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ADAM19 is tightly associated with constitutive Alzheimer's disease APP α-secretase in A172 cells

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Abstract

To elucidate whether new proteases are involved in the processing of amyloid precursor protein (APP), we examined catalytically active ADAM12 and ADAM19 as candidates α-secretases. The overexpression of ADAM19 in HEK293 cells resulted in an increase in sAPPα. Therefore, we suggest that ADAM19 has a constitutive α-secretase activity. We examined regulated α-secretase activity by adding phorbol 12-myristate 13-acetate (PMA), but no regulated activity was found. To verify that endogenous ADAM19 has an APP α-secretase activity, we examined whether the constitutive level of α-secretase activity was reduced by RNA interference with ADAM19 in A172 cells. The amount of secreted sAPPα decreased by about 21% following RNAi. These results suggest that ADAM19 has a constitutive α-secretase activity for APP.

Keywords: ADAM19; APP; α-secretase; Alzheimer's disease

One of the hallmarks of Alzheimer's disease (AD) is the formation of senile plaques composed mainly of amyloid β-peptide (Aβ), a 4 kDa small peptide. Aβ is derived from amyloid precursor protein (APP) through an amyloidogenic pathway. In this pathway, APP is cleaved proteolytically by β- and γ-secretases. In the alternative, non-amyloidogenic pathway, APP is cleaved within the Aβ sequence by α-secretase, releasing the ectodomain fragment referred to as soluble APP α (sAPPα) from the cell surface. Then the transmembrane stub is cleaved by γ-secretase to generate a soluble p3 fragment instead of Aβ. To avoid the formation of senile plaques, suppressing the generation of Aβ is important to the amyloid cascade hypothesis that the Aβ molecule initiates the pathological cascade of Alzheimer's disease [1]. The activation of α-secretase presents one potential therapeutic target.

α-Secretase belongs to a disintegrin and metalloprotease (ADAM) family; ADAM9, ADAM10, and ADAM17 are identified as putative α-secretases [2–4]. ADAMs comprise a family of type I membrane proteins that contain multiple conserved domains, including a signal peptide, prodomain, metalloprotease domain, disintegrin domain, cysteine-rich domain, EGF-like domain, transmembrane domain, and cytoplasmic domain. ADAMs mediate the ectodomain shedding of various membrane anchored signaling and adhesion proteins. The initial ectodomain shedding of ADAMs triggers the subsequent cleavage of the remaining C-terminal stubs, referred to as regulated intramembrane proteolysis (RIP), to release the intracellular domain of the protein. This fragment can induce transcription. ADAMs mediate constitutive and regulated shedding.

Abbreviations: AD, Alzheimer's disease; Aβ, amyloid β-peptide; APP, amyloid precursor protein; sAPPα, soluble APP α; ADAM, a disintegrin and metalloprotease; PMA, phorbol 12-myristate 13-acetate; TRANCE, TNF-related activation-induced cytokine.

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There is sufficient evidence that ADAMs are related to α-secretase proteolysis to produce APP. Koike et al., indicated that ADAM9 has both constitutive and regulative α-secretase activity by the coexpression of mouse ADAM9 and human APP in COS-7 cells [2]. Hotoda et al., cloned a new secreted form of human ADAM9, hADAM9s, the EGF-like domain of which contains a stop codon, then coexpressed hADAM9s with human APP in COS cells, providing evidence that hADAM9s has regulated activity [5]. On the other hand, there are no differences in Aβ and p3 in cultured hippocampal neurons from ADAM9-deficient or wild-type mice [6]. This result might be due to compensation by or redundancy with other α-secretases, or with other molecules that might be necessary to uncover a physiologically relevant function.

In 1999, the overexpression of ADAM10 in HEK293 cells indicated that ADAM10 has constitutive and regulated α-secretase activity [3]; however, APP processing is normal in embryonic fibroblast cells from ADAM10 deficient mice [7]. In spite of that, double transgenic mice expressing ADAM10 and APPV717I showed reduced formation of Aβ and no Aβ deposition in plaques, as well as an alleviation of impaired long-term potentiation and cognitive deficits [8]. These results suggest that the activation of α-secretase leads to a predominance of the non-amyloidogenic pathway and avoids Aβ deposition even in AD model mice. The mechanism of regulation of these two pathways remains unknown, but a conspicuous function for α-secretase in the amyloidogenic pathway is apparent.

Disruption of the ADAM17 gene completely abolishes the regulated but not the constitutive α-cleavage in primary embryonic fibroblasts [4]. However, the overexpression of ADAM17 in HEK293 cells shows that it also acts as a constitutive α-secretase [9]. Therefore, these contradictions were thought to be attributable to the difference in cell types.

