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Identification of Cell-Binding Site of Angiomodulin (AGM/TAF/Mac25) That Interacts With Heparan Sulfates on Cell Surface

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Abstract Angiomodulin (AGM/TAF/mac25) is a 30-kDa glycoprotein that was identified as an integrin-independent cell adhesion protein secreted by human bladder carcinoma cells. AGM is highly accumulated in small blood vessels of tumor tissues. In the present study, we attempted to identify the cell surface receptor and the cell-binding site of AGM using ECV-304 human vascular endothelial cells and BALB/c3T3 mouse fibroblasts. Heparin, heparan sulfate, and dextran sulfate, but not chondroitin sulfate, inhibited both adhesion of the two cell lines to AGM-coated plates and binding of AGM to these cells. Treatment of cells with heparinase, but not chondroitinase, inhibited both cell adhesion to AGM and AGM binding to cells. These results strongly suggested that heparan sulfates are the major receptor for AGM. Furthermore, we determined a 20-amino acid sequence within AGM molecule as its major cell-binding site. The synthetic peptide for the cell-binding sequence showed cell adhesion activity comparable to that of AGM, and the activity was inhibited by heparin and heparan sulfate. The peptide competitively inhibited cell adhesion to AGM and the binding of AGM to cells. These results indicated that AGM binds to cells through interaction of the identified cell-binding sequence with heparan sulfates on cell surface. It was also found that the heparan sulfate-binding peptide inhibited the formation of capillary tube-like structures of vascular endothelial cells in culture. J. Cell. Biochem. 75:187–195, 1999.

Key words: angiomodulin/TAF/Mac25; vascular endothelial cells, cell adhesion; heparan sulfate; angiogenesis

A variety of proteoglycans are widely distributed in organs, tissues, and body fluids. Glycosaminoglycan chains in proteoglycans bind various proteins and act as their reservoir or protect them from proteolytic degradation. In particular, heparan sulfate proteoglycans on cell surface have important roles in controlling cellular functions [Yanagishita and Hascall, 1992]. Heparin and heparan sulfates bind various growth factors, cytokines, enzymes, and inhibitors, and modulate their biological activities [Kjellen and Lindahl, 1991; Schlessinger et al., 1995]. It is well known that the interaction between fibroblast growth factors (FGFs) and heparan sulfate proteoglycans is required for their high-affinity binding to FGF receptors [Schlessinger et al., 1995; Yayon et al., 1991; Sakaguchi et al., 1991]. Heparan sulfate proteoglycans also interact with cell adhesion molecules, such as fibronectin, collagen, and laminin, to regulate cell-to-cell and cell-to-extracellular matrix adhesion [Bernfield et al., 1992; Elenius et al., 1990; Liu et al., 1998; Woods and Couchman, 1992].

Previously, we purified a cell adhesive glycoprotein of about 30 kDa from conditioned medium of human bladder carcinoma cell line EJ-1,

Abbreviations used: AGM, angiomodulin; BSA, bovine serum albumin; HSBS, heparan sulfate-binding sequence of AGM; PBS, Ca^{2+}- and Mg^{2+}-free Dulbecco's phosphate-buffered saline; TAF, tumor-derived adhesion factor.

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and tentatively named it tumor-derived adhesion factor (TAF) [Akaogi et al., 1994]. This protein stimulates adhesion of vascular endothelial cells and other types of cells to plastic substrates without interaction with integrins. TAF is highly accumulated in small blood vessels within, and adjacent to, tumor tissues in vivo and capillary tube-like structures formed by vascular endothelial cells in vitro [Akaogi et al., 1996a]. TAF is secreted by vascular endothelial cells and smooth muscle cells, as well as fibroblasts and various types of carcinoma cells. These facts suggest that TAF is involved in tumor angiogenesis or modulation of some vascular functions. Thus, we have proposed a new name, "angiomodulin (AGM)," for TAF [Akaogi et al., 1996a].

The cDNA of AGM has been cloned as mac25 from human brain leptomeningeal cells by Murphy et al. [1993]. The mac25 gene is expressed in the brain, lung, heart, skeletal muscle, testis, ovary, and pregnant uterus of normal mice. Essentially the same cDNA as mac25 has been cloned from human fibroblasts as prostacyclin-stimulating factor (PSF) [Yamauchi, 1994]. Since AGM/mac25/PSF has structural homology to insulin-like growth factor (IGF) binding proteins (IGFBPs), it is also called IGFBP-7 [Yamanaka et al., 1997]. Indeed AGM has some affinity to IGFs and insulin, but the affinity is far less than those of other IGFBPs to IGFs [Akaogi et al., 1996b]. Therefore, it is unclear whether AGM functions as an IGFBP.

