Galectin-3 Is a Novel Substrate for Human Matrix Metalloproteinases-2 and -9†

Josiah Ochieng,[‡,§,||] Rafael Fridman,[§,⊥] Pratima Nangia-Makker,[‡] David E. Kleiner,[#] Lance A. Liotta,[#] William G. Stetler-Stevenson,[#] and Avraham Raz[*,‡,⊥,○]

Metastasis Research Program, Michigan Cancer Foundation, Detroit, Michigan 48201, Departments of Pathology and Radiation Oncology, Wayne State University, Detroit, Michigan 48201, and Laboratory of Pathology, National Cancer Institute, NIH, Bethesda, Maryland 20892

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ABSTRACT: The primary structure of galectin-3, a ~30 kDa galactoside-binding protein (aka CBP-35, mL-34, hL-31, L-29, Mac-2, and ϵBP), reveals two structural domains: an amino-terminal domain consists of a Pro-Gly-rich motif, and a globular carboxyl-terminal domain containing a carbohydrate-binding site. In this study, we report that the amino-terminal domain of galectin-3 contains a cleavage site for two members of the matrix metalloproteinase family of enzymes: the 72 kDa (gelatinase A, MMP-2) and the 92 kDa (gelatinase B, MMP-9) proteinases. The major cleavage site for the gelatinases in galectin-3 is at the Ala_{62}−Tyr_{63} bond, and its hydrolysis by these enzymes was inhibited by TIMP-2. Cell-surface expression of galectin-3 was reduced following treatment of viable T47D human breast carcinoma cells with gelatinase A. These results suggest that galectin-3 may be a substrate for gelatinases and that its degradation may play a role in modulating the biological activities of galectin-3.

The 72 (gelatinase A, MMP-2)[1] and 92 (gelatinase B, MMP-9) kDa enzymes are two endopeptidases that belong to the family of matrix metalloproteinases (MMP), a family of related zinc-dependent proteinases capable of degrading extracellular matrix (ECM) components (Matrisian, 1990; Stetler-Stevenson et al., 1993; Woessner, 1991). MMP-2 and MMP-9 have been suggested to play a role in the turnover of basement membranes due to their ability to degrade collagen IV to yield $^1/_4$ N-terminal and $^3/_4$ C-terminal fragments (Collier et al., 1988; Fessler et al., 1984; Wilhelm, 1989), and they have been associated with tumor invasion and metastasis (Liotta et al., 1991). The gelatinases have been shown to degrade other ECM components including collagens V, VII, and XI, fibronectin, laminin (Collier et al., 1988; Matrisian, 1990; Wilhelm et al., 1989; Woessner, 1991), elastin (Murphy et al., 1991; Senior et al., 1991), proteoglycans (Fosang et al., 1993; Murphy et al., 1991; Nguyen et al., 1993), and entactin (Sires et al., 1993) although with different efficiencies and probably involving different cleavage sites. The gelatinases can also degrade the Gly−Leu or Gly−Ile bond of denatured collagen I; however, they can also attack a variety of peptide bonds in other ECM components including the Asn−Phe bond in the interglobular domain of cartilage aggrecan (Whitelock et al., 1991), and His_{16}−Ile_{17} and Leu_{25}−Leu_{26} in cartilage link protein (Nguyen et al., 1993). MMP-2 has also been shown to hydrolyze the Lys_{16}−Leu_{17} peptide bond of a synthetic decapeptide representing the soluble β-amyloid sequence of amino acid residues 10−20 (Miyazaki et al., 1993). Thus, the gelatinases may also degrade other extracellular proteins.

