Available online at www.sciencedirect.com
SCIENCE DIRECT®
Biochemical and Biophysical Research Communications 315 (2004) 79–84
BBRC
www.elsevier.com/locate/ybbrc

ADAM28 is activated by MMP-7 (matrilysin-1) and cleaves insulin-like growth factor binding protein-3

Satsuki Mochizuki,[a] Masayuki Shimoda,[a] Takayuki Shiomi,[a] Yutaka Fujii,[b] and Yasunori Okada[a,*]

[a] *Department of Pathology, School of Medicine, Keio University, 35 Shinanomachi, Shinjuku-ku, Tokyo 160-0016, Japan*
[b] *Department of Chemistry, Fukui Medical University, 23-3 Shimoaizuki, Matsuoka-cho, Yoshida-gun, Fukui 910-1193, Japan*

Received 17 December 2003

Abstract

ADAM28, a member of a disintegrin and metalloproteinase (ADAM) family, has two isoforms, membrane-type form (ADAM28m) and secreted form (ADAM28s). Although ADAM28 is expressed and synthesized in a precursor form (proADAM28) by lymphocytes and some cancer cells, its activation mechanism and substrates remain unclear. Here, we report that proADAM28s of 65 kDa is processed with active matrix metalloproteinase-7 (MMP-7) to 42- and 40-kDa forms which corresponds to active ADAM28s without propeptide. Processed ADAM28s digested insulin-like growth factor binding protein-3 (IGFBP-3) in both free and complex forms with IGF-I or IGF-II, and the digestion was prevented with EDTA, 1,10-phenanthroline, KB-R7785, tissue inhibitor of metalloproteinases-3 (TIMP-3), and TIMP-4. These data provide the first evidence that proADAM28s is activated by MMP-7 and ADAM28 digests IGFBP-3.

Keywords: ADAM28; MMP-7; Insulin-like growth factor binding protein-3; Insulin like growth factor; TIMP-3

A disintegrin and metalloproteinase (ADAM) family are multifunctional cell surface and secreted glycoproteins. At present, 34 members of the ADAM family have been identified in a wide variety of tissues and organism [1]. Typical functions of ADAM are ectodomain shedding of membrane proteins and adhesive property [2–5]. ADAM28 is expressed by human lymphocytes in two alternative forms, i.e., membrane-type form (ADAM28m) and secreted form (ADAM28s) [6]. ADAM28 possesses a prototypical domain structure [7], which is more closely related to snake venom metalloproteinases than other ADAMs. The previous report on catalytic activity of ADAM28 showed cleavage of myelin basic protein [8]. More recently, Fourie et al. [9] reported that ADAM28 is implicated in ectodomain shedding of CD23, a low affinity IgE receptor. In addition, disintegrin domain of ADAM28 is a ligand for leukocyte integrin $\alpha4\beta1$ [10,11]. However, information about functions of ADAM28 including substrates is still limited. Like other ADAM species, ADAM28 is synthesized as a zymogen form (proADAM28), but there is no study on the activation mechanisms of proADAM28.

Insulin-like growth factors (IGFs) I and II are potent mitogens for breast cancer cells [12] and their mitogenic activity is regulated by IGF binding proteins (IGFBPs) [13] through modulation of the ligand–receptor interactions [14]. The IGFBP family comprises six proteins with high affinity for IGFs [15] and several lower affinity IGFBP-related proteins [16]. Among them, major IGF transport function is attributed to IGFBP-3, which is the most abundant circulating IGFBP species [17]. Recent study has indicated that IGFBP-3 is cleaved by ADAM12 [18]. In addition, our preliminary studies have shown that human breast carcinoma tissues and breast carcinoma cell lines express ADAM28s (Mochizuki et al. manuscript in preparation), which are also known to express matrix metalloproteinase-7 (MMP-7) [19]. Thus, it is reasonable to speculate that ADAM28 may interact with MMP-7 and attack IGFBP-3.

* Corresponding author. Fax: +81-3-3353-3290.
E-mail address: okada@sc.itc.keio.ac.jp (Y. Okada).

0006-291X/$ - see front matter © 2004 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2004.01.022

In the present study, we examined whether pro-ADAM28s is activated by MMP-7 and IGFBP-3 is susceptible to ADAM28s. The data demonstrate for the first time that proADAM28s is processed by MMP-7 into active forms, which can digest IGFBP-3.