Although AGM displays cell adhesion activity on various types of cells and weak growth-stimulatory activity on fibroblasts, there is little information about interaction of AGM with cell surface receptors. To clarify the function of AGM, we attempted to identify cell surface receptors for AGM and the receptor-binding site of AGM.

MATERIALS AND METHODS

Materials

AGM was purified to homogeneity from the serum-free conditioned medium of human bladder carcinoma cell line EJ-1 as described previously [Akaogi et al., 1994; Miyazaki et al., 1993]. A monoclonal antibody (#88) against the purified AGM was prepared in our laboratory [Akaogi et al., 1996a]. Bovine intestinal heparan sulfate and porcine intestinal heparin were purchased from Sigma Chemical Co. (St. Louis, MO) and Nacalai Tesque (Kyoto, Japan), respectively. Bovine kidney heparan sulfate and chondroitin sulfate isoforms A, B, and C, which were derived from whale cartilage, hog skin, and shark cartilage, respectively, were obtained from Seikagaku Corp. (Tokyo, Japan). Bovine liver heparan sulfate was purified by DEAE-cellulose column chromatography (2.0 M LiCl eluate fraction) as reported [Yamane et al., 1998] and then further purified by removing chondroitin sulfate by chondroitinase ABC digestion. Heparinase I (EC 4.2.2.7), heparinase III (EC 4.2.2.8), and chondroitinase ABC (EC 4.2.2.4) were purchased from Sigma. Dextran and dextran sulfate were purchased from Wako Pure Chemicals (Osaka, Japan) and Pharmacia Biotech, respectively. Bovine plasma fibronectin was purchased from Iwaki Glass (Chiba, Japan); pepsin-treated bovine skin type I collagen was from Koken (Tokyo, Japan); and bovine serum albumin (BSA) was from Sigma. Synthetic peptides were purchased from Biologica (Aichi, Japan).

Cells and Culture Conditions

Human umbilical vein endothelial cell line ECV-304 was a kind gift from Dr. K. Takahashi (National Defense Medical College, Japan). ECV-304 and BALB/c3T3 fibroblasts were maintained in a 1:1 mixture of Dulbecco's modified Eagle's medium and Ham's F-12 medium (DMEM/F12) (Life Technologies, NY) supplemented with 0.1 mg/ml streptomycin sulfate, 100 U/ml penicillin G, 1.2 mg/ml $NaHCO_3$, and 10% (v/v) fetal calf serum (FCS) (JRH Biosciences, Australia), at 37°C in 5% CO_2/95% air. Plastic culture dishes were generous gifts from Sumibe Medical (Tokyo, Japan).

Cell Attachment Assay

The cell attachment assay was carried out by essentially the same method as described previously [Kikkawa et al., 1994]. Briefly, each well of 96-well enzyme-linked immunosorbent assay (ELISA) plates (Sumibe Medical) was coated with 50 µl of AGM or synthetic peptides in PBS (Dulbecco's phosphate-buffered saline [PBS]) at 37°C overnight and blocked with 1.2% (w/v) bovine serum albumin (BSA) in PBS. ECV-304 or BALB/c3T3 cells were trypsinized and suspended in serum-free DMEM/F12 medium; 3×10^4 cells were then plated with or without test samples into each well of the plates, followed by incubation at 37°C for 30 min. In some experiments, cells were preincubated in suspension

with 2 U/ml each of heparinase I, heparinase III, or chondroitinase ABC at room temperature for 30 min, being vibrated with Microtube Mixer EM-36 (Taitec, Saitama, Japan), before plating on ELISA plates. Cells attached to the plates were fixed with 5% (v/v) glutaraldehyde for 10 min and stained with 10 μg/ml of Hoechst33342 in 0.001% (v/v) Triton X-100 for 90 min. The fluorescent intensity of each well was measured with Cytofluor 2350 fluorometer (Millipore, Bedford, MA) with excitation at 360 nm and emission at 530 nm.

Assay of AGM Binding to Fixed Cells

Binding of AGM to fixed cells was performed by the method described previously [Akaogi et al., 1996a]. ECV-304 or BALB/c3T3 cells were plated at 1×10^4 cells per well of 96-well ELISA plates (Sumibe Medical) and incubated at 37°C for 3 days. The cells were fixed with 5% (v/v) glutaraldehyde, rinsed with PBS and then blocked with 3% (w/v) casein at 37°C for 90 min. After washing with PBS, AGM with or without test samples in PBS was added into each well and incubated at 37°C for 90 min. Unbound AGM molecules were removed by washing with PBS containing 0.05% (v/v) Tween 20. Relative amounts of AGM bound to the fixed cells were determined by the enzyme-linked immunosorbent assay (ELISA) with the anti-AGM antibody (#88), biotinylated anti-mouse IgG antibody (Vector Laboratories, Burlingame, CA), avidin-alkaline phosphatase (Vector Laboratories), and p-nitrophenylphosphate sodium salt (Wako Pure Chemicals, Osaka, Japan) as a substrate. The color development was measured at 405 nm in Immunoreader NJ-2000 (InterMed, Tokyo, Japan).

