·Basic Research·

Expression of subretinal fluid hepatocyte growth factor and proliferative vitreoretinopathy

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Abstract

• AIM: To explore the role that hepatocyte growth factor (HGF) plays in proliferative vitreoretinopathy (PVR) after retinal detachment(RD).

• METHODS: The contents of HGF in subretinal fluid (SRF) in 49 cases with RD were measured with enzyme-linked immunosorbent assay.

• RESULTS: With the worsening of PVR and vitreous opacity and prolonging of disease course, the contents of HGF increased , $P<0.05$ was considered statistically significant.

• CONCLUSION: The change of HGF in SRF had a close relationship with the occurrence and development of PVR after RD.

• KEYWORDS: hepatocyte growth factor; enzyme-linked immunosorbent assay; proliferative vitreoretinopathy

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INTRODUCTION

Retinal detachment (RD) is a clinically common disease that severely affects the vision, while proliferative vitreoretinopathy (PVR) is a common complication of RD and the cause of operation failure, and its cause is still unclear. Some people hold the idea that body fluid has a close relationship with the occurrence and development of PVR. Hepatocyte growth factor (HGF), a kind of multifunctional cytokine, has vast biologic functions. Its influences on the health and illness of eyes are gradually made known and become a study focus in the field of ophthalmology. By measuring the contents of HGF in subretinal fluid (SRF), we try to find out whether it gets involved in the occurrence and development of PVR.

MATERIALS AND METHODS

Materials Forty-nine cases with RD in one eye but without choroid detachment and the other normal eye were selected, among whom 26 were male and the other 23 were female, aging from 22 to 61 (mean 42±16.73) years, with course of disease ranging from 5 days to 36 months. In the light of the vision, the dimension and number of foramen, the scope of detachment and degree of PVR of each case, the PVR was classified into five grades: Grade A, B, C_{1-2}, C_3, and D, which are in accordance with the norms set by the Naming Commission of World Retinal Society. According to the vitreous opacity, they were classified into three groups: Light, Mild and Severe Group. Furthermore, they were categorized into five groups according to the period of course: Group within 1 month, 1-3, 3-6, 6-12 months and over 12 months. Each group was the control group of the others.

Collection of SRF SRF of operated patients was extracted at random, and the part of the sclera from which fluid would come out was fully exposed during the operation. Bleeding being stopped completely, with thorough hemostasis, the operated eye was washed. The surface of sclera was dried with cotton ball, and the part of the sclera was cut open from which fluid would come out. The choroid was impaled to let the fluid out, and then SRF was extracted from the fluid hole with 1mL injection syringe with slanting needlepoint. The blood-polluted part was removed and the sample was stored at -20℃ frozen for later measurement.

Methods The concentration of HGF was measured with ELISA (R&D, American) supplied by Shenzhen Jingmei BioTech Co. Ltd. The coefficient of variation within the batch was 5.4% and that between batches was 7.1%.

Statistical Analysis The data were analyzed with SASS8. 12 statistical software and the comparison between groups was made with analysis of variance. The relations among

lesion degrees, course of disease and HGF were analyzed with Spearman rank correlation.

RESULTS

The results of HGF in each group of SRF with different degrees of PVR (Table 1): Comparisons were made between Groups ① and ②, between ③, ② and ④, and between ④ and ⑤ (P >0.05), the difference was not obvious. Comparisons were also made between Group ④ and ⑤, Group ① and ② and Group ⑤ and ③ (P <0.05), the difference was statistically significant. As PVR became severe, the HGF increased. The correlation coefficient r between the two was 0.64, which was statistically significant.

The results of HGF in each group of SRF with different degrees of turbidity of vitreum were as follows (Table 2): Group ① was compared with Group ② and ③, and Group ② with Group ③ (P<0.05), the difference was significant. With the turbidity of vitreum becoming severe, the contents of HGF increased. The correlation coefficient r between the two was 0.61, which was statistically significant.

The results of HGF in each group of SRF with different courses were as follows (Table 3): Group ① was compared with Group ②, Group ③ with Group ④ and ⑤, and Group ④ with Group ⑤ (P>0.05), the difference was insignificant; Groups ③ and ④ were compared respectively with Group ① and ②, and Group ⑤ with Group ①, ② and ③, (P< 0.05), the difference was statistically significant. With the prolonging of courses, the contents of HGF increased. The correlation coefficient r between the two was 0.58, which was statistically significant.