The enzymatic activity of MMP-2 and MMP-9 is specifically inhibited by the tissue inhibitors of metalloproteinases (TIMPs) (Matrisian, 1990; Stetler-Stevenson et al., 1993; Woessner, 1991), a family of naturally occurring inhibitors of MMPs. TIMP-2, one member of the TIMP family, is known for its ability to form a complex with latent pro-MMP-2 (Goldberg et al., 1989; Stetler-Stevenson et al., 1989) and to inhibit active forms of both the 72 and 92 kDa enzymes (Woessner, 1991). TIMP-2 has been shown to inhibit *in vitro* tumor cell invasion (Albini et al., 1991). It has been suggested that a balance between TIMP-2 and activated gelatinases may determine the extent of ECM degradation that occurs during tumor cell invasion (Liotta et al., 1991). Several studies have shown that MMP-2 can be localized in the surface of tumor cells (Edmonard et al., 1992; Moll et al., 1990; Whitelock et al., 1991; Zucker et al., 1987). This association of gelatinase A with the cell membrane may facilitate zymogen activation (Brown et al., 1993; Strongnin et al., 1993; Ward et al., 1991) and substrate degradation in areas of cell−matrix contact. It is also possible that the broad substrate specificity of the gelatinases may allow the enzymes to attack other cell-surface proteins, thus affecting other cellular activities.

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* To whom correspondence should be addressed at the Michigan Cancer Foundation, 110 E. Warren Ave., Detroit, MI 48201. FAX: (313) 831-7518.

‡ Michigan Cancer Foundation.

§ The two authors share equal recognition for first authorship.

|| Present address: Department of Biochemistry, Meharry Medical College, School of Medicine, Nashville, TN 37211.

⊥ Department of Pathology, Wayne State University.

National Cancer Institute, NIH.

○ Department of Radiation Oncology, Wayne State University.

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[1] Abbreviations: APMA, *p*-aminophenylmercuric acetate; CBP-35, carbohydrate-binding protein 35; FACS, fluorescence-activated cell sorter; ECM, extracellular matrix; FITC, fluorescein isothiocyanate; galectin-3, ~30 kDa galactoside-binding lectin; hL-31, human galactoside-binding protein; ϵBP, IgE-binding protein; L-29, lactose-binding lectin; mAb, monoclonal antibody; MMP-2, matrix metalloproteinase-2; MMP-9, matrix metalloproteinase-9; mL-34, mouse galactoside-binding lectin; PA, plasminogen activator; PBS, phosphate-buffered saline; r, recombinant; TIMP, tissue inhibitor of metalloproteinase.

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Cells express on their surface carbohydrate-binding proteins containing the Pro-Gly-rich motif including galectin-3 (Barondes et al., 1994; Cherayil et al., 1990; Jia & Wang, 1991; Raz et al., 1989; Robertson et al., 1990). Although galectin-3 is thought to be involved in cell growth, differentiation, transformation, and metastasis, the mechanism of its *in vivo* action has not yet been fully elucidated. Galectin-3 contains two distinct structural domains: the amino-terminal half is composed of Gly-X-Y tandem repeats characteristic of collagens. The triplet repeats are modified by insertion of one or two additional amino acids which change the predicted secondary structure from the triple-helical structure of collagen to the β-sheet structure of galectin-3. Interruption in the consensus Gly-X-Y sequences has also been found in type IV collagen (Schuppan et al., 1980). The carboxy-terminal globular half which contains the carbohydrate-binding site for poly(*N*-acetyllactosamine) sequences is homologous with a lower molecular mass (~14 kDa) galactoside-binding lectin (Cherayil et al., 1990; Jia & Wang, 1988; Oda et al., 1991; Raz et al., 1989, 1991; Robertson et al., 1990). The recombinant or natural proteins are soluble or membrane-bound molecular species and can be cleaved by bacterial collagenases (Agrwal et al., 1993; Hsu et al., 1992; Ochieng et al., 1993; Raz et al., 1989). A recent study suggested that galectin-3 can be degraded by MMP-9 (Herrmann et al., 1993). However, direct demonstration of degradation of galectin-3 by this enzyme was not provided. In the present study, we demonstrate using purified MMP-2 that galectin-3 is cleaved by the gelatinases and have characterized the cleavage site.

