Differential expression of connective tissue growth factor and hepatocyte growth factor in the vitreous of patients with high myopia versus vitreomacular interface disease

Xinyi Ding[1,3†], Rong Zhang[2,3†], Shujie Zhang[2,3], Hong Zhuang[1,3*] and Gezhi Xu[1,3]

Abstract

Background: To determine the levels of connective tissue growth factor (CTGF) and hepatocyte growth factor (HGF) in the vitreous of patients with high myopia, in comparison with those with a vitreomacular interface disease (VMID).

Methods: Patients with either high myopia (high myopia group) or a VMID (VMID group) were included in this study. Each of the two groups were further subdivided into two subgroups: group A (high myopia with macular hole), group B (high myopia with macular retinoschisis), group C (idiopathic macular hole), and group D (idiopathic epiretinal membrane). Vitreal specimens were collected during vitrectomy, and enzyme-linked immunosorbent assay was used to quantitatively measure the CTGF and HGF levels in the vitreous.

Results: The average axial length was markedly longer in the high myopia group than in the VMID group. The vitreal CTGF level was significantly higher in the high myopia group than in the VMID group. Subgroup analysis revealed significantly higher vitreal CTGF in group A than in the other three subgroups. The vitreal HGF level was not significantly different between the high myopia and VMID groups, but was significantly higher in group D than in group C in the subgroup analysis. Correlation analysis showed that the vitreal CTGF level was positively correlated with the axial length.

Conclusions: The vitreal CTGF level is elevated in highly myopic eyes and may be related to the pathogenesis of high myopia, whereas increased expression of HGF may be involved in the development of idiopathic epiretinal membrane.

Keywords: Connective tissue growth factor, Hepatocyte growth factor, High myopia, Vitreomacular interface disease

Background

Myopia is prevalent worldwide, particularly in East Asian countries such as China [1–3]. According to an epidemiological study, the incidence of high myopia is increasing.[2] High myopia is characterized by progressive elongation of the eyeball, scleral thinning, and formation of posterior staphyloma. The posterior segment complications in high myopia, which include retinal detachment, choroidal neovascularization, macular retinoschisis, and macular hole, are leading causes of irreversible vision loss [4, 5].

Myopia is caused by remodeling of the scleral extracellular matrix. Animal studies indicate that cytokines and proteinases are involved in the scleral remodeling processes during the development of myopia. Previous studies have focused mainly on transforming growth factor (TGF)-β and matrix metalloproteinases (MMPs) [6–8]. We previously measured TGF-β2 and MMP-2 levels in the vitreous of patients with high myopia and

* Correspondence: zhuang_hong1008@126.com
†Xinyi Ding and Rong Zhang contributed equally to this work.
[1]Department of Ophthalmology, Eye and ENT Hospital of Fudan University, 83 Fenyang Road, Shanghai 200031, China
[3]Key Laboratory of Visual Impairment and Restoration of Shanghai and Key Laboratory of Myopia of State Health Ministry, Fudan University, Shanghai, China
Full list of author information is available at the end of the article

Ding *et al. BMC Ophthalmology* (2019) 19:25

Page 2 of 5

found that the MMP-2 level was elevated and strongly correlated with the TGF-β2 level in high myopia [9].

Other cytokines may also be involved in the development of myopia. Connective tissue growth factor (CTGF) is expressed by fibroblasts in various tissues and is involved in the synthesis of the extracellular matrix [10, 11]. Hepatocyte growth factor (HGF) upregulates the expression of MMP-2, which results in degradation of extracellular matrix [12]. Expression of HGF was increased in the scleral tissue of a myopic animal model [13]. However, the expression of CTGF and HGF has not been evaluated in human myopic eyes.

Therefore, we investigated the changes in CTGF and HGF levels in the vitreous of patients with high myopia. As a comparison, we also evaluated these vitreal cytokines in patients with the vitreomacular interface diseases (VMIDs) of idiopathic macular hole and idiopathic epiretinal membrane.

Methods

Subjects

We performed an observational study of patients with either high myopia (high myopia group) or a VMID (VMID group) who underwent vitrectomy surgery at the Eye and ENT Hospital of Fudan University, China. The present study was conducted in accordance with the principles of the Declaration of Helsinki and was approved by the Ethics Committee of the Eye and ENT Hospital of Fudan University.