Morphological Change of Cultured Endothelial Cells

The formation of cord-like structures by vascular endothelial cells was assayed by the method described previously [Akaogi et al., 1996a]. ECV-304 cells were grown to confluence in each well of 24-well plates (Sumibe Medical) containing 500 μl of DMEM/F12/10% FCS. The culture medium was replaced with 500 μl of the fresh medium supplemented with 100 μg/ml of type I collagen and with or without test samples. The next day, the culture was fixed with methanol, stained with 2.5% Giemsa solution in PBS, and photographed.

RESULTS

Interaction of AGM With Heparan Sulfates

AGM is a cell adhesion protein with heparin-binding activity. Heparin inhibits the binding of AGM to vascular endothelial cells [Akaogi et al., 1996a]. Therefore, AGM seems to bind to heparin-like molecules on cell surface. To test this possibility, we examined inhibitory effects of some sulfated glycosaminoglycans, such as heparin, heparan sulfates, and chondroitin sulfates, dextran, and dextran sulfates on the cell adhesion activity of AGM.

Human vascular endothelial cell line ECV-304 or mouse fibroblast cell line BALB/c3T3 was mixed with each sample and plated on plastic plates precoated with AGM. Like porcine heparin, heparan sulfate from bovine liver inhibited the adhesion of ECV-304 cells to AGM (Fig. 1A). However, heparan sulfates derived from bovine kidney and intestinal mucosa inhibited the cell adhesion to AGM either very weakly or hardly at all. Although dextran did not inhibit the cell adhesion, dextran sulfate was inhibitory. Essentially the same result was obtained with the mouse fibroblast cell line BALB/c3T3 (Fig. 1B). These results indicated that the negative charges of sulfonyl residues are important for the binding of AGM to sulfated glycosaminoglycans. Chondroitin sulfate isoforms A, B, and C, which were derived from whale cartilage, hog skin, and shark cartilage, respectively, did not inhibit the cell adhesion to AGM (data not shown).

To examine the interaction of AGM and sulfated glycosaminoglycans on cell surface, we tested the effects of two sulfated glycans on the binding of AGM to the fixed cells. When mixed with AGM, porcine intestinal heparin and bovine liver heparan sulfate significantly inhibited the binding of AGM to ECV-304 and BALB/c3T3 cells fixed on plastic plates by glutaraldehyde (Table I). The inhibition was more evident in BALB/c3T3 cells than in ECV-304 cells. The different inhibition might be attributed to the quantitative and/or qualitative differences in cell surface heparan sulfates between the two cell lines.

To confirm the interaction of AGM with cell surface heparan sulfates, effects of heparinase I, heparinase III, and chondroitinase ABC on the AGM-mediated cell adhesion were examined with ECV-304 and BALB/c3T3 cells. Cells were pretreated with each enzyme and then