In a previous report, Asai et al., showed that these three ADAMs act as constitutive and regulatory α-secretases in COS-7 cells, although their suppression by siRNA was incomplete with substantial activities remaining in A172 glioblastoma cells [10]. Other evidence that ADAM10 deficient mice have unchanged protein levels of ADAM9 and ADAM17 has been reported [7]. The data imply the existence of a new protease acting as an α-secretase. To elucidate whether other proteases are involved in this process, we examined catalytically active ADAM12 and ADAM19 as candidates for APP secretases.

Materials and methods

Cell culture. HEK293 (human embryonic kidney) and A172 (human glioblastoma) cells were cultured in DMEM (Sigma) supplemented with 10% fetal bovine serum (Gibco). The cells were maintained at 37 °C in a 5% CO_2 atmosphere in a tissue culture incubator.

Vector and constructs. Human ADAM12 or ADAM19 was inserted into pcDNA3.1/V5-HisA (Invitrogen), the site of which was digested by *Eco*RV and *Xba*I, after the addition of GCCACC as a Kozak sequence and excluding the stop codon. The V5 epitope tag was fused at the C-terminus.

Constitutive α-secretase activity. HEK293 cells were transiently transfected with vector, hADAM12 or hADAM19 cDNA using lipofection with FuGENE™6 Transfection Reagent (Roche). Two microgram of DNA was used to transfect one 6 cm dish of 40–50% confluent cells. Forty-eight hours later, the cells were washed twice with PBS and 1.5 ml of serum-free DMEM was added. Following incubation for 6 h, the cells and medium were collected.

Regulated α-secretase activity. HEK293 cells were transiently transfected with vector, hADAM12 or hADAM19 cDNA using lipofection with FuGENE™6 Transfection Reagent (Roche). Eight microgram of DNA was used to transfect one 10 cm dish of 50–60% confluent cells. The cells were replated in 6 cm dishes 24 h after transfection, washed twice with PBS, and 1.5 ml of serum-free DMEM was added with or without 30 nM phorbol 12-myristate 13-acetate (PMA) (Wako). Forty-eight hours after transfection, the cells and medium were collected. All samples contained 0.1% DMSO.

Cell collection and medium concentration. Cells were lysed on ice in TNE lysis buffer (25 mM Tris–HCl [pH 7.5], 150 mM NaCl, 5 mM EDTA, and 1% NP-40) containing a protease inhibitor mixture. Cell disruption was completed by freezing and thawing three times. Following centrifugation at 16,000*g* for 10 min at 4 °C, the protein concentration in the supernatant was quantified by a DC Protein Assay Kit (Bio-Rad).

The medium was concentrated by precipitation with 10% trichloroacetic acid (TCA). The total proteins obtained from medium or 20 μg of proteins from cell lysates were used for Western blot analysis.

Western blot analysis. Samples were separated by 10% SDS–PAGE, and then transferred to PVDF membranes (Immobilon-P, Millipore). The membranes were soaked in 5% non-fat dried milk in TPBS (PBS with 0.05% Tween) for 1 h, and then incubated overnight with primary antibodies dissolved in TPBS containing 0.1% BSA and 1 mM NaN_3 at 4 °C. After washing three times with TPBS for 5 min, the membranes were incubated with a horseradish peroxidase (HRP)-conjugated secondary antibody (Cell Signaling Technology) for 1 h at room temperature. The membranes were rinsed three times with TPBS for 5 min, and the antigen-antibody complex was detected by enhanced chemiluminescence using a Luminescent Image analyzer LAS-3000 (Fujifilm). The magnitude of the signal was digitized using Multi Gauge Ver.2.3 (Fujifilm).

The antibodies used in this study are as follows: 1:1000 of 6E10 (SIGNET), 1:1000 of actin (Sigma), 1:5000 of anti-V5 antibody (Invitrogen).

RNA interference. Stealth RNAs specific to hADAM19 were designed by the program offered by Invitrogen. Stealth RNA is 25 bp-blunt-end-dsRNA chemically modified to eliminate the nonspecific stress response of interferon. We confirmed the specificity of these sequences in BLAST. Fifty percentage confluent A172 cells were transfected with hADAM19 stealth RNA or control random RNA (100 pmol per 6-cm dish) using Lipofectamine 2000 (Invitrogen) and OPTI-MEM I (Gibco). The medium was exchanged 6 h after transfection. The cells were washed once with PBS, and 1.5 ml of DMEM was added 36 h after transfection; 8 h later, the cells and medium were collected. Cells were divided into two fractions for protein assay and RT-PCR, and the medium was concentrated with 10% TCA.

The target sequences used in this study are as follows:

sequence 1
sense: GGGCCAACACCUUAUUUACAGAUCU
anti-sense: AGAUCUGUAAAUAAGGUGUUGGCCC
sequence 2
sense: CCUCGUGGCUGAUUAUUUAGAGUUU
anti-sense: AAACUCUAAAUAAUCAGCCACGAGG

We used stealth RNAi Negative Control Low GC Duplex (Invitrogen) as a negative control.

