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Inhibitory Antibodies against Endopeptidase Activity of Human Adamalysin 19[1]

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Human adamalysin 19 (hADAM19)/meltrin β is a member of the ADAM (a disintegrin and metalloproteinase) family and an active metalloproteinase. It is a new metalloproteinase and disintegrin dendritic cell antigen marker. Adamalysin 19 gene was expressed in normal and transformed tissues and cells such as placenta, brain, heart, leukocytes, and colorectal adenocarcinoma SW480. To develop specific tools to investigate the functions of hADAM19, peptide antigens were rationally selected and specific polyclonal antibodies (pAbs) were developed to modulate hADAM19 activity. Anti-metalloproteinase and anti-disintegrin domain IgG molecules inhibited the α-2-macroglobulin cleavage by hADAM19; however, their pre-immune and anti-pro-domain IgG molecules did not. Since anti-disintegrin IgG also neutralized the proteolytic activity, the disintegrin domain may affect the hADAM19 protein folding and/or substrate binding. These pAbs may be used to specifically localize the hADAM19 protein in tissues and cells and elucidate its biological and pathological functions such as processing pro-growth factors. © 2001 Academic Press

Key Words: **human ADAM19/meltrin β; metalloproteinase and disintegrin; adamalysin; zinc metalloenzyme; metzincin; neuregulin processing enzyme; dendritic cell antigen marker; antibody development; enzyme inhibition; functional blocking antibodies.**

Abbreviations used: Ab, antibody; ADAM, a disintegrin and metalloproteinase; ADAMTS1 (METH-1), a disintegrin and metalloproteinase with thrombospondin-like motifs-1; α2-M, α-2-macroglobulin; BCIP, 5-bromo-4-chloro-3-indoyl phosphate; Brij-35, polyoxyethylene lauryl ether; ECM, extracellular matrix; EDTA, ethylenediaminetetraacetic acid; ELISA, enzyme-linked immunosorbent assay; hADAM19, human disintegrin and metalloproteinase 19; Hepes, *N*-2-hydroxyethylpiperazine-*N′*-2-ethane sulfonate; MDC, metalloproteinase/disintegrin/cysteine-rich; METH-1 (ADAMTS1), protein-1 with metalloproteinase and thrombospondin domains; MMPs, matrix metalloproteinases; MS, mass spectrometry; NBT, nitro blue tetrazolium; NRG, neuregulin; pAb, polyclonal antibody; RP-HPLC, reverse-phase high performance liquid chromatography; SDS–PAGE, sodium dodecyl sulfate–polyacrylamide gel electrophoresis; SH3, *Src*-homologous 3; TACE, tumor necrosis factor-α converting enzyme; TBS, Tris-buffered saline; TIMP, tissue inhibitor of metalloproteinases; TNF-α, tumor necrosis factor-α.

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A disintegrin and metalloproteinase (ADAM), also known as adamalysin or MDC (metalloproteinase/disintegrin/cysteine-rich) protein, is a member of a large family of membrane-anchored glycoproteins [1–3, http://www.med.virginia.edu/~jag6n/white-lab.html]. Most of the ADAM members have all or some of the following domain structures: a signal peptide, a propeptide domain, a metalloproteinase domain, a disintegrin domain, a cysteine-rich domain, an epidermal growth factor (EGF)-like domain, a transmembrane region, and a cytoplasmic tail. The number of genes within the ADAM family has grown rapidly, but the biological functions of most members remain to be identified. Their domain structures suggest that they may have many potential functions including proteolysis, adhesion, fusion, and intracellular signaling. Some of the proteins have been implicated in a variety of important processes, such as sperm–egg (1, 4) and muscle cell binding and fusion (5), shedding of tumor necrosis factor α (TNF-α) (6, 7), cleavage of type IV collagen (8) and aggrecan (9), cell adhesion and migration (10–16), angiogenesis inhibition (17), and signal transduction (11, 18).

Human ADAM19 (hADAM19) is a new member of the ADAM family identified by Fritsche's group and our group (19, 20). Like most other members, it has a signal sequence, a pro-domain with an unpaired cysteine residue (the "cysteine-switch" residue), a metalloproteinase domain with a zinc-binding site, a disintegrin domain, a cysteine-rich domain, an epidermal growth factor-like domain, a transmembrane domain,

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and a cytoplasmic domain with putative SH3 ligand binding sites (19). Although hADAM19 was first reported as a novel metalloproteinase and disintegrin dendritic antigen marker (MADDAM) (20), we demonstrated that its gene was widely expressed in many normal human organs, tissues, cells, and several human cancer cell lines. In particular, high levels of mRNA hADAM19 were detected in the placenta, heart, brain, lungs, bladder, spleen, appendix, colon, lymph nodes, skeletal muscle, peripheral blood leukocytes, colorectal adenocarcinoma SW480, lung carcinoma A549, and chronic myelogenous leukemia K-562 (19).