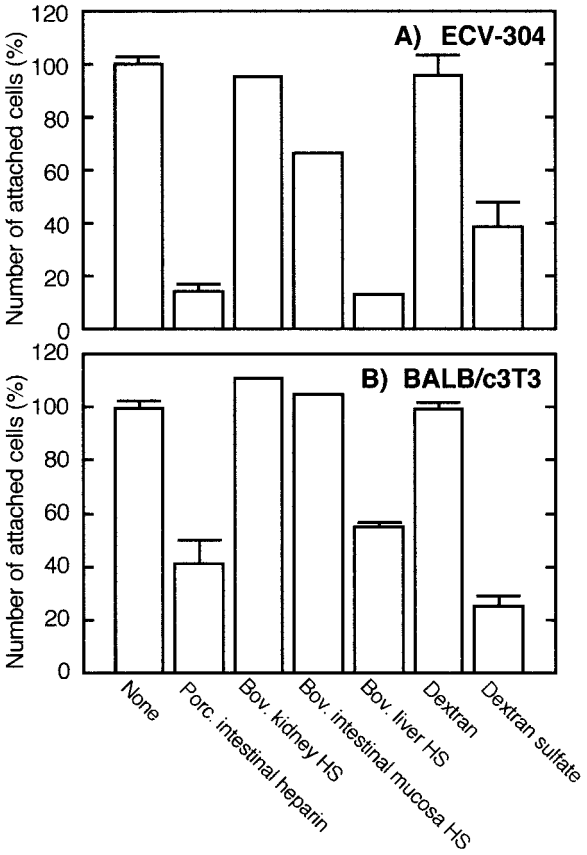

Fig. 1. Effects of sulfated glycosaminoglycans, dextran, and dextran sulfate on adhesion of ECV-304 cells and BALB/c3T3 cells to AGM. ECV-304 cells **(A)** and BALB/c3T3 cells **(B)** were mixed with each polysaccharide sample (5 μg/ml) and plated into each well of 96-well plates precoated with 2 μg/ml AGM (0.35 μg/cm^2). After incubation at 37°C for 30 min, the relative number of the attached cells was determined as described under Materials and Methods. The number of the attached cells in the control culture (None) was taken as 100%. Results are the means of duplicate wells. Bars point to the higher value in each assay. The differences between the two values are less than 15% of the respective mean values, except for dextran sulfate **(A)** (48%) and porcine intestinal heparin **(B)** (43%). Porc., porcine; Bov., bovine; HS, heparan sulfate.

plated on AGM-coated plates. In both cell lines, treatment with heparinase I decreased the cell adhesion to AGM more strongly than that with heparinase III (Table II). Since heparinases I and III act on high- and low-sulfated regions of heparan sulfates, respectively [reviewed by Yamada et al., 1998], the results suggest that the highly sulfated region, rather than the lower sulfated region, of the cell surface heparan sulfates is involved in the AGM binding. Chondroitinase ABC had no effect on BALB/c3T3 cells, but it appeared to increase the adhesion of ECV-304 cells to AGM, suggesting that in this cell line chondroitin sulfates on cell surface

TABLE I. Inhibitory Effects of Heparin and Heparan Sulfate on Binding of AGM to Fixed ECV-304 and BALB/c3T3 Cells*

	Bound AGM (%)	
Sample	ECV-304	BALB/c3T3
None	(100 ± 5)	(100 ± 9)
Heparin	68 ± 7	30 ± 6
Heparan sulfate	61 ± 4	29 ± 3

*AGM (2 μg/ml) was mixed with 1 μg/ml porcine intestinal heparin or bovine liver heparan sulfate, added to glutaraldehyde-fixed ECV-304 and BALB/c3T3 cells, and incubated at 4°C overnight. Relative amounts of AGM bound to the fixed cells were measured as described under Materials and Methods. Results are the means ± SE for triplicate wells.

TABLE II. Effects of Glycosidase Treatment of ECV-304 and BALB/c3T3 Cells on Cell Adhesion to AGM*

	No. of attached cells (%)	
Enzyme	ECV-304	BALB/c3T3
None	(100 ± 5)	(100 ± 13)
Heparinase I	31 ± 1	63 ± 4
Heparinase III	45 ± 3	77 ± 5
Chondroitinase ABC	140 ± 9	107 ± 5

*The two cell lines were treated with the indicated enzymes and then added to each well of 96-well plates pre-coated with 2 μg/ml (0.35 μg/cm^2) AGM. After incubation at 37°C for 30 min, relative numbers of attached cells were measured. Other experimental conditions are described under Materials and Methods. Results are the means ± SE for triplicate wells.

may hinder the binding of AGM to heparan sulfates.

Similarly, effects of these enzymes on the binding of AGM to glutaraldehyde-fixed cells were examined. The fixed cells were pretreated with each enzyme and then incubated with AGM. Heparinase I decreased the binding of AGM to the fixed cells more than heparinase III, but chondroitinase ABC had no effects (Table III). All these results strongly suggest that AGM binds to heparan sulfate molecules on the cell surface.

Cell-Binding Site of AGM

The results described above showed that AGM probably interacts with heparan sulfate molecules on cell surface. To identify the cell-binding site(s) of AGM, we selected two peptide sequences that are rich in basic amino acids: K^{89}SRKRRKGK^{97} and K^{195}VKRGHY^{201}. When these synthetic peptides were coated on plastic plates, the peptide "KSRKRRKGK," but not

TABLE III. Effects of Glycosidase Treatment of ECV-304 and BALB/c3T3 Cells on Binding of AGM to Fixed ECV-304 and BALB/c3T3 Cells*

	Bound AGM (%)	
Enzyme	ECV-304	BALB/c3T3
None	(100 ± 7)	(100 ± 4)
Heparinase I	75 ± 4	58 ± 6
Heparinase III	86 ± 8	84 ± 7
Chondroitinase ABC	100 ± 5	99 ± 9

*Each culture of ECV-304 and BALB/c3T3 cells was fixed with glutaraldehyde and then treated with 1 unit/ml each of the indicated enzymes at 4°C overnight. AGM (2 μg/ml) was added to the cultures and incubated at 37°C for 30 min. Relative amounts of bound AGM were measured. Other experimental conditions are described under Materials and Methods. Results are the means ± SE for triplicate wells.

