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Glucose-regulated protein 78 in the aqueous humor in diabetic macular edema patients

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Abstract

In this study, we explored the presence and elevation of glucose-regulated protein 78 (GRP78) in aqueous humor of patients with diabetic macular edema (DME).

After comparing DME patients with the controls, we analyzed GRP78 and vascular endothelial growth factor (VEGF) levels in DME patients. We examined factors associated with GRP78 levels in DME patients.

GRP78 was detected in aqueous humor with elevated levels in DME patients. Stepwise backward regression analysis showed that GRP78 levels were associated with the VEGF levels and the duration of diabetes ($P < .001$ and $P = .002$, respectively). However, no statistical significance was observed between GRP78 levels and the decrease in CST following 3 monthly anti-VEGF treatments in univariate regression analysis ($P = .695$).

We showed that GRP78 is elevated in DME patients. In addition, there is a correlation between GRP78 and VEGF levels in aqueous humor. However, GRP78 levels were not associated with the responsiveness of anti-VEGF in DME patients.

Abbreviations: BCVA = the best-corrected visual acuity, CST = central subfield thickness, DM = diabetes mellitus, DME = diabetic macular edema, LISA = enzyme-linked immunosorbent assay, ER = endoplasmic reticulum, GRP78 = glucose-regulated protein 78, IVB = intravitreal bevacizumab, KDEL = lysine-aspartate-glutamate-leucine, logMAR = logarithm of the minimum angle of resolution, NPDR = nonproliferative diabetic retinopathy, OCT = optical coherence tomography, PDR = proliferative diabetic retinopathy, VEGF = vascular endothelial growth factor.

Keywords: diabetic macular edema, GRP78, intravitreal bevacizumab injection, VEGF

1. Introduction

The endoplasmic reticulum (ER) is a eukaryotic cell organelle, which is responsible for the metabolism and synthesis of substances. The rough ER, through binding with ribosomes, synthesizes, and processes proteins. In various physiological and pathological conditions, an accumulation of unfolded or misfolded proteins in the ER is associated with cell survival.[1,2] An abnormal accumulation of these proteins, known as ER stress, can lead to apoptosis or autophagy.[3–6] Under ER stress, ER chaperone proteins are activated to facilitate folding and assembly of proteins, or to export abnormal proteins for degradation.[7]

Among the ER chaperone proteins, glucose-regulated protein 78 (GRP78), a member of the heat-shock protein 70 family, is a widely used ER stress marker protein.[6,8,9] GRP78 has multiple functions, being involved in proper protein folding and assembly, misfolded protein degradation, calcium homeostasis, and control of the activation of transmembrane ER stress sensor proteins.[8,10,11] Most studies with GRP78 have been conducted in poorly perfused solid tumors that induced ER stress because of altered glucose metabolism and microenvironmental factors, such as glucose deprivation, hypoxia, and acidosis.[7,8,10,11] The induction of GRP78 by ER stress leads not only to an increase in GRP78 in the ER but also to relocalization from the ER to the cell surface, cytoplasm, mitochondria, and nucleus.[12–15] Some studies have even reported that exocrine pancreatic cells and oviduct epithelial cells secrete GRP78.[16,17] Although studies on glaucoma have reported elevated GRP78 in trabecular meshwork cells and tissues,[18,19] there have been no reports about the secreted form of GRP78 in the eye. Based on studies showing that diabetic retinopathy also occurs under ER stress conditions,[20–22] we designed this study to investigate the presence of GRP78 in the aqueous humor of patients with diabetic macular edema (DME).

2. Methods

2.1. Study subjects

This study characterized GRP78 levels in the aqueous humor, and analyzed the association between GRP78 and vascular endothelial growth factor (VEGF) levels in the aqueous humor, of naïve DME patients with type II diabetes mellitus (DM). The study adhered to the tenets of the Declaration of Helsinki, and the protocol was approved by the Institutional Review/Ethics Board of the Catholic University of Korea. All methods were performed in accordance with the relevant guidelines and regulations by the protocol. All participants gave written informed consent to the use of their clinical records.

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Table 1

Demographics and baseline clinical characteristics of the study participants.

