Women's Medical University, 477-96, Owada-shinden, Yachiyo, Chiba 276-8524, Japan (noma-hide@umin.ac.jp).
Author Contributions: Dr Noma had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.
Conflict of Interest Disclosures: None reported.
Additional Contributions: Katsunori Shimada, PhD (Department of Biostatistics, Statz Corporation, Tokyo), provided assistance with the statistical analysis.

REFERENCES

1. Tsujikawa A, Ogura Y, Hiroshiba N, Miyamoto K, Kiryu J, Honda Y. In vivo evaluation of leukocyte dynamics in retinal ischemia reperfusion injury. *Invest Ophthalmol Vis Sci.* 1998;39(5):793-800.
2. Scott IU, Ip MS, VanVeldhuisen PC, et al; SCORE Study Research Group. A randomized trial comparing the efficacy and safety of intravitreal triamcinolone with standard care to treat vision loss associated with macular edema secondary to branch retinal vein occlusion: the Standard Care vs Corticosteroid for Retinal Vein Occlusion (SCORE) study report 6. *Arch Ophthalmol.* 2009;127(9):1115-1128.
3. Miyake K, Miyake T, Kayazawa F. Blood-aqueous barrier in eyes with retinal vein occlusion. *Ophthalmology.* 1992;99(6):906-910.
4. Noma H, Funatsu H, Mimura T, Eguchi S, Shimada K. Visual prognosis and vitreous molecules after vitrectomy for macular edema with branch retinal vein occlusion. *Clin Ophthalmol.* 2011;5:223-229.
5. Lu M, Perez VL, Ma N, et al. VEGF increases retinal vascular ICAM-1 expression in vivo. *Invest Ophthalmol Vis Sci.* 1999;40(8):1808-1812.
6. Zhang SX, Wang JJ, Gao G, Shao C, Mott R, Ma JX. Pigment epithelium–derived factor (PEDF) is an endogenous antiinflammatory factor. *FASEB J.* 2006;20(2):323-325.
7. Yoshimura T, Sonoda KH, Sugahara M, et al. Comprehensive analysis of inflammatory immune mediators in vitreoretinal diseases. *PLoS One.* 2009;4(12):e8158. doi:10.1371/journal.pone.0008158.
8. Noma H, Minamoto A, Funatsu H, et al. Intravitreal levels of vascular endothelial growth factor and interleukin-6 are correlated with macular edema in branch reti-

