Overexpression of Hepatocyte Growth Factor and Its Receptor c-Met in Nasal Polyps

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Objectives: To investigate the expression and distribution of hepatocyte growth factor (HGF) and c-Met proteins in normal nasal mucosa and in nasal polyps and to evaluate the possible effects of HGF and c-Met on the development of nasal polyps.

Design: Prospective study.

Setting: Tertiary academic institution.

Patients: Normal inferior turbinate mucosa was obtained from 20 patients undergoing surgery for augmentation rhinoplasty. Nasal polyps were obtained from 20 patients undergoing endoscopic sinus surgery for chronic polypoid sinusitis.

Interventions: Semiquantitative reverse-transcriptase polymerase chain reaction, immunohistochemistry, and Western blot analysis were performed.

Main Outcome Measures: The expression and distribution of HGF and c-Met were analyzed.

Results: Using immunohistochemistry, moderate to high levels of HGF were mainly localized in submucosal glands of nasal polyps, while c-Met was detected in submucosal glands and in epithelial cells. In normal turbinate mucosa, immunopositive HGF was detected in submucosal glands, where faint staining was found, while c-Met was noted in epithelial cells and in submucosal glands. Semiquantitative reverse-transcriptase polymerase chain reaction and Western blot analysis showed that HGF expression was increased in nasal polyps compared with that in normal turbinate mucosa. The same result was observed for c-Met.

Conclusion: Elevated c-Met expression in combination with expression of HGF in nasal polyps may enhance the proliferation of epithelial cells and submucosal glandular cells through the release of HGF, which activates c-Met receptors in nasal polyps.

Figure 1. Gene expression of hepatocyte growth factor (HGF) and c-Met analyzed using semiquantitative reverse-transcriptase polymerase chain reaction. A, Hepatocyte growth factor and c-Met messenger RNA were detected in inferior turbinate mucosa and in nasal polyps. B, The bands were quantified using densitometric scanning, and the relative amount of each gene was calculated by dividing by the internal control, GAPDH indicates glyceraldehyde-3-phosphate dehydrogenase, P and unshaded bars indicate polyp; T and shaded bars, turbinate; and asterisk, P < .05.

**METHODS**

Tissue from normal inferior turbinate was obtained from 20 patients (13 men and 7 women [age range, 20-35 years]) undergoing surgery for augmentation rhinoplasty. All of these subjects were free of nasal symptoms at the time of investigation, and rhinoscopy revealed no anatomical abnormalities or signs of mucosal injury. Nasal polyps were obtained from 20 patients (17 men and 3 women [age range, 35-50 years]) undergoing endoscopic sinus surgery for chronic polyposid sinusitis. None of these patients had a history of nasal allergy, asthma, or aspirin sensitivity. Before the tissue specimens were obtained, the protocols and informed consent form were approved by the institutional review boards for human research at our institution.

Tissue specimens (15 turbinate and 15 polyp) were frozen in liquid nitrogen and were stored at -70°C for total RNA and protein isolation. For immunohistochemistry, other samples (5 turbinate and 5 polyp) were immersed overnight in a freshly prepared fixative containing 4% paraformaldehyde in a phosphate-buffered saline solution, pH 7.4. These samples were then dehydrated in a graded series of ethanol to xylene and were embedded in paraffin wax.

**TOTAL RNA ISOLATION AND REVERSE-TRANSCRIPTASE POLYMERASE CHAIN REACTION**

The frozen tissues were homogenized in TRIzol reagent (GIBCO BRL, Grand Island, NY), and equal amounts of total RNA (1 pg) from each sample were reverse transcribed in 20 µL of a reaction mixture containing 2.5 U of M-MLV reverse transcriptase (GIBCO BRL) and 50pM random hexanucleotide at 42°C for 60 minutes. RNA integrity and the success of the reverse-transcriptase reaction were monitored by polymerase chain reaction (PCR) amplification of glyceraldehyde-3-phosphate dehydrogenase gene (GAPDH) transcripts. Negative control samples consisted of omission of the reverse-transcriptase enzyme from the complement DNA (cDNA) synthesis for each sample. The following primers were used (at 37°C annealing temperature): HGF (168 base pair [bp]) (sense: 5'-CTGGTTCCCTCCCTCAATAGCA-3' and antisense: 5'-CTCCAGGGCGTACATTGTAG-3'), c-Met (201 bp) (sense: 5'-CAGGCCAGTCCGATGTAGT-3' and antisense: 5'-GATGATTCCCTCGTGCAAG-3'), and GAPDH (502 bp) (sense: 5'-ATCTTTCCAGAGGAGGATCC-3' and antisense: 5'-ACACCTGACAGTGGAGCAG-3'). Amplified PCR products were resolved in 2% agarose gels, stained with ethidium bromide, and photographed under UV light. The identity of each PCR product was confirmed by sequencing and was found to be identical with the messenger RNA (mRNA) sequence of each gene as deposited in the National Center for Biotechnology Information database (http://www.ncbi.nlm.nih.gov/Entrez).