"KVKRGHY," promoted the adhesion of BALB/c3T3 cells to the substrate (data not shown). We synthesized a longer active peptide containing the former sequence, G^{84}MECVKSRKRRKGKAGAAAG^{103} and examined its activities in more detail.

The cell adhesion activity of the synthetic peptide was examined in the same manner as AGM. When the peptide was coated on plastic plates at various concentrations, the adhesion of both ECV-304 cells and BALB/c3T3 cells to the plates were promoted in a dose-dependent manner (Fig. 2). It is noteworthy that the peptide and native AGM supported the cell adhesion at equivalent concentrations. Inhibitory effects of heparin, heparan sulfate, and dextran sulfate on the adhesion of ECV-304 and BALB/c3T3 cells to the peptide were also examined. In this experiment, the cells were mixed with each sample and then plated on plastic plates precoated with the peptide. Porcine intestinal heparin, bovine liver heparan sulfate, and dextran sulfate significantly inhibited the adhesion of both types of cells to the substrate (Fig. 3). These inhibitory patterns of sulfated glycosaminoglycans and their analogue were similar to those on the cell adhesion to AGM shown in Figure 1, suggesting that this peptide sequence is the cell adhesion site, or the heparan sulfate-binding site, of AGM. Chondroitin sulfates, which did not inhibit the cell adhesion to AGM, had no effect on the cell adhesion to the peptide (data not shown).

Furthermore, we examined inhibitory effects of the synthetic peptide "G^{84}MECVKSRKRRKGKAGAAAG^{103}" on the AGM-mediated cell adhesion and on the binding of AGM to the

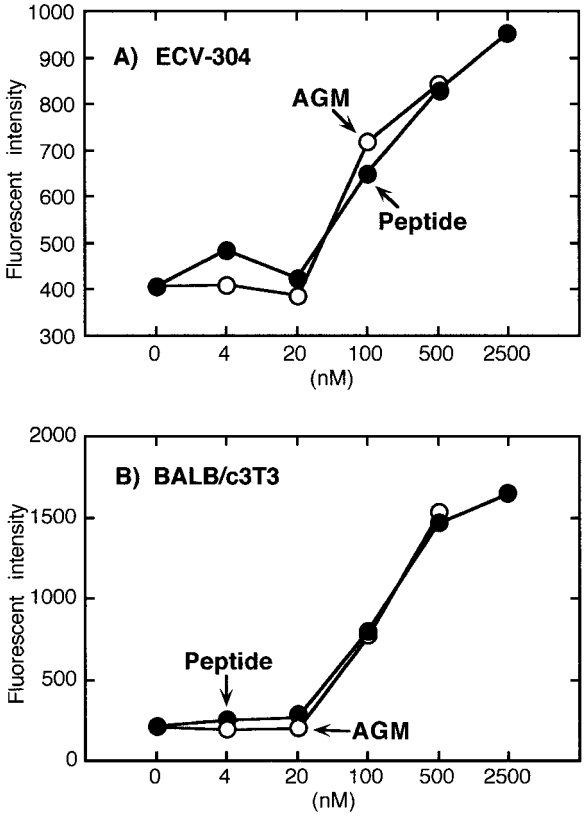

Fig. 2. Adhesion of ECV-304 cells and BALB/c3T3 cells to various concentrations of AGM and synthetic peptide. ECV-304 cells **(A)** and BALB/c3T3 cells **(B)** were added to each well of 96-well plates precoated with the indicated concentrations of AGM (○) or the synthetic peptide G^{84}MECVKSRKRRKGKAGAAAG^{103} (●). After incubation at 37°C for 30 min, attached cells were quantified as described under Materials and Methods. 100 nM AGM and 100 nM HSBS peptide correspond to 3 μg/ml (0.53 μg/cm^2) and 0.206 μg/ml (36 ng/cm2), respectively. Each point represents the mean of duplicate wells. The differences between the two values are less than 30% of the respective mean values, except for 500 nM AGM **(A)** (51%) and 500 nM peptide **(A)** (100%).

fixed cells. When ECV-304 cells were mixed with various concentrations of the peptide and plated on AGM-coated plates, cell adhesion to AGM was inhibited in a dose-dependent manner (Fig. 4A). Similarly, the peptide competitively inhibited the binding of AGM to ECV-304 cells fixed on plastic plates (Fig. 4B). Essentially the same results were obtained with BALB/c3T3 cells (data not shown). These results confirmed that this peptide sequence is a major cell-binding and heparan sulfate-binding site of AGM. Therefore, we named this 20-amino acid heparan sulfate-binding sequence "HSBS."

