The endo-β-D-glucuronidase, heparanase, is capable of specifically degrading heparan sulfate, and this activity is associated with the metastatic potential of tumor cells. The predicted amino acid sequence of heparanase includes six putative N-glycosylation sites; however, the precise biochemical role of glycosylated heparanase remains unknown. In this study, we examined the link between glycosylation and the function of heparanase in human tumor cell lines. Heparanase protein was glycosylated at six Asn residues in human tumor cell lines. Treatment with a glycosylation inhibitor demonstrated that glycosylation was not required for the activity of heparanase. However, glycosylation affected the kinetics of endoplasmic reticulum-to-Golgi transport and secretion of the enzyme.

Heparan sulfate (HS)\(^1\) and heparan sulfate proteoglycans (HSPGs) that are located in the extracellular matrix and on the external surface of cell membranes play a major role in cell-cell and cell-extracellular matrix interactions (1–3). Various molecules have been reported to interact with HS/HSPGs. These include growth factors (e.g. fibroblast growth factors), cytokines (e.g. interleukin-2), extracellular matrix proteins (e.g. collagens), factors involved in blood coagulation (e.g. heparin cofactor II), and other proteins including β-amylloid proteins (1–3).

Because of the important and multifaceted roles of HSPGs in cell physiology, their cleavage is likely to alter the integrity and functional state of tissues, and this may provide a mechanism by which cells can respond rapidly to changes in the extracellular environment (4–9). Enzymatic degradation of HS by heparanase is therefore likely to be involved in fundamental biological phenomena, ranging from pregnancy, morphogenesis, and development to inflammation, angiogenesis, and cancer metastasis. Recently, several groups independently reported the cloning of human heparanase and examined its expression in human tumors and tumor cell lines (15–18). In the case of N-linked glycans, more than 30 enzymes, located in the cytosol, the endoplasmic reticulum (ER), and the Golgi apparatus, are required to generate, attach, and process the oligosaccharides (20–23). As the nascent glycoprotein enters the ER, a preformed oligosaccharide known as the dolichol-phosphate precursor is attached co-translationally to certain asparagine residues that are part of the consensus sequence Asn-Xaa-Ser/Thr (where Xaa represents any amino acid except Pro). Many functions have been described for protein glycosylation, including promoting protein folding in the ER, stabilizing cell surface glycoproteins, and providing recognition epitopes that activate the innate immune system (19). In contrast to the N-glycosylation system, several nuclear and cytoplasmic proteins are O-glycosylated at specific serine or threonine hydroxyl groups. Moreover, O-glycosylation and phosphorylation are reciprocal, occurring at the same or adjacent hydroxyl moieties in some cases (24, 25).

In this report, we demonstrate that glycosylation of heparanase is not required for its cleavage activity; however, it is important for the kinetics of ER-to-Golgi transport and secretion in human tumor cell lines. Our data suggest that glycosylated heparanase may be responsible for heparanase-mediated tumor cell invasion and that inhibitors of glycosylation may provide effective therapeutic drugs for those human cancers that are expressing heparanase.

**EXPERIMENTAL PROCEDURES**

**Establishment of Heparanase-overexpressing Stable Cell Lines**—The human heparanase gene was cloned into the pGEM-T Easy vector (Promega, Madison, WI). An EcoRI fragment of pGEM-T Easy vector-inserted human wild-type heparanase was subcloned into pcDNA3.1Myc-His(+) vector (Invitrogen). The permanent cell lines expressing heparanase-Myc-His were established by transfecting pcDNA3.1Myc-His(+) heparanase into HepG2 cells followed by G418 selection. The cells transfected with pcDNA3.1Myc-His(+) vector were designated HepG2-Neo, and the clone expressing a high level of heparanase protein was designated HepG2-HP. To detect heparanase protein in the cell lines, we carried out Western blot analysis using anti-Myc antibody.
Construction of Heparanase Mutants—We substituted some asparagine residues that include six putative N-glycosylation sites in heparanase protein with glutamine residues by PCR site-directed mutagenesis using the technique of overlap extension (26).