Semiquantitative PCR was performed on the samples to assess for differential expression of HGF and c-Met mRNA in normal nasal mucosa and in nasal polyp tissues. The optimum number of PCR cycles for each cDNA species was determined by plotting the PCR product yield of different cycles on a semilogarithmic graph, and the number of cycles representing the exponential amplification was chosen for the final amplification. For quantification of the PCR products of all samples, the expected bands were analyzed using densitometry, and data were expressed as the ratio of HGF or c-Met cDNA to the corresponding GAPDH cDNA. Data are presented as mean ± SD. The statistical significance of differences between groups was evaluated using the t test, and the level of significance was set at P < .05.

**IMMUNOHISTOCHEMICAL AND WESTERN BLOT ANALYSES**

Immunohistochemical staining was performed using an avidin-biotin-peroxidase technique. Briefly, tissue sections were deparaffinized, rehydrated, and treated with 3% hydrogen peroxide in methanol for 10 minutes to quench endogenous peroxidase activity. After washing in a 10 mM phosphate-buffered saline solution (pH 7.4), sections were incubated with 10% normal goat serum to block nonspecific binding. Sections were then incubated overnight at 4°C with a 1:20 dilution of anti-HGF (Santa Cruz Biotechnology, Inc, Santa Cruz, Calif) or a 1:100 dilution of anti-c-Met (Santa Cruz Biotechnology, Inc, primary polyclonal rabbit antibodies. After washing in a phosphate-buffered saline solution, sections were treated with biotinylated goat anti-rabbit IgG and subsequently with an avidin-biotin-peroxidase conjugate (Vector Laboratories, Burlingame, Calif). To visualize the peroxidase activity, 0.02% diaminobenzidine hydrochloride containing 0.03% hydrogen peroxide was used as a chromogen. Negative immunohistochemical control procedures included omission of the primary antibodies and replacement of the primary antibodies by normal rabbit IgG in appropriate concentrations.

For Western blot analysis, samples frozen in liquid nitrogen were crushed into pieces and were vigorously vortexed in a buffer solution containing 50 mM Tris hydrochloride (pH 8.0), 150 mM sodium chloride, 1% Triton X-100, and 1 mM phenylmethylsulfonyl fluoride. Fifty micrograms of extracted protein was resuspended in sodium dodecyl sulfate sample buffer and boiled for 5 minutes. Equal amounts of total protein were separated on 12% sodium dodecyl sulfate–polyacrylamide gels and were transferred to Immobilon, a polyvinylidene fluoride membrane (Millipore Corporation, Bedford, Mass). The blots were reacted with the anti-HGF or anti-c-Met antibody in a Tris-buffered saline solution (20 mM Tris hydrochloride [pH 8.0], 150 mM sodium chloride, 0.05% Triton X-100, and 5% skim milk) for 2 hours at 4°C, and were then reacted with a biotin-conjugated anti–rabbit IgG antibody (Vector Laboratories) in a phosphate-buffered saline solution for 1 hour. After several washings with Tris-buffered saline, the membranes were incubated with the avidin-biotin-peroxidase complex. Antibody reactions were detected using the ECL (enhanced chemiluminescence) detection kit (Amersham Bioscience, Chalfont St Giles, England), followed by detection of chemiluminescence on x-ray film. As an internal control, β-actin expression was ana-
lyzed in parallel blots using the β-actin antibody (Sigma-Aldrich Inc, St Louis, Mo). Intensity of the detected bands was quantified using Scion Image Beta 4.0.2 software (Scion Corporation, Frederick, Md). Relative intensities of HGF and c-Met signals were obtained by dividing intensities of HGF and c-Met signals by those of β-actin signals. The statistical significance of differences was evaluated using the t test.

RESULTS

Reverse-transcriptase PCR assays were performed to detect the presence of human HGF and c-Met mRNA in human inferior turbinate mucosa and nasal polyps. As shown in Figure 1A, PCR products of the expected sizes corresponding to 168 bp for HGF and to 201 bp for c-Met were detected in human inferior turbinate mucosa and nasal polyps and showed 100% sequence homology with the published sequences. These results indicate that human nasal mucosa and nasal polyps express HGF and c-Met mRNA. The relative abundances of HGF and c-Met genes with respect to GAPDH were significantly increased in nasal polyps compared with those in normal turbinate mucosa (Figure 1B) (P<.05).