EXPERIMENTAL PROCEDURES

Cells and Culture Conditions. The human breast carcinoma T47D cell line (obtained from Dr. E. Thompson, Georgetown University, Washington, D.C.) and HeLa cells (ATCC CCL22) were grown in Dulbecco's modified Eagle's minimal essential medium supplemented with glutamine, essential and nonessential amino acids, vitamins, antibiotics, and 10% heat-inactivated fetal bovine serum for T47D and 5% horse serum for HeLa cells. The cells were maintained at 37 °C in a humidified atmosphere of 5% CO_2/95% air.

Expression and Purification of Recombinant Human Galectin-3. Recombinant human galactoside-binding protein (galectin-3) was expressed in Escherichia coli as previously described in detail (Ochieng et al., 1993).

Electrophoresis. SDS−PAGE was performed under reducing conditions, using a 15% polyacrylamide separating gel and a 3.5% stacking gel. Samples were dissolved in a sample buffer and boiled for 5 min. The gels were stained with Coomassie blue, and the protein standards were prestained [low molecular weight markers (Bio-Rad)]. For immunoblots, the ^{14}C-labeled molecular weight markers (Amersham) were mixed with prestained protein standards to help in tracking the efficiency of transfer.

Human Recombinant 72 and 92 kDa Gelatinases and TIMP-2. Pro-MMP-2, pro-MMP-9, and TIMP-2 were expressed in a vaccinia virus−mammalian cell expression (Vac/T7) system as previously described (Fridman et al., 1992, 1993). The recombinant proenzymes were purified by affinity chromatography on a gelatin−Sepharose column (Sigma) as described (Collier et al., 1988).

Enzyme Activation. The gelatinases diluted in 5 mM Tris-HCl, 150 mM NaCl, 5 mM $CaCl_2$, and 0.02% Brij-35 buffer were activated (30 min, 37 °C) with a final concentration of 1 mM *p*-aminophenylmercuric acetate (APMA). The presence of active forms and the enzyme activity of the recombinant enzymes were determined by zymography and gelatinase assays using [^3H]gelatin (Fridman et al., 1992, 1993), respectively.

Immunoblotting. The samples from the slab gels were electroblotted to either nitrocellulose or Immobilon-P membranes. The membranes were quenched in 5% non-fat dried milk in PBS for 4 h and then incubated with the first antibody (rat anti-Mac-2 monoclonal antibody, ATCC TIB-166) or with monospecific antibody (990), raised in rabbit against a synthetic peptide of the putative galactoside-binding domain of mouse galectin-3 (Raz et al., 1989) for 1 h (23 °C) in the same quenching solution. The membrane was washed 5 times (10 min each) with the quenching solution containing 0.1% Tween-20 and subsequently incubated with the secondary antibody (^{125}I-sheep anti-rat IgG or ^{125}I-goat anti-rabbit IgG, respectively) for 1 h (23 °C) and washed as above.

Protein Iodination. Affinity-purified recombinant human galectin-3 was iodinated with carrier-free $Na^{125}I$ (ICN) following the Iodo-Beads method according to the manufacturer's instruction (Pierce).

Gelatinase Digestion and Protein Microsequencing. The human galectin-3 protein was treated with proenzymes or active enzymes at an enzyme:substrate ratio of 1:20 (w/w) in the presence or absence of TIMP-2 at a 1:1 molar ratio (enzyme:TIMP-2). The reaction was stopped with 2× sample buffer, and the samples were separated by reducing SDS−PAGE. For microsequencing, the digested products were electroblotted onto Immobilon-P (Millipore) at 180 mA for 1.5 h using glycine-free CAPS buffer (10 mM CAPS/10% methanol, pH 11). After transfer, the membrane was washed in double-distilled water and stained (0.2% Coomassie brilliant blue/50% methanol). After destaining, the digested band was cut out and N-terminal-sequenced at the Wayne State University Macromolecular Core Facility using the Model 470 gas phaser−sequencer (Applied Biosystems).