Because hADAM19 contains a metalloproteinase domain with a typical zinc-binding consensus sequence (HEXGHXXGXXH), which exists in the matrix metalloproteinase (MMP) family members and plays an important role during extracellular matrix (ECM) degradation and reconstruction, there is potential that ADAMs might be involved in various physiological and pathological processes related to ECM turnover. Although the physiological substrates of hADAM19 have not been identified, we found that hADAM19 is an active metalloproteinase using the α-2-macroglobulin (α2-M) trapping assay (19).

The hADAM19 gene and the mouse ADAM19 (meltrin β) gene are 80.6% identical. The full-length hADAM19 gene encodes a 918-amino acid residue protein that is 84.1% identical to the mouse ADAM19 protein (19, 21). Based on the high identity between mADAM19 and hADAM19, hADAM19 might have similar functions as mADAM19, including osteoblast differentiation (21), peripheral neuronal cell lineages differentiation (22), and the intracellular processing of neuregulin (NRG, a member of the EGF family) (23). ADAM19 may play essential roles during mouse development, growth, and morphogenesis.

Most of the domain functions of ADAMs are not fully characterized. To investigate the hADAM19 protein cellular and tissue localizations, its biological and pathological functions, and the interactions between hADAM19 metalloproteinase domain and disintegrin domain, we have rationally selected and synthesized specific antigen peptides based on hADAM19 primary structure and used these peptides to immunize rabbits and generate polyclonal antibodies (pAbs) against the hADAM19 pro-domain, metalloproteinase domain, and disintegrin domain, respectively. All of the pAbs were characterized by enzyme-linked immunosorbent assays (ELISA) and Western blots to determine the specificity against ADAM19. Interestingly, both anti-metalloproteinase and anti-disintegrin domain IgG molecules blocked the cleavage of α2-M by hADAM19. This finding indicates that the disintegrin domain may contribute to the proteolytic process of hADAM19 by facilitating protein folding and/or substrate binding. Those Abs may have functional blocking activities against the endopeptidase activity of this enzyme and they may provide researchers with powerful tools to decipher the biological and pathological roles of hADAM19.

MATERIALS AND METHODS

Antigen peptides synthesis. Specific peptides corresponding to the unique sequences in the different hADAM19 domains were synthesized and purified according to our reported methods (24, 25). Specifically, the pro-domain, metalloproteinase domain, and disintegrin domain peptides of hADAM19 were synthesized. The sequences for the pro-domain, metalloproteinase domain, and disintegrin domain are Glu^{93}-Thr-His-Tyr-Thr-Ser-Ser-Gly-Asn-Pro-Gln-Thr-Thr-Thr-Arg-Lys-Leu-Glu-Asp^{111}-NH_2 (peptide No. 360), Glu^{222}-Phe-Gln-Lys-Asn-Arg-Arg-Asp-Gln-Asp-Ala-Thr-Lys-His-Lys^{236}-NH_2 (peptide No. 361), and Asp^{431}-Cys-Gly-Glu-Glu-Glu-Glu-Cys-Asn-Asn-Pro-Cys-Cys-Asn^{444}-NH_2 (peptide No. 362), respectively. These peptides were synthesized by Dr. Umesh Goli at the Biochemical Analysis, Synthesis and Sequencing Service Laboratory at our Department of Chemistry at Florida State University. The purity of these peptides was verified by reverse-phase high performance liquid chromatography (RP-HPLC) and mass spectrometry (MS).

Antibodies production, purification, and characterization. The pre-immunization sera were collected from New Zealand white rabbits 4 weeks before the initial injection of antigen. These peptides were coupled to keyhole limpet hemocyanin (Sigma Chemical Co., St. Louis, MO) with 2% glutaraldehyde in 0.2 M phosphate buffer, pH 7.3. One milligram of the conjugated peptide was emulsified in the complete Freund's adjuvant (Sigma) for initial injection. The rabbits were boosted with the same peptide emulsified in the incomplete Freund's adjuvant every 4–5 weeks. The blood was collected 9 days after each boost. Sodium azide (final concentration of 0.02%) was added to the sera and the sera were stored at −85°C.