In normal turbinate mucosa, immunopositive HGF was detected in submucosal glands, while faint staining was found (Figure 2A). However, c-Met immunostaining was noted in epithelial cells and in submucosal glands...
of normal turbinate mucosa (Figure 2C and E). In nasal polyps, HGF with moderate and high intensity was also localized in glandular epithelium, while c-Met was localized in surface epithelium and in glandular epithelium (Figure 2B, D, and F). In general, immunoreactivity for HGF was seen in cytoplasmic compartments in normal turbinate mucosa and in nasal polyps. c-Met staining was also mainly cytoplasmic. However, nuclear staining was frequently found.

Western blot analysis of normal turbinate mucosa and of nasal polyps showed the presence of the 69-kDa α-chain of HGF and the 145-kDa β-chain transmembrane/intracellular subunit of c-Met. Hepatocyte growth factor expression was increased in nasal polyps compared with that in normal turbinate mucosa (Figure 3) (P<.05). The same result was observed for c-Met.

**COMMENT**

In the present study, we characterized the expression and distribution of HGF and c-Met in human nasal mucosa and nasal polyps. At the protein levels, HGF and c-Met were expressed in nasal polyps and in normal turbinate mucosa. This is the first study, to the best of our knowledge, to demonstrate the expression of HGF and c-Met in normal nasal mucosa and in nasal polyps. Immunohistochemical examinations revealed that the expression of HGF was mainly localized in submucosal glands of normal nasal mucosa and nasal polyps, while its receptor c-Met was observed in submucosal glands and in epithelial layers. Western blot analysis showed that the expression levels of HGF and c-Met in nasal polyps are increased compared with those in normal nasal mucosa. Therefore, the results of our study suggest the involvement of HGF and c-Met in the pathogenesis of nasal polyps.

A role for HGF in the maintenance of normal tissues in the body is suspected because it is locally produced in areas without evidence of pathologic features. The present study confirms that normal nasal mucosa expresses HGF and c-Met, with both of these proteins produced in small amounts. Using immunohistochemistry on normal nasal mucosa, we found a weak but detectable localization of HGF in submucosal glands and of c-Met in epithelial layers and in submucosal glands. Because nasal mucosa used in the present study was in the normal state, it may be that HGF and c-Met activities were at their minimum. Naim et al. reported that healthy auditory meatal skin showed a diminished expression of HGF and of c-Met. In healthy laryngeal tissue, mild to moderate positive immunoreactivity for c-Met was observed in acinar and ductal cells in minor salivary glands. These findings are consistent with our results. On the other hand, nasal epithelium is frequently injured after exposure to various environmental agents. After injury, tissue repair is accomplished through a complex series of events consisting of inflammation, matrix deposition, repair, remodeling, and final healing. Several growth factors are candidates for nasal epithelial regeneration. Furthermore, it has been hypothesized that HGF might play an important role in regulating the regeneration of the nasal epithelia.

Therefore, it is assumed that HGF and c-Met levels in normal nasal mucosa can be modulated by injury and by factors associated with wound healing. This suggestion is supported by other data in various tissue systems. Additional work is needed to reveal a relationship between HGF activity and regeneration of wounded nasal mucosa.

Although the etiology of nasal polyps is still unknown, several pathological studies have addressed the key mechanisms leading to cellular proliferation, such as epithelial cell proliferation and angiogenesis, with the results suggesting that growth factors produced by inflammatory cells could affect cell proliferation. In those studies, insulin-like growth factor I, keratinocyte growth factor, and vascular endothelial growth factor were detected in nasal polyps and were implicated in the pathogenesis of disease. However, the expression of HGF and c-Met in nasal polyps was not investigated in the previous studies. The present data provide evidence that HGF and c-Met are overexpressed in nasal polyps. Therefore, our results support the role of HGF and c-Met as etiologic factors, suggesting that the overexpression of HGF and c-Met may contribute to the development or growth of nasal polyps. Furthermore, HGF was mainly localized in glandular epithelium, while c-Met was detected in glandular epithelium and in surface epithelium of nasal polyps. These results suggest that HGF, secreted by glandular epithelial cells, may act as an autocrine or a paracrine effector on glandular epithelium and on surface epithelium, stimulating cellular proliferation.

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REFERENCES