RT-PCR. Total RNA from each culture was isolated by extraction with guanidine thiocyanate/phenol/chloroform, treated with RNase-free DNase (TaKaRa) for 30 min at 37 °C to eliminate any contaminating DNA, extracted with phenol/chloroform, and precipitated with 2-propanol. The amount of RNA was determined spectrophotometrically by the absorbance at 260 nm. RNA was reverse transcribed using oligo (dT) primer by RT-PCR with a ThermoScript™ RT-PCR System (Invitrogen) according to the manufacturer's instructions. Primers specific for each gene were used to

detect each mRNA prepared by RT-PCR. Amplification with specific primers was carried out in a Mastercycler gradient (Eppendorf) with an Ex-*Taq* polymerase PCR kit (TaKaRa) for 40 cycles with 30 s at 96 °C denaturation, 30 s at 58 °C annealing, 30 s at 72 °C extension profile in the case of ADAM; and for 30 cycles with a 30 s at 96 °C denaturation, 30 s at 58 °C annealing, 18 s at 72 °C extension profile in the case of β-actin. Amplification of the mRNA for the housekeeping gene β-actin was used as an internal quality standard. Amplified products were electrophoresed in 8% acrylamide gels, and visualized by ethidium bromide staining.

The primer sequences were as follows:

hADAM9
sense: 5-TTGCCACAGACCCGGTATGT-3;
anti-sense: 5-CTCTCCCATCATCGTGATTC-3;
hADAM10
sense: 5-GGCAATGTGCAGGTTCTATC-3;
anti-sense: 5-GGTTTAGGAGGAGGCAACTT-3;
hADAM17
sense: 5-GTTTGAGACTGCCCAGAAGA-3;
anti-sense: 5-CAAGGAGAAAACCAGGACAG-3;
hADAM19
sense: 5-GCCCATTGACACCACTATCATC-3;
anti-sense: 5-GGAGTGTTGATCACCTTTCGC-3;
β-actin
sense: 5-GTGACATTAAGGAGAAGCTGTGC-3;
anti-sense: 5-TCTCCTTCTGCATCCTGTCGGC-3

Co-immunoprecipitation. HEK293T cells overexpressing vector or human ADAM19 were solubilized in TNE lysis buffer containing 1 mM PMSF and protease inhibitor mixture. The homogenate was passed through a 20-gauge needle (20 times). The cells were rotated for 20 min and centrifuged at 100,000*g* for 30 min at 4 °C. The same protein concentration of the supernatant was transferred to new tubes and precleared using Protein A–Sepharose beads (Amersham Biosciences) for 1 h at 4 °C and immunoprecipitated overnight using V5 antibody (Invitrogen). The antibody-bound complexes were isolated by incubation with Protein A Sepharose beads for 2 h and washed three times using the TNE lysis buffer. The protein complexes were eluted in 2× SDS sample buffer and analyzed by Western blot with a polyclonal APP antibody directed to the C-terminus of APP.

Results

Effects of ADAM12 and ADAM19 cDNA transfection on the constitutive α-secretase activity in HEK293 cells

First, we selected ADAM12 and ADAM19 as candidates for APP secretases from catalytically active human ADAMs expressed in brain. To find out whether these ADAMs have α-secretase activity, we overexpressed them in HEK293 cells. The release of sAPPα into the medium was detected by Western blot analysis. The results are shown in Fig. 1A. Quantitative analysis of an immunoblot with 6E10 showed that HEK293 cells transiently expressing ADAM19 released 2.01 times as much sAPPα as control cells (Fig. 1B). In contrast, cells expressing ADAM12 released sAPPα at a level that was almost the same as the control.

However, the percentage of the active form of ADAM12 was lower than that of ADAM19 (Fig. 1C). This conversion from the pro to active form might account for the difference in catalytic activity between ADAM12 and ADAM19.

Effects of ADAM12 and ADAM19 on the regulated α-secretase activity in HEK293 cells

A number of studies have shown that treatment with phorbol esters such as phorbol 12-myristate 13-acetate (PMA) activates PKC and results in an increase in sAPPα secretion from cells [3,4]. It has been reported that PKC-dependent α-secretase competes with β-secretase for the cleavage of APP in the *trans*-Golgi network [11]. To clarify whether these ADAMs have a regulated α-secretase activity, cells were treated with 30 nM PMA for 2 h. Consistent with previous reports, we observed an increase in sAPPα secretion by PMA treatment (Figs. 2A and B); however, there was no difference according to the ratio of PMA addition to ADAM only (Fig. 2C). These results suggest that these ADAMs do not possess a regulated α-secretase activity. We confirmed that the expression levels of active ADAMs did not change (Fig. 2D).

