Available online at www.sciencedirect.com
SCIENCE DIRECT
Biochemical and Biophysical Research Communications 347 (2006) 254–260
BBRC
www.elsevier.com/locate/ybbrc

Design and characterization of a novel cellular prion-derived quenched fluorimetric substrate of α-secretase

Moustapha Alfa Cissé [a], Céline Gandreuil [b], Jean-François Hernandez [b], Jean Martinez [b], Frédéric Checler [a,*], Bruno Vincent [a,*]

[a] *Institut de Pharmacologie Moléculaire et Cellulaire, UMR6097 CNRS/UNSA, Equipe labélisée Fondation pour la Recherche Médicale, 660 route des lucioles, 06560 Valbonne, France*
[b] *LAPP, UMR5810 CNRS/Université de Montpellier I et II, avenue Charles Flahault, 34060 Montpellier, France*

Received 30 May 2006
Available online 21 June 2006

Abstract

Under normal conditions, the cellular prion protein (PrP^c) undergoes a proteolytic attack between amino acids 111 and 112 which gives rise to the N-terminal secreted N1 fragment and its C-terminal membrane-tethered counterpart C1. Importantly, this cleavage precludes the integrity of the neurotoxic 106–126 sequence. Here, we describe an original and reliable assay based on a quenched fluorimetric substrate (JMV2770) encompassing the 111/112 sequence of PrP^c. In whole brain homogenate, the JMV2770-hydrolysing activity is optimal at neutral pH and sensitive to the metalloprotease inhibitor BB3103 but not to acidic and serine protease blockers. JMV2770 is efficiently cleaved by intact HEK293 cells and fibroblasts in culture, consistent with an hydrolysis by a typical ectoprotease. Overexpressions of α-secretases a disintegrin and metalloprotease-9 (ADAM9), ADAM10 or TACE (ADAM17) in human cells increase BB3103-sensitive JMV2770 hydrolysis, while invalidation of ADAM10 and TACE or reduced expression of ADAM9 by an antisense approach significantly reduced its cleavage. Finally, analysis of JMV2770 hydrolysis following transient transfection of ADAM10 or ADAM9 cDNA in $ADAM10^{-/-}$ fibroblasts allowed to confirm our previous data establishing that ADAM9 does not behave as a genuine α-secretase but rather acts as an important upstream regulator of ADAM10 activity.

Keywords: Disintegrins; Prion diseases; α-Secretase; Fluorimetric assay; Alzheimer's diseaseβ

In normal brain, a main cleavage occurs between amino acids 111 and 112 of the cellular prion protein (PrP^c), while an additional proteolytic attack takes place at the 90/91 site in Creutzfeldt–Jakob disease brains [1]. Apart from producing different metabolites, this leftward shift preserves the 106–126 sequence of PrP^c which has been shown to be toxic for neurons [2] and could be, at least partly, responsible for the pathogenicity associated to prion diseases. In this context, our laboratory investigated the regulation of the physiological processing of PrP^c as well as the nature of the enzymes involved. We established that several members of the disintegrin family, namely ADAM10 and TACE, were responsible for the constitutive and PKC-dependent production of N1, respectively, [3]. More recently, we demonstrated that ADAM9 also contributed to N1 formation [4]. However, unlike ADAM10 and TACE that behaved as genuine PrP^c-processing enzymes, ADAM9 only contributed indirectly to PrP^c breakdown by up-regulating ADAM10 activity, likely by enhancing its shedding [4].

Here, we describe a new enzymatic assay based on a quenched fluorimetric substrate (JMV2770) which sequence overlaps the 111–112 cleavage site of PrP^c. We show that this substrate is efficiently cleaved by both brain homogenate and intact cells only at neutral pH and that the JMV2770-hydrolysing activities are largely sensitive

* Corresponding authors. Fax : +33 4 93 95 77 08.
E-mail addresses: checler@ipmc.cnrs.fr (F. Checler), vincentb@ipmc.cnrs.fr (B. Vincent).

0006-291X/$ - see front matter © 2006 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2006.06.065

to metalloprotease inhibitors. JMV2770-hydrolysing activity was enhanced in cells overexpressing disintegrins and impaired in cells either invalidated for ADAM10 or TACE or displaying reduced ADAM9 activity. Overall, this novel assay provides a suitable and powerful means to monitor α-secretase-like disintegrins in routine and to screen for molecules able to stimulate these enzymes and therefore enhance PrP^c cleavage at the 111–112 site.

