Increased Expression of *c-met* Messenger RNA Following Acute Gastric Injury in Rats

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Received February 28, 1994

The aim of the present study was to evaluate messenger RNA expression of *c-met*, a hepatocyte growth factor receptor gene, after gastric mucosal injury in rats. Male Sprague-Dawley rats were fasted for 24 hours, received 0.6 N hydrochloric acid (HCl), and served for polyadenylated RNA extraction from the oxyntic gastric mucosa. The transcripts of rat *c-met* gene were analyzed by reverse-transcript polymerase chain reaction and Northern blotting. Although it was detected even before the HCl administration, the *c-met* expression increased 6, 24 and 48 hours after the HCl administration. Thereafter, gastric mucosal injury diminished and the *c-met* expression declined. Hepatocyte growth factor reportedly plays an important role in gastric cell proliferation. The increased *c-met* expression indicates that this gene may participate in the healing process of gastric mucosa after injury.

The human *c-met* oncogene encodes a transmembrane tyrosine kinase (p190-*c-met*) with structural and functional features of a growth-factor receptor (1,2). Northern blot analysis has shown that the high levels of *c-met* messenger RNA (mRNA) have been detected in liver, intestinal tract, thyroid and kidney (3). Western blot analysis has also shown that the levels of the *met* protein generally correspond to those of the mRNA (3). A study using monoclonal antibody to the *c-met* protein demonstrated that the immunoreactivities were distributed in normal epithelial cells lining gut and hepatocytes as well as in more than 50% of gastric cancer (4). It was reported the putative tyrosine kinase receptor encoded by the oncogene *c-*

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Abbreviations used in this manuscript:
HCl, hydrochloric acid; HGF, hepatocyte growth factor; mRNA, messenger ribonucleic acid; poly(A)+RNA, polyadenylated ribonucleic acid; RT-PCR, reverse transcription-polymerase chain reaction.

0006-291X/94 $5.00

met is activated (i.e., tyrosine-phosphorylated in vivo) in the human gastric carcinoma cell line GTL-16 (5). Recently, *c-met* protein was reported to be a receptor for hepatocyte growth factor (HGF), a potent growth factor for hepatocytes and to respond to liver regeneration (6,7). HGF significantly promoted the growth of human adenocarcinoma MKN-74 cells in a dose-dependent manner (8). These results indicate that the receptor encoded by the *met* gene is involved in growth control of hepatocytes and that increased *c-met* expression may confer a growth advantage to neoplastic cells.

Gastric mucosa has a potent ability to recover from various injuries including intraluminal acid. However, it has been obscure whether the *c-met* gene is involved in the healing process in normal gastric mucosa after injury. The aim of the present study was to evaluate how the *c-met* gene could be expressed in response to acute gastric mucosal injury induced by hydrochloric acid (HCl) administration in rats. We applied reverse transcription polymerase chain reaction (RT-PCR) for partial cloning of rat stomach *c-met* gene, which had not been available.

MATERIALS AND METHODS

Male Sprague-Dawley rats weighing 250 g, 3 animals per group, were fasted for 24 hours. Under light anesthesia with ether, the animals received 1 ml of 0.6 N HCl through an orogastric tube. Thirty minutes after the HCl administration, all of the gastric content in was aspirated with the tube. Six, 24, 48 and 96 hours after, the animals were sacrificed. The oxyntic mucosa was scraped from the 3 animals, immediately frozen in liquid nitrogen and pooled before processing in RT-PCR and Northern analysis.

RNA extraction, RT-PCR, and Northern blot analysis were carried out as previously described (9). In brief, total RNA was extracted from the rat oxyntic mucosa by denaturation in a guanidine thiocyanate solution followed by pelleting through a cesium trifluoroacetate cushion. Three micrograms of total RNA was used for polyadenylated (poly-A(+)) RNA extraction with oligo dT latex and RT-PCR procedure. In RT-PCR, 20 mer antisense and sense oligonucleotides, which were complimentary to codons 1460-1479 and the same as codons 1084-1103 of the mouse *c-met* proto-oncogene (10) respectively, were synthesized with a DNA synthesizer (Applied Biosystems Inc., Foster City, CA), were applied for RT-PCR. The PCR products were subjected to 1% agarose gel electrophoresis and visualized by ethidium bromide staining. The PCR products were extracted from gel using Gene Clean (Bio101 Inc., La Jolla, CA) under the manufacture's recommended conditions. The sequences of the PCR products were analyzed with a DNA sequencer (Applied Biosystems, CA).