Reduction in the constitutive α-secretase activity by RNA interference of hADAM19 in A172 cells

To verify that internal ADAM19 has an α-secretase activity, we examined whether the constitutive α-secretase activity was reduced by RNA interference of ADAM19 in A172 cells. In the present study, we used the same cell line as used in a previous report [10]. After reference to the program 'siDirect', we chose several effective sequences as shown in Methods. Finally three sequences were selected with the program offered by Invitrogen. To screen for effective sequences, we used HEK293 cells stably expressing hADAM19. The three RNAs effectively, but not completely, repressed the expression of hADAM19 as detected by Western blot for the V5 epitope added to the C-terminus of the ADAM19 transgene. We used two of these RNAs in this experiment. As shown in Fig. 3, the amount of secreted sAPPα was decreased in 21% by the addition of sequence 2. The same results were obtained using sequence 1 (data not shown). We determined the specificity to hADAM19 by semi-quantitative RT-PCR in Fig. 3C. These sequences did not interfere with the expression of ADAM9, 10, or 17.

Direct interaction of ADAM19 with APP

To further support that ADAM19 has an APP α-secretase activity, we confirmed the direct interaction between APP and ADAM19. HEK293T cells overexpressing ADAM19 was immunoprecipitated with anti-V5 antibody. APP was co-immunoprecipitated by the anti-V5-antibody (Fig. 4A, lane 4), which was absent in the control (Fig. 4A, lane 3). Total lysates were used as a positive control (Fig. 4, lanes 1 and 2). Immunoprecipitation was completed as shown in Fig. 4B.

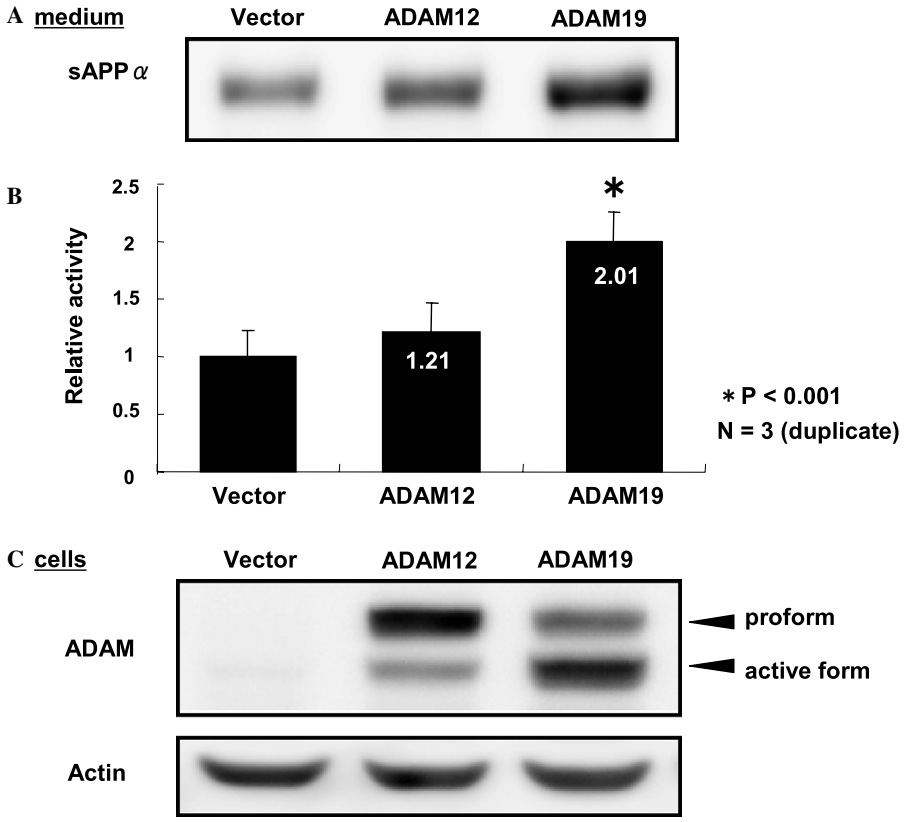

Fig. 1. Constitutive α-secretase activity in HEK293 cells. (A) Cells were transfected with control, ADAM12 or ADAM19 vectors. Following incubation for 6 h, the medium was collected and sAPPα content was determined by Western blot analysis with 6E10. (B) Relative α-secretase activity compared with the control was calculated from (A) adjusted for the protein content of the cell lysate. Values represent means ± SE Statistical significance between control and ADAM-transfected cells were determined by two-tailed Student's *t*-test, and values of $p < 0.001$ were considered significant. A value of $p = 0.06$ was obtained for ADAM12. (C) Cells were collected and the amounts of expressed ADAMs were determined by Western blot analysis with anti-V5 antibody. The same membrane was stripped and determined with anti-actin antibody.

Discussion

We have shown that the overexpression of ADAM19 increases sAPPα secretion in HEK293 cells. Moreover, the suppression of endogenous ADAM19 expression by RNAi decreases the secretion of sAPPα by A172 cells. These results suggest that ADAM19 has a constitutive α-secretase activity for APP.