Materials and methods

Antibodies and reagents. The polyclonal antibody directed against mouse ADAM9 and the mouse ADAM9 cDNA was provided by Pr. C. Blobel (New York, USA). Polyclonal anti-ADAM10 antibody was from Euromedex and the AL45 anti-TACE antiserum was kindly provided by Drs. Y. Zhang and S. Franck (Birmingham, USA). The mouse TACE cDNA as well as the TapI inhibitor and the $TACE^{-/-}$ fibroblasts were kindly supplied by Dr. R. Black (Immunex, Seattle, USA). The human ADAM10 cDNA was a kind gift from Dr. C. Lunn (Schering-Plough, Kenilworh, USA). BB3103 was kindly provided by British Biotech and the $ADAM10^{-/-}$ cells were generously provided by Drs. B. De Strooper and P. Saftig (Leuwen, Belgium). The monoclonal anti-tubulin antibody and protease inhibitors were purchased from Sigma (St. Quentin-Fallavier, France). The α-secretase commercial substrate (Mca-Pro-Leu-Ala-Gln-Ala-Val-Dpa-Arg-Ser-Ser-Ser-Arg- NH_2) was from R&D System (Minneapolis, USA).

Synthesis of the JMV2770 substrate. The intramolecularly quenched fluorimetric substrate Abz-Thr-Asn-Met-Lys-His-Met-Ala-Gly-Ala-Ala-Gln-EDDnp (JMV2770) contains the *ortho*-aminobenzoyl (Abz)/dinitrophenyl groups (EDDnp) as the donor/acceptor pair and was synthetized as previously described [5].

Cell cultures and transfection experiments. HEK293 cells and mouse embryonic fibroblasts (MEFs) were cultured as previously described [3,6]. HEK293 cells overexpressing mouse ADAM9, human ADAM10, mouse TACE or mouse ADAM9-antisense cDNAs were obtained as described elsewhere [3,4]. Transient transfections of empty pcDNA3 vector or pcDNA3 encoding mouse ADAM9 or human ADAM10 (2 μg) were carried out onto 35 mm-dishes-cultured $ADAM10^{-/-}$ fibroblasts using Lipofectamine-2000 reagent (Invitrogen, Cergy-Pontoise, France).

Western blot analysis of disintegrin expressions. HEK293 or fibroblasts were assayed for their disintegrin contents as extensively described previously [4] and the chemiluminescence was recorded using a Luminescence Image Analyzer LAS-3000 (Raytest, Courbevoie, France). Tubulin immunoreactivity was monitored in all samples as loading controls.

Fluorimetric assay in mouse brain homogenates. Whole brain from a 4-week-old C57 black mouse was homogenized in Tris/HCl, pH 7.5, and sonicated. Forty to 50 μg of proteins was resuspended in a final volume of 90 μl in 25 mM Na^+-acetate/MES/Tris/TES adjusted at pH 7.5 or 4.5 and incubated in a 96-well plate for 10 min at 37 °C. Then, 10 μl of JMV2770 was added and fluorescence was recorded at various times at 320 and 420 nm as excitation and emission wavelengths, respectively.

Fluorimetric assay on intact cells. Mouse embryonic fibroblasts or HEK293 cells were cultured in 35 mm-dishes until cell confluency reached approximatively 80%. Under basal conditions or 36 h after transient transfection, cells were washed twice with phosphate-buffered saline (PBS) and pretreated for 30 min at 37 °C with 1 ml PBS supplemented with or without the inhibitors BB3103 (10 μM), *o*-phenanthroline (100 μM), TapI (10 μM), AEBSF (10 μM) or pepstatin (10 μM). Then, JMV2770 or the commercial α-secretase substrate (10 μM) was directly added to the cultured cells for various time periods at 37 °C. At the end of each incubation time, 100 μl of medium was collected and fluorescence was recorded in a 96-well plate at 320 and 420 nm as excitation and emission wavelengths, respectively. After the last incubation time, cells were resuspended in lysis buffer (10 mM Tris/HCl, pH 7.5, 150 mM NaCl, 0.5% Triton X-100, 0.5% deoxycholate, and 5 mM EDTA), protein concentrations were determined by the Bradford method [7], and all fluorimetric values were normalized according to protein contents in samples.

