FOR THE RECORD

Are hepatocyte growth factor-like protein and macrophage stimulating protein the same protein?

JORGE A. BEZERRA, SU HAN,¹ MARY JO S. DANTON,
AND SANDRA J. FRIEZNER DEGEN

Division of Basic Science Research, Children's Hospital Research Foundation and the Department of Pediatrics,
University of Cincinnati College of Medicine, Cincinnati, Ohio 45229

(RECEIVED January 6, 1993; ACCEPTED January 12, 1993)

We have isolated and characterized a human gene that is located at the D3F15S2 locus on human chromosome 3 (3p21), a region believed to code for one or more tumor suppressor genes because this region is deleted in DNA from small cell lung carcinomas, renal carcinomas, and other forms of cancer (Han et al., 1991). The corresponding cDNA for our recently discovered gene codes for a protein with a domain structure similar to hepatocyte growth factor (HGF; Nakamura et al., 1989) in that it contains four kringle domains followed by a serine protease-like domain. Based on this similarity, we have called the protein encoded by our cDNA "HGF-like (HGFL) protein." HGF itself can act as either a growth factor, tumor suppressor, scatter factor, or morphogen depending on the target tissue or cell type (Michalopoulos & Zarnegar, 1992). However, the biological activities of HGFL protein remain to be determined.

Recently, the partial amino acid sequence of several peptides isolated from human macrophage stimulating protein (MSP) has come to our attention (Skeel et al., 1991). MSP has been found to be the stimulating signal required for resident peritoneal macrophages from C3H mice to undergo a chemotactic response in the presence or absence of a chemotactic stimulus (complement component C5a) and to cause marked macrophage spreading and morphological changes in vitro (Leonard & Skeel, 1976; Skeel et al., 1991). MSP is also a phagocytosis-activating signal for mouse resident peritoneal macrophages to ingest sheep erythrocytes opsonized with IgM anti-Forssman antibody and mouse C3b. Comparison of these sequences with the HGFL protein sequence suggests

that these proteins are the same or highly related proteins (Table 1). There are some differences, most of which can be attributed to no or a low yield during the sequencing of the peptides. In addition, several peptides isolated from the β -chain of MSP are similar to sequences determined for the α -chain of MSP and the putative α -chain

Table 1. Comparison of the translated amino acid sequence of human HGFL protein with the sequence of peptides derived from human macrophage stimulating protein (MSP)

	Amino acid residue ^a or peptide ^b	Sequence ^c
HGFL	230–247	FLDQGLDDNYCRNPDGSE
MSP	α BU-11	-----
HGFL	288–310	GEGYRGTTTACVPCQRWDAQ
MSP	α BU-7	---E---?-----?E-E?---
HGFL	326–341	DLRENFRCRNPDGSEAP
MSP	α BU-12	-----?-----S---
HGFL	388–404	GVQCQRWSAETPHKPQF
MSP	α BU-14	---?--W---P???
HGFL	484–502	VVGHHPGNSPWTVSLRNQ
MSP	β BU-9	-----S--T-?-N-
HGFL	530–551	PLTGVEVWLGTLFQNPQHGEPS
MSP	CB-5	-----?-----?--SL
HGFL	574–596	LERSVTLNQRVALICLPPEWYVV
MSP	β BU-16	-----E--V
HGFL	602–611	CEIAGWGETK
MSP	β BU-5	-----

^a Derived from translation of the cDNA coding for human hepatocyte growth factor-like (HGFL) protein (Han et al., 1991).

^b Skeel et al. (1991).

^c Hyphens represent identical residues when the two sequences are compared; underlined residues are uncertain due to low yield; and ? represent undetermined residues.

Reprint requests to: Sandra J. Friezner Degen, Division of Basic Science Research, Children's Hospital Research Foundation, Elland and Bethesda Avenues, Cincinnati, Ohio 45229-2899.

¹ Present address: Symphony Pharmaceuticals, Inc., Philadelphia, Pennsylvania 19104.

of HGFL protein (peptides BU12 and BU13 of Skeel et al. [1991]) and may be due to contamination of the two chains during their purification.