ADAM12, which is highly homologous to ADAM19, produced no statistically significant α-secretase activity. We could observe the activation of ADAM12 in this system; however, the conversion to the active form was less effective in the case of ADAM12 than ADAM19 (Fig. 1C). Since the prodomain of ADAM12 remains noncovalently associated with the rest of the molecule after cleavage [12], it is intriguing to consider whether this prodomain plays some role such as an activation regulator or a receptor.

Next, we determined the regulated activity of these ADAMs in HEK293 cells by PMA treatment. Increases in the secretion of sAPPα were observed, but were thought to be due to other α-secretases such as ADAM9, 10 or, 17. We suggest that ADAM12 and ADAM19 do not have a regulated activity. The transcriptional level of ADAM was slightly elevated by PMA treatment, although the activation of ADAM was regulated at a certain level in cells.

We demonstrated with the RNAi experiment that the constitutive APPα-secretase activity in A172 cells is due to ADAM19. The percentage (21%) is relatively low compared with other α-secretase activities, but this phenomenon is observed only in A172 glioblastoma cells. Therefore, it is difficult to say which ADAM is the true α-secretase mainly acting in normal brain. Considering that the remaining activity was about 13% in a previous study [10], ADAM19 may contribute about 20% of the α-secretase activity in A172 cells. Since purified ADAM19 did not digest α-secretase substrates (MCA-HQKLVF-FAK(DNP)-NH_2, Suc-HQK-MCA, and Suc-HHQ-MCA) *in vitro* (unpublished results), it might be possible that ADAM19 activates α-secretase activity indirectly by activating other ADAMs.

In the present study, we have shown ADAM19 as a new α-secretase for APP. This extends the availability of therapeutic targets. However, the concern remains that the activation of α-secretase could induce side effects by shedding substrates other than APP. ADAM19 is associated with