Fluorimetric assay in cell medium. $ADAM10^{-/-}$ fibroblasts were cultured in 35 mm dishes and transiently transfected with empty pcDNA3 or mouse ADAM9 and/or human ADAM10 cDNAs as described above. Forty-eight hours after transfections, cells were washed twice with PBS and incubated for 2 h with 1 ml PBS in the absence or in the presence of BB3103 (10 μM) then, the medium was collected and centrifuged for 5 min at 14,000 rpm in order to discard residual cell debris. A hundred microliters of medium was preincubated for 15 min in a 96-well plate at 37 °C then, JMV2770 was added (10 μl of a 100 μM solution, 10 μM final) for various time periods at 37 °C. Fluorescence recording, cell collection, and normalization procedure were as described above.

Statistical analysis. Statistical analyses were performed with the Prism software (Graphpad, San Diego, USA) using the unpaired *t* test for pairwise comparisons. All results are expressed as means ± SE values.

Results

The structure of JMV2770 and its corresponding region on the full-length PrP^c are illustrated in Fig. 1. The peptide backbone of the substrate consists of the TNMKHMAGAA sequence that corresponds to the 107–116 region of human PrP^c and encompasses the His/Met 111–112 peptidyl bond targeted by disintegrins [1]. An additional glutamine residue is present at the C-terminal end in order to link the EDDnp group which, in absence of cleavage, interacts with Abz, thereby quenching the fluorophore.

Fig. 2A indicates that incubation of crude mouse brain homogenate with 10 μM of JMV2770 led to a time-dependent detection of a fluorimetric signal that reached a plateau between 20 and 30 min at pH 7.5. Hydrolysis of JMV2770 was not observed at pH 4.5 (Fig. 2A). We compared the time-dependent hydrolysis of both JMV2770 and an α-secretase commercial substrate (mimicking the α-secretase site on the β-amyloid precursor protein) (R&D System). JMV2770 was more efficiently cleaved than the commercial substrate at pH 7.5 (Fig. 2B). Initial-velocity measurements at various concentrations of substrates (Fig. 2C) allowed us to derive an apparent K_m of about 18 μM for JMV2770 (Fig. 2D), an affinity slightly higher than the one obtained with the commercial substrate ($K_m = 28$ μM, not shown).

Interestingly, when incubated on intact cells, JMV2770, that is non-permeant, allows to measure putative ectoproteases, i.e. enzymes facing the extracellular space. Thus, we investigated whether mock-transfected HEK293 cells display endogenous JMV2770-hydrolysing ectoprotease activity. Indeed, intact HEK293 cells displayed a time-dependent ability to cleave the substrate. This activity was partly inhibited by the disintegrin-specific hydroxamic acid-based inhibitor BB3103 [8] (Fig. 3A). Importantly, the metalloprotease blocking agent *o*-phenanthroline and the TACE-specific inhibitor TapI [9] both reduced the JMV2770-hydrolysing activity by intact HEK293 cells by about 50% as was observed with BB3103. By contrast, AEBSF and pepstatin, which target serine and acidic proteases, respectively, had no effect (Fig. 3B). In addition, intact mouse fibroblasts also exhibited a membrane-bound JMV2770-hydrolysing activity that was partly inhibited by BB3103 (Fig. 3C), indicating that these ectoprotease activities were not cell-specific.