	DME group (n = 103)	Control group (n = 14)	*P* value
Age, years	55.41 ± 8.54	66.29 ± 11.98	<.001
Sex (male: female)	51:52	7:7	1.000
Axial length, mm	23.43 ± 0.80	23.44 ± 0.72	.982
VEGF, pg/mL	72.51 ± 69.43	22.29 ± 25.15	.001
GRP78, ng/mL	3.81 ± 3.62	0.06 ± 0.19	<.001
Duration of DM, years	10.97 ± 7.16		
HbA1c (%)	7.50 ± 1.04		
DR stage (NPDR: PDR)	36:67		

DME = diabetic macular edema, DR = diabetic retinopathy, GRP78 = glucose-regulated protein of 78 kDa, HbA1c = glycated hemoglobin, IOP = intraocular pressure, NPDR = nonproliferative DR, PDR = proliferative DR, VEGF = vascular endothelial growth factor.

We enrolled eyes with a central subfield thickness (CST) > 300 μm due to DME; eyes with cataract without DM were the controls. The aqueous humors of 103 naïve DME patients with type II DM and 14 cataract patients were analyzed. Exclusion criteria included eyes with glaucoma, retinal degeneration, and macular edema due to other causes, such as epiretinal membrane, medication, or vitreomacular traction. Eyes with concurrent diseases, such as retinal vascular occlusion, as well as eyes with a history of ocular trauma, uveitis, or prior intraocular surgery that could influence the enzyme levels of the aqueous humor, were also excluded.

We measured glycated hemoglobin levels, and all patients underwent a full ophthalmic examination that included measurement of the best-corrected visual acuity (BCVA), a dilated fundus examination, and classification of the eye according to the Early Treatment of Diabetic Retinopathy Study as mild nonproliferative diabetic retinopathy (NPDR), moderate NPDR, severe NPDR, or proliferative diabetic retinopathy. Macular thickness was measured using optical coherence tomography (OCT; Cirrus High-Definition OCT; Carl Zeiss Meditec, Dublin, CA). Axial length was measured using an IOL Master instrument (Carl Zeiss Meditec). We evaluated changes in the CST and BCVA at the 1 month visit after 3 consecutive monthly intravitreal bevacizumab (IVB) injections.[23]

2.2. Quantitation of GRP78 in the aqueous humor

The volume of the collected aqueous humor was at least 85 μL. Levels of GRP78 protein were determined using 1:1 diluted aqueous humor samples and a human GRP78 enzyme-linked immunosorbent assay (ELISA) kit (Enzo Life Sciences, Lausen, Switzerland) with a detection range of 1.4 to 4500 ng/mL. The VEGF levels were determined using 1:2 diluted samples and a human VEGF ELISA kit (DuoSet ELISA; R&D Systems, Minneapolis, MN) with a detection range of 31.20 to 2000 pg/mL. The assays were performed according to the manufacturers' instructions.

2.3. Statistical analyses

The study group patients were compared with the control group using the nonparametric Mann–Whitney *U* test and the chi-squared test. Univariate and stepwise backward regression analyses were used to identify associations of GRP78 levels and VEGF levels with age, axial length, BCVA (logMAR), and CST at the initial visit, and changes in BCVA and CST after 3 consecutive IVBs. Statistical analyses were performed using SPSS for Windows statistical software (ver. 20.0; SPSS Inc., Chicago, IL) and R software (ver. 3.2.3 [2015-2-0, Platform: x86_64-redhat-linux-gnu, R Core Team (2015)]. R is a language and environment for statistical computing (R Foundation for Statistical Computing, Vienna, Austria; https://www.R-project.org/).

3. Results

The average age of the cataract patients was 66.29 ± 11.98 years, and that of the DME patients was 55.41 ± 8.54 years ($P < .001$). There were 51 males and 52 females in the study group and 7 males and 7 females in the control group. There was no significant difference in axial length between the study and control groups. GRP78 and VEGF levels were significantly elevated in the DME group ($P < .001$ and $P = .001$, respectively; Table 1).

In univariate analyses, GRP78 levels correlated with VEGF levels and the duration of DM ($P < .001$ and $P = .005$, respectively; Table 2; Fig. 1A and B) Stepwise backward regression analysis also showed a statistically significant relationship between GRP78 levels and VEGF levels and duration of DM ($P < .001$and $P = .002$, respectively; Table 2). VEGF levels correlated with GRP78 levels in stepwise backward regression analysis ($P < .001$; Table 3). However, in a further regression analysis with the same parameters, initial CST was not associated with GRP78 or VEGF levels ($P = .956$ and $P = .984$, respectively);

Table 2

Variables associated with level of GRP78 in aqueous humor of DME patients in univariate and stepwise backward regression analyses.