In Northern blotting, poly(A)+ RNAs were selected as mRNAs from total RNA using an oligo-dT latex. These mRNAs were fractionated with 0.9% agarose formaldehyde gels and then transferred onto nitrocellulose filters. For hybridization, the fragment of the rat *c-met*, produced by RT-PCR, was labeled with ^{32}P-deoxy CTP using a multiprimer labeling kit (Amersham International, Bucks, UK). The membrane was probed sequentially with cDNA for human beta-actin.

Another series of rats, 4 animals per group, were used for macroscopic and microscopic assessment of the injury with the same experimental protocol. In brief, the animals were sacrificed and laparotomized before and 6, 24, 48 and 96 hours after the HCl-administration under ether anesthesia. The stomach was harvested and immediately fixed onto a plastic board. The area of the macroscopic hemorrhages and erosions was assessed by planimetry. Thereafter, the stomach was treated with buffered formalin and paraffin-embedded. The semi-thin section was stained with hematoxylin-eosin and observed under an Olympus microscope.

These animal experiments were performed according to the guidelines of the Committee on Experimental Animals of Osaka University.

RESULTS

A sequence for the RT-PCR product (Table 1) was homologous by 95.2 % to the corresponding part of mouse *c-met* oncogene. This sequence was complementary to the other sequence of the RT-PCR products. Figure 1 shows the expected 396 base pair products which were visualized on ethidium bromide staining. The RT-PCR showed that the rat *c-met* gene was expressed even in normal oxyntic mucosa. The *c-met* mRNA level went to the peak 48 hours after the HCl administration whereas it was continuously expressed throughout the experiment. On nitrocellulose filter containing 5 μg of polysomal poly(A)+ RNA per lane, the size of the hybridizing transcripts was 8.5 kilobases (Figure 2). This result implied that

Table 1. Partial sequences of the rat *c-met* transcript cloned by RT-PCR (the 1st row for cDNA and the 2nd for amino acids) and the corresponding parts of the mouse *c-met* (the 3rd and the 4th rows). The underlined parts indicate the differences between the two species. The italic parts are used for the primers. Please note that the sequence is a part of full sequence of *c-met* (8.5 kb).

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1/1                                     31/11
TGT GCA TTC CCC ATC AAA TAT GTC AAT GAC TTC TTC AAC AAG ATT GTC AAC AAA AAC AAC
cys ala phe pro ile lys tyr val asn asp phe phe asn lys ile val asn lys asn asn
TGT GCA TTC CCC ATC AAA TAT GTC AAT GAC TTC TTC AAC AAG ATT GTC AAC AAA AAC AAC

61/21                                   91/31
GTA CGG TGT CTC CAG CAT TTT TAT GGA CCC AAC CAC GAG CAC TGT TTC AAT AGG ACC CTG
val arg cys leu gln his phe tyr gly pro asn his glu his cys phe asn arg thr leu
GTG AAG TGT CTC CAG CAT TTT TAT GGA CCC AAC CAT GAG CAC TGT TTC AAT AGG ACC CTG
val lys                                     his

121/41                                  151/51
CTG AGA AAT TCA TCG GGC TGC GAA GTG CGC AGT GAC GAG TAC CGG ACG GAG TTT ACC ACG
leu arg asn ser ser gly cys glu val arg ser asp glu tyr arg thr glu phe thr thr
CTG AGA AAC TCT TCG GGC TGT GAA GCG CGC AGT GAC GAG TAT CGG ACA GAG TTT ACC ACG
        asn ser         cys     ala                 tyr     thr