nal vein occlusion. *Graefes Arch Clin Exp Ophthalmol.* 2006;244(3):309-315.
9. Noma H, Funatsu H, Mimura T, Harino S, Eguchi S, Hori S. Pigment epithelium-derived factor and vascular endothelial growth factor in branch retinal vein occlusion with macular edema. *Graefes Arch Clin Exp Ophthalmol.* 2010;248 (11):1559-1565.
10. Noma H, Funatsu H, Mimura T, Eguchi S, Hori S. Soluble vascular endothelial growth factor receptor-2 and inflammatory factors in macular edema with branch retinal vein occlusion. *Am J Ophthalmol.* 2011;152(4):669-677.e1. doi:10.1016 /j.ajo.2011.04.006.
11. Kaneda S, Miyazaki D, Sasaki S, et al. Multivariate analyses of inflammatory cytokines in eyes with branch retinal vein occlusion: relationships to bevacizumab treatment. *Invest Ophthalmol Vis Sci.* 2011;52(6):2982-2988.
12. Peri G, Introna M, Corradi D, et al. PTX3, a prototypical long pentraxin, is an early indicator of acute myocardial infarction in humans. *Circulation.* 2000;102(6): 636-641.
13. Latini R, Maggioni AP, Peri G, et al; Lipid Assessment Trial Italian Network (LATIN) Investigators. Prognostic significance of the long pentraxin PTX3 in acute myocardial infarction. *Circulation.* 2004;110(16):2349-2354.
14. Breviario F, d'Aniello EM, Golay J, et al. Interleukin-1–inducible genes in endothelial cells: cloning of a new gene related to C-reactive protein and serum amyloid P component. *J Biol Chem.* 1992;267(31):22190-22197.
15. Garlanda C, Bottazzi B, Bastone A, Mantovani A. Pentraxins at the crossroads between innate immunity, inflammation, matrix deposition, and female fertility. *Annu Rev Immunol.* 2005;23:337-366.
16. Alles VV, Bottazzi B, Peri G, Golay J, Introna M, Mantovani A. Inducible expression of PTX3, a new member of the pentraxin family, in human mononuclear phagocytes. *Blood.* 1994;84(10):3483-3493.
17. Introna M, Alles VV, Castellano M, et al. Cloning of mouse *ptx3*, a new member of the pentraxin gene family expressed at extrahepatic sites. *Blood.* 1996;87 (5):1862-1872.
18. Goodman AR, Levy DE, Reis LF, Vilcek J. Differential regulation of TSG-14 expression in murine fibroblasts and peritoneal macrophages. *J Leukoc Biol.* 2000; 67(3):387-395.
19. Doni A, Peri G, Chieppa M, et al. Production of the soluble pattern recognition receptor PTX3 by myeloid, but not plasmacytoid, dendritic cells. *Eur J Immunol.* 2003;33(10):2886-2893.
20. Klouche M, Peri G, Knabbe C, et al. Modified atherogenic lipoproteins induce expression of pentraxin-3 by human vascular smooth muscle cells. *Atherosclerosis.* 2004;175(2):221-228.
21. Toniatti C, Demartis A, Monaci P, Nicosia A, Ciliberto G. Synergistic *trans*-activation of the human C-reactive protein promoter by transcription factor HNF-1 binding at two distinct sites. *EMBO J.* 1990;9(13):4467-4475.
22. Pepys MB, Hirschfield GM. C-reactive protein: a critical update. *J Clin Invest.* 2003; 111(12):1805-1812.
23. Souza DG, Soares AC, Pinho V, et al. Increased mortality and inflammation in tumor necrosis factor–stimulated gene-14 transgenic mice after ischemia and reperfusion injury. *Am J Pathol.* 2002;160(5):1755-1765.
24. Souza DG, Amaral FA, Fagundes CT, et al. The long pentraxin PTX3 is crucial for tissue inflammation after intestinal ischemia and reperfusion in mice. *Am J Pathol.* 2009;174(4):1309-1318.
25. Branch Vein Occlusion Study Group. Argon laser photocoagulation for macular edema in branch vein occlusion. *Am J Ophthalmol.* 1984;98(3):271-282.
26. Otani T, Kishi S. Tomographic assessment of vitreous surgery for diabetic macular edema. *Am J Ophthalmol.* 2000;129(4):487-494.
27. Saika S, Tanaka T, Miyamoto T, Ohnishi Y. Surgical posterior vitreous detachment combined with gas/air tamponade for treating macular edema associated with branch retinal vein occlusion: retinal tomography and visual outcome. *Graefes Arch Clin Exp Ophthalmol.* 2001;239(10):729-732.
28. Stefánsson E, Novack RL, Hatchell DL. Vitrectomy prevents retinal hypoxia in branch retinal vein occlusion. *Invest Ophthalmol Vis Sci.* 1990;31(2):284-289.
29. Stefánsson E. Ocular oxygenation and the treatment of diabetic retinopathy. *Surv Ophthalmol.* 2006;51(4):364-380.
30. Okunuki Y, Usui Y, Katai N, et al. Relation of intraocular concentrations of inflammatory factors and improvement of macular edema after vitrectomy in branch retinal vein occlusion. *Am J Ophthalmol.* 2011;151(4):610-616.e1. doi:10.1016 /j.ajo.2010.09.030.
31. Yamamoto S, Saito W, Yagi F, Takeuchi S, Sato E, Mizunoya S. Vitrectomy with or without arteriovenous adventitial sheathotomy for macular edema associated with branch retinal vein occlusion. *Am J Ophthalmol.* 2004;138(6):907-914.
32. Kumagai K, Furukawa M, Ogino N, Uemura A, Larson E. Long-term outcomes of vitrectomy with or without arteriovenous sheathotomy in branch retinal vein occlusion. *Retina.* 2007;27(1):49-54.
33. Funatsu H, Noma H, Mimura T, Eguchi S, Hori S. Association of vitreous inflammatory factors with diabetic macular edema. *Ophthalmology.* 2009;116(1): 73-79.
34. Deban L, Russo RC, Sironi M, et al. Regulation of leukocyte recruitment by the long pentraxin PTX3. *Nat Immunol.* 2010;11(4):328-334.
35. Miyamoto K, Khosrof S, Bursell SE, et al. Vascular endothelial growth factor (VEGF)–induced retinal vascular permeability is mediated by intercellular adhesion molecule-1 (ICAM-1). *Am J Pathol.* 2000;156(5):1733-1739.
36. Ishida S, Usui T, Yamashiro K, et al. VEGF164 is proinflammatory in the diabetic retina. *Invest Ophthalmol Vis Sci.* 2003;44(5):2155-2162.
37. Xia P, Aiello LP, Ishii H, et al. Characterization of vascular endothelial growth factor's effect on the activation of protein kinase C, its isoforms, and endothelial cell growth. *J Clin Invest.* 1996;98(9):2018-2026.
38. He H, Venema VJ, Gu X, Venema RC, Marrero MB, Caldwell RB. Vascular endothelial growth factor signals endothelial cell production of nitric oxide and prostacyclin through flk-1/KDR activation of c-Src. *J Biol Chem.* 1999;274(35): 25130-25135.
39. Wu LW, Mayo LD, Dunbar JD, et al. Utilization of distinct signaling pathways by receptors for vascular endothelial cell growth factor and other mitogens in the induction of endothelial cell proliferation. *J Biol Chem.* 2000;275(7):5096-5103.
40. Olivera A, Edsall L, Poulton S, Kazlauskas A, Spiegel S. Platelet-derived growth factor–induced activation of sphingosine kinase requires phosphorylation of the PDGF receptor tyrosine residue responsible for binding of PLCγ. *FASEB J.* 1999; 13(12):1593-1600.
41. Ghosh S, Baltimore D. Activation in vitro of NF-κB by phosphorylation of its inhibitor IκB. *Nature.* 1990;344(6267):678-682.
42. Matsusaka T, Fujikawa K, Nishio Y, et al. Transcription factors NF-IL6 and NF-κB synergistically activate transcription of the inflammatory cytokines, interleukin 6 and interleukin 8. *Proc Natl Acad Sci U S A.* 1993;90(21):10193-10197.
43. Ledebur HC, Parks TP. Transcriptional regulation of the intercellular adhesion molecule-1 gene by inflammatory cytokines in human endothelial cells: essential roles of a variant NF-κB site and p65 homodimers. *J Biol Chem.* 1995;270 (2):933-943.
44. Wrighton CJ, Hofer-Warbinek R, Moll T, Eytner R, Bach FH, de Martin R. Inhibition of endothelial cell activation by adenovirus-mediated expression of IκBα, an inhibitor of the transcription factor NF-κB. *J Exp Med.* 1996;183(3):1013-1022.
45. Baldwin AS Jr. The NF-κB and IκB proteins: new discoveries and insights. *Annu Rev Immunol.* 1996;14:649-683.
46. Marumo T, Schini-Kerth VB, Fissithaler B, Busse R. Platelet-derived growth factor–stimulated superoxide anion production modulates activation of transcription factor NF-κB and expression of monocyte chemoattractant protein 1 in human aortic smooth muscle cells. *Circulation.* 1997;96(7):2361-2367.
47. Boyle EM Jr, Kovacich JC, Canty TG Jr, et al. Inhibition of nuclear factor-κB nuclear localization reduces human E-selectin expression and the systemic inflammatory response. *Circulation.* 1998;98(19)(suppl):II282-II288.
48. Barnes PJ, Karin M. Nuclear factor-κB: a pivotal transcription factor in chronic inflammatory diseases. *N Engl J Med.* 1997;336(15):1066-1071.
49. Ghosh S, May MJ, Kopp EB. NF-κB and Rel proteins: evolutionarily conserved mediators of immune responses. *Annu Rev Immunol.* 1998;16:225-260.
50. Perkins ND. Integrating cell-signalling pathways with NF-κB and IKK function. *Nat Rev Mol Cell Biol.* 2007;8(1):49-62.
51. Carnevale KA, Cathcart MK. Protein kinase C β is required for human monocyte chemotaxis to MCP-1. *J Biol Chem.* 2003;278(28):25317-25322.
52. Hong KH, Ryu J, Han KH. Monocyte chemoattractant protein-1–induced angiogenesis is mediated by vascular endothelial growth factor-A. *Blood.* 2005;105 (4):1405-1407.
53. Koga J, Matoba T, Egashira K, et al. Soluble *Flt-1* gene transfer ameliorates neointima formation after wire injury in flt-1 tyrosine kinase–deficient mice. *Arterioscler Thromb Vasc Biol.* 2009;29(4):458-464.
54. Hao Q, Wang L, Tang H. Vascular endothelial growth factor induces protein kinase D–dependent production of proinflammatory cytokines in endothelial cells. *Am J Physiol Cell Physiol.* 2009;296(4):C821-C827.
55. Kim I, Moon SO, Kim SH, Kim HJ, Koh YS, Koh GY. Vascular endothelial growth factor expression of intercellular adhesion molecule 1 (ICAM-1), vascular cell adhesion molecule 1 (VCAM-1), and E-selectin through nuclear factor-κB activation in endothelial cells. *J Biol Chem.* 2001;276(10):7614-7620.
56. Yao JS, Zhai W, Young WL, Yang GY. Interleukin-6 triggers human cerebral endothelial cells proliferation and migration: the role for KDR and MMP-9. *Biochem Biophys Res Commun.* 2006;342(4):1396-1404.
57. Basile A, Sica A, d'Aniello E, et al. Characterization of the promoter for the human long pentraxin *PTX3*: role of NF-κB in tumor necrosis factor–α and interleukin-1β regulation. *J Biol Chem.* 1997;272(13):8172-8178.
58. Ferrara N, Davis-Smyth T. The biology of vascular endothelial growth factor. *Endocr Rev.* 1997;18(1):4-25.
59. Claesson-Welsh L. Signal transduction by vascular endothelial growth factor receptors. *Biochem Soc Trans.* 2003;31(pt 1):20-24.
60. Forsythe JA, Jiang BH, Iyer NV, et al. Activation of vascular endothelial growth factor gene transcription by hypoxia-inducible factor 1. *Mol Cell Biol.* 1996; 16(9):4604-4613.
61. Pagès G, Pouysségur J. Transcriptional regulation of the vascular endothelial growth factor gene—a concert of activating factors. *Cardiovasc Res.* 2005;65(3):564-573.
62. Noma H, Funatsu H, Yamasaki M, et al. Aqueous humour levels of cytokines are correlated to vitreous levels and severity of macular oedema in branch retinal vein occlusion. *Eye (Lond).* 2008;22(1):42-48.