	Univariate analysis†		Stepwise regression analysis†	
	ß ± SE	*P* value	ß ± SE	*P* value
Axial length, mm	0.243 ± 0.448	.588		
DR stage (mild NPDR~PDR)	0.237 ± 0.431	.584		
DM duration, years	0.139 ± 0.048	.005	0.144 ± 0.046	.002
HbA1c (%)	0.262 ± 0.347	.453		
Preoperative BCVA (logMAR)	−0.717 ± 1.351	.597		
Change of BCVA (logMAR)	−1.546 ± 2.109	.465		
Preoperative CST, μm	0.002 ± 0.004	.956		
Reduction of CST, μm	−0.002 ± 0.004	.695		
VEGF level, pg/mL	0.018 ± 0.005	<.001	0.019 ± 0.005	<.001

BCVA = best-corrected visual acuity, CST = central subfield thickness, DME = diabetic macular edema, DR = Diabetic retinopathy, GRP78 = glucose-regulated protein of 78 kDa, HbA1c = glycated hemoglobin, IOP = intraocular pressure, NPDR = nonproliferative DR, PDR = proliferative DR, VEGF = Vascular endothelial growth factor.

† Adjusted by age and sex.

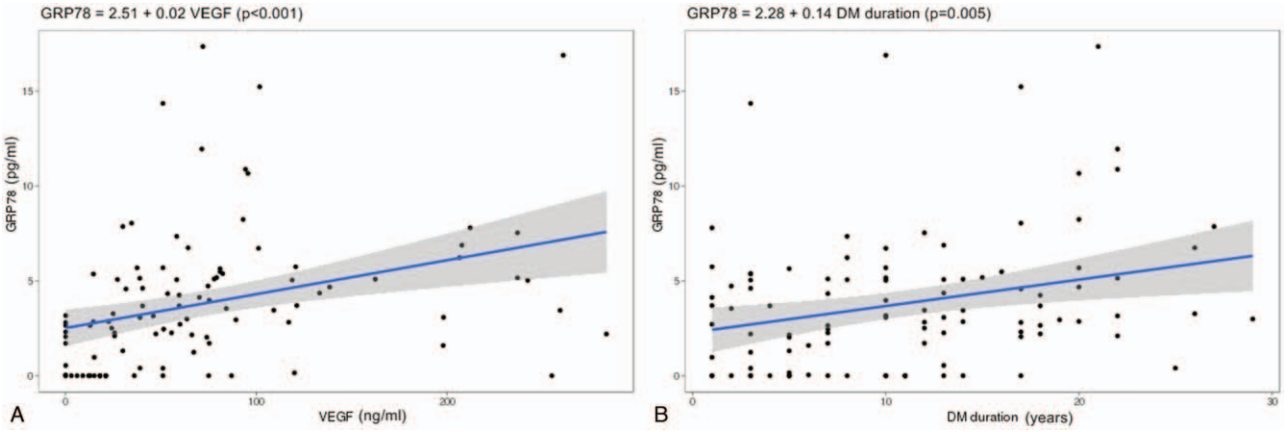

Figure 1. (A) Scatter plot showing the correlation between GRP78 and vascular endothelial growth factor (VEGF) levels in the aqueous humor. (B) Scatter plot showing the correlation between GRP78 levels and duration of diabetes mellitus in univariate regression analyses. Both correlations were statistically significant ($P < .001$ and $P = .005$, respectively). GRP78 = glucose-regulated protein of 78 kDa, VEGF = vascular endothelial growth factor

the initial BCVA was also not associated with the level of either of these proteins ($P = .597$ and $P = .883$, respectively).

4. Discussion

ER stress occurs during inflammation, ischemia, and tumor conditions, and is associated with cell survival.[1,2] The early pathogenesis of DME is usually characterized by damage to the inner blood-retinal barrier due to hypoxia and inflammation.[24,25] We therefore designed this study to explore the possible correlation between ER stress and DME, by determining the level of GRP78 in the aqueous humor of DME patients.