High myopia was defined as a spherical equivalent ≤ − 6.0 diopter and axial length ≥ 26 mm. A total of 33 eyes of 33 patients with high myopia who received vitrectomy for macular hole or macular retinoschisis were enrolled in this study. Among these eyes, 19 had macular hole and 14 had macular retinoschisis. Exclusion criteria included those high myopic patients with choroidal neovascularization, proliferative vitreoretinopathy, and any history of laser treatment or ocular surgery.

As a comparison, patients with VMID were also enrolled in this study. A total of 33 eyes of 33 patients with VMID were evaluated: 18 eyes with idiopathic macular hole and 15 with idiopathic epiretinal membrane. None of these eyes had high myopia or any history of ocular trauma or intraocular inflammation.

All patients received a comprehensive ocular examination, including an ophthalmoscopic examination, A- and B-scan ultrasonography, and optical coherence tomography. Axial length was assessed by A-scan ultrasonography and posterior staphyloma by B-scan ultrasonography in each patient with high myopia. The diagnoses of macular retinoschisis, macular hole, and epiretinal membrane were made using optical coherence tomography.

Samples of undiluted vitreous fluid (300–500 μl) were collected in sterile tubes at the time of vitrectomy before intraocular infusion and then immediately stored at − 80 °C until assayed.

Measurement of CTGF and HGF levels

Enzyme-linked immunosorbent assay kits were used to measure the concentration of CTGF (Assay Biotech, Losangeles, USA) and HGF (R&D Systems, Minneapolis, USA) according to each manufacturer's protocol. In brief, the samples were added to the wells of a 96-well plate coated with a monoclonal antibody and incubated for 2 h, after which the plate was washed and an enzyme-labeled antibody added. After further incubation, the plate was washed again, and the substrate was added. Color development was terminated using the stop solution provided in the kits, and the optical density was read at 450 nm and 630 nm using a multimode microplate reader (Synergy H1, BioTek Instruments, USA). The 450 nm readings corrected by the 630 nm readings were used for evaluation. A standard curve was generated by measuring the optical densities of serially diluted protein solutions of known concentrations. The concentrations of CTGF and HGF in the vitreous samples were calculated based on the standard curve.

Statistical analysis

Statistical analysis was performed using Stata 11.0 statistical software (Stata Corporation, College Station, TX, USA). Differences between the high myopia and VMID groups were analyzed using the Wilcoxon rank-sum test. Each of the two groups (high myopia and VMID groups) were further subdivided into two subgroups: group A (high myopia with macular hole), group B (high myopia with macular retinoschisis), group C (idiopathic macular hole), and group D (idiopathic epiretinal membrane). Differences among the four subgroups were analyzed using the Kruskal–Wallis test. The correlations between two parameters were analyzed using Spearman's rank correlation coefficient. A two-tailed P value < 0.05 was considered statistically significant.

Results

Patient characteristics

The high myopia group included 24 females and 9 males, and the VMID group included 25 females and 8 males. Thus, both groups comprised mainly females, and there was no difference in sex distribution between the two groups. The average patient age was significantly lower in the high myopia group (55.3 ± 9.9 years) than in the VMID group (61.8 ± 5.6 years; $P < 0.01$). The average axial length was markedly longer in the high myopia group (28.3 ± 1.7 mm) than in the VMID group

Table 1 Patient characteristics and vitreal cytokine concentrations in four subgroups

Parameter	Group A	Group B	Group C	Group D
Gender (female/male)	12/7	12/2	14/4	11/4
Age (years)	54.4 ± 10.6	56.6 ± 9.2	61.2 ± 5.0	62.5 ± 6.3
Axial length (mm)	28.0 ± 1.6	28.7 ± 1.8	23.3 ± 0.7	23.0 ± 1.2
CTGF (pg/ml)	247.6 ± 89.6	182.8 ± 50.2	172.7 ± 48.2	167.7 ± 48.5
HGF (ng/ml)	16.3 ± 7.3	17.8 ± 12.9	12.2 ± 5.5	18.7 ± 4.8

Group A (high myopia with macular hole), Group B (high myopia with macular retinoschisis), Group C (idiopathic macular hole), and Group D (idiopathic epiretinal membrane)

$(23.2 \pm 1.0 \, \mathrm{mm}, \ P < 0.0001)$. The subgroup analysis of axial length showed no difference between groups A and B or between groups C and D. The patient characteristics and vitreal cytokine concentrations in the four subgroups are listed in Table 1.