Experimental Eye Research 116 (2013) 17–26

Contents lists available at ScienceDirect

Experimental Eye Research

journal homepage: www.elsevier.com/locate/yexer

Review

The role of SIRT1 in ocular aging

Tatsuya Mimura [a,*], Yuichi Kaji [b], Hidetaka Noma [c], Hideharu Funatsu [c], Shinseiro Okamoto [d]

[a] Department of Ophthalmology, Tokyo Women's Medical University Medical Center East, 2-1-10 Nishiogu, Arakawa-ku, 116-8567 Tokyo, Japan
[b] Department of Ophthalmology, Institute of Clinical Medicine, University of Tsukuba, Tsukuba, Ibaraki, Japan
[c] Department of Ophthalmology, Yachiyo Medical Center, Tokyo Women's Medical University, Yachiyo, Chiba, Japan
[d] Okamoto Eye Clinic, Yamato, Kanagawa, Japan

ARTICLE INFO

Article history:
Received 7 August 2012
Accepted in revised form 16 July 2013
Available online 26 July 2013

Keywords:
review
SIRT1
eye

ABSTRACT

The sirtuins are a highly conserved family of nicotinamide adenine dinucleotide (NAD+)-dependent histone deacetylases that helps regulate the lifespan of diverse organisms. The human genome encodes seven different sirtuins (SIRT1-7), which share a common catalytic core domain but possess distinct N- and C-terminal extensions. Dysfunction of some sirtuins have been associated with age-related diseases, such as 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 polyphenolic SIRT1 activator that has been shown to increase the lifespan and to protect various organs against aging. A number of animal studies have been conducted to examine the role of sirtuins in ocular aging. Here we review current knowledge about SIRT1 and ocular aging. The available data indicate that SIRT1 is localized in the nucleus and cytoplasm of cells forming all normal ocular structures, including the cornea, lens, iris, ciliary body, and retina. Upregulation of SIRT1 has been shown to have an important protective effect against various ocular diseases, such as cataract, retinal degeneration, optic neuritis, and uveitis, in animal models. These results suggest that SIRT1 may provide protection against diseases related to oxidative stress-induced ocular damage, including cataract, age-related macular degeneration, and optic nerve degeneration in glaucoma patients.

