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ULEX EUROPAEUS I LECTIN INDUCES ACTIVATION OF MATRIX-METALLOPROTEINASE-2 IN ENDOTHELIAL CELLS

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In this report, we show that the lectin Ulex europaeus agglutinin I (UEA I), which binds to α-linked fucose residues on the surface of endothelial cells, mediates activation of the 72-kDa matrix metalloproteinase-2 (MMP-2). A dose-dependent increase in the active 62-kDa form of MMP-2 was observed in conditioned medium from monkey aortic endothelial cells (MAEC) following incubation with concentrations of UEA I ranging from 2 to 100 μg/ml. The increase in the 62-kDa MMP-2 gelatinolytic activity was not reflected by a rise in MMP-2 gene expression. The UEA I-mediated activation of MMP-2 was blocked by L-fucose, which competes with UEA I for binding to α-fucose. These findings may suggest that a similar *in vivo* mechanism exists, whereby adhesive interactions between tumor cell lectins and endothelial cells can mediate MMP-2 activation.

Adhesion of circulating tumor cells to microvascular endothelium may be one of the major determining factors for organ-specific metastasis (1). EC possess galactose-binding lectins which have been implicated in colonization by tumor cells (2,3). Furthermore, the endothelial adhesion molecules, ELAM-1 and GMP-140, were found to have sequence homology with C-type mammalian lectins (4). The lectin UEA I binds selectively to EC of human and nonhuman primate origin due to its high binding affinity for α-linked fucose residues on the EC surface (5-7).

We have previously demonstrated tumor cell mediated stimulation of polarized EC secretion of matrix metalloproteinase (MMP) and plasminogen activator (PA) activity which resulted in *in situ* degradation of the subendothelial basement membrane (BM) (8). MMPs are secreted as latent proenzymes and upon activation, they undergo 6-10 kDa reduction in molecular mass through removal of a N-terminal fragment of the enzyme (9). *In vitro* studies have shown that MMP-2 can be activated by a variety of agents such as TGFß (10), type I collagen (11), integrins and concanavalin A (12,13).

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In this report, we show evidence for UEA I-mediated activation of MMP-2, a proteinase which is constitutively expressed by a variety of normal and malignant cell types and has been implicated in tumor invasion and neovascularization (14).

MATERIALS AND METHODS

Isolation and Culture of Endothelial Cells. MAEC were isolated as previously described (15). Briefly, the aortas were dissected from its surrounding connective tissues, washed and then cut into 5-mm cubes and suspended in collagenase. The cell preparation was subjected to immunoseparation using Evonymous europaeus agglutinin-coated Dynabeads. The cells attached to the coated beads were collected with a magnetic particle concentrator, washed and finally cultured in endothelial growth medium consisting of: Ham's F12K medium (Biofluids, Rockville, MD), 30% fetal bovine serum (FBS), 2% glutamine (Biofluids), 0.4% bovine brain extract, 0.1% epidermal growth factor, 0.1% hydrocortisone and 0.1% GA-1000 (all from Clonetics Co., San Diego, CA). At confluence, the cell monolayers were rinsed three times in phosphate buffered saline (PBS) and incubated in serum-free medium containing 2, 20, 40 or 100 μg/ml of UEA I lectin (Vector Lab., Burlingame, CA) for 12 h. Other experiments were carried on in the presence of 0.1 M L-fucose. Culture supernatants were collected, and cell debris was removed by centrifugation.

Labelling with Fluorescein-UEA I. After seven days in culture, the MAEC were exposed to 25 μg of fluorescein-labelled UEA I in Ham's F12K medium with 10% FBS for 20 h. After three washes with PBS, the cell cultures were fixed with 4% paraformaldehyde in PBS and photographed.

Western Blots. Western blot analysis was carried out as previously described, using an MMP-2 (1:1000) specific primary antibody and a secondary alkaline phosphate-conjugated anti-rabbit antibody (1:1000) (16).

Densitometric Analysis. Densitometric quantitation of gelatinolytic activity was performed by measuring optical density with a scanning densitometer (Scanmaster 3, Hudson, NH). The results were interpreted with Quantity One software (Protein Database Inc., Huntington Station, NY).

Substrate Gel Electrophoresis. Substrate SDS-PAGE gels containing 0.1% gelatin were prepared and used for assessing MMP activities as previously described (10).

Northern Blot Analysis. Total RNA was extracted from the UEA I treated and untreated MAEC cultures, using RNAzol kit (TEL-TEST Inc., TX) according to the procedure recommended by the supplier. Northern blot analysis was performed using 15 μg of total RNA. After the electrophoresis, the RNA was immobilized onto nylon membrane (Schleicher & Schuell, Keene, NH) and hybridized with 1 x 10^6 cpm/ml of nick-translated, [^{32}P]cDNA probes of MMP-2 and ß-actin, which were obtained as described previously (18).