The effect of HSBS peptide on the formation of the cord-like structure by ECV-304 vascular endothelial cells was also examined. When 100

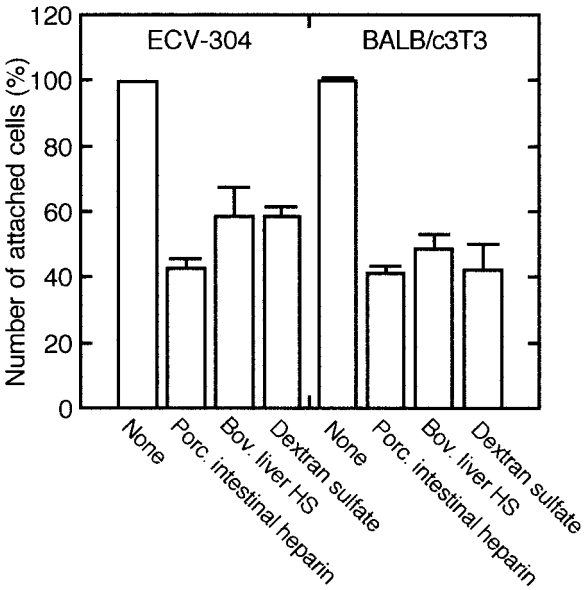

Fig. 3. Effects of sulfated glycosaminoglycans and dextran sulfate on adhesion of ECV-304 cells and BALB/c3T3 cells to synthetic peptide. ECV-304 and BALB/c3T3 cells were mixed with each sample (2 μg/ml) and plated into each well of 96-well plates precoated with 100 nM (36 ng/cm^2) synthetic peptide (G^{84}MECVKSRKRRKGKAGAAAG^{103}). Other experimental conditions are the same as in Figure 1. Results are the means ± SE (bars) for triplicate wells. Porc., porcine; Bov., bovine; HS, heparan sulfate.

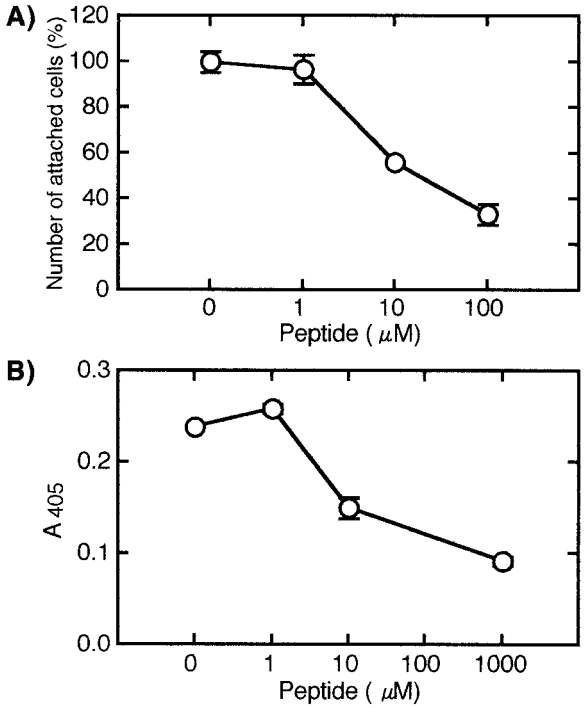

Fig. 4. Inhibitory effects of synthetic peptide on adhesion of ECV-304 cells to AGM and on binding of AGM to fixed ECV-304 cells. **A:** ECV-304 cells were mixed with the indicated concentrations of the synthetic peptide G^{84}MECVKSRKRRKGKAG AAAG^{103} and plated into each well of 96-well plates precoated with AGM. After incubation at 37°C for 30 min, the relative number of the attached cells was determined as described in Fig. 1. Each point represents the mean ± SE (bar) for triplicate wells. **B:** ECV-304 cells were fixed with glutaraldehyde on 96-well plates. AGM (3μg/ml) was mixed with the indicated concentrations of the synthetic peptide, added to the fixed cells, and incubated at 4°C overnight. The relative amount of AGM bound to the fixed cells was measured as described under Materials and Methods. Each point represents the mean ± SE (bar) for triplicate wells.

μM (206 μg/ml) HSBS peptide at a final concentration was added to the confluent monolayer culture of ECV-304 cells in the presence of type I collagen, the formation of the network of fibrillar array or cord-like structures of sprouted cells was inhibited as in the case of heparin (Fig. 5). This suggested that this sequence of AGM might be involved in the morphological change of ECV-304 cells.