The antisera were incubated at 56°C for 30 min. Then, the IgG was purified following the instructions of the Pierce ImmunoPureIgG (protein A) purification kit. Briefly, the serum was diluted with binding buffer and added to the equilibrated protein A column. The column was washed with 15 ml ImmunoPureIgG binding buffer, and then eluted with 5 ml ImmunoPureIgG elution buffer. The concentration of IgG in the elution fractions was monitored by absorbance at 280 nm. Then, the protein A elution fractions were desalted by adding them to an Excellulose column. TBS buffer (100 mM Tris–HCl, pH 7.5, 150 mM sodium chloride) was added to wash the column. The final purified IgG concentration was calculated by OD_{280} times 0.8 mg/ml. The purity of the IgG was verified by silver stain. The pre-immune IgG for each rabbit was also purified by this procedure for a negative control.

An enzyme-linked immunosorbent assay (ELISA) was used to assess the strength and specificity of the rabbit polyclonal antibodies as described (24, 25). Polyvinyl micro-ELISA plates were coated with 50 μl/well of different concentrations of the synthetic peptide antigens for hADAM19 and protein-1 with metalloproteinase and thrombospondin domains (METH-1/ADAMTS1) and purified METH-1 (residues 1–510) protein in borate-buffered saline. Alkaline phosphatase-conjugated goat anti-rabbit IgG (Sigma) was used as the secondary antibody. *p*-nitrophenyl phosphate (1 mg/ml) in 1.0 M diethenolamine buffer and 0.5 M $MgCl_2$, pH 9.8, was used as the substrate. After incubating for 30 min, the plates were read at 405 nm with a Titertek Multiscan MC-340 automatic microplate reader. The different peptide antigens and anti-METH-1 antibodies were used to test the specificities and cross-reactivities of these antibodies. Pre-immune IgG molecules of different antibodies were also tested as negative controls.

Western blotting was performed according to standard procedures (24, 25). Briefly, the samples were electrophoresed on 10% SDS–polyacrylamide gels, and the proteins were electrophoretically transferred to nitrocellulose membranes in 25 mM Tris, 192 mM glycine, and 20% (v/v) methanol at 90 V and 4°C for 2 h. After blocking with

5% BSA (Sigma Chemical Co.), the membranes were incubated with the rabbit anti-hADAM19 pro-domain IgG (pAb360), the rabbit anti-hADAM19 metalloproteinase domain peptide IgG (pAb361), anti-hADAM19 disintegrin domain peptide IgG (pAb362), and anti-METH-1 metallo-domain IgG (pAb332), pre-immune control IgG for pAb361 or pre-immune control IgG for pAb362. The final concentration of primary Ab was 1 μg/ml. The membrane was then washed and incubated in the secondary antibody, alkaline phosphatase-conjugated goat anti-rabbit IgG (Sigma Chemical Co.). Immunoreactive bands were visualized by adding nitro blue tetrazolium (NBT) and 5-bromo-4-chloro-3-indoyl phosphate (BCIP).

hADAM19 binding to and cleavage of α2-M. The purified pFlag-hADAM19 ectodomain of the protein (residues 26–701) was prepared by the mammalian expression system (293T cells) as described in our previous report (19). Protein molecular weight markers were from Amersham Pharmacia Biotechnology, Co. Bovine plasma α-2-macroglobulin (α2-M, Boehringer Mannheim, Germany) was dissolved in 1× Hepes buffer, which contains 50 mM Hepes, pH 7.5, 0.2 M NaCl, 10 mM $CaCl_2$, 25 μM $ZnCl_2$ and 0.05% Brij-35. The concentration of the α2-M solution was 0.2 units/ml. 30 μl of purified hADAM19 protein was mixed with 70 μl of α2-M and incubated at 37°C for one to five days. 20 μl aliquots of reaction solution were removed on each day of the 5 days and the reaction was stopped by adding 4× zymography sample buffer (8% SDS, 40% glycerol, 250 mM Tris–HCl pH 6.8, and 0.04% bromophenol blue, without EDTA and β-mercaptoethanol) and incubating at 37°C for 30 min. The samples were loaded onto 5% SDS–polyacrylamide gels. After electrophoresis, the gels were stained with 0.2% Coomassie blue and destained with a methanol/water (45%/55%, v/v) solution.