Vitreal levels of CTGF and HGF

The vitreal level of CTGF was significantly higher in the high myopia group $(220.1 \pm 81.3 \, \mathrm{pg/ml})$ than in the VMID group $(170.4 \pm 47.7 \, \mathrm{pg/ml}, \ P < 0.01)$; in the subgroup analysis, vitreal CTGF was significantly higher in group A than in the other three subgroups (Fig. 1a). There was no significant difference in the vitreal level of HGF between the high myopia $(17.0 \pm 9.9 \, \mathrm{ng/ml})$ and VMID $(15.2 \pm 6.1 \, \mathrm{ng/ml})$ groups. However, the subgroup analysis showed a significantly higher HGF level in group D than in group C $(P < 0.05)$ (Fig. 1b).

In the correlation analyses, the CTGF level showed no correlation with age but a significant positive correlation with axial length (Spearman's rho = 0.41, $P < 0.01$). The level of HGF was not significantly correlated with age or axial length.

Discussion

Myopia is a highly prevalent eye disease, and its pathogenesis is a focus of ophthalmology research. Previous studies have shown that TGF-β and MMPs play important roles in the pathogenesis of myopia [6–9]. Alterations in TGF-β2 expression is associated with scleral remodeling in myopia development [14]. MMP-2 upregulation was shown to precede myopia development [15], whereas reduced expression of the tissue inhibitor of metalloproteinase was associated with myopia development [16]. But, the associations of CTGF and HGF with myopia are still unclear.

In this study, we explored the expression levels of CTGF and HGF in the vitreous humor of highly myopic eyes. Patients with high myopia complicated with macular hole or macular retinoschisis were included in this study because vitreous specimens could be collected from these patients during vitrectomy. Importantly, we had to exclude certain conditions that potentially disrupt the blood–retinal barrier, such as choroidal neovascularization, proliferative vitreoretinopathy, and any history of laser treatment or ocular surgery. Patients with idiopathic
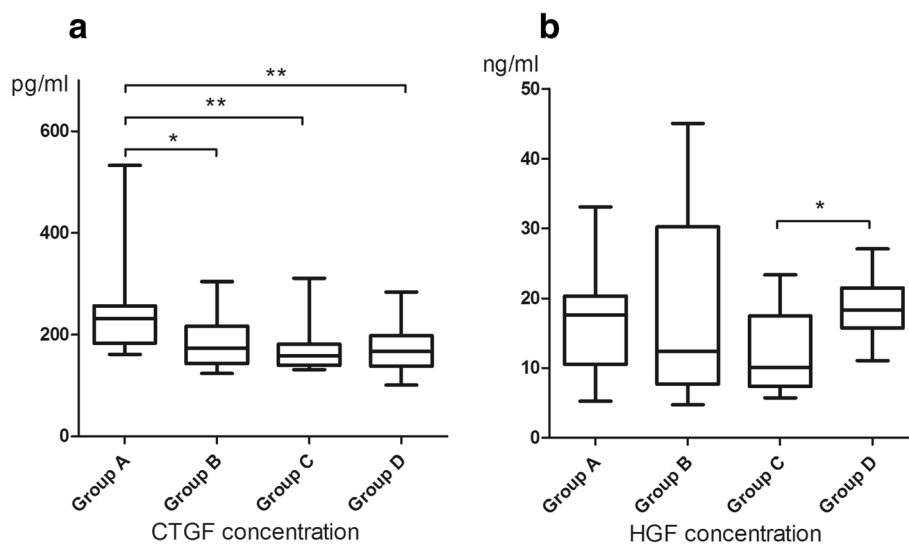

Fig. 1 Comparative analysis of vitreal cytokine levels in four subgroups. **a.** The vitreal level of CTGF; **b.** The vitreal level of HGF. (* $P < 0.05$, ** $P < 0.01$)

Ding *et al. BMC Ophthalmology*   (2019) 19:25

Page 4 of 5

macular hole or idiopathic epiretinal membrane were selected as the control group because these VMIDs involve relatively few factors that interfere with components of the vitreous. Furthermore, vitreous specimens are easily obtained from patients with idiopathic macular hole and idiopathic epiretinal membrane, which are two of the most commonly diagnosed VMIDs. None of these eyes with VMIDs had high myopia. This study confirmed that the axial length was significantly shorter in the VMID than in the high myopia group.