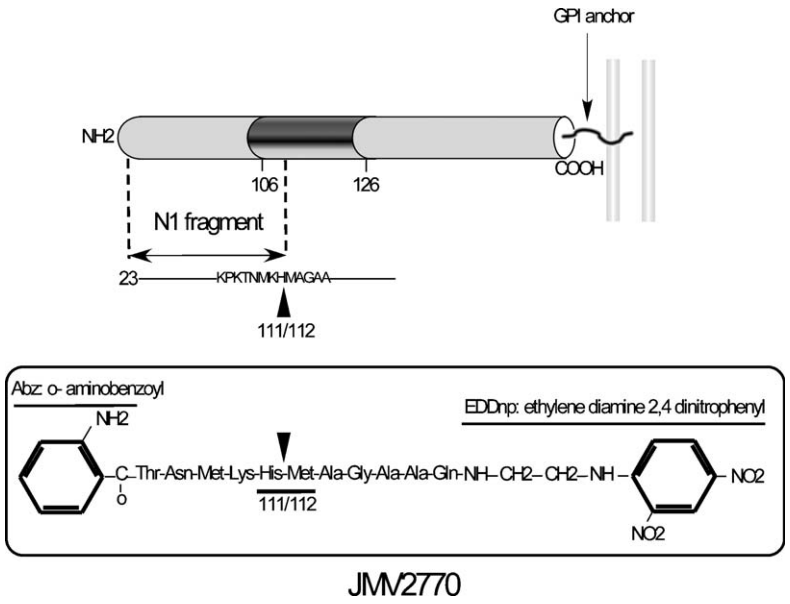

Fig. 1. Structure of the JMV2770 quenched fluorimetric substrate and the prion protein corresponding fragment. The cellular prion (PrP^c) is a glycosylphosphatidylinositol (GPI)-anchored protein. According to the human sequence, the mature protein starts with the 23rd amino acid after the removal of the signal peptide. The putative toxic core is highlighted by the black box encompassing residues 106–126. After reaching the plasma membrane, PrP^c undergoes a physiological cleavage between residues 111 and 112 by the disintegrins ADAM10 and TACE. This cleavage yields a fragment termed "N1" which is secreted in the extracellular space. JMV2770 mimicks this cleavage site as well as the surrounding amino acid sequence of PrP^c. The peptidic sequence of the substrate consists of the TNMKHMAGAA sequence that corresponds to the 107–116 region of human PrP^c and encompasses the His/Met 111–112 peptidyl bond targeted by disintegrins.

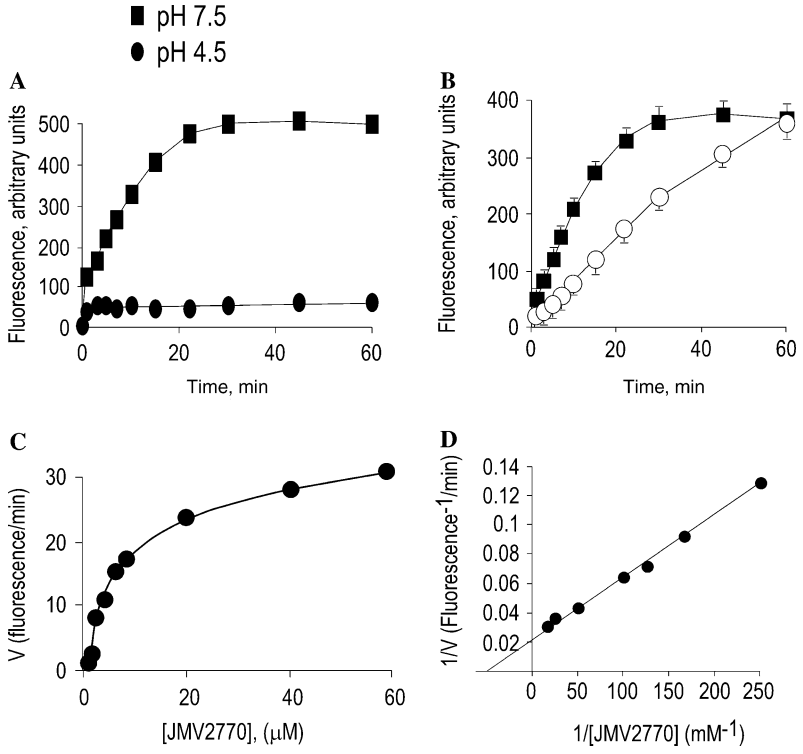

Fig. 2. Kinetic parameters of JMV2770 hydrolysis by mouse brain homogenate. (A) Mouse brain homogenate (50 μg of proteins) was incubated in 25 mM Na^+-acetate/MES/Tris/TES at pH 7.5 or 4.5 with the JMV2770 substrate (10 μM) at 37 °C for the indicated times. (B) Forty micrograms of mouse brain homogenate was incubated at 37 °C, pH 7.5, for the indicated times with either JMV2770 (black squares) or the α-secretase commercial substrate (white circles) at a concentration of 10 μM in the presence or in the absence of BB3103 (10 μM), and the fluorescence was recorded as described in Materials and methods. The curves represent the BB3103-sensitive fluorescence and are the means ± SEM of four independent determinations. (C) The reaction rate as a function of substrate concentration and (D) the Lineweaver–Burk plot of JMV2770 hydrolysis obtained with the indicated substrate concentrations.