The hADAM19 cleavage of α2-M was performed according to our previous report (19). Briefly, 10 μl of purified hADAM19 (50 μg/ml) was mixed with 23.76 μl α2-M (0.2 unit/ml) in 66.24 μl 1× Hepes buffer and incubated at 37°C 1–5 days. A 20 μl aliquot of reaction solution was removed on each of the 5 days, and the reaction was stopped by adding 2× sample buffer (4% SDS, 20% glycerol, 120 mM Tris–HCl, pH 6.8, 100 mM EDTA, 5% β-mercaptoethanol, and 0.02% bromophenol blue) and boiled for 5 min. The samples were loaded onto 10% SDS–polyacrylamide gels. After electrophoresis, the bands were visualized by silver staining.

Neutralizing hADAM19 proteinase activity by two specific polyclonal antibodies. The pre-immune IgG molecules, anti-domain-specific hADAM19 antibodies, rabbit anti-MMP-7/matrilysin metalloproteinase domain IgG, and rabbit anti-METH-1 metalloproteinase domain IgG were produced and purified as described above. The IgG molecules or 1,10-phenanthroline (10 mM) were preincubated with hADAM19 in 1× Hepes buffer at room temperature (25°C) for 2 h. Then, α2-M was added, and the mixture was incubated at 37°C for 18 h. The final IgG concentration in each reaction system was 11.8 μM except for anti-MMP-7 metalloproteinase domain IgG, which had a final concentration of 50 μM. The reaction was stopped by adding 2× sample buffer and boiled for 5 min. Trypsin (6.6 μM) was mixed with the same molar concentration of α2-M as a positive endopeptidase control. Anti-MMP-7 catalytic domain IgG and anti-METH-1 metalloproteinase domain IgG (pAb332) were also used as negative controls to test if they have functional blocking capability toward hADAM19.

RESULTS AND DISCUSSION

Antibodies against hADAM19 are specific. The sequence homology comparisons of the three antigen peptides were performed against all the current protein data banks by using basic local alignment search tool (BLAST) programs (National Center for Biotechnology Information, NCBI, Bethesda, MD, http://www.ncbi.nlm.nih.gov). The programs were used to perform database searches and rigorous statistical analyses for evaluation of the significance of the matches. Both the pro-domain antigen and metallo-domain antigen had sequence similarities with mouse ADAM19, thus, the anti-pro-domain and metallo-domain antibodies might cross-react with mouse ADAM19. None of the known proteins in the databanks searched had identities greater than 45% compared to the disintegrin-domain antigen peptide.

The Abs against the hADAM19 pro-domain peptide, metalloproteinase domain peptide, and disintegrin domain peptide were characterized by ELISA (Fig. 1; data for anti-hADAM19 propeptide domain Ab, pAb360 are not shown). The optimal dilution range for the anti-hADAM19 metalloproteinase domain peptide IgG (pAb361) and anti-hADAM19 disintegrin domain peptide IgG (pAb362) was from 1:100 to 1:800. The specificity of the Abs was determined by testing their cross-reactivity with purified recombinant METH-1 (residues 1–510) protein. At the METH-1 protein concentrations up to 500 nM and primary Abs dilutions of 1:200, no cross-reactivity was detected (data not shown). In addition, the pre-immune IgG molecules of the same rabbits did not react with the peptide antigens (data not shown).

The Abs directed against the hADAM19 peptides were further characterized by Western blots using purified hADAM19 protein, the cell culture media of hADAM19 transfected 293T cells, and purified recombinant METH-1 protein (Fig. 2). The results showed that the pre-immune control IgG molecules for pAb361 and pAb362 did not recognize hADAM19 protein. The pAb360 recognized 86- and 35-kDa fragments in the purified hADAM19. Both pAb361 and pAb362 recognized the 86-, 56-, 45-, and 40-kDa bands in the purified protein. The major band recognized by pAb361 and pAb362 in the media lane was 56 kDa. None of the three anti-hADAM19 Abs had cross-reactions with the purified METH-1 protein. The anti-METH-1 metalloproteinase domain IgG (pAb332) only recognized the METH-1 protein. Therefore, the polyclonal Abs against hADAM19 are very specific.

