Inhibition of Tumor Growth by Antibody to ADAMTS1 in Mouse Xenografts of Breast Cancer

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Abstract. *Background: A disintegrin and metalloproteinase with thrombospondin motifs-1 (ADAMTS1), a member of the ADAMTS family of proteases, is involved in the shedding of epidermal growth factor (EGF)-like ligands such as amphiregulin, which activate the EGF receptor. Since ADAMTS1 has been implicated in aggressive breast carcinogenesis, we examined potential antitumor effects of antibody to ADAMTS1 in a mouse model of breast cancer. Materials and Methods: BALB/c female mice were inoculated with syngenic 4T1 breast cancer cells and treated with anti-ADAMTS1 antibody or control IgG. Tumor volume and weight were evaluated. Results: Mouse 4T1 cells expressed ADAMTS1 and its substrates amphiregulin and heparin-binding EGF. Treatment with antibody to ADAMTS1 inhibited tumor growth without any adverse effects. Conclusion: ADAMTS1 could be a promising molecular target for immunotherapy of breast cancer.*

A disintegrin and metalloproteinase with thrombospondin motifs-1 (ADAMTS1), a member of the ADAMTS family of proteases (1), is composed of propeptide, metalloproteinase, disintegrin, and cysteine-rich domains, domains containing thrombospondin type I-like motifs, and a spacer region (2). ADAMTS1 has been shown to be up-regulated in breast cancer cell lines with elevated metastatic activity (3). Overexpression of ADAMTS1 promotes pulmonary metastasis of TA3 mammary carcinoma and Lewis lung carcinoma cells (4). ADAMTS1 and matrix metalloproteinase-1 synergistically promote bone metastasis of breast cancer cells by shedding epidermal growth factor (EGF)-like ligands, including heparin-binding EGF (HB-EGF), amphiregulin, and transforming growth factor α, from tumor cells, and subsequent activation of the EGF receptor (EGFR) (5). In human breast samples, *ADAMTS1* mRNA is expressed in non-neoplastic mammary tissues, predominantly in stromal fibroblasts, and is significantly down-regulated in breast carcinomas (6). The erythroblastic leukemia viral oncogene homolog (ERBB) family of receptor tyrosine kinases, comprising EGFR (or ERBB1), ERBB2 (or HER2), ERBB3 and ERBB4, have been implicated in the development and progression of many types of human cancer, including breast cancer (7). EGF, transforming growth factor α and amphiregulin bind specifically to EGFR, while HB-EGF binds to both EGFR and ERBB4. None of the EGF family of peptides bind to ERBB2, and ERBB2 has been suggested to function in a dimer with other ERBB receptors. Amphiregulin and HB-EGF are up-regulated in breast cancer (8, 9). ADAMTS1 sheds amphiregulin and HB-EGF to soluble forms, and these EGF-like ligands can promote breast tumor growth and progression by functioning as autocrine and/or paracrine growth factors (4, 5). Several antibodies and tyrosine kinase inhibitors against ERBB receptors have been developed and some of them are effective breast cancer treatments (7). Since inhibition of ADAMTS1 activation or expression blocks EGFR signaling (4, 5), ADAMTS1 is suggested to be a promising target for breast cancer therapy. In this study, we examined the effects of an antibody to ADAMTS1 on tumor growth in a mouse model of breast cancer.

Materials and Methods

Cell culture. Murine breast cancer 4T1 cells (American Type Culture Collection, Manassas, VA, USA), derived from BALB/c mice (10), were culture in RPMI-1640 medium containing 10 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid, 1 mM sodium