Materials and methods

Materials. Recombinant human IGFBP-3, EDTA, 1,10-phenanthroline, aprotinin, IGF-I, IGF-II, 4-aminophenylmercuric acetate (APMA), and *N*-ethylmaleimide were purchased from Sigma (St. Louis, MO). Tissue inhibitor of metalloproteinases-1 (TIMP-1), TIMP-2, TIMP-3, and TIMP-4 were provided by Dr. Kazushi Iwata in Daiichi Fine Chemical (Takaoka, Japan). KB-R7785 was a kind gift by Dr. Koichiro Yoshino in Carnabiosience (Kobe, Japan). ProMMP-7 was purified from culture media of CaR-1 cells according to our methods described previously [20].

Expression and purification of recombinant proADAM28s. cDNA fragment encoding the full-length proADAM28s was amplified by PCR from human lung cDNA library (Clontech, Tokyo, Japan) using a set of primers: 5′-GGAATTCCCCAGCATGTTGCAAGGTCTC-3′ and 5′-ACGCGTCGACTCTGAAATGATTTTCCTTCGC-3′. The PCR fragment encoding proADAM28s was digested with *Eco*RI and *Sal*I, and then cloned into pCMV-Tag4a vector (Stratagene, La Jolla, CA). The pCMV-Tag4a construct was further digested with *Eco*RI and *Kpn*I, and the cDNA encoding the full-length proADAM28s with FLAG tag was cloned into pFASTBac1 vector, (Invitrogen, Tokyo, Japan), generating pFASTBac1/proADAM28s. The vector was transfected into DH10Bac cells (Invitrogen). The bacmids were isolated and then used to generate baculovirus particles in insect cells according to the manufacturer's instructions. Sf9 cells (about 1.0×10^6/ml) were infected with pFASTBac1/proADAM28s and cultured for 2 days and the media were harvested. FLAG-tagged proADAM28s was purified by the immunoaffinity column chromatography of anti-FLAG M2-agarose affinity gels (Sigma, St. Louis, MO) by eluting with 6 M urea in 50 mM Tris–HCl buffer, pH 7.5, 3 M NaCl, 10 mM $CaCl_2$, 0.05% Briji 35, and 0.02% NaN_3. The eluate was extensively dialyzed against 50 mM Tris–HCl buffer, pH 7.5, 0.15 M NaCl, 10 mM $CaCl_2$, 0.05% Briji 35, and 0.02% NaN_3 (TNC buffer).

Processing of proADAM28s by MMP-7. Purified recombinant proADAM28s was iodinated using iodogen-coated tube (Pierce, Rockford, IL) according to our previous methods [21]. ^{125}I-labeled proADAM28s was incubated with active MMP-7 in molar ratios of 5–30:1 (proADAM28s:MMP-7) for 0–8 h at 37 °C in TNC buffer. Five-molar excess amount of TIMP-1 was added to the reaction mixtures and incubated for 5 h to completely block MMP-7 activity. Processing of proADAM28s molecule was visualized by autoradiography after SDS–PAGE (12.5% total acrylamide). Similarly, activation of ^{125}I-labeled proADAM28s with APMA or *N*-ethylmaleimide was monitored by autoradiography according to the previous methods [20,22].

Degradation of IGFBP-3 by ADAM28s. IGFBP-3 was first iodinated and ^{125}I-IGFBP-3 (100 ng) was reacted with ADAM28s in molar ratios of 5–30:1 (IGFBP-3:ADAM28s) for 0–16 h at 37 °C in TNC buffer. Active ADAM28s was prepared by incubating with MMP-7 in a molar ratio of 10:1 (proADAM28s:MMP-7) for 1 h at 37 °C and by blocking MMP-7 activity with 5-M excess amount of TIMP-1. Proteinase inhibitors including EDTA, 1,10-phenanthroline, aprotinin, TIMPs, and KB-R7785 were incubated with activated ADAM28s for 30 min (or for 1 h for TIMPs) at 37 °C prior to incubation with ^{125}I-IGFBP-3. Similarly, ^{125}I-labeled IGF-I or IGF-II was incubated with IGFBP-3 or TNC buffer for 1 h and then digested with ADAM28s for 16 h at 37 °C. The reaction was terminated by boiling for 10 min with 2× SDS-sample buffer containing 5 mM EDTA and then analyzed by SDS–PAGE (12.5% total polyacrylamide). Degradation products of ^{125}I-IGFBP-3 or ^{125}I-IGFs were visualized by autoradiography.