Table 1 HGF contents of SRF in different groups of PVR

(mean ± SD)

Group order	Grade	Number of cases	HGF	Group order				
				1	2	3	4	5
1	A	11	7.21 ± 4.07					
2	B	10	8.04 ± 4.65					
3	C_{1-2}	9	12.35 ± 6.41	a				
4	C_3	9		a	a			
5	Above D	10	14.87 ± 6.26	a	a	a		
			19.62 ± 7.23					

HGF：hepatocyte growth factor

Table 2 HGF contents of SRF with different degrees of turbidity of vitreum (mean ± SD)

Group order	Degree of turbidity	Number of cases	HGF	Group order		
				1	2	3
1	Light	17	7.33 ± 4.11			
2	Mild	16	12.72 ± 6.37	a		
3	Sever	16	19.46 ± 7.39	a	a	

HGF：hepatocyte growth factor

Table 3 HGF contents of SRF in groups with different courses

(mean ± SD)

Group order	Course	Case No.	HGF	Group order				
				1	2	3	4	5
1	Within 1mo	11	7.27 ± 4.10					
2	1-3mo	10	8.35 ± 4.56					
3	3-6mo	11	13.49 ± 6.41	a	a			
4	6-12mo	9	15.78 ± 6.33	a	a			
5	Over 1a	8	20.62 ± 7.35	a	a	a		

HGF：hepatocyte growth factor

DISCUSSION

There has been PVR in the whole process of RD and the forming of PVR was the main cause for the failure of operations. Experiments and clinical studies confirmed that cellular proliferation and contraction on the surface of retina was the basic pathological process of PVR, and pigment epithelial cell played an important role in the occurrence and development of PVR, for it was not only the main cell causing proliferation membrane to form and contract, but also producing chemokine, attracting fibroglia cell and fibroblast to participate in the formation of proliferation membrane[1].

Hepatocyte growth factor (HGF)[2,3] is the growth factor with multi-effect originating from mesenchymal and has powerful function of causing cleavage and tissue constitution, inducing transition and raiding of epithelial cells, and evoking vascularization. Combined with its specific receptor c-Met, HGF caused a series of enzymatic reaction of signal transduction protein and corresponding biologic effects. HGF played an important role in the occurrence and development of many tissue organs, plerosis of trauma, and adjusting the function of endothelial cells and inducing the formation of blood vessels. It is also an important regulator for the growth and differentiation of eye cells that can stimulate the growth and transmigration of corneal, endothelial, iris, retinal pigment epithelial, lens epithelial and trabecula cells.

Experimental studies confirm that blood endothelial cells, fibroblast, neuroglial and RPE cells can produce and release HGF and RPE has been found to have c-Met[4, 5] (the HGF receptor) while RPE cells are the key constitutional part of PVR fiber proliferative membranes. Among patients with PVR, when the layer of retina nerve fiber was separated from the RPE layer, cells in RPE proliferated and migrated. Experiments *in vitro* reported that the form of RPE cells

tended to turn into that of fibroblast. It was estimated that HGF and its receptor c-Met might, in early PVR pathogenesis, activate RPE cells and cause the change of RPE form and movement, and the move of RPE cells would create some factors after active mitosis, including HGF [6]. Researches in the last two years show that culturing human RPE cells can express HGF receptors that, when stimulated by HGF, become tyrosine phosphoric acidulation and, as mitogen and chemokine, cause RPE to proliferate and migrate [3, 4]. The expression of HGF receptors increased remarkably when cells were stimulated by inflammatory factors. HGF, when combined with its receptors, could help the infiltration of monocytes and convert into macrophag-ocyte [7]. We can thus suppose that HGF could play an important role in PVR pathogenesis.

Examination of SRF of the 49 RD patients showed that, with the development of PVR pathologic process, the content of HGF in SRF increased gradually (P<0.05). RD caused impairment to the blood-retina barrier and the strengthening of RPE cells' reaction to growth factor, which would enhance the effusion from blood vessels in the eye on one hand increase the endogenous and ectogenous growth factor in SRF, improve the chemotectic ability and the activity of mitosis, and on the other hand the impairment to blood-retina barrier would expand the interspace in the vascular endothelial cells to let infected cells come into SRF. It was found that with PVR worsening, the content of HGF in the SRF of patients increased significantly and the degree of PVR and turbidity of vitreum had medium-and-high positive correlation with the content of HGF, indicating that HGF had a close relation ship with the occurrence and development of PVR. In addition, this experiment also indicated that with the prolonging of the disease course, the expression of HGF in SRF increased greatly, showing a positive correlation with the course. Some scholars think that, except for the endogenous HGF resulting from self-secretion of the eye tissue, the prolonging of the disease course would worsen endlessly the damage of the blood-retina barrier, making it easier for HGF to permeate directly into the vitreum and SRF, and also for inflammatory cells to come directly into eyes and release HGF locally [8], which can be one of the causes for the high density of SRF. Therefore, the content of HGF in SRF increased with the development of PVR and the prolonging of the disease course.

The above study showed that the density of HGF in retina had an up-going expression with the development of the pathological process of hyperplastic vitreous retina, indicating that HGF has a close relationship with the occurrence and development of PVR after the RD. The study also further investigated the causes of disease for PVR to occur after RD, which is significant for further study on how to block up or alleviate the function of HGF and how to hinder or reduce the occurrence and development of PVR.

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