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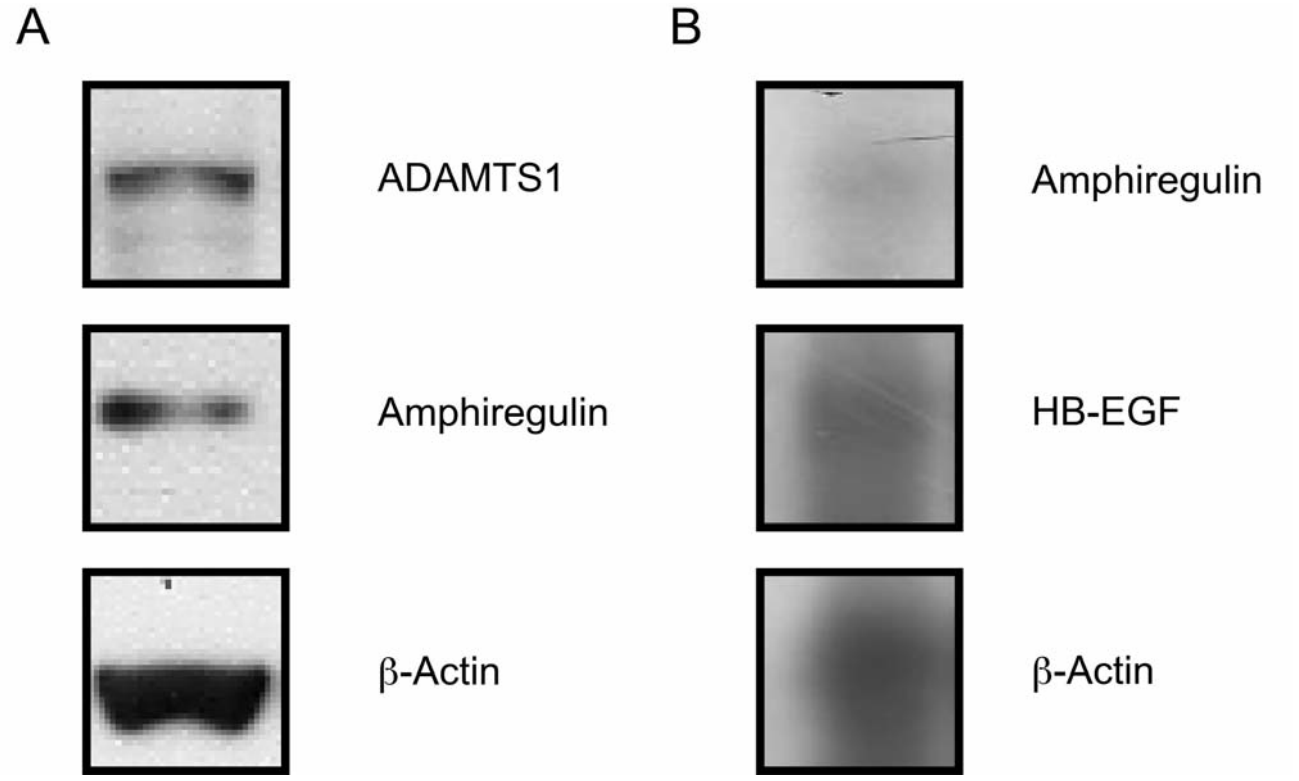

Figure 1. *Expression of ADAMTS1 and EGF-like ligands in mouse breast cancer 4T1 cells. A: Western immunoblotting analysis of ADAMTS1, amphiregulin and β-actin. B: Northern analysis of amphiregulin, HB-EGF and β-actin mRNA.*

pyruvate and 10% fetal bovine serum at 37˚C in a humidified 5% CO_2 atmosphere.

Northern analysis. Total RNA from cells was prepared by guanidium isothiocyanate lysis followed by cesium chloride gradient ultracentrifugation and probed as described previously (11). cDNA probes included mouse amphiregulin (nucleotides 189-960; GenBank accession no. BC009138) and HB-EGF (nucleotides 222-909; GenBank accession no. NM_010415), which were generated from a mouse spleen cDNA library (OriGene Technologies, Inc., Rockville, MD, USA) by polymerase chain reaction and labeled with [α-^{32}P]dCTP.

Western immunoblotting analysis. Total cell lysates (10 μg for each lane) from 4T1 cells were electrophoresed on a sodium dodecyl sulfate-polyacrylamide gel and transferred to a polyvinylidene fluoride membrane for immunoblotting. Western blot analysis was performed using antibodies to ADAMTS1 (Sigma-Aldrich, St. Louis, MO, USA), amphiregulin (Thermo Fisher Scientific Inc., Rockford, IL, USA), or β-actin (Sigma-Aldrich), visualized with an ECL Plus detection system (GE Healthcare, Chalfont St. Giles, UK).

Production of antibody to ADAMTS1. Full-length cDNA encoding mouse ADAMTS1 (2) was inserted into pcDNA3.1 with histidine and flag tags at the C-terminal site. Recombinant ADAMTS1 protein was expressed in HEK293T cells (American Type Culture Collection) and purified with a nickel column. New Zealand White rabbits (Oriental Yeast Co., Ltd., Tokyo, Japan) were immunized subcutaneously with ADAMTS1 repeatedly. The antiserum was obtained 38 days after immunization and purified with a Protein A Sepharose column chromatography (GE Healthcare).