RESULTS AND DISCUSSION

Lectins are not only present on plants and microorganisms, but are also found on the surface of mammalian cells (19). There is experimental evidence that mammalian lectins may play a role in tumor cell dissemination (20). It was reported recently that tumor cells of

high metastatic potential have more abundant cell surface lectins than the less metastatic cells (21). Microvascular EC lectins, serving as ligands for N-linked oliogosaccharides on the surface of circulating tumor cells, have also been shown to contribute to metastasis formation (3). There is emerging evidence that nonmalignant cell types, such as fibroblasts and EC, can contribute to the proteolytic tissue degradation, associated with tumor invasion (8,22).

In the present report, we demonstrated that binding of the lectin UEA I to α-fucose residues on the surface of MAEC resulted in activation of MMP-2. Fluorescein-conjugated UEA I was found to have high binding affinity for MAEC in culture, where all of the cells displayed cytoplasmic fluorescence after 20 h incubation with UEA I (Fig. 1).

MMP-2 activation was evaluated by zymography, using a gelatin substrate incorporated into SDS-PAGE. The active form of MMP-2 was visualized as a gelatinolytic band of approximately 62 kDa. Since SDS is known to activate matrix metalloproteinases (23), it was possibly responsible for activating the 72 kDa form of MMP-2 which was also observed as a gelatinolytic band in the zymograms. Serum-free conditioned media, collected from MAEC incubated with increasing concentrations (2, 20, 40, 100 μg/ml) of UEA I for 24 h, were subjected to gelatin SDS-PAGE. A dose-dependent increase in gelatinolytic

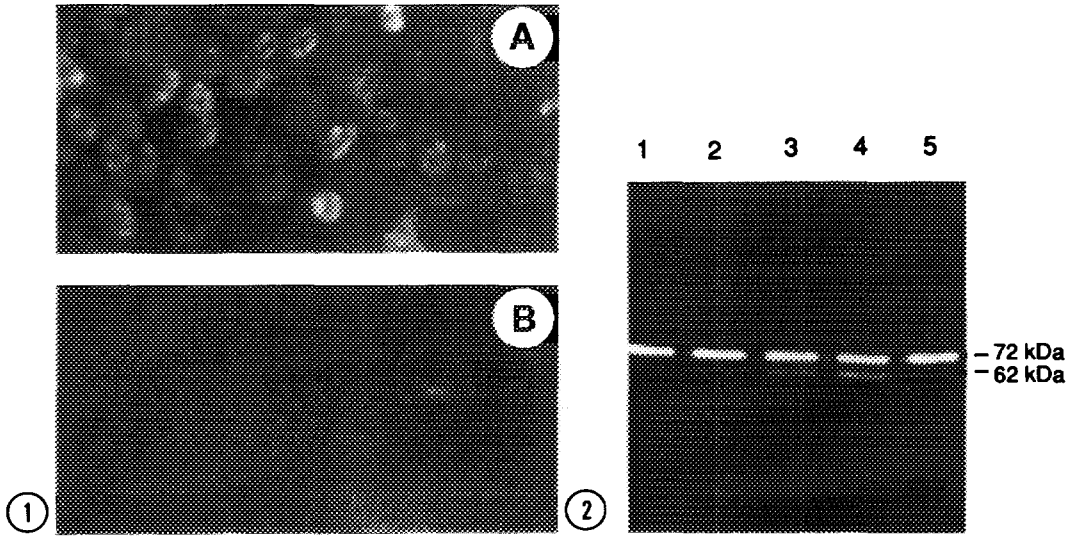

Figure 1. *Binding of UEA I to MAEC.* Cytoplasmic binding is present following incubation of MAEC with fluorescein-conjugated UEA I for 20 h (A). The untreated control is negative (B).

Figure 2. *Gelatin zymography of conditioned media from MAEC treated with increasing doses of UEA I.* Gelatinolytic activities at molecular weights of approximately 72 and 62 kDa correspond to the latent and active forms of MMP-2. Dose responsive increase of gelatinolytic band, corresponding to the active form of MMP-2 is seen in the conditioned media from MAEC treated with the following doses of UEA I: 1: 2 μg/ml; 2: 20 μg/ml; 3: 40 μg/ml; 4: 100 μg/ml; 5: untreated control.

activity was observed at a molecular weight of approximately 62 kDa, which was consistent with that of the activated form of MMP-2 (Fig. 2). Densitometric analysis of the 62 kDa gelatinolytic activity in the conditioned media from the MAEC treated with 100 μg/ml revealed a 10-fold increase, compared to the untreated control. However, the density of the 72 kDa gelatinolytic band did not change following UEA I treatment.

