Human Hepatocyte Growth Factor (HGF) in the Aqueous Humor

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Abstract: Using an enzyme-linked immunosorbent assay, we measured the concentration of hepatocyte growth factor (HGF) in human aqueous humor and serum from 36 eyes of 33 patients and analyzed the relationship between HGF and several parameters of corneal endothelial cells. Aqueous HGF concentrations ranged from 0.020-0.194 ng/mL (average: 0.101 ± 0.044 ng/mL) and was correlated positively with corneal endothelial cell density ($r = 0.343, P = 0.04$). The aqueous HGF level did not correlate with other corneal endothelial cell parameters or the serum HGF level. The HGF receptor, c-Met, was found in the corneal endothelial cell membranes. This study suggests that the aqueous humor HGF is a factor which maintains an integrity of corneal endothelial cells. Jpn J Ophthalmol 1997;41:409-413 © 1997 Japanese Ophthalmological Society

Key Words: Aqueous humor, c-MET, cell density, corneal endothelial cell, hepatocyte growth factor.

Introduction

Human endothelial cells exhibit a limited potential for proliferation in vivo and in vitro.1-3 Once they are severely impaired, a cascade of events can lead to reduced corneal transparency and eventual bullous keratopathy. Given this poor prognosis, it would be helpful to discover some of the factors influencing the ability to maintain the integrity of the corneal endothelial cells.

Newly recognized as a growth factor, hepatocyte growth factor (HGF) has several important functions as a mitogen, motogen, and morphogen and as a tumor suppressor and a scatter factor. Numerous target cells are responsive to HGF.4-6 Wilson et al7 reported that epithelial, stromal, and endothelial layers of human cornea possess the HGF messenger, RNA, and the HGF receptor, c-Met. He also noted that exogenous HGF stimulated proliferation of corneal epithelial and endothelial cells in vitro in a dose-dependent manner. Wilson concluded that HGF may contribute to corneal preservation and wound healing in both a paracrine and an autocrine fashion. Since some growth factors (basic fibroblast growth factor [bFGF], 23-25 platelet-derived growth factor,26 endothelin-1,25 and epidermal growth factor [EGF]27) have previously been found to have mitogenic and motogenic effects on corneal endothelial cells, HGF might possess similar characteristics.

We speculated that the aqueous humor, which continuously bathes the endothelial cells, may contain HGF and could be involved in maintaining the integrity of endothelial function. In this study, we first identified HGF in the aqueous humor and then examined its relationship to corneal endothelial cells in vivo.

Materials and Methods

This study was conducted in compliance with the recommendations of the World Medical Association Declaration of Helsinki; permission was also re-
ceived from the Medical Ethics Committee of Kinki Central Hospital. Written informed consent was obtained from all subjects.

Patients in this study ranged in age from 60-90 years (average: 74.25); there were 13 men and 20 women. Samples of aqueous humor and serum were taken from 36 eyes of the 33 healthy subjects who had no significant medical conditions or eye disease except cataract. Just prior to cataract extraction, aqueous humor specimens were obtained through the limbus, using a 26-gauge needle. Serum samples were obtained at the same time.

The human HGF (hHGF) concentrations were determined using an hHGF assay kit (Otsuka Pharmaceutical, Tokyo): 15-μL aliquots of standard hHGF, or samples, were placed into 96 wells coated with an anti-hHGF mouse monoclonal antibody, followed by 0.05 mL of 10 mM sodium phosphate buffer (pH = 7.4) containing 0.2% (wt/vol) BSA, 2 M NaCl, 0.2% (wt/vol) CHAPS, and 0.1% (vol/vol) Tween 20 (Nippon Bio-Rad, Tokyo). The plate was incubated for 1 hour, washed three times, and a 100-μL aliquot of anti-hHGF polyclonal antibody diluted to 1:1000 with sodium phosphate buffer was added to each well. The plate was incubated again for 1 hour. After being washed three times, 0.1 mL goat (anti-rabbit IgG) IgG-peroxidase conjugates were reacted for 1 hour, and the plate was again washed. Next, 0.1 mL of 0.25% 0-phenylenediamine was added to each well and allowed to stand for 10 minutes. The reaction was stopped by addition of 0.1 mL of 1N H2SO4. Absorbance was read at 492 nm by an automatic plate reader (E-max, Molecular Devices Co. Ltd., Menlo Park, CA, USA) with a reference wavelength of 690 nm and the lowest detection level limit of 0.010 ng/mL.