Animal studies. Six-week-old BALB/c female mice (CLEA Japan, Inc., Tokyo, Japan) were inoculated subcutaneously with 4T1 cells (1×10^6). Starting the next day, mice were injected with anti-ADAMTS1 antibody or control IgG (100 μg) *via* the tail vein twice a week (n=7 per group), and body weights and tumor volumes were measured as described previously (11). All animal experiments were conducted in accordance with Animal Care and Use Committee guidelines of Kureha Chemical Industry and Japanese governmental guidelines for animal experiments.

Results

We first examined the expression of ADAMTS1 in breast cancer 4T1 cells, which are aggressive and can metastasize to bone and other tissues (12). As reported in human breast cancer cells with highly metastatic activity (3), ADAMTS1 was detected in 4T1 cells (Figure 1A). Amphiregulin and HB-EGF are EGF-like ligands that are shed and activated by ADAMTS1 (4, 5). 4T1 cells produced amphiregulin protein (Figure 1A). Expression of amphiregulin and HB-EFG mRNA was also detected in 4T1 cells (Figure 1A and B).

We determined the effects of anti-ADAMTS1 antibody on tumor growth of 4T1 cells in syngenic BALB/c mice. Tumor

formation was detected in all the mice inoculated with 4T1 cells at day 3 (Figure 2A). Treatment with anti-ADAMTS1 antibody tended to inhibit tumor growth at day 7 and day 10 and exhibited a significant inhibition at day 14 when compared to the control antibody (Figure 2A). A similar trend was observed at day 17 although it was at the limit of significance (p=0.057). Tumor weight at day 17 in mice treated with anti-ADAMTS antibody was significantly decreased, being 0.77±0.13 g, which was 63% that of control mice (1.23±0.41 g) (Figure 2B). Although abnormal phenotypes of *ADMATS1*-null mice indicate the existence of a physiological function of ADAMTS1 (13), mice treated with anti-ADAMTS1 antibody gained body weight as well as control mice (Figure 2C) and did not exhibit any adverse reactions to treatment.

Discussion

ADAMTS1 is expressed in both metastatic breast cancer cells and normal mammary tissues (6, 14). EGF-like ligands, including amphiregulin and HB-EGF, are substrates of ADAMTS1 and soluble forms of these ligands play a role in bone metastasis of breast cancer (5). Amphiregulin is required for mammary gland development (15), and activation of ERBB receptors is involved in breast carcinogenesis (16). 4T1 breast cancer cells expressed ADAMTS1, amphiregulin and HB-EGF (Figure 1). 4T1 cells also express EGFR and treatment with EGF induces strong phosphorylation of EGFR and extracellular regulated protein kinase (17). Overexpression of ADAMTS1 increases phosphorylation of EGFR and ERBB2 in a protease-dependent manner (4). Our results show that treatment with antibody to ADAMTS1 suppressed tumor growth of 4T1 cells in mice (Figure 2), suggesting that the antibody used here inhibits ADAMTS1 functions, such as the shedding of amphiregulin and/or HB-EGF and subsequent activation of ERBB receptors. Amphiregulin is also cleaved by other proteases such as ADAM17 (18). Further studies are needed to determine whether antibody to ADAMTS1 can inhibit function of other ADAM and ADAMTS proteases. ADAMTS1 promotes cancer cell migration by modulating the extracellular matrix and inducing a stromal reaction (19, 20). The antibody to ADAMTS1 may inhibit tumor growth and progression by multiple mechanisms. The underlying molecular and cellular mechanisms remain to be elucidated. In addition to the successful use of ERBB-targeted antibodies such as trastuzumab, immunotherapy against ADAMTS1 might be promising in the treatment of breast cancer.