1. Introduction

The recognition of homeostasis and homeostatic mechanisms dates back to the 19th century French physiologist Claude Bernard, who established the concept of homeostasis or internal environment in the body. Walter Bradford Cannon expanded on Claude Bernard's concept of homeostasis (Cannon, 1932). Epigenetic mechanisms have recently been recognized for their essential contribution in maintaining homeostasis by regulating gene expression and chromatin structure. In part, gene expression is regulated by the reversible methylation of DNA and acetylation of histones, thus controlling a wide variety of cellular functions. Histone deacetylases (HDACs) are enzymes that deacetylate histones, and non-histone proteins, such as the p53 tumor suppressor protein, α-tubulin and forkhead transcription factors (Luo et al., 2001; Vaziri et al., 2001). Class III HDACs, which are known as sirtuins, catalyze deacetylation of the acetyl-lysine residues of histones using nicotinamide adenine dinucleotide (NAD+) as a cofactor. In addition to deacetylase activity, sirtuins also possess ADP-ribosyl transferase activity. They control energy homeostasis in response to stress but promote cellular aging when homeostasis fails thereby avoiding genomic instability (Rodriguez and Fraga, 2010). Thus, the sirtuins play an essential role in helping maintain cell homeostasis via regulation of both epigenetic and non-epigenetic mechanisms.

Silent information regulator 2 (Sir2), first described in yeast, was the first gene discovered in the sirtuin family (Shore et al., 1984; Gottlieb and Esposito, 1989). Sir2 shows a high level of evolutionary conservation and is an important regulator of senescence (Langley et al., 2002; van der Veer et al., 2007), cell differentiation (Takata and Ishikawa, 2003; Blander and Guarente, 2004; Anastasiou and Krek, 2006; Prozorovski et al., 2008; Wojcik et al., 2009), stress tolerance (Blander and Guarente, 2004; Anastasiou and Krek, 2006; Wojcik et al., 2009), metabolism (Blander and Guarente, 2004; Anastasiou and Krek, 2006; Wojcik et al., 2009), and cancer (Pruitt et al., 2006; Oberdoerffer et al., 2008; Han et al., 2013). Sirtuins have been suggested to have a role in aging (Gotta et al., 1997; Guarente and Kenyon, 2000), calorie restriction (Cohen et al., 2004; Nemoto et al., 2004;

* Corresponding author. Tel.: +81 33810 1111x7765, fax: +81 33894 0282.
E-mail address: mimurat-tky@umin.ac.jp (T. Mimura).

0014-4835/$ – see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.exer.2013.07.017

Rodgers et al., 2005; Nisoli et al., 2005; Corton and Brown-Borg, 2005; Civitarese et al., 2007; Milne et al., 2007; Bordone et al., 2007; Mulligan et al., 2008; Coppari et al., 2009; Satoh et al., 2010; Baur et al., 2010; Qiu et al., 2010; Gesing et al., 2011; Takemori et al., 2011; Radak et al., 2013), and inflammation (Yeung et al., 2004; Qiu et al., 2010), and apoptosis (Bhattacharya et al., 2012; Liu et al., 2012). Overexpression of Sir2 prolongs the lifespan of various organisms, whereas deletion or mutations of Sir2 leads to a shorter lifespan (Kaeberlein et al., 1999; Tissenbaum and Guarente, 2001; Rogina and Helfand, 2004). Seven human Sir2 homologues, generally known as sirtuins, have been identified to date, and these are designated as SIRT1 to SIRT7 (Frye, 1999, 2000).

There have been numerous reports suggesting that sirtuins are important anti-aging molecules and may have a role in preventing several age-related ocular diseases. These age-related changes of the eye are listed in Table 1. The process of ocular aging may be influenced by various factors, which include aging itself, ultraviolet (UV) radiation, oxidative stress (Fletcher, 2010), systemic diseases (such as diabetes, hypertension (DellaCroce and Vitale, 2008), or metabolic syndrome), and lifestyle factors (Fig. 1). Interaction of these various etiological and environmental factors during the aging process causes or exacerbates various age-related ocular diseases, such as pinguecula (Panchapakesan et al., 1998; Pham et al., 2005a, 2005b; Fotouhi et al., 2009; Mimura et al., 2011, 2012b), conjunctivochalasis (Di Pascuale et al., 2004; Mimura et al., 2009, 2012a), cataract (Oliver, 1906), spheroid degeneration (Fraunfelder et al., 1972), age-related macular degeneration (AMD) (Vinding, 1989), and glaucoma (Segal and Skwierczynska, 1967).

This article reviews current knowledge about the mechanisms underlying the actions of sirtuins in ocular tissues, with an emphasis on Sirt1, which is the best characterized family member. Modulation of sirtuin-related signal transduction and downstream effects may have potential applications in managing ocular diseases associated with aging, such as cataract, AMD, and optic nerve degeneration in glaucoma patients.

2. The sirtuin family

Sirtuins belongs to a family of histone deacetylases (HDACs) that have been divided into four groups (Fremont, 2000; Frye, 2000; Bastianetto and Quirion, 2002; Borra et al., 2005; Sinclair et al., 2006; Baur and Sinclair, 2006). HDACs from classes I, II, and IV share common features, as all of these molecules are zinc-dependent and exhibit some sequence similarities, while class III HDACs are NAD+-dependent enzymes that show no homology with the other HDACs. Sirtuins are Class III HDACs, which are essential for maintaining the silence of chromatin during histone deacetylation (Frye, 2000; Borra et al., 2005; Denu, 2005; Kaeberlein et al., 2005).

The mammalian sirtuin family has seven members, designated as SIRT1–7 (Frye, 1999, 2000; Michan and Sinclair, 2007) (Fig. 2). Structurally, sirtuins share significant sequence homology, with all of them containing a conserved catalytic domain of 275 amino acids and a nicotinamide adenine dinucleotide (NAD+)-binding domain, as well as unique additional N-terminal and/or C-terminal sequences of variable length (Frye, 1999; Sherman et al., 1999; Imai et al., 2000; Finnin et al., 2001; Yamamoto et al., 2007).