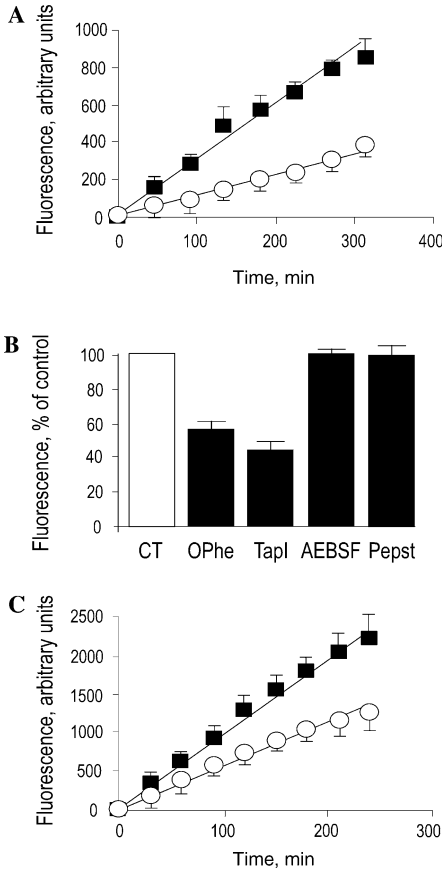

Fig. 3. JMV2770 hydrolysis by intact HEK293 cells and fibroblasts. Intact HEK293 cells (A,B) or fibroblasts (C) were monitored for their capacity to hydrolyse the JMV2770 substrate (10 μM) at 37 °C. Assays were performed at pH 7.5 in PBS in the absence (black squares) or in the presence (white circles) of BB3103 (10 μM) for the indicated times (A,C) or for 90 min with *o*-phenanthroline (100 μM), TapI (10 μM), AEBSF (10 μM) or pepstatin (10 μM) (B). Fluorescence was recorded at 320 and 420 nm as excitation and emission wavelengths, respectively, as described in Materials and methods. Values are means ± SEM of four independent determinations.

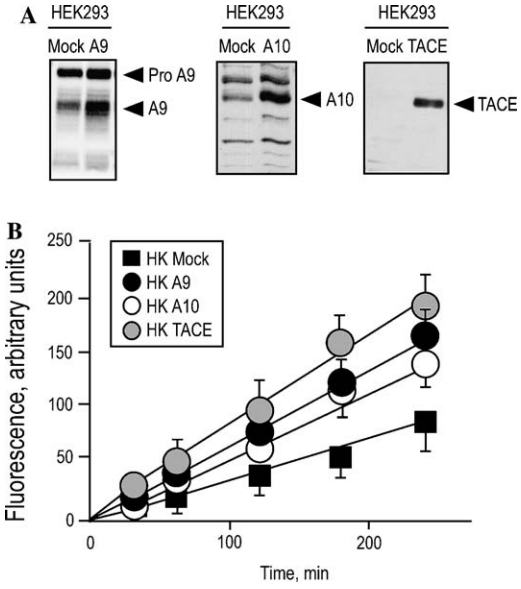

Fig. 4. JMV2770 hydrolysis by ADAM9- ADAM10- or TACE-overexpressing HEK293 cells. (A) Intact HEK293 cells stably transfected with either empty pcDNA3 vector (Mock), ADAM9 (A9), ADAM10 (A10) or TACE were assayed for their JMV2770-hydrolysing activities. (B) Cells grown in 6-well plates were pretreated for 30 min at 37 °C with PBS containing or not BB3103 (10 μM) then incubated with JMV2770 at a final concentration of 10 μM. Media (100 μl) were taken out at the indicated time points and the fluorescence was recorded. The values represent the BB3103-sensitive activities normalized according to the protein concentrations in each cell types and are means ± SEM of three independent determinations.

