EMMPRIN (CD147), an Inducer of Matrix Metalloproteinase Synthesis, Also Binds Interstitial Collagenase to the Tumor Cell Surface

Huiming Guo, Rongsong Li, Stanley Zucker, and Bryan P. Toole

Department of Anatomy and Cellular Biology, Tufts University School of Medicine, Boston, Massachusetts 02111 [H. G., R. L., B. P. T.], and Departments of Research and Medicine, Veteran Affairs Medical Center, Northport, New York 11768 [S. Z.]

Abstract

Extracellular matrix metalloproteinase inducer (EMMPRIN), also known as basigin or CD147, is a glycoprotein that is enriched on the surface of tumor cells and stimulates production of several matrix metalloproteinases by adjacent stromal cells. In this study, we have found that EMMPRIN not only stimulates the production of interstitial collagenase (MMP-1) but also forms a complex with MMP-1 at the tumor cell surface. Complex formation was demonstrated by phage display, affinity chromatography, and immunocytochemistry. Presentation of MMP-1 complexed to EMMPRIN at the tumor cell surface may be important in modifying the tumor cell pericellular matrix to promote invasion.

Introduction

MMPs have been implicated in several aspects of tumor progression, including invasion through basement membranes and interstitial matrices, angiogenesis, and tumor cell growth (1–3). Strong support for the involvement of MMPs at some step in tumor progression comes from experiments in which tissue inhibitors of MMPs or synthetic inhibitors of metalloproteinases have been shown to reduce tumor growth and metastasis (4, 5). Over the past several years, it has become increasingly apparent that tumor cells create a pericellular environment in which MMPs and other proteases become concentrated, thus enhancing the ability of tumor cells to invade extracellular matrices (6–8). Previous studies from this laboratory have demonstrated that EMMPRIN, a member of the immunoglobulin superfamily that is enriched on the surface of most tumor cells, stimulates stromal cells to produce elevated levels of several MMPs, including MMP-1 (9–11). We have now found that tumor cell EMMPRIN not only stimulates MMP-1 production by fibroblasts but also binds MMP-1 to the surface of tumor cells, thus adding to the complement of proteases on the tumor cell surface that may promote invasion.

Materials and Methods

Phage Display Library. mRNA was prepared from human fibroblasts with the Oligotex mRNA kit (Qiagen, Valencia, CA) and used for cDNA synthesis with the Directional RH primer cDNA synthesis kit (Novagen, Madison, WI). After second-strand synthesis, the cDNA ends were flushed with T4 DNA polymerase and ligated to EcoRI/HindIII directional linkers. The cDNA was then digested with EcoRI and HindIII and ligated to T7Select-1-1b vector arms (Novagen). The ligated DNA was packaged into bacteriophage T7 using the T7 Select primer (GGAGCTGTCGTATTCCAGTC) and the T7 Select-Down primer (AACCCTCAAGACCCGTTTA; Novagen).

The wells were coated with immunopurified EMMPRIN protein (Ref. 12; 1 μg/ml in Tris-buffered saline) at 4°C overnight and washed with Tris-buffered saline five times. Unreacted sites were blocked with 5% blocking reagent overnight at 4°C and washed. In the first round of screening, the phage lysate was applied to the EMMPRIN-coated plate (0.5 ml lysate/well) for 30 min at room temperature. The plate was then washed five times with Tris-buffered saline. The bound phages were eluted by adding 0.5 ml of elution buffer (1% SDS) at room temperature for 20 min. The eluted phages were then added to a culture of the host cells (BLT 5403) in LB media and incubated at 37°C with shaking for 3 h, at which time lysis was observed. The lysed culture was centrifuged, and the supernatant was collected for the next round of phage screening. A total of five rounds of screening was carried out. DNA from the phages isolated during the final round of screening was purified and sequenced using the T7 SelectUp primer (GGAGCTGTCGTATTCCAGTC) and the T7 Select-Down primer (AACCCTCAAGACCCGTTTA; Novagen).

Immunoaffinity and Ligand Affinity Chromatography. EMMPRIN was isolated from extracts of membranes from LX-1 human lung carcinoma cells by immunoaffinity chromatography using E11F4 monoclonal antibody against EMMPRIN immobilized on Sepharose beads, as described previously (12).

For manufacture of the ligand affinity medium, EMMPRIN protein (0.5 mg) was first dissolved in coupling buffer [0.1 m NaHCO3 and 0.5 m NaCl (pH 8.3) containing 0.5% NP40]. The coupling solution was then mixed with CNBr-activated Sepharose 4B gel (Pierce; 0.25 g of dried powder swelled and washed in 1 m Tris buffer (pH 8.3)). The gel was washed three times with 5 ml of coupling buffer, followed by incubation in 0.1 m Tris-HCl (pH 8) for 2 h to block any remaining active groups. Then the gel was washed using three cycles of 0.1 m acetate buffer, 0.5 m NaCl (pH 4), and 0.1 m Tris and 0.5 m NaCl (pH 8). After washing, the gel was resuspended in 5 ml of 10 mm Tris buffer (pH 8.3).