DISCUSSION

We have previously reported that both cell-binding and cell adhesion activities of the tumor-derived adhesion factor TAF/AGM are inhibited by heparin [Akaogi et al., 1996a,b]. In this study, we further examined the interaction between some sulfated glycosaminoglycans and AGM. Their inhibitory effects on cell adhesion activity of AGM, as well as the inhibitory effects of heparinase treatments of cells, strongly suggest that the cell surface receptor of AGM is heparan sulfate moieties of proteoglycans. Almost the same results were obtained with two types of cell lines, ECV-304 vascular endothelial cells and BALB/c3T3 fibroblasts. In addition, we identified a cell-binding and heparan sulfate-binding sequence (HSBS) of AGM that consists of 20-amino acid residues including 7 basic amino acids. Since AGM and the synthetic HSBS peptide show almost the same cell adhesion activity, HSBS is thought to be the major cell-binding site that interacts with heparan sulfates on cell surface.

Of the sulfated glycosaminoglycans tested in this study, porcine heparin and bovine liver heparan sulfate inhibited the cell adhesion to AGM, whereas heparan sulfates derived from bovine kidney and intestinal mucosa and chondroitin sulfates very weakly or hardly inhibited the cell adhesion. It is known that the sites of sulfation are irregularly distributed in heparin and heparan sulfate chains as compared with chondroitin sulfates. There are highly sulfated

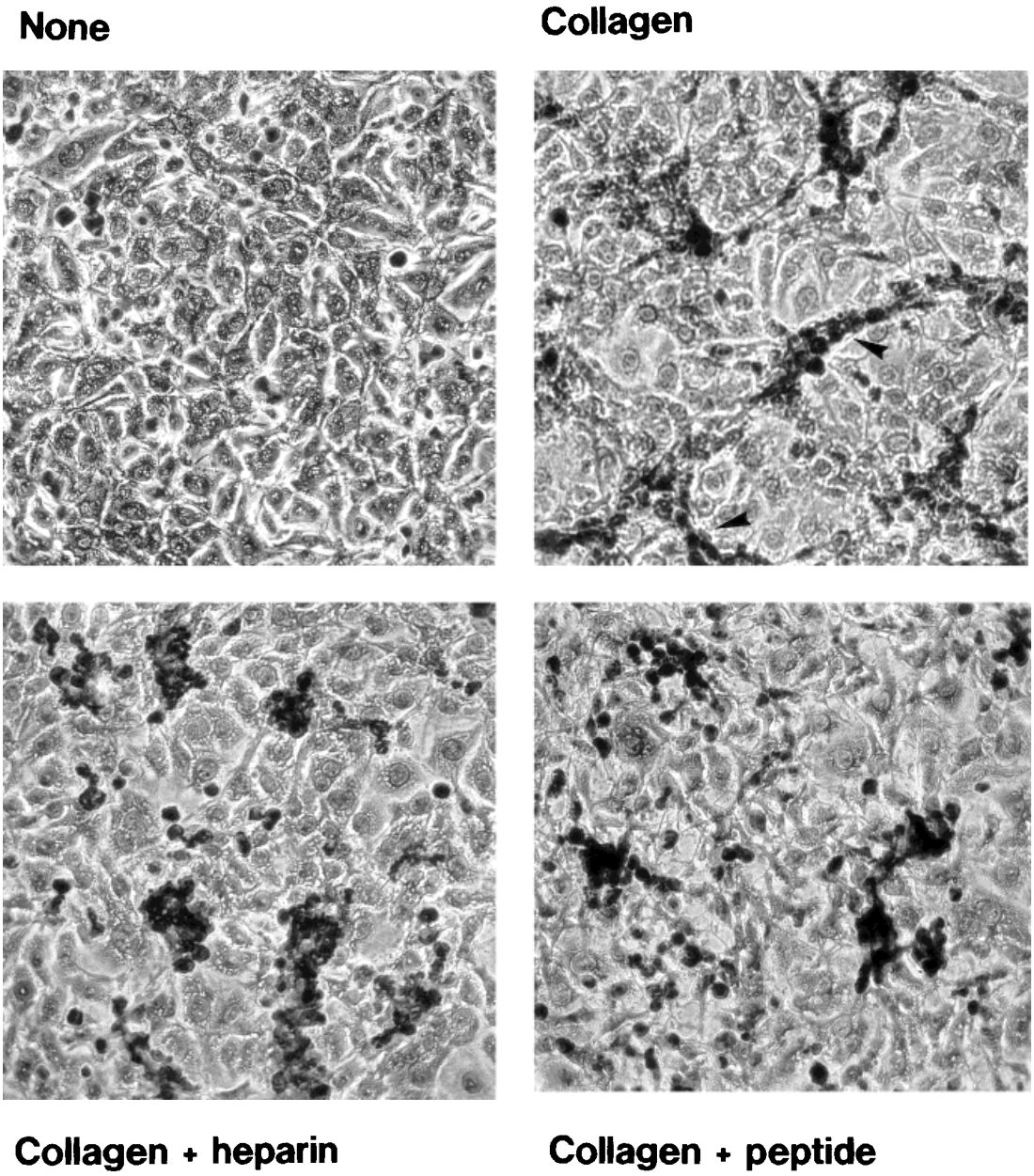

Fig. 5. Effect of synthetic peptide HSBS on formation of cord-like structures by cultured endothelial cells. ECV-304 cells were incubated in culture medium with or without 100 μg/ml type I collagen, 20 μg/ml heparin, and 100 μM (206μg/ml) HSBS peptide. The cultures were fixed and stained with Giemsa. Other experimental conditions are described under Materials and Methods. Arrowheads, cord-like structures of sprouted endothelial cells.