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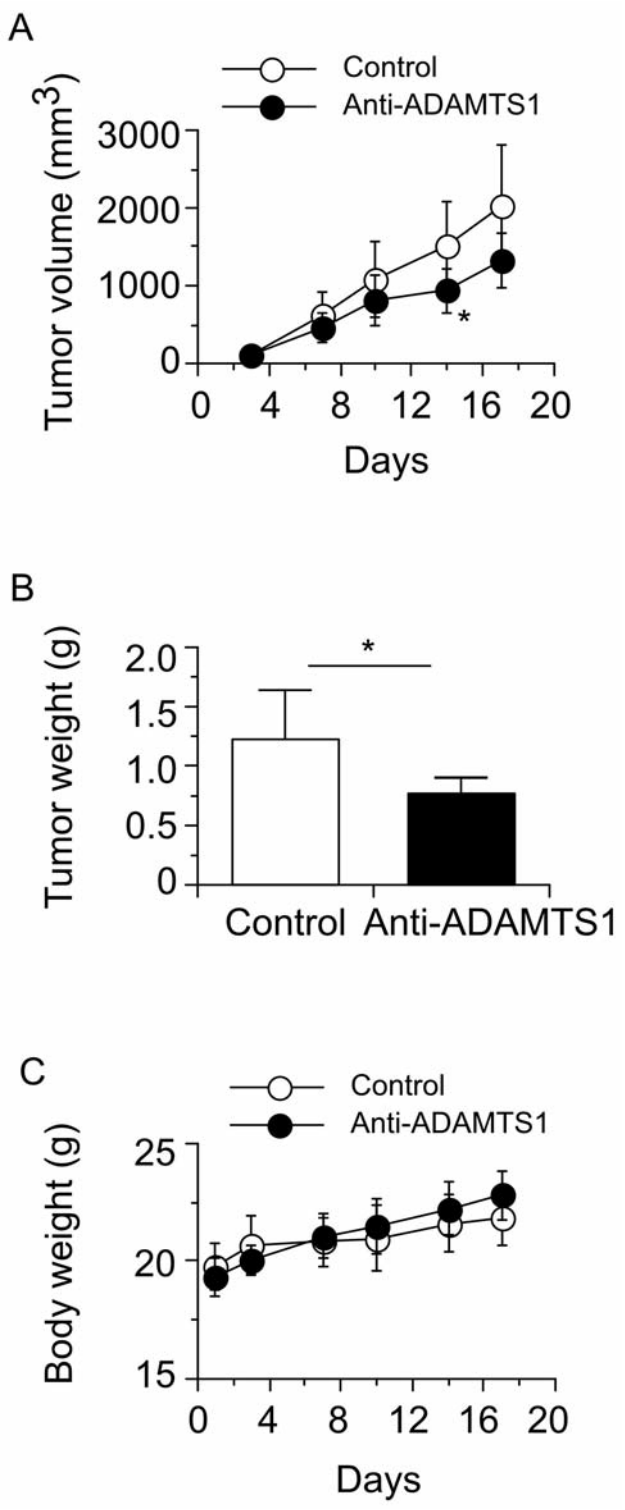

Figure 2. *Effect of antibody to ADAMTS1 on tumor growth of 4T1 cells in xenografts. A: Time course of tumor volume. B: Tumor weight at day 17. C: Body weight. BALB/c female mice inoculated with syngenic breast cancer 4T1 cells were treated with control antibody (Control) or antibody to ADAMTS1 (anti-ADAMTS1) as described in the Materials and Methods. *p<0.05 compared with control, one-way ANOVA.*

References

1 Porter S, Clark IM, Kevorkian L and Edwards DR: The ADAMTS metalloproteinases. Biochem J *386*: 15-27, 2005.

2 Kuno K, Kanada N, Nakashima E, Fujiki F, Ichimura F and Matsushima K: Molecular cloning of a gene encoding a new type of metalloproteinase-disintegrin family protein with thrombospondin motifs as an inflammation associated gene. J Biol Chem *272*: 556-562, 1997.

3 Kang Y, Siegel PM, Shu W, Drobnjak M, Kakonen SM, Cordon-Cardo C, Guise TA and Massague J: A multigenic program mediating breast cancer metastasis to bone. Cancer Cell *3*: 537-549, 2003.

4 Liu Yj, Xu Y and Yu Q: Full-length ADAMTS-1 and the ADAMTS-1 fragments display pro- and antimetastatic activity, respectively. Oncogene *25*: 2452-2467, 2005.

5 Lu X, Wang Q, Hu G, Van Poznak C, Fleisher M, Reiss M, Massague J and Kang Y: ADAMTS1 and MMP1 proteolytically engage EGF-like ligands in an osteolytic signaling cascade for bone metastasis. Genes Dev *23*: 1882-1894, 2009.