GRP78 has several functions in the ER, including acting as a chaperone under non-stress conditions.[26,27] Under excessive ER stress or pathological conditions, GRP78 can translocate to the cell surface or be secreted.[27] GRP78 at the cell surface controls cellular proliferation, apoptosis, and/or immune functions.[27–29] The secreted form of GRP78 is not as well known as other forms. All chaperone proteins have a lysine-aspartate-glutamate-leucine (KDEL) motif at the C terminus, functioning as an ER retention signal. When KDEL receptors are saturated or defective, GRP78 is thought to be secreted.[6] Although the secreted form of GRP78 has been mainly studied in cancer research, some studies have shown its presence in the oviduct or pancreatic exocrine samples.[16,17,30] Recent studies have even reported that blood GRP78 levels are associated with metabolism in the polycystic ovary, obesity and physical activity.[30,31] Secreted GRP78 may therefore be involved in biological processes in both pathological and physiological conditions.[6] We showed that GRP78 was also present in the aqueous humor, and that it was elevated in DME patients.

An association between ocular disease and ER stress has been reported in several studies. Studies of glaucoma patients reported that GRP78 levels were elevated in trabecular meshwork cells and tissues.[18,19] Other studies reported that the unfolded protein response occurred in retinal vascular diseases, and that VEGF levels correlated with ER stress.[22,32–35] However, because investigation with human retinal tissue is very difficult, most studies involved *in vitro* samples or animal models. We therefore characterized ER stress in the aqueous humor, which reflects the retinal status and is easily obtained during ocular surgery.[36–38] Our multivariate regression results showed that GRP78 levels in the aqueous humor are positively correlated with VEGF levels and the duration of diabetes significantly (Table 2). Similarly, aqueous VEGF levels were associated with GRP 78 levels (Table 3). Some other studies have reported that GRP78 levels were correlated with the degree of neovascularization, as well as with elevated VEGF levels.[39–41] These results all together suggest that ER stress and angiogenesis be associated.

Table 3

Variables associated with level of VEGF in aqueous humor of DME patients in univariate and stepwise backward regression analyses.

	Univariate analysis*		Stepwise regression analysis*	
	ß ± SE	*P* value	ß ± SE	*P* value
Axial length, mm	−1.796 ± 8.591	.835		
DMR stage (mild NPDR~PDR)	15.973 ± 8.115	.052	13.839 ± 7.688	.075
DM duration, years	−1.024 ± 0.959	.288		
HbA1c (%)	1.250 ± 6.667	.852		
Preoperative BCVA (logMAR)	3.824 ± 25.925	.994		
Change of BCVA (logMAR)	40.435 ± 40.306	.318		
Preoperative CST, μm	−0.002 ± 0.075	.984		
Reduction of CST, μm	−0.100 ± 0.074	.178	−0.081 ± 0.069	.241
GRP78 level, ng/mL	6.558 ± 1.791	<.001	6.302 ± 1.770	<.001

BCVA = best-corrected visual acuity, CST = central subfield thickness, DME = diabetic macular edema, DR = diabetic retinopathy, GRP78 = glucose-regulated protein of 78 kDa, HbA1c = glycated hemoglobin, IOP = intraocular pressure, NPDR = nonproliferative DR, PDR = proliferative DR, VEGF = vascular endothelial growth factor.

* Adjusted by age and sex.

There were some limitations to this study. First, although the mean GRP78 level in the aqueous humor was significantly higher in the study group, the sample size was relatively small to investigate the functional and anatomical relationships with GRP78 levels. Second, there was insufficient sample volume to determine the possible presence of inflammatory cytokines, such as interleukins and adhesion molecules, or of other growth factors that act as biomarkers for DME.[36] In future studies, we plan to characterize vitreous samples using other techniques, such as the Luminex x MAP suspension array technology (Luminex Corp., Austin, TX). Third, we measured only GRP78 levels in the aqueous humor, and did not determine which cells or tissues are involved in GRP78 secretion. As mentioned previously, human ocular tissue is difficult to obtain, so our future studies will involve animal models or *in vitro* experiments. Fourth, breakdown of blood retinal or aqueous barrier could lead to elevation of overall protein concentration in aqueous humor. Therefore, normalization of GRP78 concentration to total protein concentration in aqueous humor needs, and this normalized data should be compared with GRP78 concentration from blood samples.

In conclusion, this study is the first to reveal increased GRP78 levels in DME patients. And the GRP 78 levels in aqueous had positive correlations with the aqueous VEGF levels and duration of diabetes. However, this protein levels showed no association with the degree of responsiveness to anti-VEGF therapy. Future studies should aim to explore the role and origin of this ER chaperone in the aqueous humor.