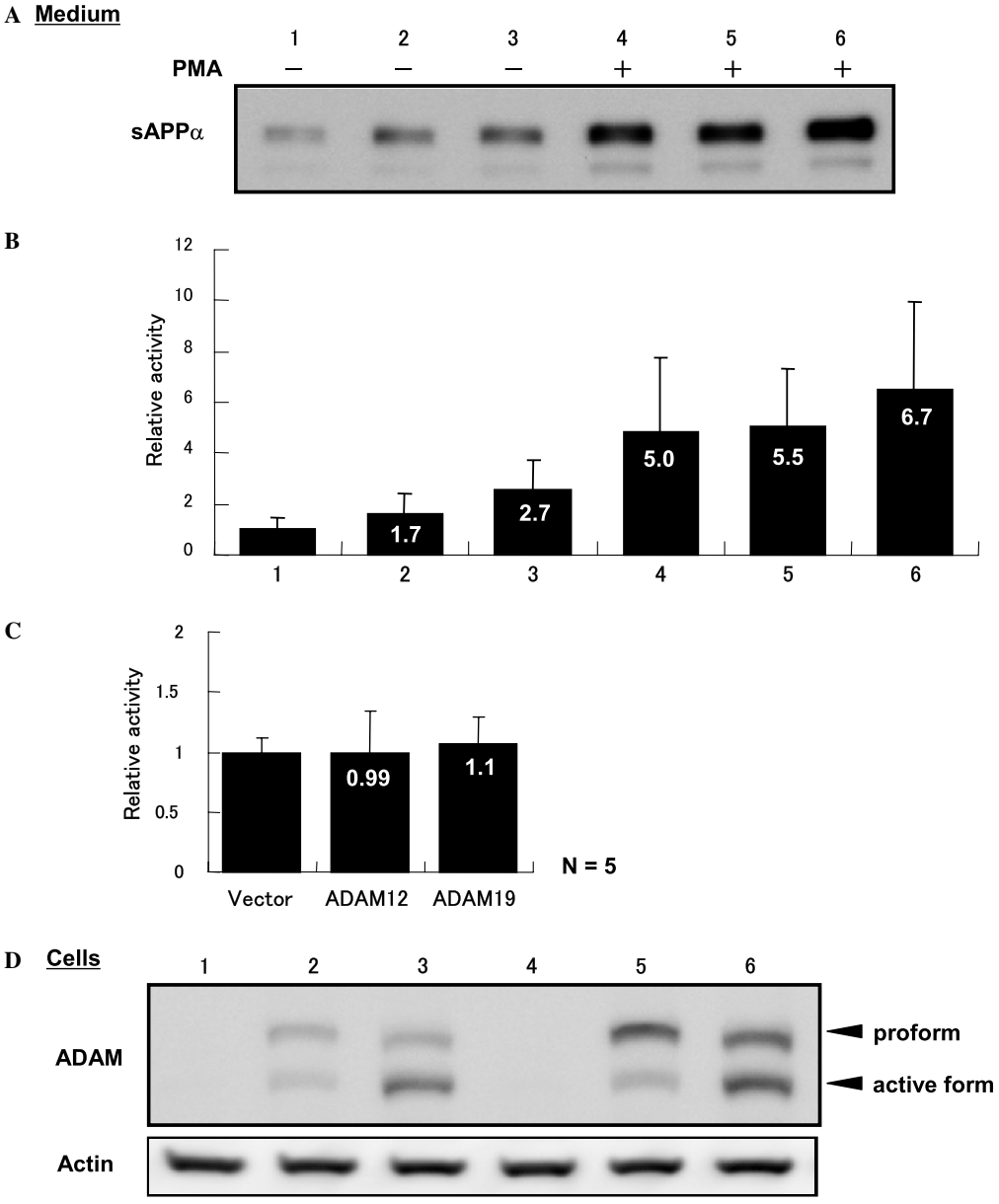

Fig. 2. Regulated α-secretase activity in HEK293 cells. (A) Cells were transfected with control (lanes 1 and 4), ADAM12 (lanes 2 and 5) or ADAM19 (lanes 3 and 6) vectors, and incubated for 2 h with (lanes 4–6) or without (lanes 1–3) 30 nM PMA in serum-free DMEM. All samples contained 0.1% DMSO. The medium was collected and sAPPα content was determined by Western blot analysis with 6E10. (B) The relative α-secretase activity compared with the untreated control is shown in (A) adjusted for the protein content of the cell lysate. Values represent means ± SE of five experiments. (C) The influence of PMA treatment on the increase in sAPPα secretion was compared with the control (A). Values represent means ± SE of five experiments. Statistical significance between the control and ADAM were determined by use of two-tailed Student's *t*-test; the results were not significant. (D) Cells were collected and the expressed ADAMs were measured by Western blot analysis with anti-V5 antibody. The same membrane was stripped and determined with anti-actin antibody.

the proteolytic processing of neuregulin [13] and TNF-related activation-induced cytokine (TRANCE) [14]. Neuregulin and ADAM19 are simultaneously expressed in the nervous system during development, and ADAM19 participates in the proteolytic processing of β-type neuregulin, which is involved in neurogenesis and synaptogenesis [13]. Wong et al., have reported a potential role for ADAM19 in shedding TRANCE in cells where both molecules are highly expressed [14]. TRANCE is expressed most highly in thymus and lymph nodes, but not in non-lymphoid tissues [15]. These findings suggest that the upregulation of ADAM19 is also available as a therapeutic target in adult brain. Recent study has shown that acetylcholinesterase inhibitors promote the trafficking of ADAM10, 17, and APP to the cell membrane and enhance α-secretase activity with few side effects [16]. This implies the importance of the location that APP shedding occurs.