Western Blotting—Cells were untreated or treated with various concentrations of tunicamycin A (Calbiochem) and lysed in lysis buffer (10 mM HEPES, 142.5 mM KCl, 5 mM MgCl₂, 1 mM EGTA, 0.2% Nonident P-40, 0.1% aprotinin, and 1 mM phenylmethylsulfonyl fluoride, pH 7.2) at 4°C with sonication. The lysates were centrifuged at 14,000 rpm for 15 min, and the amount of protein in each lysate was measured by staining with Coomassie Brilliant Blue G-250 (Bio-Rad). Loading buffer (42 mM Tris-HCl, pH 6.8, 10% glycerol, 2.3% SDS, 5% 2-mercaptoethanol, and 0.002% bromphenol blue) was then added to each lysate, which was subsequently boiled for 3 min and electrophoresed on an SDS-polyacrylamide gel. Proteins were transferred to polyvinylidene difluoride membrane and immunoblotted with anti-heparanase (BD Biosciences, San Jose, CA), anti-Myc (Babco, Richmond, CA) and anti-α-tubulin (Sigma) antibodies. Detection was performed with enhanced chemiluminescence reagent (Pierce).

In Vitro Glicosidase Treatment—The cells were lysed in TENV buffer (27, 28). One-ml aliquots of cell lysates were incubated with anti-Myc antibody at 4°C for 2 h. The immune complexes in the cell lysates were precipitated by centrifugation, and the pellets were boiled for 3 min to inactivate endogenous enzymes and subsequently incubated with either 50 milliunits of endoglycosidase H (Endo-H; Roche Applied Science) or 5 units of peptide N-glycosidase F (PNGase-F; Roche Applied Science) at 37°C for 18 h. The samples were electrophoresed and immunoblotted with anti-Myc antibody (Medical and Biological Laboratories, Nagoya, Japan).

Measurement of Heparanase Activity—The cells were washed, suspended in PBS (pH 6.0), and disrupted by four cycles of freezing at −70°C and thawing at 37°C. The lysates were centrifuged at 10,000 rpm for 10 min at 4°C, and the amount of protein in cell lysates was measured with Coomassie Brilliant Blue G-250 (Bio-Rad). A mixture of 90 μl of cell lysate (2.0 mg/ml) and 10 μl of HS solution (10 mg/ml in PBS, pH 6.0) was incubated for 24 h at 37°C. Following the addition of 20 μl of sampling solution (36% glycerol, 1% bromphenol blue), 10 μl of each reaction mixture was subjected to SDS-PAGE (20%). The electrophoresed gel was soaked in water for 2 h to remove SDS, stained with 0.1% methylene blue in 50% ethanol for 1 h, and then destained with water for 3 h (29, 30).

Fluorescence Microscopy—Transient transfection of HeLa cells using vectors that encode Myc-tagged wild-type or mutant form of heparanase was used to generate material for examination by fluorescence microscopy. After 24 h, transfected cells grown on coverslips were fixed with 4% paraformaldehyde in PBS. After washing with PBS, they were incubated in 0.1% Triton X-100 in PBS for 5 min, washed once with PBS, and incubated with an anti-Myc antibody for 30 min. Alexa 488-conjugated anti-mouse IgG (Molecular Probes, Inc., Eugene, OR) was used as secondary antibody. After washing three times with PBS and were examined using a fluorescence microscope (Olympus, Tokyo, Japan). Intracellular organelles were labeled with anti-Lamp-1 (lyosomes; BD Transduction, Lexington, KY), anti-GM130 (Golgi apparatus; BD Transduction), anti-BiP/GRP78 (ER; BD Transduction), or anti-EEA1 (endosomes; BD Transduction) antibodies.

Assessment of Secreted Protein—Exponentially growing cells, seeded at the same cell density, were washed and cultured with serum-free medium for 24 h. The conditioned media were collected by centrifugation at 15,000 rpm for 15 min at 4°C, and loading buffer was added to the supernatant, which was subsequently boiled for 3 min and electrophoresed on an SDS-polyacrylamide gel. The cell lysates were prepared as described before. The amounts of heparanase proteins in both cell lysates and culture media were measured by Western blotting using anti-Myc antibody. Band intensities were quantified using an MD Scanning Imager equipped with MD ImageQuant Software Version 3.22 (Amersham Biosciences). Apolipoprotein E (apoE), α₁-antitrypsin (AT), and albumin were monitored as the controls of the secreted proteins in HepG2 cells (32, 33), measured by Western blotting using anti-apoE antibody (Santa Cruz Biotechnology, Inc., Santa Cruz, CA), anti-AT antibody (Organon Teknika Corp., Durham, NC), and anti-albumin antibody (Organon Teknika Corp.), respectively. The indexes of secretions are expressed by the band intensity of conditioned medium divided by that of cell lysate.