Release of IGF-I from the IGF-I/IGFBP-3 complex after digestion with ADAM28s. Microtiter plates with 96-wells (Nalge Nunc International, Rochester, NY) were coated with 50 μl IGFBP-3 (100 ng/well) for overnight at 4 °C. Then the plates were washed twice with phosphate-buffered saline (PBS) containing 0.05% Brij 35 and subsequently blocked with 1% bovine serum albumin (BSA) in PBS for 2 h at room temperature. ^{125}I-labeled IGF-I (3×10^5 cpm, 100 ng/well) was incubated in each well for 24 h at 4 °C to make the ^{125}I-IGF-I/IGFBP-3 complex. After washing twice with PBS containing 0.05% Brij 35, the complex was digested with ADAM28s (10 ng/well) in TNC buffer for 8 or 16 h at 37 °C. Supernatants were collected and the complex remaining on the wells was dissociated by treatment with 1 N NaOH. Radioactivity in the supernatants and NaOH-dissociated fractions was counted by γ-counter ARC-600 (Aloka, Tokyo, Japan).

NH_2-terminal sequence analysis. ProADAM28s (4 μg) was incubated with MMP-7 in a molar ratio of 10:1 (proADAM28s:MMP-7) for 1 h at 37 °C and MMP-7 activity was blocked with 5-M excess amount of TIMP-1. IGFBP-3 (2 μg) was digested with ADAM28s in a molar ratio of 10:1 (IGFBP-3:ADAM28s) for 16 h at 37 °C. The digestion products were subjected to SDS–PAGE after termination of the reaction with 5 mM EDTA. Proteins in the gels were transferred onto polyvinylidene difluoride membranes and located by staining with 0.1% Coomassie brilliant blue R-250. The bands were excised and sequenced by Edman degradation using Procise 491 Protein Sequencer (Perkin–Elmer Life Sciences, Yokohama, Japan).

Results

Processing of proADAM28s by MMP-7

Purified proADAM28s showed a single band of 65 kDa on the silver stained gel, which corresponds to the predicted size for the precursor form (data not shown). When ^{125}I-proADAM28s was incubated with active MMP-7 in a molar ratio of 10:1 at 37 °C for 0–8 h, it was processed to lower molecular weight species of 42 and 40 kDa (Fig. 1A). The NH_2-terminal sequence analysis of the 42 kDa band showed the sequence D^{196}RKVQEHEKYI, indicating the cleavage of the Lys^{195}–Asp^{196} bond at a position three amino acids upstream from the metalloproteinase domain. On the

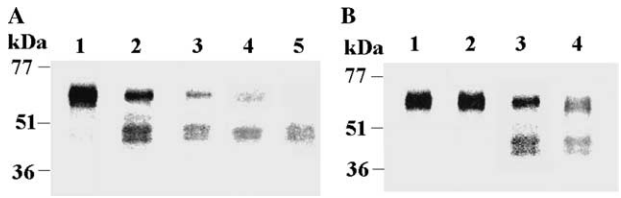

Fig. 1. Activation of proADAM28s by MMP-7. (A) ^{125}I-labeled pro-ADAM28s (100 ng) was incubated with active MMP-7 in a molar ratio of 10:1 (proADAM28s:MMP-7) at 37 °C. Lanes 1–5, proADAM28s reacted with MMP-7 for 0, 1, 2, 3, and 8 h, respectively. (B) ^{125}I-labeled proADAM28s was incubated with active MMP-7 at 37 °C for 1 h. Lane 1, proADAM28s incubated with buffer alone. Lanes 2–4, pro-ADAM28s reacted with MMP-7 in molar ratios of 30:1, 10:1, and 5:1 (proADAM28s:MMP-7), respectively.

other hand, the NH_2-terminal sequence of the 40 kDa band could not be determined because of a small amount of the protein. As shown in Fig. 1B, pro-ADAM28s was processed to the doublets of 42 and 40 kDa after 1-h incubation with MMP-7 in molar ratios of 10:1 and 5:1, but not 30:1. These results demonstrate that proADAM28s is processed by MMP-7 into the lower molecular weight species without the propeptide domain. We also tested whether pro-ADAM28s can be processed by incubation with APMA or *N*-ethylmaleimide, both of which are known to activate proMMPs [23]. However, no changes of proADAM28s were observed during the incubation with these chemicals (data not shown).