181/61                                  211/71
GCG CTG CAG CGT GTG GAT TTA TTC ATG GGC CGG CTC AAC CAT GTA CTC TTG ACG TCT ATC
ala leu gln arg val asp leu phe met gly arg leu asn his val leu leu thr ser ile
GCT TTG CAG CGC GTC GAC TTA TTC ATG GGC CGG CTT AAC CAA GTC CTC CTG ACG TCT ATC
ala leu     arg val asp                     leu     gln val     leu

241/81                                  271/91
TCT ACC TTC ATC AAA GGT GAC CTC ACC ATT GCT AAT CTA GGG ACA TCA GAA GGT CGC TTC
ser thr phe ile lys gly asp leu thr ile ala asn leu gly thr ser glu gly arg phe
TCT ACC TTC ATC AAA GGT GAC CTC ACC ATT GCT AAT CTA GGG ACA TCA GAA GGT CGC TTC

301/101                                 331/111
ATG CAG GTG GTG CTC TCT CGC ACA GCA CAT TTC ACC CCC CAT GTG AAT TTC CTC CTG GAT
met gln val val leu ser arg thr ala his phe thr pro his val asn phe leu leu asp
ATG CAG GTG GTG CTC TCT CGC ACA GCA CAT TTC ACC CCC CAT GTG AAT TTC CTC CTG GAT

361/121                                 391/131
TCC TAT CCT GTG TCT CCA GGG GCT GCA GGA ATT CGA
ser tyr pro val ser pro gly ala ala gly ile arg
TCC TAT CCT GTG TCT CCA GGG GCT GCA GGA ATT CGA
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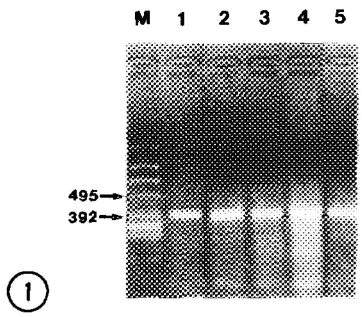

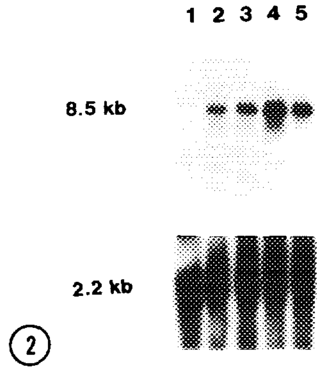

Figure 1. RT/PCR products using total RNA extracted from gastric mucosa for detection of the transcripts of the *c-met* gene. Three micrograms of total RNA extracted from rat gastric mucosa was used for reverse transcription into the cDNA with the antisense 20 mer primer(4057-4101 of the mouse *c-met* proto-oncogene). Amplification of cDNA was performed with sense 20 mer primer (codons 1084-1103 of the mouse *c-met* proto-oncogene) with 25 cycles of PCR. PCR products were subjected to 1% agarose gel electrophrosis and the visualized by ethidium bromide staining. 1 denoted normal gastric mucosa and 2, 3, 4, and 5 denoted 6, 24, 48, and 96 hours after 0.6 N HCl administration, respectively. M lane indicated X174-Hinc II digest DNA size markers.

Figure 2. Representative Northern blot analysis showing the expression of 8.5 kb *c-met* mRNA in regenerating rat gastric mucosa following acute mucosal damage. Five micrograms of poly(A)+ RNA per lane was applied to detect the mRNAs using the cDNA fragment labeled with ^{32}P. 1 denotes normal gastric mucosa, and 2, 3, 4, and 5 denote 6, 24, 48, and 96 hours after 0.6N HCl administration, respectively.

the transcripts of the rat *c-met* gene could be detected. It is also shown that *c-met* mRNA was detected even in the control group (before the HCl administration), and that *c-met* mRNA significantly increased 6 hours after the HCl administration. Forty eight hours later, *c-met* mRNA level got to the peak. The results obtained from Northern blot analysis were consistent with those from RT-PCR.