Sirtuins differ in their cellular localization, activity, and function, and are subdivided into four classes (I–IV) (Table 2) (Frye, 2000; North and Verdin, 2004; Carafa et al., 2012). Class I contains human SIRT1, SIRT2, and SIRT3, as well as all yeast sirtuins. Class I is divided in three subclasses (a, b and c). SIRT1 is positioned in Class Ia with yeast Sir2 and Hst1, while SIRT2 and SIRT3 belong to Class Ib together with yeast Hst2. Class II contains human SIRT4 along with sirtuins from bacteria, insects, nematodes, moulds, fungi, and protozoa. Class III contains human SIRT5, while class IV contains SIRT6 and SIRT7 in two different subclasses (IVa and IVb, respectively) (Frye, 2000). The high conservation among species confirms the extreme importance of this protein family. Class U consists of all bacterial sirtuins.

Seven sirtuin members (SIRT1–7) have distinct subcellular localizations: SIRT1 protein is localized in both the nucleus and cytoplasm of cells (Langley et al., 2002; Michishita et al., 2005),

Table 1
Summary of changes associated with ocular aging.

	Changes
Eyelids	Blepharochalasis, Blepharoptosis, Ectropion/Entropion, Eyelid malposition, Orbital fat prolapse, and Meibomian gland dysfunction.
Tears	Lacrimal obstruction, Dry Eyes/Watery eyes.
Conjunctiva	Pinguecula, Pterygium, Conjunctivochalasis.
Cornea	**Shape and structure:** Change of corneal curvature (shift from with-the-rule to against-the-rule astigmatism), Decreased corneal luster, Increased corneal fragility, and Increased thickness of Descemet's membrane. **Diseases:** Deposits (Arcus senilis, Hudson-Stahli line Hassall-Henle bodies), Limbal epithelium stem cell deficiency, Reduced endothelial cell density (Guttata, Fuchs endothelial degeneration), and Decreased corneal sensitivity.
Iris	Smaller pupil, Decreased light reaction of the iris, and loss of iris pigment.
Trabecular meshwork	Increased pigmentation of the trabecular meshwork, Increased resistance to the outflow of aqueous humor.
Lens/accommodation	Decreased lens elasticity, Lens fiber differentiation, and Lens capsule deterioration. Cataract, Presbyopia.
Vitreous	**Structure:** Vitreous liquefaction, Condensation of the vitreous fluid, Enhanced fibrillary structure of the vitreous, Increased mobility of fibrillary structures, Formation of lacunae, and Posterior vitreous detachment (PVD). **Diseases:** Floating PVD, Flashes, Retinal tears, and Retinal detachment caused by traction on due to PVD, Epiretinal membrane, Macular hole, and Incidental vitreoretinal hemorrhage after PVD.
Retina	**Structure:** Changes of visual function (decreased visual field sensitivity, reduced contrast sensitivity, and increased dark adaptation threshold), Retinal vascular changes (dilatation, tortuosity, elongation, and neovascularization), Neurosensory retinal changes (loss of retinal pigment epithelial cells which is vital for integrity of the rods and cones, photoreceptors, and ganglion cell), Decreased melanin content, Increased lipofuscin content, and Decreased cytoplasmic volume. **Diseases:** Epiretinal membrane, Retinal tear, Retinal detachment, Senile tigroid fundus, Retinal degenerations, Drusen, Central/branch retinal vein occlusion, Central/branch retinal artery occlusion, and Diabetic retinopathy.
Macular region	**Structure:** Decrease in number of foveal ganglion cells, Decrease in retinal macular microcirculation (Retinal ischaemia) **Diseases:** Drusen, Age related macular degeneration, Subretinal neovascular membrane, Epiretinal membrane, Macular hole, Cystoid macular edema, Pigment epithelial detachment
Optic nerve	**Structure:** Decrease of axons, Axonal swelling at the lamina cribrosa, Thickening of the lamina cribrosa, and Increase of elastic fibers. **Diseases:** Glaucoma

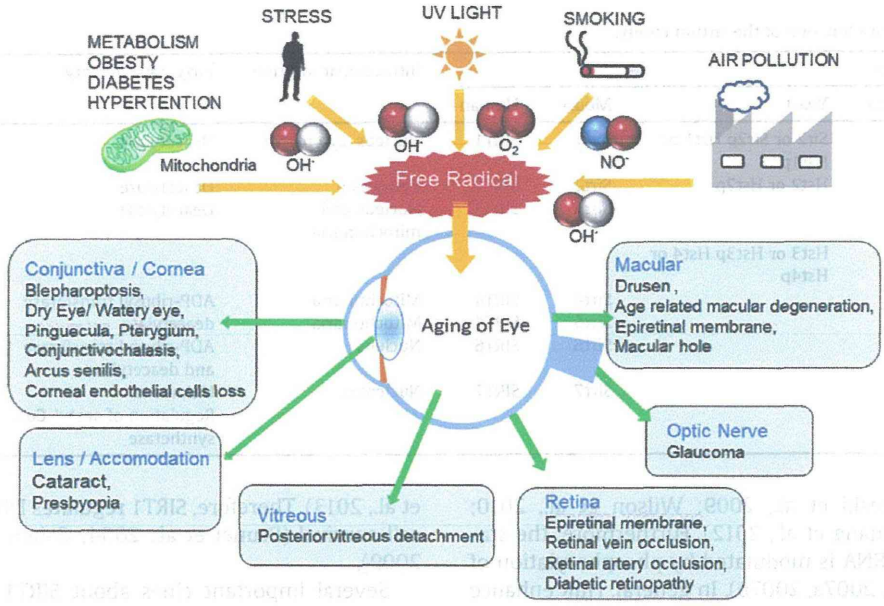

Fig. 1. Environmental factors that influence ocular aging. Ocular aging is influenced by various factors, including aging, systemic diseases (such as diabetes, hypertension, or metabolic syndrome), lifestyle factors, ultraviolet (UV) radiation, oxidative stress, smoking, and air pollution. These environmental factors produce free radicals and accumulation of free radical damage affects the ocular tissues. In addition, metabolic end products (such as advanced glycation end products) can accumulate in the ocular tissues. The normal aging process, including these environmental and psychological factors, causes various age-related ocular diseases.

while SIRT2 predominately resides in the cytoplasm (Frye, 1999; North et al., 2003). SIRT3 is primarily localized in the nucleus and mitochondria (Schwer et al., 2002). Studies have shown that SIRT3 stimulates mitochondrial biogenesis (Kong et al., 2010). Along with SIRT3, SIRT4 and SIRT5 are also localized in mitochondria and are implicated in regulating mitochondrial function (Houtkooper et al., 2012). In additional to SIRT1, SIRT6 and SIRT7 are mainly localized in the nucleus. In the nucleus, a large fraction of SIRT1 is associated with euchromatin, whereas SIRT6 is associated with heterochromatin and SIRT7 is found in the nucleolus (Michishita et al., 2005). The details of the localization, chief functions, and substrates of the sirtuins (SIRT1 to SIRT7) are given in Table 2 (Frye, 2000; North and Verdin, 2004; Carafa et al., 2012).