Processed ADAM28s cleaves IGFBP-3 by its metalloproteinase activity

IGFBP-3 showed two protein bands of 45 and 40 kDa, which are glycosylated forms of 29 kDa protein [13]. When IGFBP-3 was incubated for 0–16 h with processed ADAM28s, both bands of IGFBP-3 were degraded with time into lower molecular weight species of 22 and 18 kDa (Fig. 2A). A similar digestion pattern was obtained in a dose-dependent manner after incubation in different substrate–enzyme ratios of 30–5:1 (Fig. 2B). NH_2-terminal sequence analysis of the 45-, 40-, and 18-kDa bands of IGFBP-3 showed the same amino acid sequence of G^1ASSGGLGPV, indicating the NH_2-terminus of human IGFBP-3 [24]. On the other hand, the 22-kDa protein band had an NH_2-terminus of A^{98}YLLPAPPAP, demonstrating the cleavage at the central domain between the Arg^{97}–Ala^{98} bond of IGFBP-3. These results suggest that IGFBP-3 is cleaved by ADAM28s at one site of the Arg^{97}–Ala^{98} bond. In order to further study that the IGFBP-3 cleavage is caused by the ADAM28s activity, we carried out the inhibition studies. As shown in Fig. 2C, the digestion was completely inhibited with the divalent cation chelator, EDTA, and 1,10-phenanthroline, but not with an inhibitor of serine proteinases, aprotinin. Importantly, the activity was also completely blocked with KB-R7785 (Fig. 2C, lane 4), a synthetic ADAM inhibitor [4]. Although MMP-7 alone digested IGFBP-3 (data not shown), MMP-7 activity was completely inhibited with 5-M excess amount of TIMP-1, the condition used for activation of proADAM28s (Fig. 2D, lane 4). In addition, purified proADAM28s itself had no activity to IGFBP-3, excluding the possibility of contamination of other proteinases in the preparation (Fig. 2D, lane 2). Thus, these data demonstrate that the metalloproteinase activity of ADAM28s activated by MMP-7 is prerequisite for the IGFBP-3 degradation.

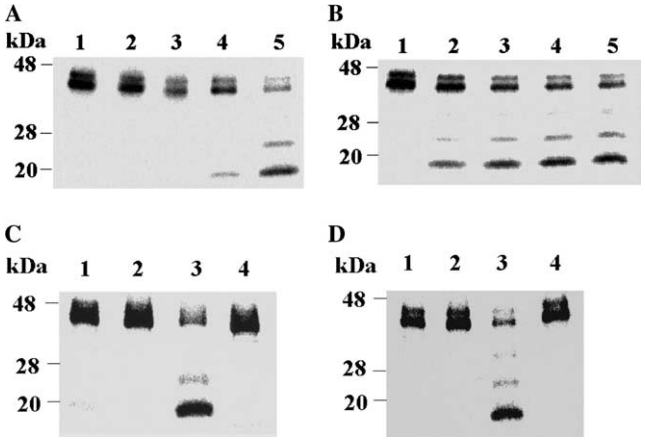

Fig. 2. Cleavage of IGFBP-3 by ADAM28s. (A) Time course digestion of IGFBP-3 by ADAM28s. ^{125}I-labeled IGFBP-3 (100 ng) was incubated with ADAM28s in a molar ratio of 10:1 (IGFBP-3:ADAM28s) at 37 °C. Lanes 1–5, IGFBP-3 reacted with ADAM28s for 0, 1, 5, 8, and 16 h, respectively. (B) Dose-dependent digestion of IGFBP-3 by ADAM28s. ^{125}I-labeled IGFBP-3 was incubated with ADAM28s at 37 °C for 16 h. Lane 1, IGFBP-3 incubated with buffer alone. Lanes 2–5, IGFBP-3 reacted with ADAM28s in molar ratios of 30:1, 20:1, 10:1, and 5:1 (IGFBP-3:ADAM28s), respectively. (C) ADAM28s was reacted with several proteinase inhibitors for 30 min and then incubated with ^{125}I-IGFBP-3 for 16 h. Lanes 1–4, ADAM28s reacted with 10 mM EDTA, 100 μM 1,10-phenanthroline, 100 μM aprotinin, and 1 μM KB-R7785, respectively. (D) ^{125}I-labeled IGFBP-3 was incubated with buffer alone (lane 1), proADAM28s (IGFBP-3:proADAM28s = 10:1, lane 2), ADAM28s (IGFBP-3:ADAM28s = 10:1, lane 3) or a mixture of MMP-7 and TIMP-1, which were reacted in a molar ratio of 1:5 prior to the incubation (IGFBP-3:MMP-7 in the mixture = 10:1, lane 4), for 16 h at 37 °C.

Inhibition of ADAM28s by TIMPs and ADAM28s cleavage of IGFBP-3 in the complex with IGF-I or IGF-II

Since the metalloproteinase activities of ADAM10, ADAM12, ADAM17, and ADAMTS4 are inhibited by TIMP-1 or TIMP-3 [18,25–27], we examined the inhibitor activities of TIMP-1, TIMP-2, TIMP-3, and TIMP-4 to ADAM28s. As shown in Fig. 3A, the degradation of IGFBP-3 by ADAM28s was completely inhibited by TIMP-3 and TIMP-4, but not by TIMP-1 or TIMP-2. We then tested if ADAM28s digests IGFBP-3 in the complex forms with IGF-I or IGF-II. Fig. 3B shows that IGFBP-3 in the complex was cleaved by ADAM28s into the 22- and 18-kDa fragments, an identical pattern to that obtained by the digestion of free IGFBP-3 with ADAM28s. Under this condition, neither IGF-I nor IGF-II was susceptible to the digestion by ADAM28s, since there were no changes in the molecular weights of these IGFs after the incubation with ADAM28s (Fig. 3C).