RESULTS

Glycosylation of Heparanase—The primary amino acid sequence of heparanase contains six predicted N-linked glycosylation consensus sequences (Fig.1A). To clarify the role of glycosylation of heparanase for its functions, initially we established a C-terminal Myc- His-tagged heparanase-overexpressing cell line, HepG2-HP, and examined whether heparanase is

FIG.1. Glycosylation of heparanase in the cells. A, schematic diagram of human heparanase. A black box denotes signal peptide (SP), and a hatched bar denotes the propeptide (PP). The location of the six putative N-linked glycosylation sites (Asn-162, Asn-178, Asn-200, Asn-217, Asn-238, and Asn-459) are indicated by solid circles, and the putative catalytic proton donor (Glul-225), and nucleophile (Glu-343) residues are indicated by asterisks. The domain boundaries are numbered. B, inhibition of heparanase glycosylation by the treatment with TM. Exponentially growing HepG2-Neo and HepG2-HP cells were treated with various concentrations of TM for 24 h. Aliquots of cell lysates were immunoblotted with the indicated antibodies (Ab). C, deglycosylation of heparanase by glycosidases in vitro. HepG2-HP cells were lysed, and aliquots of cell lysates were immunoprecipitated (IP) with anti-Myc antibody. The resulting immunoprecipitates were incubated with Endo-H or PNGase-F for 18 h. The samples were electrophoresed and immunoblotted with anti-Myc antibody. D, glycosylation of endogenous heparanase in HeLa cells. Exponentially growing HeLa cells were treated with various concentrations of TM for 24 h. Aliquots of cell lysates were immunoblotted with anti-heparanase or anti-α-tubulin antibodies.

Glycosylation of Heparanase—The primary amino acid sequence of heparanase contains six predicted N-linked glycosylation consensus sequences (Fig.1A). To clarify the role of glycosylation of heparanase for its functions, initially we established a C-terminal Myc- His-tagged heparanase-overexpressing cell line, HepG2-HP, and examined whether heparanase is
glycosylated in these cells. Treatment of the HepG2-HP cells with tunicamycin A (TM), an inhibitor of glycosylation, resulted in a size reduction of heparanase as detected by electrophoresis and Western blotting using anti-Myc antibody (Fig. 1B). One μg/ml of TM resulted in a partial inhibition of N-glycosylation, whereas 10 μg/ml of TM inhibited N-glycosylation completely (Fig. 1B). Similar results were observed in Myc-His-tagged heparanase-overexpressing HT1080 cells (data not shown). To further examine whether heparanase was indeed glycosylated in the HepG2-HP cells, anti-Myc immunoprecipitates were incubated with Endo-H or PNGase-F in vitro (Fig. 1C). Endo-H selectively removes high mannose-type oligosaccharides from glycoproteins, but it does not cleave complex glycans. Because the conversion of high mannose oligosaccharides into complex glycans occurs in the medial-Golgi compartment, resistance to Endo-H is an indication that a glycoprotein has reached this compartment. The sensitivity to PNGase-F, an enzyme that removes all types of N-linked oligosaccharides from glycoprotein, is an indication of N-linked protein glycosylation. Endo-H treatment resulted in a smear of bands suggesting that heparanase contained a mixture of both ER-derived high mannose residues and also Golgi-derived complex oligosaccharides (Fig. 1C). Complex glycosylation of heparanase was confirmed by PNGase-F treatment. PNGase-F treatment generated a major 50-kDa band, which is consistent with the molecular weight deduced from the amino acid sequence of heparanase (unglycosylated form). Endogenous heparanase of HeLa cells was also reduced in molecular size by the treatment with TM (Fig. 1D). Thus, these data suggest that heparanase is glycosylated in human tumor cell lines.

Glycosylation of Heparanase at Six Putative Glycosylation Sites—To identify the glycosylation site(s) within the heparanase, we constructed several mutant forms of the protein in which asparagines were substituted by glutamines. The electrophoretic migration of heparanase prepared from HepG2-HP/N162Q, HepG2-HP/N178Q, HepG2-HP/N200Q, HepG2-HP/N217Q, HepG2-HP/N238Q, HepG2-HP/N459Q, HepG2-HP/6NQ, and HepG2-HP/N203Q cells were untreated or treated with 10 μg/ml TM for 24 h, and lysed. Aliquots of cell lysates were immunoblotted with anti-Myc or anti-α-tubulin antibodies (Ab).