The mitochondrial localization of SIRT3, 4, and 5 suggests their potential involvement in ocular disease precipitated by mitochondrial dysfunction. Mitochondrial DNA (mtDNA) point mutations are associated with Leber's hereditary optic neuropathy (Holt et al., 1988; Wallace et al., 1988). The clinical presentation of severe neonatal neurodegeneration of the central retina and early-onset optic atrophy is consistent with Leber congenital amaurosis and suggests that the mutations affect neuroprotection of photoreceptor cells (Perrault et al., 2012). Common neuro-ophthalmic manifestations of mitochondrial dysfunction include optic neuropathy, chronic progressive external ophthalmoplegia (CPEO), pigmentary retinopathy, and retrochiasmal visual loss (Biousse and Newman, 2003; Fraser et al., 2010). Mitochondrial dysfunction has recently been found to be associated with age-related retinal disease including macular degeneration (Jarrett et al., 2008) and glaucoma (Kong et al., 2009). It has been reported that SIRT1 or SIRT1 activators has neuroprotective effects as described in Sections 4–6, but the neuroprotective effects of the three mitochondrial sirtuins – SIRT3, SIRT4, and SIRT5 against mitochondrial eye diseases was unknown.

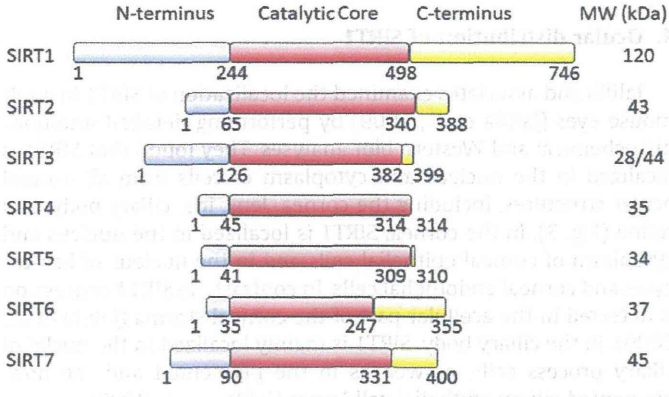

Fig. 2. Schematic representation of human sirtuins 1-7 (SIRT1-7). All have a nicotinamide adenine dinucleotide (NAD+)-dependent catalytic core domain (red). This structurally conserved core domain is flanked by N-terminal (gray) and C-terminal extensions. Molecular weights are in kDa. Molecular weight (kDa) of each protein as well as the amino acids comprising each domain is indicated.

SIRT6 regulates aging and genome stability (Mostoslavsky et al., 2006; Tennen and Chua, 2011; Kaidi et al., 2010; Polyakova et al., 2012), promotes DNA repair under stress by activating poly [adenosine diphosphate (ADP)–ribose] polymerase 1 (PARP1) (Mao et al., 2011), prevents obesity (Schwer et al., 2010; Xiao et al., 2010; Zhong et al., 2010; Dominy et al., 2012; Lerrer and Cohen, 2013) and cardiomyopathy (Webster, 2012) by modulating neural chromatin structure and gene activity, and acts as a potent tumor suppressor (Sebastián et al., 2012; Lyssiotis and Cantley, 2012). The peroxisome proliferator-activated receptor gamma (PPAR-γ) agonist also increase Sirt6 protein levels in the mouse model of Huntington's disease, which is a devastating genetic neurodegenerative disease (Jin et al., 2013). SIRT6 protects vascular endothelial cells from premature senescence and telomere and DNA damage (Cardus et al., 2013; Shen et al., 2013). However, there has been no data to suggest that SIRT6 have protective function in the development of age-related eye diseases.

SIRT1 deacetylate and affect the activity of both members of the PPAR-γ co-activator-1 alpha (PGC-1 alpha)/estrogen receptor related alpha (ERR-alpha) complex, which are essential metabolic regulatory transcription factors (Rodgers et al., 2005; Nemoto et al., 2005; Lagouge et al., 2006; Liu et al., 2008; Rodgers et al., 2008;

Table 2
Classification, cellular location and function of the sirtuin family.

Class	Sub-class	Species				Intracellular location	Enzymatic activity	Function
		Bacteria	Yeast	Mouse	Human			
I	a		Sir2 or Sir2p Hst1 or Hst1p	Sirt1	SIRT1	Nucleus, cytoplasm	Deacetylase	Metabolism inflammation
	b		Hst2 or Hst2p	Sirt2	SIRT2	Cytoplasm	Deacetylase	Cell cycle tumorigenesis
				Sirt3	SIRT3	Nucleus and mitochondria	Deacetylase	Metabolism
	c		Hst3 or Hst3p Hst4 or Hst4p					
II				Sirt4	SIRT4	Mitochondria	ADP-ribosyl transferase	Insulin secretion
III				Sirt5	SIRT5	Mitochondria	deacetylase	Ammonia detoxification
IV	a			Sirt6	SIRT6	Nucleus	ADP-ribosyl transferase and deacetylase	DNA repair, metabolism
	b			Sirt7	SIRT7	Nucleolus	Unknown	rDNA transcription
U		cobB					Regulation of acetyl-CoA synthetase	Metabolism