The propeptide domain of hADAM19 has an unpaired cysteine residue (cysteine switch residue) and a furin cleavage site ($RMKR^{203}$) at the end of this domain, which indicates that the enzyme was synthesized as a zymogen that might be processed by a proprotein convertase such as furin in transit of the secretory pathway. Furin is a serine endopeptidase found mainly in the *trans*-Golgi and expressed in a variety of cells. It cleaves a wide range of precursor proteins, including MDC15 and ADAMTS1 (26, 27). This study showed that anti-propeptide IgG recognized two recombinant hADAM19 protein species at about 86 and 35 kDa (Fig. 2A). The 35 kDa is likely to be a proteolytical fragment containing the propeptide domain. These data implicate that the propeptide domain

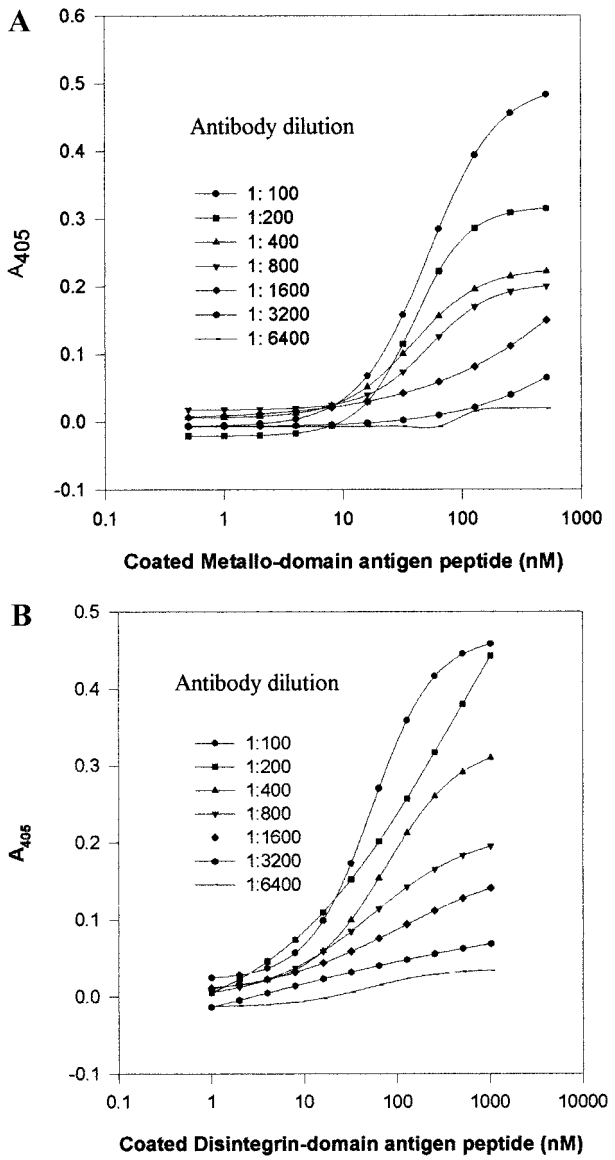

FIG. 1. Characterization of the anti-hADAM19 metalloproteinase and disintegrin domain peptide Abs against hADAM19 peptides by ELISA. ELISA plates were coated with various concentrations of (A) hADAM19 metalloproteinase domain peptide (residues 222–236) and (B) hADAM19 disintegrin domain peptide (residues 431–444). The primary Abs were the rabbit anti-hADAM19 metalloproteinase domain peptide IgG (pAb361) and the rabbit anti-hADAM19 disintegrin domain peptide IgG (pAb362), respectively. The Abs were purified by the ImmunoPureIgG (protein A) purification kit. The Ab dilutions are indicated on the diagram. The secondary Ab was goat anti-rabbit IgG conjugated with alkaline phosphatase.

of the extracellular domain of the hADAM19 protein is partially removed in the secretory pathway. Therefore, some of the ectodomain of hADAM19 were in an active form after secretion.

Antibodies against metallo-domain and disintegrin-domain of hADAM19 are functional neutralizing Abs. α2-M is about 720 kDa under nonreducing conditions (Fig. 3A). Complexes of higher molecular weight formed after incubating with purified hADAM19 for 1–5 days, while the 720-kDa bands became much weaker. The complex was detected only in the presence of α2-M. This indicates that hADAM19 was trapped by α2-M to form a new complex. To test if the hADAM19 has endopeptidase activity, the purified hADAM19 was incubated with α2-M. The α2-M was cleaved and generated new 98- and 76-kDa fragments as detected by SDS–PAGE and silver staining under reducing and denaturing conditions (Fig. 3B). The110- and 114-kDa bands disappeared after incubation for only one day. The time-course for hydrolysis of α2-M by hADAM19 showed that both the 720- and 70-kDa bands became weaker with the time of incubation. In addition, a high band (about 800 kDa) was visualized in each lane. The