FACS Analysis. The anti-Mac-2 TIB-166 mAb, in 0.02% sodium azide, was added to 10^6/mL untreated and activated MMP-2-treated T47D cells (1 μg/mL activated enzyme for 30 min at 37 °C). The control cells were incubated identically without the mAb. After 1 h at 0 °C, the cells were washed twice with PBS, and then FITC-conjugated anti-rat antibody (Zymed; 1:10) was used as the secondary antibody. After 1 h at 4 °C, the cells were washed twice in PBS, and cell-surface fluorescence was analyzed using a FACS (FACScan; Becton Dickinson, Mountain View, CA). A scatter window was set to eliminate dead cells and cell debris. The frequency and fluorescence profiles of the stained cells were determined using a laser output of 125 mV.

RESULTS

Gelatinase Degradation of Galectin-3. The ability of mammalian gelatinases to cleave human galectin-3 was investigated here. To this end, [^{125}I]galectin-3 (20 ng/reaction) was incubated (30 min, 37 °C) with either latent or APMA-activated 72 and 92 kDa gelatinases (1 ng/reaction). As shown in Figure 1, both enzymes were able to cleave [^{125}I]galectin-3, generating a major cleavage product

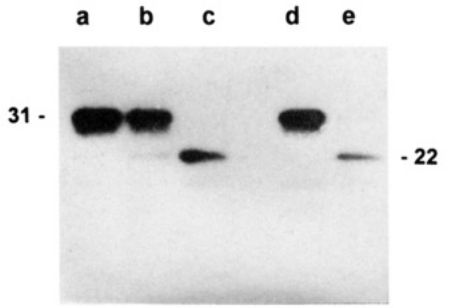

FIGURE 1: Cleavage of human recombinant galectin-3 with recombinant gelatinases. Latent 72 (lane b) or 92 kDa (lane d) progelatinases or APMA-activated 72 (lane c) or 92 kDa (lane e) gelatinases (1 ng/reaction) were incubated (30 min, 37 °C) with [^{125}I]galectin-3 (20 ng/reaction) in collagenase buffer as described under Experimental Procedures. The reaction products were electrophoresed under reducing conditions in a 15% SDS−PAGE and visualized by autoradiography. As control, [^{125}I]galectin-3 was incubated alone in collagenase buffer as indicated by its 31 kDa molecular mass (lane a). Lanes c and e show the major 22 kDa cleavage products by the activated enzymes.

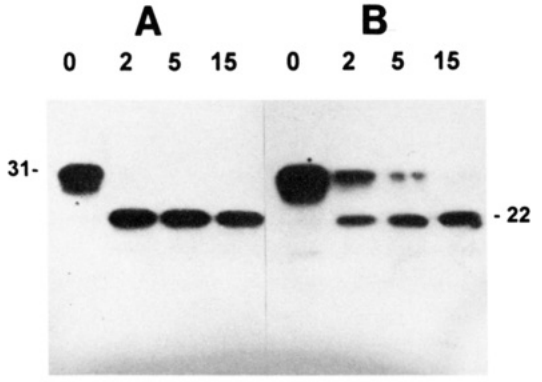

FIGURE 2: Time course degradation of human galectin-3 by activated gelatinases. [^{125}I]Galectin-3 was incubated with activated 72 kDa gelatinase (A) or 92 kDa gelatinase (B) as described in Figure 1. Aliquots were removed at the indicated times (minutes, on top), diluted with sample buffer, and electrophoresed in a 15% SDS−PAGE under reducing conditions.

of 22 kDa, an obvious difference from the degradation products reported with bacterial collagenase (Agrwal et al., 1993; Hsu et al., 1992; Ochieng et al., 1993; Raz et al., 1989, 1991; Schuppan et al., 1980). Degradation of [^{125}I]galectin-3 required enzyme activation since the progelatinases were not active against galectin-3 (Figure 1b,d). Time course experiments showed that complete degradation of [^{125}I]-galectin-3 to the 22 kDa protein with the 72 kDa enzyme was achieved as early as 2 min after incubation (Figure 2A) whereas the 92 kDa enzyme required a somewhat longer time (5−15 min) to generate the 22 kDa product (Fig 2B).