Release of IGF-I from the IGF-I/IGFBP-3 complex after digestion with ADAM28s

When ^{125}I-IGF-I was incubated in wells coated with IGFBP-3 or BSA, a large amount of ^{125}I-IGF-I bound to IGFBP-3-coated wells but not BSA-coated ones (Fig. 4). Radioactivity of ^{125}I-IGF-I bound to IGFBP-3 on the

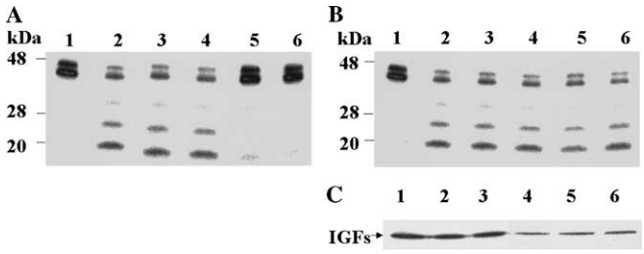

Fig. 3. Inhibition of ADAM28s activity by TIMPs and ADAM28s digestion of IGFBP-3 in the complex with IGF-I or IGF-II. (A) Inhibition activity of TIMPs against ADAM28s was examined by incubating ADAM28s with two molar excess amount of TIMP-1 (lane 3), TIMP-2 (lane 4), TIMP-3 (lane 5) or TIMP-4 (lane 6) for 1 h prior to digestion of ^{125}I-labeled IGFBP-3 for 16 h at 37 °C. Lanes 1 and 2, ^{125}I-IGFBP-3 incubated with buffer alone and ADAM28s, respectively. (B) Digestion of IGFBP-3 in the complex by ADAM28s. ^{125}I-labeled IGFBP-3 was complexed by incubating with 5 mM IGF-I (lane 3), 50 mM IGF-I (lane 4), 5 mM IGF-II (lane 5) or 50 mM IGF-II (lane 6) for 30 min at 37 °C and then digested with ADAM28s in a molar ratio of 10:1 (IGFBP-3:ADAM28s) for 16 h at 37 °C. Lanes 1 and 2, free ^{125}I-IGFBP-3 incubated with buffer alone and ADAM28s, respectively. (C) Insusceptibility to digestion of IGF-I and IGF-II by ADAM28s. Free ^{125}I-IGF-I (50 mM) and ^{125}I-IGF-II (5 mM) or ^{125}I-IGFs/IGFBP-3 complex were digested with ADAM28s in a molar ratio of 10:1 (IGFs:ADAM28s) for 16 h at 37 °C. Lanes 1 and 4, free ^{125}I-IGF-I and ^{125}I-IGF-II incubated with buffer alone, respectively. Lanes 2 and 5, digestion of the ^{125}I-IGF-I /IGFBP-3 and ^{125}I-IGF-II/IGFBP-3 complexes with ADAM28s, respectively. Lanes 3 and 6, digestion of free ^{125}I-IGF-I and ^{125}I-IGF-II with ADAM28s, respectively.

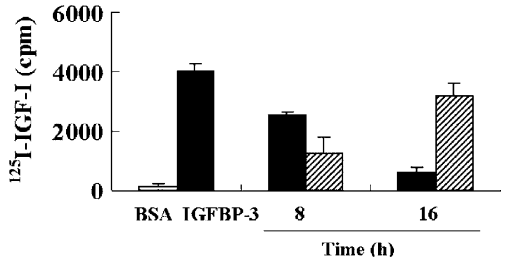

Fig. 4. Release of IGF-I from the IGF-I/IGFBP-3 complex after digestion with ADAM28s. Specific binding of ^{125}I-IGF-I to IGFBP-3 was confirmed by incubating ^{125}I-IGF-I in the wells coated with BSA or IGFBP-3. The ^{125}I-IGF-I/IGFBP-3 complex was digested with ADAM28s for 8 and 16 h at 37 °C. The radioactivity of ^{125}I-IGF-I was measured by γ-counter. Closed and hatched columns are radioactivity in NaOH-dissociated fractions and supernatants, respectively. Bars, SD of triplicate assays.

wells decreased with time after digestion with ADAM28s and that in the supernatants increased (Fig. 4). The sum of the radioactivity was nearly equal to the total radioactivity bound to IGFBP-3 on the wells. Thus, these data demonstrate that IGF-I is released from the IGF-I/IGFBP-3 complex after digestion with ADAM28s.