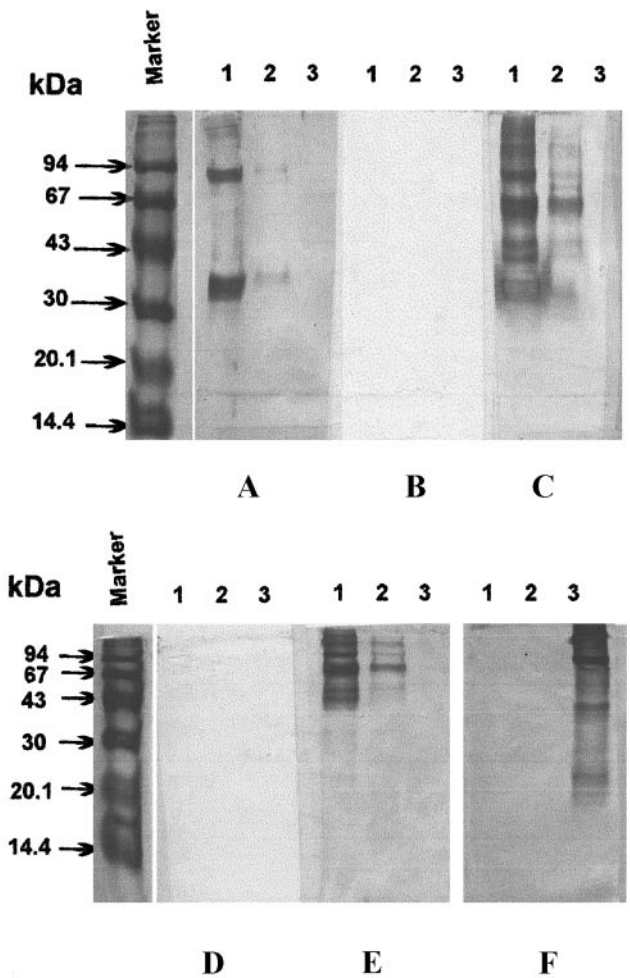

FIG. 2. Specificity of the anti-hADAM19 metalloproteinase and disintegrin domain peptide Abs by immunoblotting. The samples added in lanes 1, 2, and 3 are purified hADAM19, cell culture media of hADAM19 transfected 293T cells, and purified METH-1, respectively. The primary Abs were (A) the rabbit anti-hADAM19 prodomain IgG (pAb360), (B) the preimmune IgG of pAb361, (C) pAb361, (D) the preimmune IgG of pAb362, (E) pAb362, and (F) the rabbit anti-METH-1 metalloproteinase domain IgG (pAb332). The secondary Ab was goat anti-rabbit IgG conjugated with alkaline phosphatase.