CTGF is a key player in the regulation of extracellular matrix remodeling, fibrosis, and angiogenesis. CTGF is widely expressed in various ocular tissues such as the cornea, trabecular, choroid, and sclera [17]. Previous studies have found that CTGF is associated with corneal wound healing, proliferative diabetic retinopathy, and choroidal neovascularization [18–20]. This study showed, for the first time, that the CTGF level was increased in the vitreous humor of eyes with high myopia, especially in eyes with high myopia complicated with macular hole. This finding suggests that the cytokine CTGF is likely involved in remodeling of the scleral extracellular matrix in myopia and in the pathogenesis of myopic retinopathy. Regarding the potential underlying mechanisms, CTGF expression is regulated by TGF-β [21] and has been correlated with MMP levels in some disease models [22–24]. CTGF regulates matrix production via the ERK (p42/p44 MAPK) and p38 MAPK signaling pathways in certain scenarios [25]. The specific role and the mechanism of CTGF in myopia development requires further study.

HGF was first recognized as a factor that stimulates the proliferation of hepatocytes. It was found soon thereafter that HGF also acts on epithelial cells, vascular endothelial cells, and fibroblasts. HGF has multiple functions in the regulation of wound healing, and angiogenesis [26, 27]. In the present study, the HGF level in the vitreous was measured, for the first time, in highly myopic eyes, but no significant difference was found compared with those without high myopia.

Subgroup analysis unexpectedly showed that the vitreal HGF level was significantly higher in eyes with idiopathic epiretinal membrane than in those with idiopathic macular hole. Iannetti et al. reported elevated levels of TGF-β2 and nerve growth factor in the vitreous humor of patients with idiopathic epiretinal membrane [28]. Zandi et al. revealed differences in vitreal cytokine profiles between eyes with epiretinal membrane and those with macular hole [29]. Our findings also suggested that vitreal cytokine expression differs between epiretinal membrane and macular hole. HGF may be produced in the eye by retinal cells such as Müller cells [26], and it has been demonstrated to induce proliferation and migration of retinal pigment epithelial (RPE) cells [30, 31]. Previous studies found that HGF is associated with proliferative diabetic

retinopathy and proliferative vitreoretinopathy [32, 33]. Similarly, HGF-induced Müller cell activation and RPE cell migration may contribute to the formation of epiretinal membrane. Thus, we speculate that HGF is involved in the pathogenesis of idiopathic epiretinal membrane.

Conclusion

The CTGF level in the vitreous differs between eyes with and without high myopia, and increased CTGF expression may be related to the pathogenesis of high myopia. In addition, increased expression of HGF may be involved in the development of idiopathic epiretinal membrane.

Abbreviations
CTGF: connective tissue growth factor; HGF: hepatocyte growth factor; VMID: vitreomacular interface disease; TGF: transforming growth factor; MMP: matrix metalloproteinase

Availability of data and materials
The data used in the current study is available from the corresponding author (Hong Zhuang) upon request.

Authors' contributions
XD and RZ conducted the research and drafted the manuscript. SZ and HZ analyzed and interpreted the data. HZ and GX conceived and designed the study, and also made critical revision to the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate
The present study was conducted in accordance with the principles of the Declaration of Helsinki and was approved by the Ethics Committee of the Eye and ENT Hospital of Fudan University. Written informed consent was obtained from each patient.

Consent for publication
Not applicable

Competing interests
The authors declare that they have no competing interest.

Publisher's Note
Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Author details
[1]Department of Ophthalmology, Eye and ENT Hospital of Fudan University, 83 Fenyang Road, Shanghai 200031, China. [2]Eye Institute, Eye and ENT Hospital of Fudan University, Shanghai, China. [3]Key Laboratory of Visual Impairment and Restoration of Shanghai and Key Laboratory of Myopia of State Health Ministry, Fudan University, Shanghai, China.

References
1. Chen M, Wu A, Zhang L, Wang W, Chen X, Yu X, Wang K. The increasing prevalence of myopia and high myopia among high school students in

Ding et al. BMC Ophthalmology   (2019) 19:25

Page 5 of 5

Fenghua city, eastern China: a 15-year population-based survey. BMC Ophthalmol. 2018;18(1):159.