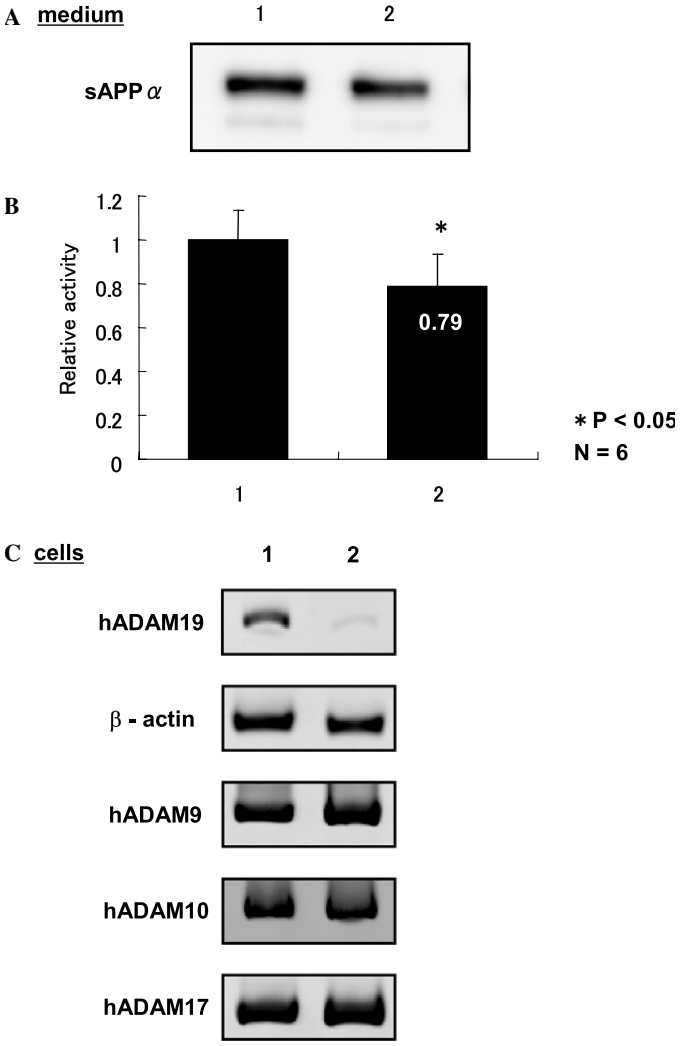

Fig. 3. RNA interference of ADAM19 in A172 cells. (A) Cells were transfected with 100 nM negative control RNA (lane 1) or stealth RNA sequence 2 (lane 2). The medium was treated for 8 h, collected and the sAPPα content was determined by Western blot analysis with 6E10. (B) The relative α-secretase activity compared with the control from (A) adjusted for the amount of protein in the cell lysate. Values represent means ± SE of six experiments. Statistical significance between negative control and RNAi samples were determined by use of two-tailed Student's *t*-test, with a value of $p < 0.05$ considered significant. (C) The transcriptional levels of ADAMs were determined by semi-quantitative RT-PCR analysis of RNA isolated from cells transfected with negative control RNA (lane 1) or stealth RNA (lane 2). Results are shown for hADAM19 (30 cycles), β-actin (20 cycles), hADAM9, 10, and 17 (30 cycles). Amplified products were electrophoresed in 8% acrylamide gels.

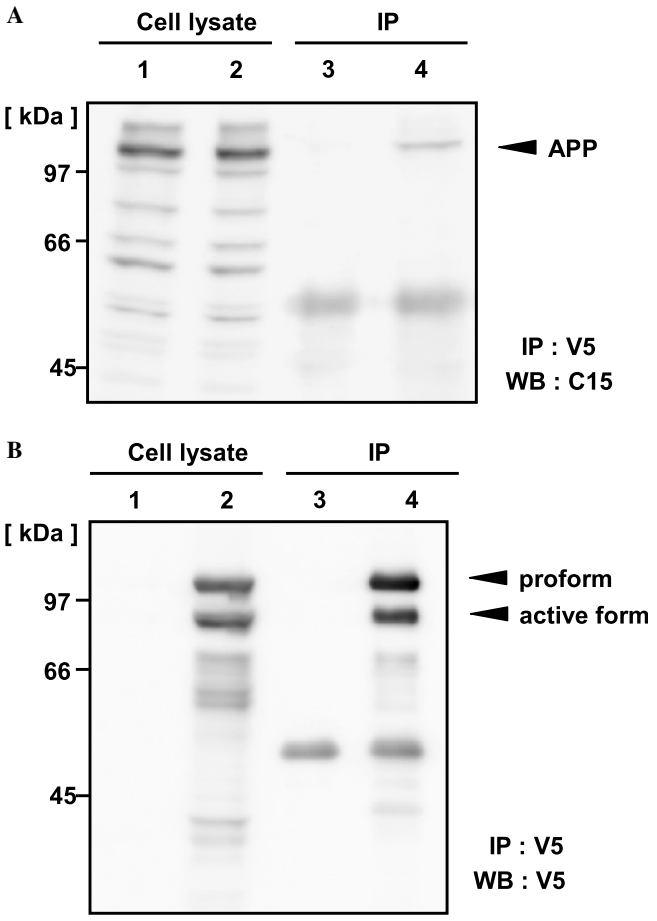

Fig. 4. Direct interaction of ADAM19 with APP. (A) HEK293T cells were immunoprecipitated with anti-V5 antibody and Western blotted with anti APP (lanes 1 and 3, mock transfection; lanes 2 and 4, with ADAM19 cDNA transfection). (B) Expression of ADAM19 was confirmed by the Western blotting with anti-V5 (arrowhead). Lanes are the same as (A).

It has been reported that part of APP and BACE1 exists in lipid rafts, a cholesterol and sphingolipid enriched fraction. Cholesterol depletion affects APP proteolysis and reduces Aβ generation [17]. The localization of mADAM19 in lipid rafts is critical for its ectodomain shedding [18]. Human ADAM19 and its mouse analog are highly similar sharing 84.6% identity in protein sequence. Therefore, hADAM19 probably exists in lipid rafts. We expect that ADAM19 is involved in APP proteolysis in lipid rafts.

Acknowledgments

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References

[1] J.A. Hardy, G.A. Higgins, Alzheimer's disease: the amyloid cascade hypothesis, Science 256 (1992) 184–185.
[2] H. Koike, S. Tomioka, H. Sorimachi, T.C. Saido, K. Maruyama, A. Okuyama, A. Fujisawa-Sehara, S. Ohno, K. Suzuki, S. Ishiura, Membrane-anchored metalloprotease MDC9 has an alpha-secretase activity responsible for processing the amyloid precursor protein, Biochem. J. 343 (1999) 371–375.
[3] S. Lammich, E. Kojro, R. Postina, S. Gilbert, R. Pfeiffer, M. Jasionowski, C. Haass, F. Fahrenholz, Constitutive and regulated alpha-secretase cleavage of Alzheimer's amyloid precursor protein by a disintegrin metalloprotease, Proc. Natl. Acad. Sci. USA 96 (1999) 3922–3927.