Cantó et al., 2009; Wareski et al., 2009; Wilson et al., 2010; Hayashida et al., 2010; Huang et al., 2012). Furthermore, the stabilization of the SIRT1 mRNA is modulated by phosphorylation of HuR (Abdelmohsen et al., 2007a, 2007b). In general, HuR enhance stabilization of its target mRNAs in response to cellular stress. However, in the case of SIRT1, cell stress induced by hydrogen peroxide leads to the dissociation of HuR/SIRT1 mRNA complexes and mRNA decay (Hinman and Lou, 2008). In addition, both HuR and HuD are methylated by coactivator-associated arginine methyltransferase 1 (CARM1) (Li et al., 2002; Fujiwara et al., 2006). This regulatory pathway of HuR-SIRT1 contributes to the maintenance of genomic integrity (Gorospe and de Cabo, 2008).

3. Biological role of SIRT1 in mammals

Of the seven mammalian sirtuin proteins, SIRT1 has been the most extensively characterized. To date, several studies have assessed the ocular role of SIRT1, including its relationship with cataract (Zheng and Lu, 2011; Lin et al., 2011), retinal degeneration (Cheng et al., 2003; Anekonda and Adamus, 2008; Jaliffa et al., 2009; Chen et al., 2009; Kubota et al., 2010; Ozawa et al., 2010; Peng et al., 2011; Geng et al., 2011), optic neuritis (Shindler et al., 2007), and uveitis (Kubota et al., 2009). The most important role of SIRT1 in the eye may be protection of the retina and optic nerve against degeneration. Peng et al. reported that the expression of SIRT1, which decreases in rats and human aged eyes, is associated with self-renewal capacity in retinal stem cells (Peng et al., 2010). These data suggest that SIRT1 may play a role in self-renewal and aging process of ocular stem cells.

SIRT1 has been shown to play a prominent role in the anti-aging effects of calorie restriction (Bonda et al., 2011), the protective effect of calorie restriction against neurodegenerative disease (Chen et al., 2008), and the enhancement role of proliferative state of neuronal stem cells in the rat hippocampus (Torres et al., 2011), as well as being involved in protection against cellular oxidative stress and DNA damage (Gorenne et al., 2013). SIRT1 has many physiological roles outside of the eye- and these studies are categorized into 10 main disease/physiological categories (Rahman and Islam, 2011), as summarized in Table 3.

Aging occurs systematically in close association with mitochondrial DNA instability and DNA stability by stimulating sirtuins (Ozawa et al., 2010). These aging processes depend on (1) E2F1 phosphorylation by ataxia telangiectasia mutated (ATM), which is a prerequisite for E2F1-mediated regulation of Sirt1 gene expression, (2) SIRT1-mediated activation of Ku70 which is a nonhomologous end-joining DNA repair protein, and (3) SIRT1 protein regulates p53 acetylation and p53-dependent apoptosis (Cheng et al., 2003; Pang et al., 2013). Therefore, SIRT1 regulates DNA stability and promotes cell survival (Brunet et al., 2004; Cohen et al., 2004; Chen et al., 2009).

Several important clues about SIRT1 function have emerged from studies of knockout mice (KO) and transgenic mice. For example, SIRT1-deficient mice are smaller than normal at birth (McBurney et al., 2003). Additionally, all SIRT1-deficient mice fail to open one or both eyes (McBurney et al., 2003). In general, SIRT1 KO mice show neither the increase in physical activity nor many of the physiological changes (Chen et al., 2005; Boily et al., 2008; K. Li, 2008; Y. Li, 2008). SIRT1 KO mice show impaired insulin secretion in response to glucose (Bordone et al., 2006). Neuron-specific SIRT1 KO mice display reduced growth hormone level, which results in the impairment of body growth (Cohen et al., 2009). SIRT1 KO mice exhibited aggravated lung vascular leakage and inflammation after ambient particulate matter exposure (Wu et al., 2012). SIRT1 KO mice also show decreased levels of deacetylated phosphatidylinositol-4-phosphate 5-kinase (PIP5K) gamma, phosphatidylinositol-4,5-bisphosphate ($PI(4,5)P_2$), and reduced the secretion of thyroid-stimulating hormone (TSH) from pituitary cells. These results indicate that the control of TSH release by the SIRT1-PIP5Kc pathway play an important role in regulating the metabolism of the whole body (Akieda-Asai et al., 2010). Knockdown of SIRT1 prevent the effect of pyruvate, a nutrient signal activating SIRT1, on the abolishment of synergistic induction of cytochrome P450 (CYP) 3A11 by pregnenolone-16α -carbonitrile (PCN) and PGC-1α (Buler et al., 2011). SIRT1 transgenic mice have reduced levels of blood cholesterol and adipokines (Bordone et al., 2007).

4. Ocular distribution of SIRT1

Jaliffa and associates examined the localization of SIRT1 in adult mouse eyes (Jaliffa et al., 2009) by performing detailed immunohistochemical and Western blot analyses. They found that SIRT1 is localized in the nucleus and cytoplasm of cells from all normal ocular structures, including the cornea, lens, iris, ciliary body, and retina (Fig. 3). In the cornea, SIRT1 is localized in the nucleus and cytoplasm of corneal epithelial cells and in the nucleus of keratocytes and corneal endothelial cells. In contrast, no SIRT1 expression is detected in the acellular part of the corneal stroma (Jaliffa et al., 2009). In the ciliary body, SIRT1 is mainly localized in the nuclei of ciliary process cells, as well as in the pigmented and the non-pigmented ciliary epithelial cell layers (Jaliffa et al., 2009).

In the adult mouse lens, SIRT1 is principally localized in the nuclei of epithelial and fiber cells, while no of SIRT1 is detected in the lens capsule (Jaliffa et al., 2009). In the mouse retina, SIRT1 is localized in the nuclei of retinal pigment epithelium (RPE) and

Table 3
The physiological role of SIRT1.