An antibody raised against MMP-2 recognized both the latent 72 kDa and the active 62 kDa forms of MMP-2 by Western blot analysis of the conditioned medium (Fig. 3). There was an apparent inconsistency between the sharp rise in the intensity of the immunostained 62 kDa MMP-2 band observed with the highest dose of UEA I by Western blotting and the modest increase in gelatinolytic activity obtained with the same dose. However, Western blots are not well suited for the assessment of protein concentration, whereas the zymographic method, although semiquantitative, reflects more closely the actual amount of protein present. We have previously demonstrated that gelatin zymography is sensitive enough to detect the activity of MMP-2 and MMP-9 in nanogram quantities (16).

It was examined if the UEA I-mediated effect on MMP-2 activation was associated with an increase in MMP-2 gene expression. Northern blot analysis revealed no differences in MMP-2 transcripts between the MAEC treated with 100 μg/ml and the untreated control (Fig. 4). This suggests that MMP-2 activation took place by a mechanism not requiring MMP-2 transcriptional response. In comparing the action of UEA I with that of the lectin ConA, the latter was shown to both stimulate mRNA expression and activate MMP-2 (12). Furthermore, ConA-mediated activation of MMP-2 in a human breast carcinoma cell line was shown to concur with up-regulation of a newly discovered membrane-type matrix metalloproteinase (MT-MMP) (24). We found that binding of UEA I to the α-fucose residues on the MAEC surface was essential for MMP-2 activation. When the MAEC were incubated with UEA I in the presence of L-fucose, which competes with UEA I for binding to α-fucose (Fig. 5), activation was completely blocked. Morphological changes and cytoskeletal reorganization have been linked to MMP-2 activation, mediated by ConA and

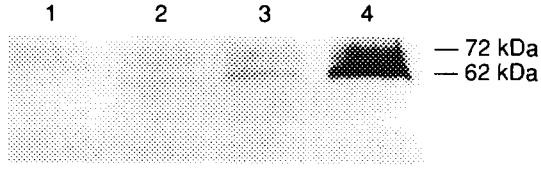

Figure 3. *Western blot analysis of MMP-2 present in conditioned medium from UEA I-treated MAEC.* The cells were treated with the following doses of UEA I: 1: 2 μg/ml; 2: 20 μg/ml; 3: 40 μg/ml; 4: 100 μg/ml. MMP-2 is seen as two immunoreactive bands at approximately 72 and 62 kDa.

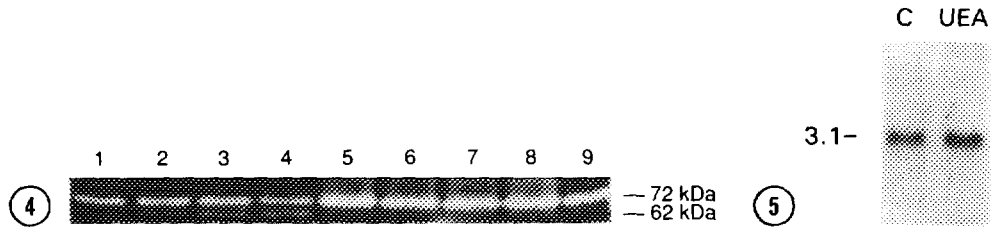

Figure 4. *Gelatin zymography of conditioned media from MAEC treated with increasing doses of UEA I in the presence or absence of L-fucose.* The MAEC were treated with UEA I as follows: 1 and 5: 2 μg/ml; 2 and 6: 20 μg/ml; 3 and 7: 40 μg/ml; 4 and 8: 100 μg/ml; 9: untreated control. The activated form of MMP-2 is apparent in the absence (lanes 1-4), but not in the presence of 0.1 M L-fucose (lanes 5-9).

Figure 5. *Northern blot analysis of MMP-2 expression in UEA I-treated MAEC.* Total RNA from MAEC, untreated (C), and treated with 100 μg/ml of UEA I was analyzed.

type I collagen (11-13). In our experiments, however, no morphological changes were detected in the MAEC exposed to UEA I (not shown).

When considering the results reported here and the findings of others on ConA-mediated activation of MMP-2, it appears that a specific binding of lectin to cell surface carbohydrates triggers complex molecular events, that lead to the MMP activation. Since activation of MMPs is essential for tissue degradation during tumor invasion, it is important to examine further if tumor cell lectins play a role in MMP activation *in vivo*.

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