In view of the protein sequence identity between HGFL protein and MSP, preliminary experiments were performed to determine whether HGFL protein was present in blood, since MSP had been purified from this source. A polyclonal antibody was obtained against fusion protein purified from *Escherichia coli* transformed with a 960-bp cDNA fragment coding for 321 amino acids of the carboxy-terminal end of the second kringle, kringles 3 and 4, and part of the serine protease-like domain of HGFL protein, and cloned in the correct reading frame at the carboxy-terminal end of the β -galactosidase gene. The antibody was immunopurified against the fusion protein and used in Western analysis of human, mouse, and rat plasma.

Western analysis of human plasma using the affinity-purified polyclonal antibody indicates that the antibody reacts with a polypeptide of the size predicted for HGFL protein on reducing sodium dodecyl sulfate (SDS)-polyacrylamide gels (Fig. 1A). This polypeptide is very likely HGFL protein, because its size is similar to that predicted based on the amino acid sequence deduced from translation of the cDNA, including additional glycosylation (90 kDa; Han et al., 1991). The antibody does not cross-react with prothrombin and plasminogen, two proteins that are homologous to HGFL protein, but does react with HGF (data not shown). Using a polyclonal antibody specific for human placental HGF (Bhargava et al., 1992), we have shown that this antibody does not react with the

same size polypeptide in human plasma that the antibody to HGFL protein recognizes (data not shown). It is not surprising that the polyclonal antibody to HGFL protein reacts with HGF, because the two proteins are 47% identical in the region expressed in the fusion protein (residues 275–585 in human HGF; Han et al., 1991).

Two polypeptides of 95 and 62 kDa react with the antibody to HGFL protein in mouse plasma (Fig. 1B). The 95-kDa polypeptide probably represents the single-chain uncleaved form of the protein. The 62-kDa polypeptide is the expected size of the heavy or α -chain. The antibody to human HGFL protein reacts with a single polypeptide in rat plasma of the same size observed in human plasma (Fig. 1C). Based on these observations, it appears that apparently healthy humans and rats have the mature, uncleaved form of HGFL protein, whereas activation may have occurred in the plasma of mice. The reason for this difference remains to be assessed.

Human MSP has been purified to apparent homogeneity from human plasma using immunoaffinity and high-pressure liquid ion exchange chromatography (Skeel et al., 1991). It was determined that this protein had a molecular weight of 70 kDa on nonreducing SDS-polyacrylamide gel electrophoresis, whereas under reducing conditions two polypeptides were observed, at 47 and 22 kDa. These sizes are smaller than those predicted for HGFL protein (Han et al., 1991). In addition, the uncleaved precursor polypeptide of HGFL protein is present in human plasma. Although these results are not consistent with HGFL protein and MSP being the same protein, it is possible that proteolytic cleavage of MSP occurred during its purification.

Based on the similarity in structure of HGFL protein/MSP and HGF it is reasonable to propose that there is a family of receptors homologous to the HGF receptor (the c-met proto-oncogene product with tyrosine kinase activity; Bottaro et al., 1991; Naldini et al., 1991). It is likely that the receptor for HGFL protein/MSP is present on the surface of macrophages. Further purification of the protein and identification of this receptor and its localization in other cell types will allow us to further understand the biological function of HGFL protein and MSP.

Acknowledgments

We are grateful to Dr. Eliot Rosen for his valuable advice and contribution of the human HGF antibodies. This work was supported in part by the American Cancer Society—Ohio Division. S.J.F.D. is an Established Investigator of the American Heart Association.

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Fig. 1. Hepatocyte growth factor-like (HGFL) protein is present in plasma. Western analysis of human (A), mouse (B), and rat (C) plasmas using an affinity-purified polyclonal antibody raised against the fusion protein β -galactosidase/human HGFL protein. Plasma proteins were electrophoresed on 10% SDS-polyacrylamide gels under reducing conditions and transferred to Immobilon membranes. Pre, preimmune serum; Ab, anti- β -galactosidase/human HGFL protein polyclonal antibody; lanes 1 and 3, 0.2 μ L plasma; lanes 2 and 4, 0.5 μ L plasma. Asterisks indicate polypeptides specifically detected by the affinity-purified antibody for HGFL protein. The sizes of molecular weight markers are indicated.

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