Extracts of human fibroblasts [106 cells in 5 ml of 10 mm Tris, 0.15 m NaCl, and 0.5% NP40 (pH 8.3)] were added to the EMMPRIN-coupled gel and incubated at 4°C overnight with rotation. The gel was then washed with 10 mm Tris and 0.15 m NaCl containing 30 mm octyl glucoside until the A280 nm was less than 0.05. Binding proteins were eluted with 0.1 m glycine buffer (pH 2.5) containing 30 mm octyl glucoside. The eluate was neutralized to pH 7 by the addition of 1 m Tris (pH 9.5) and concentrated for further analysis.

ELISA of MMP-1. MMP-1 protein was measured in the eluates from EMMPRIN-Sepharose and in immunopurified EMMPRIN preparations using a commercial ELISA system (Amersham, Piscataway, NJ) according to the manufacturer’s instructions. Briefly, 5 or 10 μl of eluate were added to microtiter plates coated with antibody to MMP-1 and incubated for 2 h at 25°C. The plates were washed with phosphate buffer and incubated with anti-MMP-1 antiserum for 2 h. After washing, the plates were incubated with peroxidase-conjugated secondary antibody for 1 h, and processed for color development and measurement at A450 nm in a microplate spectrophotometer. The concentration of MMP-1 in the eluate was estimated from a standard curve.

Received 11/18/99; accepted 1/300.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Supported by United States Army Grants DAMD17-95-1-5017 and DAMD17-99-1-5043.

To whom correspondence should be addressed. Fax: (617) 636-0380; E-mail: btower@infoonet.tufts.edu.

The abbreviations used are: MMP, matrix metalloproteinase; EMMPRIN, extracellular matrix metalloproteinase inducer; MMP-1, interstitial collagenase; MMP-2, gelatinase A; MT-MMP, membrane-type MMP; LB, Luria-Bertani.
SDS-PAGE, Silver Staining, and Western Blotting. Proteins were dissolved in SDS sample buffer containing 0.1 M DTT and heated at 95°C for 5 min. The samples were then subjected to electrophoresis on 10% SDS polyacrylamide gels. The gels were either stained using the Sterling silver staining system (National Diagnostics, Atlanta, CA) or electroblotted onto nitrocellulose membranes and incubated with antibody against EMMPRIN (E11F4; Ref. 12) or against MMP-1 (Calbiochem, La Jolla, CA) for 1 h at room temperature.

Immunocytochemistry. LX-1 human lung carcinoma cells were seeded into chamber culture slides and cultured for 48 h at 37°C in 5% CO2 air. The cells were then washed with PBS, fixed in 1% paraformaldehyde in PBS for 45 min at room temperature, quenched with 0.1 M Tris (pH 7.4), and blocked with 1% BSA, 1% goat serum, and 2% nonfat milk in PBS at room temperature for 1 h. The LX-1 cells were then incubated with monoclonal antibody against MMP-1 (Calbiochem) for 1 h at room temperature, followed by Cy3-conjugated Texas red goat antimouse IgG. The cells were washed with PBS, mounted with coverslips, and then observed and photographed using a Zeiss Axioskop-20 microscope.

Results

Phage Display Reveals MMP-1 as an EMMPRIN-binding Protein. We used the T7Select Phage Display System (Novagen) to identify EMMPRIN-binding protein(s) encoded by a cDNA library prepared from human fibroblasts, as described in “Materials and Methods.” In this method, each phage becomes coated with a fusion protein comprised of the phage coat protein and a protein generated from the cDNA library used. Phages coated with putative EMMPRIN-binding protein were selected by repeated panning over 24-well plates coated with EMMPRIN. Five rounds of biopanning were carried out, and the final lysate was used for plaque assay, PCR amplification, and sequencing. Eight clones were obtained from the procedure described above. All eight of the inserts were of identical size, i.e., 0.8 kb, and were found to have identical sequences corresponding exactly to a portion of the human MMP-1 sequence (Fig. 1).

MMP-1 Binds to EMMPRIN-Sepharose. To confirm the binding of EMMPRIN to fibroblast-produced MMP-1, we performed ligand chromatography over Sepharose conjugated with immunopurified EMMPRIN. Fibroblast extracts were mixed with the EMMPRIN-Sepharose, which was then washed and eluted as described in “Materials and Methods.” The eluates were subjected to SDS-PAGE, followed by silver staining. On silver staining, a prominent protein band at \( M_r \) 55,000 was observed, as well as a weaker band at \( M_r \), 67,000 (Fig. 2A); in some cases a \( M_r \), 45,000 band could also be seen.