Previously it has been shown that the activity of the recombinant gelatinases against gelatin and collagen type IV is inhibited by TIMP-2 in a stoichiometric manner (Fridman et al., 1992, 1993). As shown in Figure 3, preincubation of activated 72 kDa gelatinase with an equal molar amount of TIMP-2 significantly reduced the ability of the enzyme to degrade [^{125}I]galectin-3 (Figure 3c). A similar result was observed with the 92 kDa enzyme (not shown). The addition of lactose (100 mM) to the reaction mixture had no effect on the degradation of galectin-3, suggesting that the occupancy of its sugar-binding domain does not affect the degradation by gelatinases.

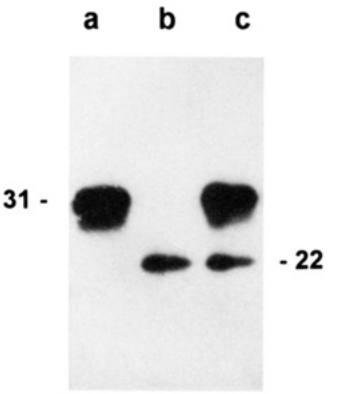

FIGURE 3: Effect of TIMP-2 on degradation of galectin-3 by 72 kDa gelatinase. Latent r72 progelatinases (1 ng/reaction) was activated with 1 mM APMA as described under Experimental Procedures and then incubated (15 min, 22 °C) with an equal molar amount of rTIMP-2. The enzyme−inhibitor complex (lane c) or the activated 72 kDa gelatinase (lane b) was then incubated with ^{125}I-labeled human galectin-3 as described in Figure 1. Degradation products separated in a 15% SDS−PAGE under reducing conditions were visualized by autoradiography. Lane a, untreated ^{125}I-labeled human galectin-3.

Determination of the Gelatinase Cleavage Site of Galectin-3. The cDNA sequences of the human Mac-2 antigen were determined (Cherayil et al., 1990) [hL-31 (Raz et al., 1991), ϵBP (Robertson et al., 1990)]. Although the three sequences were found to be highly homologous, they were at variance in the collagen-like amino-terminal domain of the molecule at the region from amino acid residue 32 to amino acid residue 78. Subsequently, L-29 was cloned (Oda et al., 1991), and more recently Mac-2 was recloned by RNA−PCR (Lotz et al., 1993). The two latter sequences were found to be identical to the ϵBP sequence except for allelic variation (Lotz et al., 1993) which promoted the need to reevaluate the hL-31 cDNA sequence for probable sequencing error. Using new sequencing kits and Long Ranger Acrylamide Gel Solution (J. Baker, Inc.), it becomes apparent that the stretch in question (amino acid residues 32−78) is rich in compressed GC domains which previously resulted in gel reading errors leading to a frame shift in that region. Thus, the deposit of hL-31 to the GenBank (Accession Number M64303) was corrected.