Effect of Glycosylation on Heparanase Activity—We next examined the effect of glycosylation on heparanase activity based on the digestion of HS and assessment of activity by fragment separation in an SDS-polyacrylamide gel. HepG2-Neo and HepG2-HP cells were treated or untreated with TM, and cell extracts were prepared and incubated with HS from bovine kidney. As shown in Fig. 3, the sample incubated with extract from HepG2-HP cells showed broader smears and higher levels of partially digested HS migrating toward the bottom of the gel compared with the samples from HepG2-Neo cells. The extracts from the TM-treated and -untreated HepG2-HP cells possessed similar heparanase activities, suggesting that glycosylation of heparanase is not involved in activation and activity (Fig. 3).

Effect of Glycosylation on Intracellular Trafficking of Heparanase—In order to characterize the intracellular trafficking of heparanase, we carried out double staining analysis in transiently transfected HeLa cells. Endosome-specific (anti-EEA1), lysosome-specific (anti-Lamp-1), Golgi apparatus-specific (anti-GM130), and ER-specific (anti-BiP/GRP78) antibodies were used to label organelles. Heparanase protein stained with anti-Myc antibody was detected in perinuclear organelle and diffused within the cells (Fig. 4E). Co-staining with anti-Myc antibody and organelle-specific antibodies demonstrated that heparanase inside HeLa cells resided within the ER and the Golgi apparatus (Fig. 4, R and V), suggesting that intracellular trafficking of heparanase protein might be ER-to-Golgi apparatus in human tumor cell lines. It is possible that transient expression is accompanied by an extreme overexpression of the molecule, which potentially changes its intracellular trafficking; however, treatment with 0.1 μg/ml brefeldin A, an inhibitor of intracellular transport of secretory proteins (34), in HepG2-HP and HT1080-HP cells, which are stably transfected, inhibited secretion of heparanase protein into the culture media (data not shown). Thus, these data revealed that intracellular trafficking of heparanase is ER-to-Golgi apparatus in these cell lines.

To identify the glycosylation site(s) of heparanase that enable the protein to be transported to the Golgi apparatus, we examined the location of the point-mutated heparanase proteins described previously (Fig. 2). Wild-type HP, HP/N162Q, HP/N178Q, HP/N200Q, HP/N217Q, and HP/N238Q were localized within the ER and Golgi apparatus (Fig. 5, 8, 11, 14, 17, and 20). In contrast, the amounts of HP/N459Q and HP/6NQ proteins in the Golgi apparatus were reduced compared with that of wild-type heparanase (Fig. 5, 23 and 26).

Effect of Glycosylation on Secretion of Heparanase—Subse-
quently, to examine the effect of glycosylation on secretion of heparanase, we compared the amount of heparanase protein in cell lysate with that of conditioned media in wild-type and mutant heparanase-expressing cells. As shown in Fig. 6A, in HP/N162Q-, HP/N178Q-, HP/N238Q-, HP/N459Q-, and HP/6NQ-expressing cells, trace amounts of heparanase proteins in culture medium were observed, and the indexes of secretion were less than 0.1; however, the secretion of HP/N200Q and HP/N217Q was partially suppressed (0.24 and 0.35 for HP/N200Q and HP/N217Q, respectively) (Fig. 6B). AT, apoE, and albumin are known to be secreted in culture medium in HepG2 cells (32, 33). The secretion levels of AT, apoE, and albumin were not affected by expression of wild-type and mutant heparanase proteins (Fig. 6A and data not shown), suggesting that expression of mutant heparanase did not affect the kinetics of general trafficking system in the cells. Moreover, the stabilities of wild-type and deglycosylated form heparanase in culture medium were observed, and the indexes of secretion were less than 0.1; however, the secretion of HP/N200Q and HP/N217Q was partially suppressed (0.24 and 0.35 for HP/N200Q and HP/N217Q, respectively) (Fig. 6B). AT, apoE, and albumin are known to be secreted in culture medium in HepG2 cells (32, 33). The secretion levels of AT, apoE, and albumin were not affected by expression of wild-type and mutant heparanase proteins (Fig. 6A and data not shown), suggesting that expression of mutant heparanase did not affect the kinetics of general trafficking system in the cells. Moreover, the stabilities of wild-type and deglycosylated form heparanase in culture medium were almost the same (data not shown). Since expression of mutant heparanase did not affect the general trafficking system and the stability of mutant heparanase in the culture media was similar compared with wild-type, it is revealed that deglycosylation at Asn-200 and Asn-217 is partially affected, but deglycosylation at Asn-162, Asn-178, Asn-238, and Asn-459 is dramatically affected for the kinetics of the secretion of heparanase.