Discussion

Among the ADAM family members, ADAM9 [28], ADAM12 [22], and ADAM17 [29] have a furin-recognition site at the end of their prodomains and can be activated intracellularly by furin and furin-like convertases. On the other hand, activation mechanisms of other ADAM species including ADAM28 are unknown. Previous studies have shown that proADAM8 and proADAM28 are spontaneously activated during the storage at 4 °C and proADAM12 is activated by the treatment with *N*-ethylmaleimide [9,22]. In the present study, however, proADAM28s was not activated spontaneously or by the treatment with *N*-ethylmaleimide or APMA, which is a common activator of proMMPs. In contrast, proADAM28s could be activated by the incubation with MMP-7. This activation was associated with removal of the propeptide of proADAM28, which was determined by the NH_2-terminal sequence analysis. In addition, it was time- and dose-dependent on active MMP-7. These data demonstrate that the activation is due to the proteolytic action of MMP-7 to proADAM28s and provide the direct evidence of a novel proADAM activation cascade by MMP. ProADAM28s was most efficiently activated by incubation with active MMP-7 in a molar ratio of 10:1 for 1 h, but activated ADAM28s form of 42 kDa appeared to be further degraded after longer incubations or with higher concentrations of MMP-7. Thus, this activation may require subtle balance between proADAM28s and MMP-7.

The present study is the first to demonstrate that ADAM28 cleaves IGFBP-3 at one site between the Arg^{97}–Ala^{98} bond present in the central domain of the molecule. ADAM12s has been reported to degrade IGFBP-3 into several fragments of 10–20 kDa, but no information is available for the cleavage sites [18]. IGFBP-3 is also susceptible to MMP-1, MMP-2, and MMP-3, all of which commonly cleave the Tyr^{99}–Leu^{100} bond with other minor cleavage sites at the Leu^{96}–Arg^{97} and Leu^{141}–His^{142} bonds by MMP-1 and MMP-2 [30] or at the Asn^{109}–Ala^{110} and Glu^{176}–Ser^{177} bonds by MMP-3 [30]. Since all these cleavage sites are located in the central domain of IGFBP-3 consisting of amino acid residues Asn^{89}–Asn^{172} between the NH_2- and COOH-terminal domains, the central domain may form a ternary structure susceptible to proteolytic attacks by MMPs and ADAM28s. The present study has also demonstrated that ADAM28s digests selectively IGFBP-3 in the complex forms with IGF-I or IGF-II without attacking IGFs. In addition, our study showed that IGF-I is released from the complex after digestion of IGFBP-3 by ADAM28s. These data suggest the possibility that ADAM28s can reactivate the activity of IGFs in the complex with IGFBP-3 through selective digestion of IGFBP-3.

Although TIMPs are common inhibitors to MMPs, TIMP-3 is known to inhibit ADAM10 [24], ADAM17 [25], and ADAM12 [18] as well as ADAMTS4 [26]. ADAM10 is also inhibited by TIMP-1 in addition to

TIMP-3 [24]. In our study, the IGFBP-3-degrading activity of ADAM28 was inhibited by both TIMP-3 and TIMP-4, but not TIMP-1 or TIMP-2. Although the molecular mechanism of the inhibition of ADAM species by TIMPs is not well understood, the present study provides the first data that in addition to TIMP-3, TIMP-4 is an inhibitor to ADAM28.

Previous studies [12,13] have shown that proliferation of the human breast cancer cells is regulated at least in part by the IGF signaling. Our parallel studies also showed that human breast carcinomas and carcinoma cell lines express ADAM28s and IGF-induced breast carcinoma cell proliferation is inhibited under the culture conditions in the presence of TIMP-3 or KB-R7785 (Mochizuki et al. manuscript in preparation). Breast cancer cells and tissues are known to express MMP-7 [19] and release the IGFBP-3 fragments with the NH_2-terminus starting with Ala^{98} [31], which can be produced through the cleavage of the Arg^{97}–Ala^{98} bond by the action of ADAM28s. Thus, it is of interest to speculate that ADAM28 activated by MMP-7 is implicated in the proliferation of the breast cancer cells by releasing IGF-I from the IGF-I/IGFBP-3 complex, although such hypothesis remains to be demonstrated by further work at the cellular and tissue levels.