As seen in Figure 1, a major breakdown product of gelatin-3 after treatment with the 72 kDa enzyme is a 22 kDa polypeptide. Amino acid analysis revealed that the amino-terminal sequence of the 22 kDa product is Tyr-His-Gly-Ala-Pro-Ala-Tyr-Pro-Gly, suggesting that the MMP-2 cleavage site is at a locus in the middle of the NH_2-terminal half of the molecule between the Ala_{62}−Tyr_{63} bond. No secondary contaminating sequences were detected by this analysis. This site is different from the bacterial collagenase D and A cleavage sites, which led to the generation of polypeptides with the following NH_2 ends: Ala-Gly-Pro-Tyr-Gly-Val and Gly Pro-Leu-Val, respectively (Agrwal et al., 1993; Hsu et al., 1992). To the best of our knowledge, the only other report for 72 kDa cleavage at a X−Tyr site is the autocleavage site of the 72 kDa proenzyme during generation of the active species (Stetler-Stevenson et al., 1989). A similar degradation profile of galectin-3 was obtained with a natural human 72 kDa gelatinase A isolated from conditioned media of tumor cells (Stetler-Stevenson et al., 1989). Immunoblot analysis of gelatinase A treated

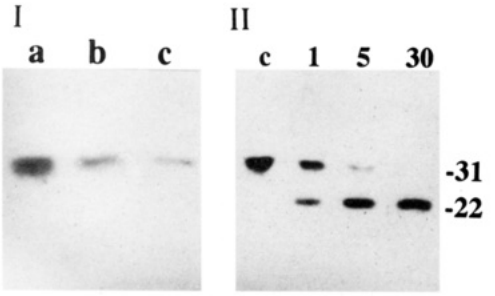

FIGURE 4: (I) Western blot analysis of cleaved recombinant human galectin-3 with APMA-activated native MMP-2. Equal amounts of galectin-3 were loaded in each lane. The migration of galectin-3 and its degradative products was determined using the TIB-166 anti-Mac-2 mAb as described under Experimental Procedures. a, control, untreated galectin-3; b, 5 min; c, 30 min duration of enzyme treatment. (II) Western blot analysis of the time course of degradation of galectin-3 by active natural MMP-2 at a 1:20 ratio of enzyme to galectin-3. Numbers on the top represent in minutes the duration of enzyme treatment. Enzyme degradation proceeded for the indicated times (minutes, on top): aliquots were diluted with sample buffer, separated by SDS−PAGE, and electroblotted onto Immobilon-P. The migration of galectin-3 and its degradative product was determined using the 990 monospecific polyclonal antibody as described under Experimental Procedures. c, control.

and untreated galectin-3 was performed using the TIB-166 anti-Mac-2 mAb. As shown in Figure 4Ia, TIB-166 recognizes intact galectin-3 as one band. Following enzyme treatment, the intensity of the 31 kDa band was markedly reduced after 5 (Figure 4Ib) and 30 min (Figure 4Ic) of enzyme treatment. Apparently, the TIB-166 mAb failed to recognize any lower molecular mass species than the 31 kDa cleaved by the enzyme. These results suggest that the TIB-166 mAb antigenic recognition site on galectin-3 is at or near the MMP-2 cleavage site in the collagen-like domain of the molecule.

We have previously generated a monospecific polyclonal antibody against the putative galactoside-binding domain of galectin-3, which resides in the carboxy terminus of the molecules (Raz et al., 1989). Such an antibody should be useful for the evaluation of galectin-3 species generated by MMPs cleavage of the collagen-like domain of the amino terminus of human galectin-3. The natural MMP-2 active enzyme was incubated with excess amounts of the substrate galectin-3 for 1, 5, and 30 min at 37 °C, and the reactions were terminated by boiling in SDS−PAGE sample buffer. The 1 min incubation period is the minimal time needed to raise the reaction mixture temperature from 0 to 37 °C. As shown in Figure 4II, the enzyme cleaved galectin-3, generating, in a time-dependent manner, a single immunodetectible cleavage product of 22 kDa as shown in Figure 1. These results suggest that the amino-terminal end of truncated galectin-3 is not further processed by gelatinase A.