We have recently set up stably transfected human HEK293 overexpressing the disintegrins ADAM9, ADAM10 or TACE (Fig. 4A) [3,4]. The incubation of the substrate with intact HEK293 cells overexpressing each of these enzymes led to a significant increase in BB3103-sensitive hydrolysing activity when compared with mock-transfected cells (Fig. 4B). The invalidation of ADAM10 or TACE in fibroblasts allowed us to assess the endogenous contribution of these enzymes in JMV2770-hydrolysing activity. ADAM10$^{-/-}$ fibroblasts did not reveal any ADAM10 immunoreactivity (Fig. 5A) and displayed a reduced BB3103-sensitive capability to hydrolyse JMV2770 when compared to wild-type cells (Fig. 5B). Similarly, in TACE$^{-/-}$ fibroblasts (Fig. 6A), the TapI-sensitive JMV2770-hydrolysing activity was lowered when compared to wild-type cells (Fig. 6B).

The endogenous contribution of ADAM9 in JMV2770 hydrolysis was investigated by means of HEK293 cells in which ADAM9 content was reduced by an antisense approach [4]. The results showed that all clones displaying reduced amounts of enzyme (Fig. 7A) exhibited lowered BB3103-sensitive JMV2770-hydrolysing activity (Fig. 7B).

We recently demonstrated that ADAM9 did not directly act as an α-secretase-like activity but rather operated upstream to ADAM10 that was responsible for the genuine α-secretase cleavage [4]. We measured the BB3103-sensitive JMV2770 hydrolysis in ADAM10$^{-/-}$ fibroblasts following single or double transfections with ADAM9 and/or ADAM10 cDNAs (Fig. 8A). ADAM9 cDNA transfection only did not modify the JMV2770-hydrolysing activity exhibited by intact mock-transfected ADAM10$^{-/-}$ fibroblasts while ADAM10 cDNA transfection potentiated JMV2770 hydrolysis (Fig. 8B). Interestingly, ADAM9/ADAM10 doubly transfected fibroblasts further increased the JMV2770-degrading activity (Fig. 8B).

In an attempt to determine whether ADAM9 influenced ADAM10 shedding in this paradigm, we measured the JMV2770-hydrolysing activity in the conditioned media of ADAM10$^{-/-}$ fibroblasts. Here again, transfection of ADAM9 cDNA only did not modify the activity observed in the conditioned medium of mock-transfected cells (Fig. 8C) while ADAM10 cDNA transfection increased JMV2770 hydrolysis in conditioned medium (Fig. 8C). Co-expression of ADAM9 and ADAM10 led to an additional increase in the ability of conditioned medium to

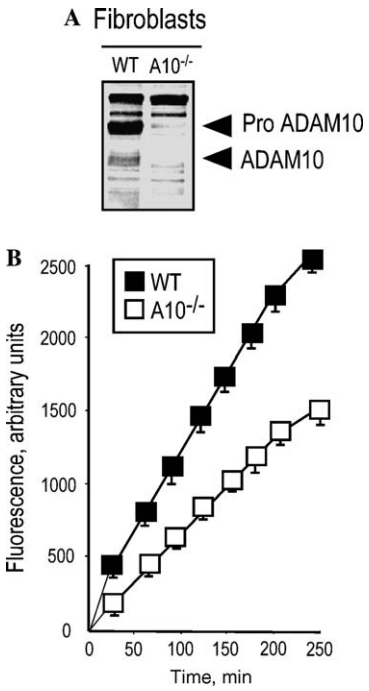

Fig. 5. JMV2770 hydrolysis is lowered in fibroblasts invalidated for ADAM10. (A) Fibroblasts derived from wild-type and ADAM10-knock-out mice were analysed for their ADAM10 contents by Western blot as described in Materials and methods. The same intact cells were monitored for their JMV2770-hydrolysing activities as described in the legend of Fig. 4. The values represent the BB3103-sensitive activities normalized according to the protein concentrations in the two cell types and are means ± SEM of three independent experiments.