DISCUSSION

Invasion and secondary spread through the blood and lymphatic system are characteristic features of malignant tumors. Invasion represents one of the greatest impediments to curing cancer. Many mechanisms are involved in this complex process, and it is thought that cell adhesion molecules and cell surface HSPGs are important factors in the regulation of cell differentiation, morphology, and migration (1–5). Heparanase is an endoglucuronidase that specifically degrades heparan sulfate, and its activity is associated with the metastatic potential of tumor cells. High levels of heparanase mRNA expression are observed in many human tumors and tumor cell lines (15–18); however, the molecular mechanisms regulating heparanase activity have not been fully elucidated.

In this report, we demonstrate that heparanase is glycosylated at six asparagine residues (Figs. 1 and 2). Although it is known that glycosylation is required for the activity of certain proteins, such as MD-2 (35), it has been reported that deglycosylation of baculovirus-produced heparanase by treatment with PNGase-F had no detectable effect on enzymatic activity (11). Thus, two possible explanations had been suggested: 1) once heparanase is glycosylated and activated, the glycosylation itself is no longer required for enzyme activity, or 2) glycosylation is not required for either activation or activity. We showed that treatment of cells with TM did not affect the heparanase activity (Fig. 3). Therefore, we conclude that glycosylation of heparanase does not contribute to either activation or activity. We also examined the effect of deglycosylation of heparanase on substrate specificity against other glycosaminoglycans, such as heparin and chondroitin sulfate. Heparanase partially digested heparin but did not cleave chondroitin sulfate (data not shown). The TM-treated cells were identical to nontreated cells in the degree of digestion of heparin and of chondroitin sulfate (data not shown), indicating that glycosylation of heparanase does not affect substrate specificity.

**Fig. 4. Intracellular trafficking of heparanase in HeLa cells.** HeLa cells were transiently transfected with pcDNA3.1Myc-His(1100)-Mock (A–C) or pcDNA3.1Myc-His(1100)+heparanase (Heparanase) (D–V) for 24 h. The cells were fixed, stained with anti-Myc (green) (B, E, I, M, Q, and U) and anti-Lamp-1 (red) (K), anti-GM130 (red) (O), anti-BiP/GRP78 (red) (S), or anti-EEA1 (red) (G) antibodies and Hoechst 33258 (blue) (A, D, H, L, P, and T), and observed under fluorescence microscopy. Areas of staining overlap are represented in yellow when the images are superimposed (C, F, J, N, R, and V). Bar, 10 μm.
The human heparanase cDNA contains an open reading frame that encodes a polypeptide of 543 amino acids (4–14). The active heparanase purified from various tissues has been found to lack the N terminus 157 amino acids downstream of the initiation codon, suggesting that there is some form of post-translational proteolysis of the heparanase polypeptide (4–14). Indeed, both proheparanase (65 kDa) and active heparanase (50 kDa) can be detected in heparanase-overexpressing cell lines (36–38). In our assay system, the heparanase-Myc-His fusion plasmids were transfected into human tumor cell lines, and the cells exhibited heparanase activity when incubated with HS (Fig. 3). Extracts from these cell lines were subjected to Western blot analysis to determine whether the Myc-His tag was cleaved from heparanase. A single 70-kDa protein was obtained, representing the 65-kDa heparanase fused to the 4-kDa Myc-His tag (Fig. 1B). Fast migrating bands were not detected with the anti-Myc antibody, indicating that the heparanase-Myc-His fusion protein was not processed. Similar results have been reported with a heparanase-green fluorescence protein fusion protein that is also detected as a single band (39). Thus, it is possible either that the C-terminal fusion suppresses the processing of heparanase or that heparanase could be processed at the nearby C-terminal end. Further studies are necessary to determine why the fusion at the C terminus of heparanase results in a lack of detecting the processed heparanase with tags.