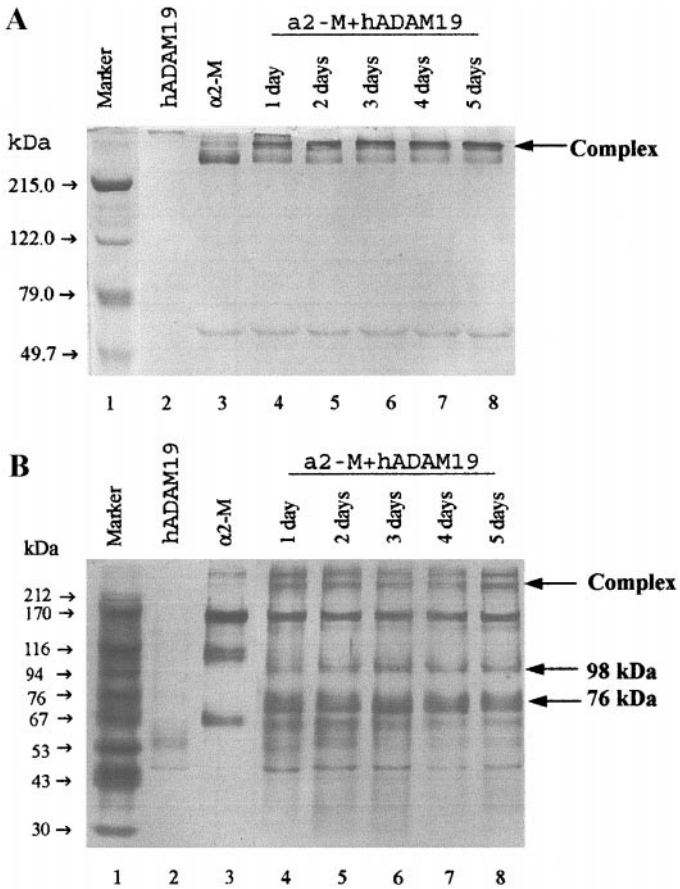

FIG. 3. Detection of hADAM19 binding to and cleavage of α2-M by SDS–PAGE and silver staining. (A) hADAM19 and α2-M complex formation as detected under nonreducing and nonheating conditions. The high-molecular-weight markers are shown in lane 1. hADAM19 and α2-M alone are shown in lanes 2 and 3, respectively. hADAM19 was incubated with α2-M at 37°C for 1–5 days (lanes 4–8) as described under Materials and Methods. The complex is indicated with an arrow. (B) α2-M cleavage by hADAM19 as detected under reducing and denaturing conditions. The combination of high- and low-molecular-weight markers is shown in lane 1. hADAM19 and α2-M are shown in lanes 2 and 3, respectively. The two arrows point to the new 98- and 76-kDa cleavage products of α2-M by hADAM19.

complexes formed between hADAM19 and α2-M were very stable and resisted heat-denaturing and reducing conditions, suggesting that the binding between hADAM19 and α2-M is very tight.

This study also confirmed our previous report that hADAM19 has a metalloproteinase domain and is catalytically active (19). ADAMs 1, 8, 10, 12, 13, 15, 17, 19, and 20 are predicted to be active proteinases (28). However, the proteinase activities of some of those ADAMs remain to be verified experimentally.

Three domain-specific hADAM19 pAbs were developed as tools to investigate the minimum hADAM19 domain structure required for the proteolytic activity. Both the anti-hADAM19 metallo-domain IgG (pAb361) and anti-hADAM19 disintegrin domain IgG (pAb362) completely inhibited the cleavage of α2-M by hADAM19 (Fig. 4). However, the pre-immune control IgG molecules for pAb361 and pAb362 did not block hADAM19 proteolytic activities. Moreover, the anti-hADAM19 pro-domain IgG (pAb360), anti-matrilysin/MMP-7 metallo-domain IgG, and anti-METH-1/ADAMTS1 metallo-domain IgG (pAb332) did not inhibit hADAM19 cleav-