Effect of MMP-2 on the Cell-Surface Expression of Galectin-3. Thus far, we have shown that MMPs can cleave soluble galectin-3; next we question whether the cell-surface-bound galectin-3 is also accessible to MMP-2. To determine this, we analyzed the cell-surface expression of galectin-3 on T47D human breast carcinoma cells. The cells (10^6) were pretreated with 1 μg/mL activated natural gelatinase A (30 min at 37 °C), immunofluorescently labeled with TIB-166 mAb and FITC−goat anti-rat IgG, and quantitatively analyzed by FACS. As seen in Figure 5, gelatinase A treatment significantly reduced the cell-surface expression of galectin-3. It should be noted that this difference is not due to nonspecific binding of the FITC-conjugated anti-rat secondary antibody, since the binding patterns of anti-rat antibody to the untreated control and MMP-2-treated cells without the first mAb were indistinguishable (Figure 5).

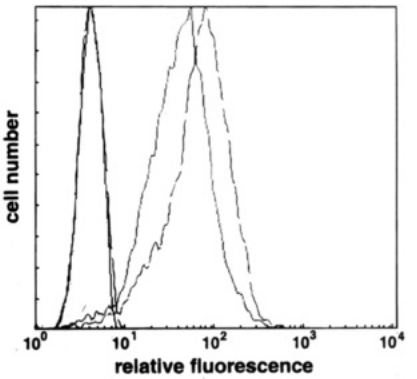

FIGURE 5: Cell-surface staining with TIB-166 mAb by flow cytometry of T47D human breast carcinoma cells. Cells were labeled with TIB-166 mAb at 4 °C, washed and reincubated with FITC−rabbit anti-rat IgG, and analyzed in the FACScan. A scatter window was set to eliminate dead cells and cell debris. (- - -) Control untreated cells; (—) cells treated with APMA-activated native MMP-2. The two overlapping peaks to the left represent control and enzyme-treated cells incubated with only the second antibody.

DISCUSSION

Carbohydrate-binding proteins present on the cell surface or in the extracellular matrices are important in the specification of cell−cell recognition and cell−ligand(s) interactions [for a review, see Sharon and Lis (1989)]. Among them are galectin-3 [mL-34 (Raz et al., 1989), hL-31 (Raz et al., 1991), ϵBP (Robertson et al., 1990), CBP-35 (Jia & Wang, 1988), Mac-2 (Woo et al., 1990), and L-29 (Oda et al., 1991)]. These molecules were localized in the cytosol, in the nucleus, and on the cell surface and are thought to be involved in cell growth, differentiation, and metastasis. The exact function of galectin-3 *in vivo* remains unknown. Galectin-3 is composed of two distinct halves: an amino-terminal domain containing a collagen-like sequence characterized by Gly-X-Y tandem repeats and a globular carboxy-terminal domain containing the carbohydrate recognition site. It has recently been shown that exhaustive bacterial collagenase degradation of both the murine and the human recombinant homologues of galectin-3 generated a carboxy-terminal polypeptide capable of maintaining the sugar-binding properties of the uncleaved molecule (Agrwal et al., 1993; Hsu et al., 1992; Ochieng et al., 1993; Raz et al., 1989). On the basis of the studies, it was concluded (a) that galectin-3 has one sugar-binding site in the carboxy-terminal domain, (b) sugar binding is independent of the thiol-reducing condition, (c) the amino-terminal domain is not essential for carbohydrate binding, and (d) the collagen-like amino-terminal domain is probably involved in the dimerization of the molecule. Previous reports (Agrwal et al., 1993; Hsu et al., 1992; Ochieng et al., 1993; Raz et al., 1989) have shown that galactin-3 has multiple bacterial collagenase cleaving sites and that exhaustive digestion of the molecule results in the appearance of a single polypeptide of ~18 kDa which maintains its carbohydrate-binding properties. These observations led to the question of whether galectin-3 can also be