We showed that heparanase protein is localized preferentially within the ER and Golgi apparatus and that it is subsequently secreted into the culture medium (Figs. 4 and 6). Analysis of the human heparanase amino acid sequence indicates the presence of a hydrophobic 35-amino acid region at the N terminus (Fig. 1A and Refs. 7–14), which may function as a signal peptide, directing the protein to the ER and Golgi apparatus and subsequently through the secretory pathway. It has been proposed that many proteins that are destined for secretion exit the trans-Golgi network to be packaged into immature specialized secretory granules. When the mature granules fuse with the plasma membrane, soluble proteins are secreted from the cell (40). The secretion of heparanase as well as AT, apoE, and albumin in HepG2-HP cells was inhibited by the treatment with brefeldin A (data not shown). Moreover, we observed that heparanase is localized within the ER, Golgi apparatus, and the culture media in human tumor cell lines; this suggests that intracellular trafficking of heparanase protein is ER-to-Golgi apparatus. Recently, it has been reported that human heparanase protein is localized within lysosomes and Golgi apparatus in human cell lines, such as MDA-231 cells, which are not able to secrete heparanase into culture medium (39). Thus, it is possible that the subcellular localization of heparanase changes from within the lysosome to secretion into the extracellular space due to an alternation of metastatic potential. Similarly, subcellular localization of cathepsin B and D in
Fig. 6. Effect of glycosylation on secretion of heparanase. A, inhibition of secretion of glycosylation-deficient forms of heparanase. HepG2-Neo, HepG2-HP, HepG2-HP/N162Q, HepG2-HP/N178Q, HepG2-HP/N200Q, HepG2-HP/N217Q, HepG2-HP/N238Q, HepG2-HP/N459Q, and HepG2-HP/N69Q cells were cultured in fetal bovine serum-free medium for 24 h. Samples from conditioned media (upper) and aliquots of cell lysates (lower) were electrophoresed and immunoblotted with the indicated antibodies (Ab). B, densitometric quantitation expressed as a percentage of the amount of heparanase protein in conditioned medium (a) or in cell lysate (b). The indexes of secretion (a/b) were calculated from the amount of heparanase protein in conditioned medium (a) divided by that of cell lysate (b). If the index of secretion was less than 1.00, the level of secretion was low compared with that of wild-type heparanase.

breast and bladder cancer cells changes from within lysosomes to the plasma membrane with increasing metastatic potential (41, 42). At present, we cannot rule out the involvement of secretory lysosome (43) for secretion of heparanase into culture medium.

We identified the residue Asn-459 as the glycosylation site of heparanase, and the residue is important determining the kinetics of ER-to-Golgi transport (Fig. 5, 22, 23, and 24). Interestingly, the Asn-162, Asn-217, and Asn-459 residues of human heparanase are conserved in chicken (36). Therefore, the Asn-459 residue of heparanase may be a common and pivotal glycosylation site determinative for the kinetics of the ER-to-Golgi transport in mammalian cells; however, we cannot exclude the possibility that deglycosylation of heparanase at the Asn-459 residue accelerated the retrograde trafficking from Golgi apparatus to ER, thereby reducing the level of heparanase protein in the Golgi apparatus. Further studies are necessary to explore the above problem. Moreover, our data indicated that the levels of secreted heparanase were partially influenced by deglycosylation at Asn-200 and Asn-217, and were dramatically influenced by deglycosylation at Asn-162, Asn-178, Asn-238, and Asn-459 (Fig. 6A); however, the stability of heparanase proteins in conditioned medium was not regulated by glycosylation (data not shown), indicating the important glycosylation sites at Asn-162, Asn-178, Asn-238, and Asn-459 residues determinative for the kinetics of heparanase secretion into the culture medium. Glycosylation is known to be required for secretion of certain proteins, such as meprin (44); however, there are proteins such as the intestinal brush-border enzyme aminopeptidase N that is secreted by a carbohydrate-independent mechanism (45). Thus, rather than the glycosylation itself being essential for secretion, the effect of glycosylation on the structure of a specific protein that is determinative for movement through the secretory pathway may be critical.

Cell surface expression and secretion of heparanase markedly promote tumor angiogenesis and metastasis (36, 46). Thus, the regulation of the expression level, activity, and intracellular trafficking of heparanase may represent a critical determinant for tumor invasiveness. Moreover, glycosylation inhibitors and glycosidase inhibitors are known to suppress tumor invasion and migration in vitro and in vivo (47–50). Therefore, heparanase inhibitor may provide a novel therapeutic approach for tumors expressing heparanase.

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