Disease	Physiological role of SIRT1 (author and year)
1) Obesity-associated metabolic diseases	• SIRT1 increases insulin secretion (Bordone et al., 2006; Lee et al., 2009) • Promotes fat metabolism (Bordone et al., 2006) • Protects β-cells (Banks et al., 2008; Pfluger et al., 2008) • Decreases lipid levels (Li et al., 2007; Nomiyama and Bruemmer, 2008) • Protects against atherosclerosis (Li et al., 2007; Walker et al., 2010) • Prevention of leptin programming (Wang et al., 2011; Trotta et al., 2011)
2) Cancer	• SIRT1 is involved in epigenetic silencing of tumor suppressor genes (Pruitt et al., 2006; Zhao et al., 2008; Kim et al., 2008) • Inhibits the transcriptional activity of p53 (Vaziri et al., 2001) • Influences the repair of DNA damage (Wang et al., 2008) • Promotes deacetylation of β-catenin (Firestein et al., 2008) • Decreases the stability of c -Myc (Yuan et al., 2009) • Influences tumor suppression (Haigis and Sinclair, 2010) • Maintains genomic stability (K. Li, 2008; Y. Li, 2008).
3) Adipose tissue	• SIRT1 decreases lipid accumulation in adipocytes (Picard and Guarente, 2005) • Increases insulin sensitivity (Sun et al., 2007) • Promotes lipolysis (Picard et al., 2004)
4) Cellular aging:	• SIRT1 suppresses the replication of extrachromosomal rDNA circles in yeast (Sinclair et al., 1998; Kaeberlein et al., 1999; Guarente, 1999) • Maintains telomere stability (Palladino et al., 1993; Cockell et al., 1995; Guarente, 1999; Mantel and Broxmeyer, 2008) • Promotes lifespan extension by calorie restriction in mice (Guarente and Picard, 2005)
5) Cellular senescence	• SIRT1 rescues primary mouse embryonic fibroblasts (Langley et al., 2002) • Deacetylates and decreases the transcriptional activity of pro-apoptotic p53 (Langley et al., 2002)
6) Cardiac aging and stress	• SIRT1 protects against oxidative stress (Hsu et al., 2008) • Reduces Forkhead-dependent apoptosis (Motta et al., 2004) • Promotes FoxO1- and FoxO3-induced cell cycle arrest (Brunet et al., 2004) • Inhibits FoxO1- and FoxO3-induced cell death (Brunet et al., 2004)
7) Neurodegenerative disease	• SIRT1 delays axonal degeneration during Wallerian degeneration (Araki et al., 2004) • Protects against Alzheimer's disease, Huntington's disease, and amyotrophic lateral sclerosis (Parker et al., 2005; Qin et al., 2006; Kim et al., 2007; Donmez and Guarente, 2010) • Delays the onset of prion disease (Aguzzi et al., 2007; Chen et al., 2008)
8) Inflammatory signaling in response to environmental stress	• SIRT1 provides protection against chronic inflammation (Schug et al., 2010) • Limits the replicative lifespan in response to chronic genotoxic stress (Chua et al., 2005)
9) Developmental effects	• SIRT1 promotes hormonal efficiency (Cheng et al., 2003; McBurney et al., 2003) • Modulates transcriptional repression (Takata and Ishikawa, 2003)
10) Placental effects	• SIRT1 promotes placental cell survival by increasing resistance to oxygen deficiency (Michan and Sinclair, 2007)

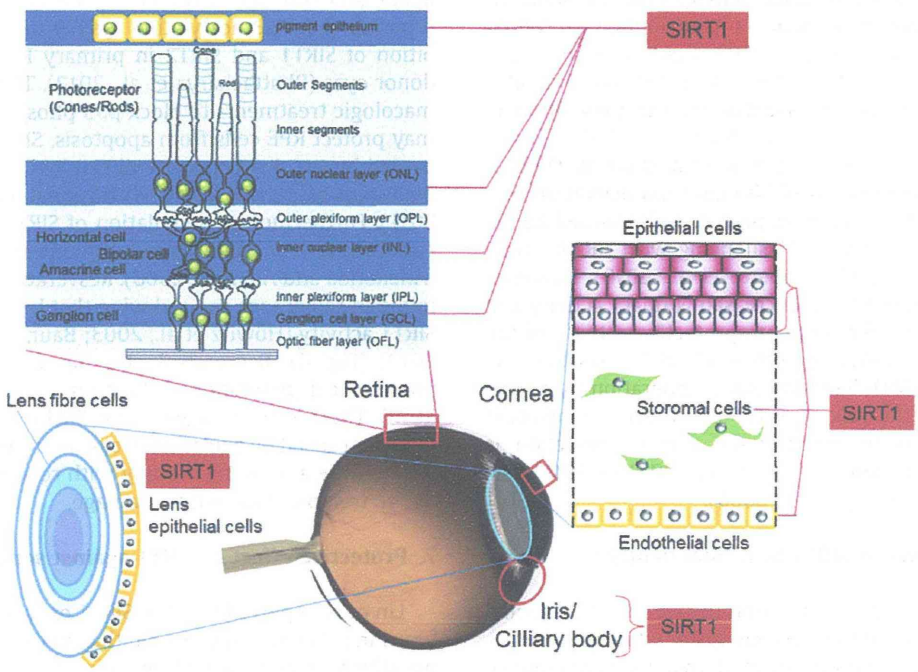

Fig. 3. Localization of SIRT1 in the mouse eye (Jaliffa et al., 2009). SIRT1 is expressed in almost all of the ocular tissues, including the cornea, lens (epithelial cells), iris, ciliary body, and retina. In the cornea, SIRT1 is localized in the corneal epithelial cells, keratocytes, and corneal endothelial cells. In the retina, SIRT1 is found in the retinal pigment epithelium (RPE), outer nuclear layer (ONL), inner nuclear layer (INL), and ganglion cell layer (GCL).