Before cataract extraction, the central corneal endothelial cells were photographed with a contact specular microscope (SP-580, Konan Medical Co. Ltd., Hyogo, Japan). The photographs were traced and analyzed by a computer-assisted color digitizer (Sun Contact, Kyoto, Japan). Parameters including endothelial cell density, coefficient of variation for cell density, and hexagonality of endothelial cells were determined. The aqueous HGF concentration was compared with these parameters using paired t-tests.

A human cornea was prepared from an eye-bank eye of a 58-year-old woman; the whole cornea was frozen and immediately embedded in OCT compound (Tissue-Tek, Miles, Elkhart, IN, USA); 10 μm sections were prepared and studied immunohistochemically. Anti-MET rabbit polyclonal antibody was purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). For negative controls, incubation with an irrelevant rabbit polyclonal antibody of the same IgG subclass was used instead of the primary antibody. The remnant of corneal tissue from an eye-bank eye after transplantation was used for this experiment. This work is considered to be indispensable for understanding corneal cell biology and will provide useful information for clinical treatment.

**Results**

Aqueous humor HGF concentrations ranged from 0.020–0.194 ng/mL (average 0.101 ± 0.044 ng/mL). HGF concentration was significantly correlated with corneal endothelial cell density (simple regression analysis, r = 0.343, P = 0.04) (Figure 1). Figure 2 shows representative corneal endothelial cells. Cells exposed to higher concentrations of HGF (Figure 2A) were well regulated, and the cell density was higher than in those endothelial cells exposed to lower concentrations of HGF (Figure 2B). There was no correlation between HGF concentration and the coefficient of variation or the incidence of hexagonal cells. Aging also had no effect on concentrations of aqueous HGF. Although the average serum concentration of HGF was slightly higher (0.162 ± 0.069 ng/mL) than in the aqueous humor (0.101 ± 0.044 ng/mL), there was no significant relationship.

Immunopositive staining of anti-MET antibody was seen in normal corneal epithelial and endothelial cells. The immunopositive structures were chiefly lo-

![Figure 1](image-url)  
**Figure 1.** The correlation of aqueous humor hepatocyte growth factor (HGF) concentration and endothelial cell density is statistically significant at P < 0.05. The horizontal axis indicates the concentration of HGF (ng/mL) in aqueous humor that was sampled at the time of cataract extraction, and the vertical axis indicates cell density of corneal endothelium (cells/mm²).
Figure 2. Representative presentations of specular microscopic photographs of corneal endothelial cells analyzed by a color digitizer. Note that cells exposed to higher amounts of hepatocyte growth factor (HGF) (A: 0.137 ng/mL) were well regulated and smaller (blue; cell density = 4005.52 cells/mm²), whereas cells exposed to lower amounts of HGF (B: 0.028 ng/mL) were larger (yellow; cell density = 2781.29 cells/mm²). Bar = 100 μm.

calized in the cell membranes (Figure 3). There was no positive staining in the negative controls.

Discussion

This study is the first demonstration that human aqueous humor contains HGF in a steady state and that the HGF is correlated to the corneal endothelial cell density but not to the coefficient of variation or to the incidence of hexagonal cells. We found concentrations ranging from 0.020–0.194 ng/mL. Other investigators have reported other growth factors in the human aqueous humor. Parelman et al. reported finding 0.5–1.4 ng/mL EGF in 1990; Tripathi et al. in 1991, found no EGF but did find bFGF at 0.48–1.44 ng/mL. Namiki et al. found neither EGF nor bFGF in normal aqueous humor but did detect them at 1 ng/mL in some pathological states.
The low levels of HGF detected in this study might dramatically increase in some pathological conditions.

The origin of the HGF in the aqueous humor has not been identified; it is not, however, from the serum. There was no relationship between serum and aqueous humor levels. Also, the blood-aqueous barrier was apparently intact in our subjects, since they were free of any significant eye disease. Therefore, the HGF in the aqueous humor may be derived from cells of the anterior chamber.

Also unclear is the nature of the correlation of HGF level and high endothelial cell density: Which is the cause and which the effect? Previous studies could imply that HGF causes the increased density in vivo. Exogenous HGF induced endothelial cell proliferation in the cornea and umbilical cord vein in vitro; Wilson et al reported, using polymerase chain reaction of mRNA, that HGF is produced by stromal rather than endothelial cells. The present study indicates that HGF influences the ability to maintain the integrity of corneal endothelial cells via the c-MET receptor we identified on these cells. Future studies must also examine the aqueous humor HGF of bullous keratopathy and other endothelial disturbances.

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References