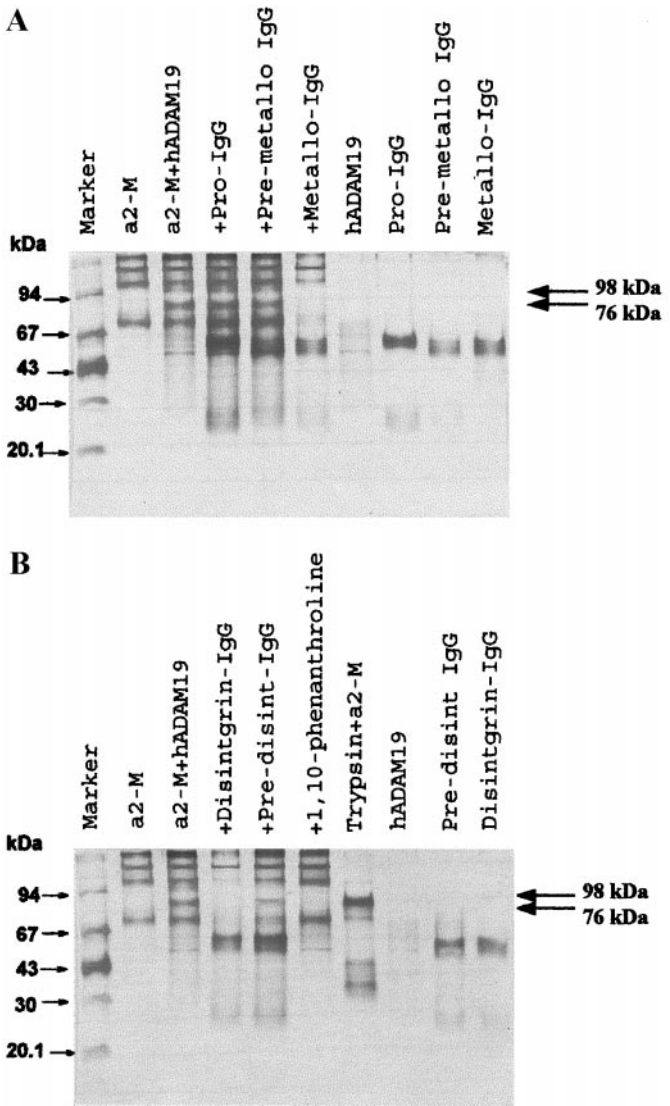

FIG. 4. Blockage of the hADAM19 cleavage of α2-M by (A) anti-metallo domain (pAb361) and (B) anti-disintegrin domain (pAb362) polyclonal Abs as detected by SDS–PAGE silver staining. The low-molecular-weight markers are shown in lane 1. α2-M alone is shown in lane 2. hADAM19 was incubated with α2-M at 37°C for 18 h (lane 3). hADAM19 was preincubated with pAb360 (pro-IgG, A, lane 4), pre-immune IgG of pAb361 (A, lane 5), pAb361 (metallo-IgG, A, lane 6), pAb362 (disintegrin-IgG, B, lane 4), pre-immune IgG of pAb362 (B, lane 5), 1,10-phenanthroline (10 mM) (B, lane 6) at room temperature for 2 h and then incubated with α2-M at 37°C for 18 h. hADAM19 is in A, lane 7, and B, lane 8. The pAb360, pre-immune IgG of pAb361 and pAb361 are shown in A, lanes 8–10, respectively. The pAb362 and pre-immune IgG of pAb362 are shown in B, lanes 9 and 10, respectively. Trypsin was also added as a positive control for an endopeptidase cleavage of α2-M (B, lane 7).

age of α2-M (Fig. 4 and data not shown). A transition metal chelator, 1,10-phenanthroline, completely inactivated the cleavage of α2-M by hADAM19 (Fig. 4B).

The disintegrin domains of ADAMs were regarded as mediators of cell-cell adhesion by binding with integrins (11, 16, 29). Our results indicated that the disintegrin domain may be involved in the process of hydrolysis of the substrate by hADAM19, possibly by facilitating hADAM19 protein folding and/or substrate binding. The cooperation between the metalloproteinase domain and disintegrin domain may be essential for the hADAM19 proteinase activity. These data supply a piece of direct evidence that the substrate may interact with both the active site and disintegrin domain of hADAM19, which is consistent with the hypothesis proposed by Black and White for ADAM17 (30)

Similar to other zinc metalloproteinases, hADAM19 proteolytic activity was inactivated by EDTA (19) and 1,10-phenanthroline; however, it was not inhibited by tissue inhibitors of metalloproteinases (TIMP-1 and TIMP-2) (19), two of the endogenous MMP inhibitors. In addition, its activity was not inhibited by the two synthetic MMP inhibitors tested (19). Although hADAM19 did not cleave the typical matrix metalloproteinase (MMP) substrates such as type I collagen and gelatin, type IV collagen, casein, laminin, and four peptide substrates, and a peptide substrate of tumor necrosis factor-α converting enzyme (TACE) (ADAM17), the purified extracellular domain of hADAM19 did show catalytic activity using the classic α2-M binding assay (19). This catalytic activity was inhibited by our polyclonal antibodies pAb361 and pAb362. Therefore, it is possible that our specific Abs, pAb361 and pAb362, might have functional blocking activity in cellular systems.

The human ADAM19 gene and the mouse ADAM19 gene are 80.6% identical and their proteins are 84.1% identical. Because the hADAM19 protein sequence is highly similar to that of mADAM19, hADAM19 might have similar functions as reported for mADAM19, which participates in osteoblast differentiation (21), peripheral neuronal cell lineage differentiation (22), and the intracellular processing of neuregulin (23). It may also be involved in cell adhesion and migration by binding to integrins through its disintegrin domain in an RGD-independent manner (11, 16, 27). Furthermore, high levels of hADAM19 mRNA were detected in colorectal adenocarcinoma SW480, lung carcinoma A549, and chronic myelogenous leukemia K-562 (19). hADAM19 may be involved in cancer progression, invasion, and metastasis by proteolytically processing cell surface and ECM proteins. By binding to the metalloproteinase and disintegrin domain of ADAM19, our polyclonal antibodies pAb361 and pAb362 may be specific inhibitors to be used to verify those predicted functions and identify novel functions of hADAM19 in biological and pathological processes.

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