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References

[1] Logue SE, Cleary P, Saveljeva S, et al. New directions in ER stress-induced cell death. Apoptosis 2013;18:537–46.
[2] Raciti M, Lotti LV, Valia S, et al. JNK2 is activated during ER stress and promotes cell survival. Cell Death Dis 2012;3:e429.
[3] Deegan S, Saveljeva S, Logue SE, et al. Deficiency in the mitochondrial apoptotic pathway reveals the toxic potential of autophagy under ER stress conditions. Autophagy 2014;10:1921–36.
[4] Rashid HO, Yadav RK, Kim HR, et al. ER stress: autophagy induction, inhibition and selection. Autophagy 2015;11:1956–77.
[5] Song S, Tan J, Miao Y, et al. Crosstalk of autophagy and apoptosis: involvement of the dual role of autophagy under ER stress. J Cell Physiol 2017;232:2977–84.
[6] Ni M, Zhang Y, Lee AS. Beyond the endoplasmic reticulum: atypical GRP78 in cell viability, signalling and therapeutic targeting. Biochem J 2011;434:181–8.
[7] Lee AS. GRP78 induction in cancer: therapeutic and prognostic implications. Cancer Res 2007;67:3496–9.
[8] Li Z, Li Z. Glucose regulated protein 78: a critical link between tumor microenvironment and cancer hallmarks. Biochim Biophys Acta 2012;1826:13–22.
[9] Wang J, Lee J, Liem D, et al. HSPA5 Gene encoding Hsp70 chaperone BiP in the endoplasmic reticulum. Gene 2017;618:14–23.
[10] Lee AS. The glucose-regulated proteins: stress induction and clinical applications. Trends Biochem Sci 2001;26:504–10.
[11] Li X, Zhang K, Li Z. Unfolded protein response in cancer: the physician's perspective. J Hematol Oncol 2011;4:8.
[12] Zhang Y, Tseng CC, Tsai YL, et al. Cancer cells resistant to therapy promote cell surface relocalization of GRP78 which complexes with PI3K and enhances PI(3,4,5)P3 production. PLoS One 2013;8:e80071.
[13] Ni M, Zhou H, Wey S, et al. Regulation of PERK signaling and leukemic cell survival by a novel cytosolic isoform of the UPR regulator GRP78/BiP. PLoS One 2009;4:e6868.
[14] Sun FC, Wei S, Li CW, et al. Localization of GRP78 to mitochondria under the unfolded protein response. Biochem J 2006;396:31–9.
[15] Reddy RK, Mao C, Baumeister P, et al. Endoplasmic reticulum chaperone protein GRP78 protects cells from apoptosis induced by topoisomerase inhibitors: role of ATP binding site in suppression of caspase-7 activation. J Biol Chem 2003;278:20915–24.
[16] Takemoto H, Yoshimori T, Yamamoto A, et al. Heavy chain binding protein (BiP/GRP78) and endoplasmin are exported from the endoplasmic reticulum in rat exocrine pancreatic cells, similar to protein disulfide-isomerase. Arch Biochem Biophys 1992;296:129–36.
[17] Marin-Briggiler CI, Gonzalez-Echeverria MF, Munuce MJ, et al. Glucose-regulated protein 78 (Grp78/BiP) is secreted by human oviduct epithelial cells and the recombinant protein modulates sperm-zona pellucida binding. Fertil Steril 2010;93:1574–84.
[18] Peters JC, Bhattacharya S, Clark AF, et al. Increased endoplasmic reticulum stress in human glaucomatous trabecular meshwork cells and tissues. Invest Ophthalmol Vis Sci 2015;56:3860–8.
[19] Kasetti RB, Phan TN, Millar JC, et al. Expression of mutant myocilin induces abnormal intracellular accumulation of selected extracellular matrix proteins in the trabecular meshwork. Invest Ophthalmol Vis Sci 2016;57:6058–69.
[20] Zhong Y, Li J, Chen Y, et al. Activation of endoplasmic reticulum stress by hyperglycemia is essential for Muller cell-derived inflammatory cytokine production in diabetes. Diabetes 2012;61:492–504.
[21] Chen Y, Wang JJ, Li J, et al. Activating transcription factor 4 mediates hyperglycaemia-induced endothelial inflammation and retinal vascular leakage through activation of STAT3 in a mouse model of type 1 diabetes. Diabetologia 2012;55:2533–45.
[22] Zhang SX, Ma JH, Bhatta M, et al. The unfolded protein response in retinal vascular diseases: implications and therapeutic potential beyond protein folding. Prog Retin Eye Res 2015;45:111–31.
[23] Sugimoto M, Ichio A, Nunome T, et al. Two year result of intravitreal bevacizumab for diabetic macular edema using treat and extend protocol. Medicine (Baltimore) 2017;96:e6406.
[24] Das A, McGuire PG, Rangasamy S. Diabetic macular edema: pathophysiology and novel therapeutic targets. Ophthalmology 2015;122:1375–94.
[25] Tang J, Kern TS. Inflammation in diabetic retinopathy. Prog Retin Eye Res 2011;30:343–58.
[26] Wadhwa R, Taira K, Kaul SC. An Hsp70 family chaperone, mortalin/mthsp70/PBP74/Grp75: what, when, and where? Cell Stress Chaperones 2002;7:309–16.
[27] Lee AS. Glucose-regulated proteins in cancer: molecular mechanisms and therapeutic potential. Nat Rev Cancer 2014;14:263–76.
[28] Takahashi H, Wang JP, Zheng HC, et al. Overexpression of GRP78 and GRP94 is involved in colorectal carcinogenesis. Histol Histopathol 2011;26:663–71.
[29] Thornton M, Aslam MA, Tweedle EM, et al. The unfolded protein response regulator GRP78 is a novel predictive biomarker in colorectal cancer. Int J Cancer 2013;133:1408–18.
[30] Khadir A, Kavalakatt S, Abubaker J, et al. Physical exercise alleviates ER stress in obese humans through reduction in the expression and release of GRP78 chaperone. Metabolism 2016;65:1409–20.
[31] Banuls C, Rovira-Llopis S, Martinez de Maranon A, et al. Metabolic syndrome enhances endoplasmic reticulum, oxidative stress and leukocyte-endothelium interactions in PCOS. Metabolism 2017;71:153–62.