Macroscopically, hemorrhagic streaks were persisted during the 24 hours after the HCl administration. Then the mucosal damage decreased and little mucosal hemorrhage remained 96 hours after the HCl administration (Table 2). Histologically, severe and diffuse mucosal

Table 2. Changes in macroscopic lesion after the orogastric administration with 1 ml of 0.6N HCl. Data are shown as mean ± SEM, and analyzed by Kruskal-Wallis test with Conover's multiple comparison. Lesion index is the percentile of lesion area to total area of the glandular stomach.

Time (hours)	n	Lesion Area (mm^2)	Lesion Index (%)
0	4	0.0 ± 0.0	0.00 ± 0.00
6	4	26.3 ± 7.1 [a]	3.19 ± 0.30 [a]
24	4	38.5 ± 1.6 [b]	3.56 ± 0.28 [b]
48	4	3.5 ± 2.4	0.99 ± 0.60
96	4	0.5 ± 0.5	0.26 ± 0.25

[a] $p<0.01$ vs. the 0 hour group, and $p<0.05$ vs. the 48 and 96 hours groups.
[b] $p<0.01$ vs. the 0 and 96 hours groups, and $p<0.05$ vs. the 48 hours group.

hemorrhagic erosion were formed, from 6 to 24 hours after the HCl administration. Although there were some small erosions even after 96 hours after HCl administration, the regenerative mucosa were recognized at 24 hours after the HCl-induced mucosal injury.

DISCUSSION

In the present paper, we examined gene expression for an HGF receptor, *c-met*, in gastric mucosa in the healing process after HCl-induced injury. Since a DNA probe for rat *c-met* gene was not available, we applied RT-PCR for cloning a part of its sequence and detecting its expression. The sequences of the resulted RT-PCR products were analyzed to be highly homologous to the corresponding part of mouse *c-met* gene (10). Furthermore, the Northern blotting indicated the size of the hybridized transcript was 8.5 kb, as expected from that of the mouse *c-met*. Considering these results together, we concluded that a part of the rat stomach *c-met* was cloned by the RT-PCR.

The RT-PCR made it possible to analyze *c-met* expression semi-quantitatively in rat gastric mucosa. The Northern analysis also confirmed the results obtained by the RT-PCR. Both of the results show that *c-met* is expressed in rat gastric mucosa even before the injury. The observation agrees to the previous reports on *c-met* messenger RNA and its product in human gut (3,4). These results indicate that the stomach has *c-met*/HGF receptor and is one of the important targets of HGF.

Furthermore, the results demonstrate that the expression of *c-met*/HGF receptor mRNA increases during the mucosal regeneration following to the HCl-induced injury. Although precise roles of *c-met* in gastric mucosal healing remain obscure, Shibamoto *et al.* reported that HGF significantly promoted the growth of human adenocarcinoma MKN-74 cells in a dose-dependent manner (8). Ponzetto *et al.* reported that *c-met* was activated in cancer cell line (5). Moreover, HGF has mitogenic activity for rat gastric mucosal epithelial cells (11) as well as epidermal melanocytes, keratinocytes and mature hepatocytes (12). HGF mRNA and HGF activity increase markedly in the liver after various liver injuries (12). Since HGF mRNA increases markedly in the kidney after various renal injuries, HGF may act not only as a hepatotropic factor but also as a renotropic factor (13). From this context, HGF and its receptor might play a mitogenic role in normal gastric mucosal cells and contribute to gastric mucosal healing through increased epithelial cell proliferation.

It is worth noting, however, that HGF strongly enhances motility of epithelial cells as a motogen and induces epithelial tubular formation as a morphogen (13). HGF has a potent ability to promote cell migration in certain epithelial cells, including normal human keratinocytes (12). Thus, over-expression of *c-met* after the injury may also be related to migration and re-epithelization of the mucosal cells to the erosion or maturation of the proliferated cells.

In conclusion, the expression of *c-met*/HGF receptor gene increased in rat gastric mucosa after an acid-induced injury. The results indicate that HGF/HGF-receptor system is a novel and important regulator not only in liver but also in normal gastric mucosa after injury. The precise roles of the interaction between HGF and its receptor remain to be examined in the further study.

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