2. Holden BA, Fricke TR, Wilson DA, Jong M, Naidoo KS, Sankaridurg P, Wong TY, Naduvilath TJ, Resnikoff S. Global prevalence of myopia and high myopia and temporal trends from 2000 through 2050. Ophthalmology. 2016;123(5):1036–42.

3. Qin Y, Zhu M, Qu X, Xu G, Yu Y, Witt RE, Wang W. Regional macular light sensitivity changes in myopic Chinese adults: an MP1 study. Invest Ophthalmol Vis Sci. 2010;51(9):4451–7.

4. Crim N, Esposito E, Monti R, Correa LJ, Serra HM, Urrets-Zavalia JA. Myopia as a risk factor for subsequent retinal tears in the course of a symptomatic posterior vitreous detachment. BMC Ophthalmol. 2017;17(1):226.

5. Xu L, Li Y, Wang S, Wang Y, Wang Y, Jonas JB. Characteristics of highly myopic eyes: the Beijing eye study. Ophthalmology. 2007;114(1):121–6.

6. Jobling AI, Nguyen M, Gentle A, McBrien NA. Isoform-specific changes in scleral transforming growth factor-beta expression and the regulation of collagen synthesis during myopia progression. J Biol Chem. 2004; 279(18):18121–6.

7. Jobling AI, Wan R, Gentle A, Bui BV, McBrien NA. Retinal and choroidal TGF-beta in the tree shrew model of myopia: isoform expression, activation and effects on function. Exp Eye Res. 2009;88(3):458–66.

8. Siegwart JT Jr, Norton TT. Selective regulation of MMP and TIMP mRNA levels in tree shrew sclera during minus lens compensation and recovery. Invest Ophthalmol Vis Sci. 2005;46(10):3484–92.

9. Zhuang H, Zhang R, Shu Q, Jiang R, Chang Q, Huang X, Jiang C, Xu G. Changes of TGF-beta2, MMP-2, and TIMP-2 levels in the vitreous of patients with high myopia. Graefes Arch Clin Exp Ophthalmol. 2014;252(11):1763–7.

10. Grotendorst GR. Connective tissue growth factor: a mediator of TGF-beta action on fibroblasts. Cytokine Growth Factor Rev. 1997;8(3):171–9.

11. Heng NH, Zahlten J, Cordes V, Ong MM, Goh BT, N'Guessan PD, Pischon N. Effects of enamel matrix derivative and transforming growth factor-beta1 on connective tissue growth factor in human periodontal ligament fibroblasts. J Periodontol. 2015;86(4):569–77.

12. Cui Q, Wang Z, Jiang D, Qu L, Guo J, Li Z. HGF inhibits TGF-beta1-induced myofibroblast differentiation and ECM deposition via MMP-2 in Achilles tendon in rat. Eur J Appl Physiol. 2011;111(7):1457–63.

13. Li XJ, Yang XP, Wan GM, Wang YY, Zhang JS. Expression of hepatocyte growth factor and its receptor c-met in lens-induced myopia in Guinea pigs. Chin Med J. 2013;126(23):4524–7.

14. Chen BY, Wang CY, Chen WY, Ma JX. Altered TGF-beta2 and bFGF expression in scleral desmocytes from an experimentally-induced myopia guinea pig model. Graefes Arch Clin Exp Ophthalmol. 2013;251(4):1133–44.

15. Zhao F, Zhou Q, Reinach PS, Yang J, Ma L, Wang X, Wen Y, Srinivasalu N, Qu J, Zhou X. Cause and effect relationship between changes in scleral matrix Metallopeptidase-2 expression and myopia development in mice. Am J Pathol. 2018;188(8):1754–67.

16. Liu HH, Kenning MS, Jobling AI, McBrien NA, Gentle A. Reduced scleral TIMP-2 expression is associated with myopia development: TIMP-2 supplementation stabilizes scleral biomarkers of myopia and limits myopia development. Invest Ophthalmol Vis Sci. 2017;58(4):1971–81.

17. van Setten GB, Trost A, Schrödl F, Kaser-Eichberger A, Bogner B, M v S, Heindl LM, Grabner G, Reitsamer HA. Immunohistochemical detection of CTGF in the human eye. Curr Eye Res. 2016;41(12):1571–9.

18. Gibson DJ, Pi L, Sriram S, Mao C, Petersen BE, Scott EW, Leask A, Schultz GS. Conditional knockout of CTGF affects corneal wound healing. Invest Ophthalmol Vis Sci. 2014;55(4):2062–70.