6 Porter S, Scott SD, Sassoon EM, Williams MR, Jones JL, Girling AC, Ball RY and Edwards DR: Dysregulated expression of adamalysin-thrombospondin genes in human breast carcinoma. Clin Cancer Res *10*: 2429-2440, 2004.

7 Hynes NE and Lane HA: ERBB receptors and cancer: the complexity of targeted inhibitors. Nat Rev Cancer *5*: 341-354, 2005.

8 LeJeune S, Leek R, Horak E, Plowman G, Greenall M and Harris AL: Amphiregulin, epidermal growth factor receptor, and estrogen receptor expression in human primary breast cancer. Cancer Res *53*: 3597-3602, 1993.

9 Ito Y, Takeda T, Higashiyama S, Noguchi S and Matsuura N: Expression of heparin-binding epidermal growth factor-like growth factor in breast carcinoma. Breast Cancer Res Treat *67*: 81-85, 2001.

10 Aslakson CJ and Miller FR: Selective events in the metastatic process defined by analysis of the sequential dissemination of subpopulations of a mouse mammary tumor. Cancer Res *52*: 1399-1405, 1992.

11 Hirose K, Hakozaki M, Nyunoya Y, Kobayashi Y, Matsushita K, Takenouchi T, Mikata A, Mukaida N and Matsushima K: Chemokine gene transfection into tumour cells reduced tumorigenicity in nude mice in association with neutrophilic infiltration. Br J Cancer *72*: 708-714, 1995.

12 Lelekakis M, Moseley JM, Martin TJ, Hards D, Williams E, Ho P, Lowen D, Javni J, Miller FR, Slavin J and Anderson RL: A novel orthotopic model of breast cancer metastasis to bone. Clin Exp Metastasis *17*: 163-170, 1999.

13 Shindo T, Kurihara H, Kuno K, Yokoyama H, Wada T, Kurihara Y, Imai T, Wang Y, Ogata M, Nishimatsu H, Moriyama N, Oh-hashi Y, Morita H, Ishikawa T, Nagai R, Yazaki Y and Matsushima K: ADAMTS-1: a metalloproteinase-disintegrin essential for normal growth, fertility, and organ morphology and function. J Clin Invest *105*: 1345-1352, 2000.

14 Minn AJ, Kang Y, Serganova I, Gupta GP, Giri DD, Doubrovin M, Ponomarev V, Gerald WL, Blasberg R and Massague J: Distinct organ-specific metastatic potential of individual breast cancer cells and primary tumors. J Clin Invest *115*: 44-55, 2005.

15 McBryan J, Howlin J, Napoletano S and Martin F: Amphiregulin: role in mammary gland development and breast cancer. J Mammary Gland Biol Neoplasia *13*: 159-169, 2008.

16 Yotsumoto F, Yagi H, Suzuki SO, Oki E, Tsujioka H, Hachisuga T, Sonoda K, Kawarabayashi T, Mekada E and Miyamoto S: Validation of HB-EGF and amphiregulin as targets for human cancer therapy. Biochem Biophys Res Commun *365*: 555-561, 2008.

17 Du WW, Yang BB, Shatseva TA, Yang BL, Deng Z, Shan SW, Lee DY, Seth A and Yee AJ: Versican G3 promotes mouse mammary tumor cell growth, migration, and metastasis by influencing EGF receptor signaling. PLoS ONE *5*: e13828, 2010.

18 Sternlicht M and Sunnarborg S: The ADAM17–amphiregulin–EGFR axis in mammary development and cancer. J Mammary Gland Biol Neoplasia *13*: 181-194, 2008.

19 Esselens C, Malapeira J, Colome N, Casal C, Rodriguez-Manzaneque JC, Canals F and Arribas J: The cleavage of semaphorin 3C induced by ADAMTS1 promotes cell migration. J Biol Chem *285*: 2463-2473, 2010.

20 Rocks N, Paulissen Gv, Quesada-Calvo F, Munaut C, Gonzalez M-LA, Gueders M, Hacha J, Gilles C, Foidart J-M, Noel As and Cataldo DD: ADAMTS-1 metalloproteinase promotes tumor development through the induction of a stromal reaction *in vivo*. Cancer Res *68*: 9541-9550, 2008.

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