blocks in heparan sulfate molecules on cell surface [Turnbull et al., 1991]. It is very likely that the positive charges of HSBS interact with the negative charges of the highly sulfated blocks of heparan sulfate proteoglycans such as syndecans [Bernfield et al., 1992]. The different inhibitory activity shown by heparan sulfates from bovine liver and from kidney and intestinal mucosa may be attributed to the differences in the level and distribution of sulfation between the two groups of heparan sulfates. It has been reported that syndecan-1 molecules from two cell lines show different affinity to collagen due to the different fine structures of the two molecules [Sanderson et al., 1994].

The physiological functions of AGM are unknown. As shown in this discussion and in past studies, AGM has cell adhesion activity. It has

weak affinity to IGFs and insulin [Akaogi et al., 1996b] and shows synergistic growth stimulatory activity with IGFs or insulin toward fibroblasts [Akaogi et al., 1996b]. However, these biological activities are not prominent as compared with those of integrin-dependent cell adhesion proteins or growth factors. Mac25/AGM gene is expressed in normal leptomeningeal cells, but its expression is decreased or absent in meningioma cells [Murphy et al., 1993]. The level of mac25/AGM mRNA is high in nondividing, senescent mammary epithelial cells but absent in breast cancer cell lines [Swisshelm et al., 1995]. These studies suggested that mac25/AGM might play a tumor-suppressive or antiproliferative role. However, Damon et al. [1997] recently reported that the expression of mac25/AGM mRNA was higher in dividing myoblasts than in nondividing myotubes. We have found that AGM is highly secreted by human bladder carcinoma cells [Akaogi et al., 1994] and other cancer cell lines (unpublished data). Thus, the relationship between AGM expression and cell growth varies depending on cell types. On the other hand, we have reported that AGM is densely accumulated in blood vessels of various human cancer tissues and in capillary tube-like structures of cultured vascular endothelial cells [Akaogi et al., 1996a]. This finding suggests that AGM affects vascular structures and functions and may be involved in some pathological processes of vasculatures such as tumor angiogenesis and abnormal permeability of tumor blood vessels.

New blood vessels are produced under various pathological processes including inflammation, tumor growth and metastasis, as well as some physiological processes [Folkman et al., 1992; Folkman, 1995]. The neovascularization, or angiogenesis, is a complex process that requires the proteolytic degradation of extracellular matrix proteins, and the migration, proliferation, and differentiation of vascular endothelial cells [Bischoff, 1995; Strömblad et al., 1996]. These steps are regulated by growth factors including vascular endothelial cell growth factor [Plate et al., 1992; Breier et al., 1996] and basic fibroblast growth factor (bFGF) [Shing et al., 1984], matrix metalloproteinases [Vu et al., 1998], extracellular matrix (ECM) components such as fibronectin, laminin, and type IV collagen [Bischoff, 1995; Strömblad et al., 1996], and cell surface receptors including integrins, heparan sulfate proteoglycans and selectin [Bischoff, 1995; Strömblad et al., 1996; Friedlander et al., 1995; Sasisekharan et al., 1994; Koch et al., 1995]. Among these factors, cell adhesion molecules are expected to play important roles in the morphological differentiation of endothelial cells at the last step of angiogenesis.

In the present study, heparin and the heparin-binding peptide of AGM (HSBS), both of which could block the cell binding of AGM, inhibited the formation of capillary tube-like structures of cultured vascular endothelial cells. It seems likely that the inhibition of the morphological change of endothelial cells by HSBS peptide is associated with the decreased binding of AGM to heparan sulfate proteoglycans on the cell surface. However, it is also possible that the peptide, as well as heparin, might inhibit the interaction between other angiogenic cytokines or adhesion proteins and heparan sulfate proteoglycans, preventing the morphological change of endothelial cells. Although further studies are needed to clarify the physiological roles of AGM in vascular functions, the present study strongly suggests that the interaction of AGM with heparan sulfates on cell surface or extracellular matrix is important in the expression of its biological activity. The identification of the heparan sulfate-binding site of AGM in this study seems useful for the future studies.

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