Western blots were also performed on the eluates from EMMPRIN-Sepharose using antibody against human MMP-1. The protein band at \( M_r \), 55,000 (the approximate size of pro-MMP-1, which is \( M_r \), 52,000) reacted with anti-MMP-1 antibody (Fig. 2B), confirming our results from the phage display. ELISA measurements also revealed MMP-1 in the eluates from EMMPRIN-Sepharose (data not shown). The identities of the \( M_r \), 67,000 and \( M_r \), 45,000 proteins are not yet known.

EMMPRIN Forms a Complex with MMP-1 on the Surface of Tumor Cells. Some tumor cells themselves produce small amounts of MMP-1. Thus, we also determined whether, in addition to binding isolated EMMPRIN protein, MMP-1 forms a complex with EMMPRIN present on the surface of LX-1 human lung carcinoma cells. We immunopurified EMMPRIN from extracts of LX-1 cell membranes using monoclonal antibody E11F4 covalently bound to Sepharose beads and tested whether MMP-1 was present in the eluted EMMPRIN preparation. Fig. 3 shows a Western blot of such an
EMMPRIN preparation with antibody against MMP-1. A strong band at \( M_r 55,000 \), corresponding approximately in size to pro-MMP-1, reacted with the antibody, indicating the presence of MMP-1 in the EMMPRIN preparation. A weaker band at \( M_r 45,000 \), which is not seen consistently, is most likely activated MMP-1 (\( M_r 42,000 \)).

Quantitation of the MMP-1 content by ELISA gave 2.1 \( \mu \)g of MMP-1 per 5 \( \mu \)g of total protein in the EMMPRIN preparation. Because EMMPRIN and pro-MMP-1 have molecular weights of \( \sim 58,000 \) and \( \sim 52,000 \), respectively, this result suggests that EMMPRIN and MMP-1 are complexed in an equimolar ratio.

The presence of MMP-1 at the surface of LX-1 human lung carcinoma cells was confirmed by immunocytochemistry using antibody against MMP-1 (Fig. 4).

**Discussion**

Many recent studies have highlighted the importance of the pericellular milieu surrounding tumor cells in their proliferative and invasive behavior (6–8). This milieu is modified by a number of proteases, especially MMPs and tissue serine proteases, many of which are produced by tumor-associated stromal cells rather than tumor cells themselves (13, 14) and subsequently become concentrated at the tumor cell surface via interaction with specific binding
sites. For example, MMP-2 binds to the tumor cell surface via a tissue inhibitor of MMPs-2-MT-MMP complex (15, 16). MMP-2 is activated by the MT-MMP, and the complex is targeted to invasive domains of the tumor cell membrane (sometimes termed “invadopodia”) via specific docking of MT-MMP at these sites (17). Although MT-MMPs activate soluble MMP-2 as well as plasma membrane-retainend MMP-2, membrane-bound enzyme is required for tumor cell invasion (17). A similar mechanism of activation and retention at the cell surface has been described for collagenase 3 (18). Other cell surface binding sites have been described for gelatinase B, i.e., CD44 (19) and the α5(IV) chain of collagen (20), and for MMP-2, i.e., α5β1 integrin (21). These sites also appear to be important in tumor cell invasion.

Evidence for association of MMP-1 with the surface of a human pancreatic carcinoma cell line has been published previously (22), but the mechanism whereby MMP-1 binds to these cells has not been described. In the present study, we show that MMP-1 binds to EMMPRIN, a tumor cell surface glycoprotein previously shown to induce synthesis of MMP-1 and other MMPs by fibroblasts (9–11) and endothelial cells.4 We have also shown that an EMMPRIN-MMP-1 complex can be isolated from LX-1 human lung carcinoma cell membranes and that MMP-1 is present on the LX-1 cell surface. A preliminary report has been published suggesting that EMMPRIN becomes localized to invadopodia in human breast carcinoma cells (23). Tumor cell surface EMMPRIN may then be responsible for targeting MMP-1 to invadopodia, thus adding MMP-1 to the impressive list of proteases associated with these invasive structures (6, 17).

Although other proteases have been shown to be important in tumor growth and invasion under a variety of conditions, it is likely that MMP-1 is crucial for penetration of fibrous tissues because of its ability to degrade fibrillar collagen as shown, for example, in endothelial cell invasion (24) and tumor cell invasion (25) of collagen gels. Thus localization of MMP-1 on the tumor cell surface via interaction with EMMPRIN would facilitate these invasive processes.

References


4 Unpublished observations.

EMMPRIN (CD147), an Inducer of Matrix Metalloproteinase Synthesis, Also Binds Interstitial Collagenase to the Tumor Cell Surface

Huiming Guo, Rongsong Li, Stanley Zucker, et al.

Cancer Res 2000;60:888-891.

Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/60/4/888

Cited articles
This article cites 24 articles, 14 of which you can access for free at:
http://cancerres.aacrjournals.org/content/60/4/888.full.html#ref-list-1

Citing articles
This article has been cited by 14 HighWire-hosted articles. Access the articles at:
/content/60/4/888.full.html#related-urls

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.