[32] Nashine S, Liu Y, Kim BJ, et al. Role of C/EBP homologous protein in retinal ganglion cell death after ischemia/reperfusion injury. Invest Ophthalmol Vis Sci 2014;56:221–31.
[33] Miyagi H, Kanemoto S, Saito A, et al. Transcriptional regulation of VEGFA by the endoplasmic reticulum stress transducer OASIS in ARPE-19 cells. PLoS One 2013;8:e55155.
[34] Ma JH, Wang JJ, Zhang SX. The unfolded protein response and diabetic retinopathy. J Diabetes Res 2014;2014:160140.
[35] Pollreisz A, Afonyushkin T, Oskolkova OV, et al. Retinal pigment epithelium cells produce VEGF in response to oxidized phospholipids through mechanisms involving ATF4 and protein kinase CK2. Exp Eye Res 2013;116:177–84.
[36] Jung SH, Kim KA, Sohn SW, et al. Association of aqueous humor cytokines with the development of retinal ischemia and recurrent macular edema in retinal vein occlusion. Invest Ophthalmol Vis Sci 2014;55:2290–6.
[37] Cheung CM, Vania M, Ang M, et al. Comparison of aqueous humor cytokine and chemokine levels in diabetic patients with and without retinopathy. Mol Vis 2012;18:830–7.
[38] Kwon JW, Choi JA, Jee D. Matrix metalloproteinase-1 and matrix metalloproteinase-9 in the aqueous humor of diabetic macular edema patients. PLoS One 2016;11:e0159720.
[39] Yoo SA, You S, Yoon HJ, et al. A novel pathogenic role of the ER chaperone GRP78/BiP in rheumatoid arthritis. J Exp Med 2012;209:871–86.
[40] Roybal CN, Marmorstein LY, Vander Jagt DL, et al. Aberrant accumulation of fibulin-3 in the endoplasmic reticulum leads to activation of the unfolded protein response and VEGF expression. Invest Ophthalmol Vis Sci 2005;46:3973–9.
[41] Matsui A, Kaneko H, Kachi S, et al. Expression of vascular endothelial growth factor by retinal pigment epithelial cells induced by amyloid-beta is depressed by an endoplasmic reticulum stress inhibitor. Ophthalmic Res 2015;55:37–44.