Acknowledgments

We are grateful to Dr. Kazushi Iwata in Daiichi Fine Chemical Co. Ltd. and Dr. Koichiro Yoshino in Carnabiosience for providing us with TIMPs and a synthetic ADAM inhibitor, respectively. This work was supported by the Ministry of Education, Science and Culture of Japan (Grant-in-Aid for scientific research on priority area B2-11240206) to Y.O.

References

[1] D.F. Seals, S.A. Courtneidge, The ADAMs family of metalloproteases: multidomain proteins with multiple functions, Genes Dev. 17 (2003) 7–30.
[2] R.A. Black, C.T. Rauch, C.J. Kozlosky, J.J. Peschon, J.L. Slack, M.F. Wolfson, B.J. Castner, K.L. Stocking, P. Reddy, S. Strinivasan, et al., A metalloproteinase disintegrin that releases tumour-necrosis factor-alpha from cells, Nature 385 (1997) 729–733.
[3] J.R. Allport, H.T. Ding, A. Ager, D.A. Steeber, T.F. Tedder, F.W. Luscinskas, L-selectin shedding does not regulate human neutrophil attachment, rolling, or transmigration across human vascular endothelium in vitro, J. Immunol. 158 (1997) 4365–4372.
[4] M. Asakura, M. Kitakaze, S. Takashima, Y. Liao, F. Ishikura, T. Yoshinaka, H. Ohmote, K. Node, K. Yoshino, H. Ishiguro, et al., Cardiac hypertrophy is inhibited by antagonism of ADAM12 processing of HB-EGF: metalloproteinase inhibitors as a new therapy, Nat. Med. 8 (2002) 35–40.
[5] D. Nath, P.M. Slocombe, A. Webster, P.E. Stephens, A.J. Docherty, G. Murphy, Meltrin gamma (ADAM-9) mediates cellular adhesion through alpha(6)beta(1) integrin, leading to a marked induction of fibroblast cell motility, J. Cell Sci. 113 (2000) 2319–2328.
[6] C.M. Roberts, P.H. Tani, L.C. Bridges, Z. Laszik, R.D. Bowditch, MDC-L, a novel metalloprotease disintegrin cysteine-reich protein family member expressed by human lymphocytes, J. Biol. Chem. 274 (1999) 29251–29259.
[7] L. Howard, R.A. Maciewicz, C.P. Blobel, Cloning and characterization of ADAM28: evidence for autocatalytic pro-domain removal and for cell surface localization of mature ADAM28, Biochem. J. 348 (2000) 21–27.
[8] L. Howard, Y. Zheng, M. Horrocks, R.A. Maciewicz, C. Blobel, Catalytic activity of ADAM28, FEBS Lett. 498 (2001) 82–86.
[9] A.M. Fourie, F. Coles, V. Moreno, L. Karlsson, Catalytic activity of ADAM8, ADAM15, and MDC-L (ADAM28) on synthetic peptide substrates and in ectodomain cleavage of CD23, J. Biol. Chem. 278 (2003) 30469–30477.
[10] L.C. Bridges, P.H. Tani, K.R. Hanson, C.M. Roberts, M.B. Judkins, R.D. Bowditch, The lymphocyte metalloprotease MDC-L (ADAM28) is a ligand for the integrin alpha4beta1, J. Biol. Chem. 277 (2002) 3784–3792.
[11] L.C. Bridges, K.R. Hanson, P.H. Tani, T. Mather, R.D. Bowditch, Integrin alpha4beta1-dependent adhesion to ADAM28 (MDC-L) requires an extended surface of the disintegrin domain, Biochemistry 42 (2003) 3734–3741.
[12] B. Dufourny, J. Alblas, H.A. van Teeffelen, F.M. van Schaik, B. van der Burg, P.H. Steenbergh, J.S. Sussenbach, Mitogenic signaling of insulin-like growth factor I in MCF-7 human breast cancer cells requires phosphatidylinositol 3-kinase and is independent of mitogen-activated protein kinase, J. Biol. Chem. 272 (1997) 31163–31171.
[13] S.M. Firth, R.C. Baxter, Cellular actions of the insulin-like growth factor binding protein, Endocr. Rev. 23 (2002) 824–854.
[14] V. Hwa, Y. Oh, R.G. Rosenfeld, The insulin-like growth factor-binding protein (IGFBP) superfamily, Endocr. Rev. 20 (1999) 761–787.
[15] R.C. Baxter, Insulin-like growth factor (IGF)-binding proteins: interactions with IGFs and intrinsic bioactivities, Am. J. Physiol. Endocrinol. Metab. 278 (2000) E967–E976.
[16] I.H. Hamelers, P.H. Steenbergh, Interactions between estrogen and insulin-like growth factor signaling pathways in human breast tumor cells, Endocr. Relat. Cancer. 10 (2003) 331–345.
[17] R.C. Baxter, Circulating-binding proteins for the insulin like growth factors, Trends Endocrinol. Metab. 4 (1993) 91–96.
[18] F. Loechel, J.W. Fox, G. Murphy, R. Albrechtsen, U.M. Wewer, ADAM 12-S cleaves IGFBP-3 and IGFBP-5 and is inhibited by TIMP-3, Biochem. Biophys. Res. Commun. 278 (2000) 511–515.
[19] M.M. Pacheco, M. Mourao, E.B. Mantovani, I.N. Nishimoto, M.M. Brentani, Expression of gelatinases A and B, stromelysin-3 and matrilysin genes in breast carcinomas: clinico-pathological correlations, Clin. Exp. Metastasis 16 (1998) 577–585.
[20] K. Imai, Y. Yokohama, I. Nakanish, E. Ohuchi, Y. Fuji, N. Nakai, Y. Okada, Matrix metalloproteinase 7 (matrilysin) from human rectal carcinoma cells. Activation of the precursor, interaction with other matrix metalloproteinases and enzymic properties, J. Biol. Chem. 270 (1995) 6691–6697.
[21] I. Inoki, T. Shiomi, G. Hashimoto, H. Enomoto, H. Nakamura, K. Makino, E. Ikeda, S. Takata, K. Kobayashi, Y. Okada, Connective tissue growth factor binds vascular endothelial growth factor (VEGF) and inhibits VEGF-induced angiogenesis, FASEB J. 16 (2002) 219–221.
[22] F. Loechel, B.J. Gilpin, E. Engvall, R. Albrechtsen, U.M. Wewer, Human ADAM12 (meltrin alpha) is an active metalloprotease, J. Biol. Chem. 273 (1998) 16993–16997.
[23] R. Visse, H. Nagase, Matrix metalloproteinases and tissue inhibitors of metalloproteinases, structure, functions, and biochemistry, Circ. Res. 92 (2003) 827–829.
[24] W.I. Wood, G. Cachianes, W.J. Henzel, G.A. Winslow, S.A. Spencer, R. Hellmiss, J.L. Martin, R.C. Baxter, Cloning and expression of the growth hormone-dependent insulin-like growth