19. Hinton DR, Spee C, He S, Weitz S, Usinger W, LaBree L, Oliver N, Lim JI. Accumulation of NH2-terminal fragment of connective tissue growth factor in the vitreous of patients with proliferative diabetic retinopathy. Diabetes Care. 2004;27(3):758–64.

20. Watanabe D, Takagi H, Suzuma K, Oh H, Ohashi H, Honda Y. Expression of connective tissue growth factor and its potential role in choroidal neovascularization. Retina. 2005;25(7):911–8.

21. Liu Y, Liu H, Meyer C, Li J, Nadalin S, Königsrainer A, Weng H, Dooley S, ten Dijke P. Transforming growth factor-beta (TGF-beta)-mediated connective tissue growth factor (CTGF) expression in hepatic stellate cells requires Stat3 signaling activation. J Biol Chem. 2013;288(42):30708–19.

22. Lin SC, Chou HC, Chiang BL, Chen CM. CTGF upregulation correlates with MMP-9 level in airway remodeling in a murine model of asthma. Arch Med Sci. 2017;13(3):670–6.

23. Muromachi K, Kamio N, Narita T, Annen-Kamio M, Sugiya H, Matsushima K. MMP-3 provokes CTGF/CCN2 production independently of protease activity and dependently on dynamin-related endocytosis, which contributes to human dental pulp cell migration. J Cell Biochem. 2012;113(4):1348–58.

24. Tan TW, Lai CH, Huang CY, Yang WH, Chen HT, Hsu HC, Fong YC, Tang CH. CTGF enhances migration and MMP-13 up-regulation via alphavbeta3 integrin, FAK, ERK, and NF-kappaB-dependent pathway in human chondrosarcoma cells. J Cell Biochem. 2009;107(2):345–56.

25. Nagai N, Klimava A, Lee WH, Izumi-Nagai K, Handa JT. CTGF is increased in basal deposits and regulates matrix production through the ERK (p42/p44mapk) MAPK and the p38 MAPK signaling pathways. Invest Ophthalmol Vis Sci. 2009;50(4):1903–10.

26. Nishimura M, Ushiyama M, Nanbu A, Yoshimura M. Neovascularization and HGF: neovascularization in proliferative diabetic retinopathy and intraocular HGF. Rinsho Byori. 1998;46(11):1156–61.

27. Colombo ES, Menicucci G, McGuire PG, Das A. Hepatocyte growth factor/scatter factor promotes retinal angiogenesis through increased urokinase expression. Invest Ophthalmol Vis Sci. 2007;48(4):1793–800.

28. Iannetti L, Accorinti M, Malagola R, Bozzoni-Pantaleoni F, Da Dalt S, Nicoletti F, Gradini R, Traficante A, Campanella M, Pivetti-Pezzi P. Role of the intravitreal growth factors in the pathogenesis of idiopathic epiretinal membrane. Invest Ophthalmol Vis Sci. 2011;52(8):5786–9.

29. Zandi S, Tappeiner C, Pfister IB, Despont A, Rieben R, Garweg JG. Vitreal cytokine profile differences between eyes with Epiretinal membranes or macular holes. Invest Ophthalmol Vis Sci. 2016;57(14):6320–6.

30. Wang L, Dong F, Reinach PS, He D, Zhao X, Chen X, Hu DN, Yan D. MicroRNA-182 suppresses HGF/SF-induced increases in retinal pigment epithelial cell proliferation and migration through targeting c-met. PLoS One. 2016;11(12):e0167684.

31. Liou GI, Matragoon S, Samuel S, Behzadian MA, Tsai NT, Gu X, Roon P, Hunt DM, Hunt RC, Caldwell RB, et al. MAP kinase and beta-catenin signaling in HGF induced RPE migration. Mol Vis. 2002;8:483–93.

32. Katsura Y, Okano T, Noritake M, Kosano H, Nishigori H, Kado S, Matsuoka T. Hepatocyte growth factor in vitreous fluid of patients with proliferative diabetic retinopathy and other retinal disorders. Diabetes Care. 1998;21(10):1759–63.

33. Mitamura Y, Takeuchi S, Matsuda A, Tagawa Y, Mizue Y, Nishihira J. Hepatocyte growth factor levels in the vitreous of patients with proliferative vitreoretinopathy. Am J Ophthalmol. 2000;129(5):678–80.