[4] J.D. Buxbaum, K.N. Liu, Y. Luo, J.L. Slack, K.L. Stocking, J.J. Peschon, R.S. Johnson, B.J. Castner, D.P. Cerretti, R.A. Black, Evidence that tumor necrosis factor alpha converting enzyme is involved in regulated alpha-secretase cleavage of the Alzheimer amyloid protein precursor, J. Biol. Chem. 273 (1998) 27765–27767.

[5] N. Hotoda, H. Koike, N. Sasagawa, S. Ishiura, A secreted form of human ADAM9 has an alpha-secretase activity for APP, Biochem. Biophys. Res. Commun. 293 (2002) 800–805.

[6] G. Weskamp, H. Cai, T.A. Brodie, S. Higashyama, K. Manova, T. Ludwig, C.P. Blobel, Mice lacking the metalloprotease-disintegrin MDC9 (ADAM9) have no evident major abnormalities during development or adult life, Mol. Cell. Biol. 22 (2002) 1537–1544.

[7] D. Hartmann, B. de Strooper, L. Serneels, K. Craessaerts, A. Herreman, W. Annaert, L. Umans, T. Lubke, A. Lena Illert, K. von Figura, P. Saftig, The disintegrin/metalloprotease ADAM10 is essential for Notch signalling but not for alpha-secretase activity in fibroblasts, Hum. Mol. Genet. 11 (2002) 2615–2624.

[8] R. Postina, A. Schroeder, I. Dewachter, J. Bohl, U. Schmitt, E. Kojro, C. Prinzen, K. Endres, C. Hiemke, M. Blessing, P. Flamez, A. Dequenne, E. Godaux, F. van Leuven, F. Fahrenholz, A disintegrin-metalloproteinase prevents amyloid plaque formation and hippocampal defects in an Alzheimer disease mouse model, J. Clin. Invest. 113 (2004) 1456–1464.

[9] B.E. Slack, L.K. Ma, C.C. Seah, Constitutive shedding of the amyloid precursor protein ectodomain is up-regulated by tumour necrosis factor-α converting enzyme, Biochem. J. 357 (2001) 787–794.

[10] M. Asai, C. Hattori, B. Szabo, N. Sasagawa, K. Maruyama, S. Tanuma, S. Ishiura, Putative function of ADAM9, ADAM10, and ADAM17 as APP α-secretase, Biochem. Biophys. Res. Commun. 301 (2003) 231–235.

[11] D.M. Skovronsky, D.B. Moore, M.E. Milla, R.W. Doms, V.M. Lee, Protein kinase C-dependent alpha-secretase competes with beta-secretase for cleavage of amyloid-beta precursor protein in the trans-golgi network, J. Biol. Chem. 275 (2000) 2568–2575.

[12] N. Kawaguchi, X. Xu, R. Tajima, P. Kronqvist, C. Sundberg, F. Loechel, R. Albrechtsen, U.M. Wewer, ADAM12 protease induces adipogenesis in transgenic mice, Am. J. Pathol. 160 (2002) 1895–1903.

[13] K. Shirakabe, S. Wakatsuki, T. Kurisaki, A. Fujisawa-Sehara, Roles of Meltrin beta/ADAM19 in the processing of neuregulin, J. Biol. Chem. 276 (2001) 9352–9358.

[14] V. Chesneau, J.D. Becherer, Y. Zheng, H. Erdjument-Bromage, P. Tempst, C.P. Blobel, Catalytic properties of ADAM19, J. Biol. Chem. 278 (2003) 22331–22340.

[15] B.R. Wong, J. Rho, J. Arron, E. Robinson, J. Orlinick, M. Chao, S. Kalachikov, E. Cayani, F.S. Bartlett 3rd, W.N. Frankel, S.Y. Lee, Y. Choi, TRANCE is a novel ligand of the tumor necrosis factor receptor family that activates c-Jun N-terminal kinase in T cells, J. Biol. Chem. 272 (1997) 25190–25194.

[16] M. Zimmermann, F. Gardoni, E. Marcello, F. Colciaghi, B. Borroni, A. Padovani, F. Cattabeni, M. Di Luca, Acetylcholinesterase inhibitors increase ADAM10 activity by promoting its trafficking in neuroblastoma cell lines, J. Neurochem. 90 (2004) 1489–1499.

[17] R. Ehehalt, P. Keller, C. Haass, C. Thiele, K. Simons, Amyloidogenic processing of the Alzheimer beta-amyloid precursor protein depends on lipid rafts, J. Cell Biol. 160 (2003) 113–123.

[18] S. Wakatsuki, T. Kurisaki, A. Sehara-Fujisawa, Lipid rafts identified as locations of ectodomain shedding mediated by Meltrin beta/ADAM19, J. Neurochem. 89 (2004) 119–123.