a substrate for mammalian metalloproteinases. In a recent study (Herrmann et al., 1993), it was suggested that the 92 kDa gelatinase can degrade galectin-3 isolated from rat lungs. However, direct evidence using purified enzyme was not provided. Here we show that galectin-3 is cleaved by both purified MMP-2 and MMP-9, and that its degradation is inhibited by TIMP-2, a specific MMP inhibitor. The cleavage site was identified following microsequencing of the generated peptide and was found to be between the Ala_{62}–Tyr_{63} bond which is different from the cleavage site of bacterial collagenases [X-Gly-Pro, X-Gly-Ala, or Gly-X-Pro-Gly (Agrwal et al., 1993; Hsu et al., 1992)]. This site is also different from that reported by Herrmann et al. (1993) after using a partially purified preparation of rat galectin-3 and a copurified metalloproteinase, furthermore, Phe_{119}–Gly_{120} bond cleaved in the rat galectin-3 is not present in the human galectin-3 sequence (Oda et al., 1991; Lotz et al., 1993; M64303). The cleavage of non-collagen Gly-X-X-Gly repeat polypeptides by the gelatinases is supported by (1) the autocleave domain of the enzyme between the Asn_{80}–Tyr_{81} bond for enzymic conversion from latent to active (Stetler-Stevenson et al., 1989), (2) the cleavage of the membrane-bound precursor protein of the amyloid β-protein at Lys_{16}–Leu_{17} bonds (Miyazaki et al., 1993), and (3) the cleavage of the Asn–Phe bond in aggrecan (Fosang et al., 1993). The cleavage of galectin-3 was not restricted to recombinant gelatinases since the natural MMP-2 effectively cleaved galectin-3 and generated the same degradation products. Time course analysis of galectin-3 degradation revealed that the protein is degraded to a 22 kDa species after 1 min incubation, that no other protein bands were detected, and that, most probably, the Ala_{62}–Tyr_{63} bond is the initial MMP cleavage site and the newly generated amino terminus is not further processed. These results probably exclude the possibility of multiple cleavage sites nearer to the N-terminal end of the intact molecule, since otherwise additional bands larger than 22 kDa should have been detected. Whether or not the amino-terminal polypeptide is further degraded by the enzymes is unknown. An ancillary observation is presented in Figure 4I and reveals that the epitope of the widely used TIB-166 anti-Mac-2 mAb is at or near Ala_{62}–Tyr_{63} as the antibody recognized only the intact molecule. In addition, cells expressing membrane-bound galectin-3 show a reduced immunoreactivity of galectin-3 after exposure to MMP-2, suggesting that the cell-surface galectin-3 may serve as a substrate for MMP-2.

Several other carbohydrate-binding proteins, such as the mannose-binding protein, the lung surfactant protein A, and conglutinin, were reported to have similar structural similarities to galectin-3. They contain a short NH_2-terminal of non-collagen-like sequence, followed by a collagenous region of repeating Gly-X-Y sequence, and a carboxy-terminal containing the carbohydrate recognition site (Voss et al., 1988). All of these molecules were found to be recognized by the complement subcomponent Clq receptor through their collagenous domain (Voss et al., 1988). Thus, it is possible that these molecule and galectin-3 can also serve as substrates for enzymes recognizing collagen-like sequences which may regulate their activity *in vivo*.

The presence of MMP-2 on the cell surface of tumor cells (Emonard et al., 1992; Moll et al., 1990; Whitelock et al., 1991; Zucker et al., 1987) may facilitate the degradation of cell-surface proteins such as galectin-3 as shown here. Since expression of galectin-3 has been shown to be associated with cell–cell and cell–matrix interactions and with malignant transformations and metastasis formation, proteolytic cleavage of galectin-3 may modulate the biological functions of this protein affecting a variety of cellular processes. If that is the case, then it may be suggested that an additional function for gelatinases is as a regulator of the activity of galectin-3. The relationship between gelatinases and galectin-3 in metastasis formation is now under investigation.

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