factor-binding protein, Mol. Endocrinol. 2 (1988) 1176–1185.

[25] A. Amour, C.G. Knight, A. Webster, P.M. Slocombe, P.E. Stephens, V. Knauper, A.J. Docherty, G. Murphy, The in vitro activity of ADAM-10 is inhibited by TIMP-1 and TIMP-3, FEBS Lett. 473 (2000) 275–279.

[26] A. Amour, P.M. Slocombe, A. Webster, M. Butler, C.G. Knight, B.J. Smith, P.E. Stephens, C. Shelley, M. Hutton, V. Knauper, A.J. Docherty, G. Murphy, TNF-alpha converting enzyme (TACE) is inhibited by TIMP-3, FEBS Lett. 435 (1998) 39–44.

[27] M. Kashiwagi, M. Tortorella, H. Nagase, K. Brew, TIMP-3 is a potent inhibitor of aggrecanase 1 (ADAM-TS4) and aggrecanase 2 (ADAM-TS5), J. Biol. Chem. 276 (2001) 12501–12504.

[28] M. Roghani, J.D. Becherer, M.L. Moss, R.E. Atherton, H. Erdjument-Bromage, J. Arribas, R.K. Blackburn, G. Weskamp, P. Tempst, C.P. Blobel, Metalloprotease-disintegrin MDC9: intracellular maturation and catalytic activity, J. Biol. Chem. 274 (1999) 3531–3540.

[29] H.R. Clarke, M.F. Wolfson, C.T. Rauch, B.J. Castner, C.P. Huang, M.J. Gerhart, R.S. Johnson, D.P. Cerretti, R.J. Paxton, V.L. Price, R.A. Black, Expression and purification of correctly processed, active human TACE catalytic domain in *Saccharomyces cerevisiase*, Protein Expr. Purif. 13 (1998) 104–110.

[30] J.L. Fowlkes, J.J. Enghild, K. Suzuki, H. Nagase, Matrix metalloproteinases degrade insulin-like growth factor-binding protein-3 in dermal fibroblast cultures, J. Biol. Chem. 269 (1994) 25742–25746.

[31] H. Salahifar, S.M. Firth, R.C. Baxter, J.L. Martin, Characterization of an amino-terminal fragment of insulin-like growth factor binding protein-3 and its effects in MCF-7 breast cancer cells, Growth Horm. IGF Res. 10 (2000) 367–377.