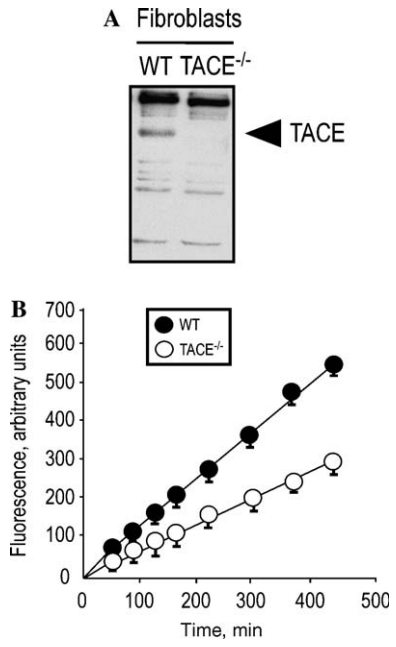

Fig. 6. JMV2770 hydrolysis is impaired in fibroblasts invalidated for TACE. (A) Fibroblasts derived from wild-type and TACE-knock-out mice were analysed for their TACE contents by Western blot as described in Materials and methods. The same intact cells were monitored for their JMV2770-hydrolysing activities as described in the legend of Fig. 4 except that cells were pretreated (or not) with the TACE-specific inhibitor TapI (10 μM). (B) The values represent the TapI-sensitive activities normalized according to the protein concentrations in the two cell types and are means ± SEM of three independent experiments.

degrade JMV2770 (Fig. 8C). These results strongly reinforced our previous demonstration that ADAM9 indirectly contributes to α-secretase-like activity by acting as an upstream regulator of ADAM10.

Discussion

The first demonstration that PrP^c underwent proteolytic attacks came from *in vivo* experiments performed with human brains [1]. In normal brain, a main cleavage occurs at the 111/112 site and gives rise to a secreted fragment named N1 and its membrane-tethered C-terminal counterpart C1. We previously established that two members of the disintegrin family of proteins behaved as genuine PrP^c-cleaving enzymes. Thus, ADAM10 was mainly responsible for the constitutive secretion of the N1 fragment in cells while ADAM17 was involved in the PKC-regulated cleavage of PrP^c [3,6]. In addition, a third disintegrin, namely ADAM9, rather participated indirectly in the normal metabolism of PrP^c by activating ADAM10 [4]. A recent *in vivo* study strongly supported a physiological implication of ADAM10 in PrP^c processing since the rate of active form of ADAM10 in human brain correlates with the level of C1 fragment [10].

The identification of disintegrins as α-secretases and the well-characterized regulation of this cleavage opened a new area of investigation that might prove useful for the treatment of prion diseases as was recently suggested for Alzheimer's disease [11]. However, if numerous techniques have been developed for rapid and reliable measurements of Aβ production only few are available concerning the α-secretase pathway. Here, we described a fluorimetric assay to specifically and routinely monitor this activity. The full characterization presented here matches perfectly with the data previously obtained in our studies on ADAM-mediated secretion of N1. Thus, ADAM10 and TACE behaved as genuine PrP^c-cleaving proteases whereas ADAM9 displayed ADAM10-mediated activating properties. In addition, a comparative analysis of the data collected, under the same conditions, with either the βAPP-mimicking α-secretase commercial substrate or JMV2770, reinforced the statement that this cleavage is actually not dependent on the targeted amino acid sequence of the substrate but is rather driven by an α-helical conformation that leads to cleavages at 12–13 residues from the plasma membrane [12]. This further supported the very strong analogy between βAPP and PrP^c normal metabolism and made this novel tool a reliable and rapid disintegrin assay.

Interestingly, JMV2770, but not the α-secretase commercial substrate, was able to detect endogenous shedding activities in the conditioned medium after complementation of $ADAM10^{-/-}$ fibroblasts by ADAM10 cDNA transfection. Thus, when the α-secretase commercial

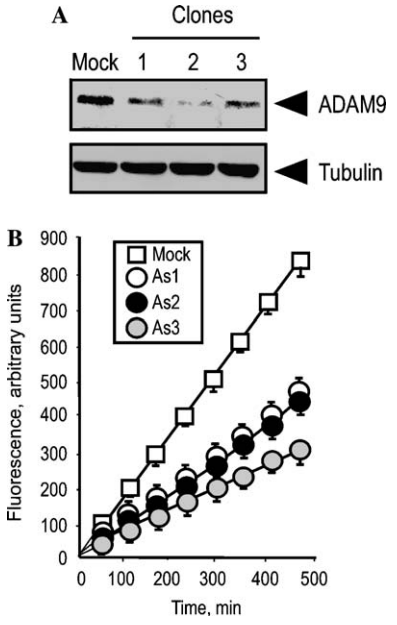

Fig. 7. JMV2770 hydrolysis is lowered in human HEK293 cells displaying reduced ADAM9 expression. (A) HEK293 cells stably transfected with either empty pcDNA3 vector (Mock) or with antisense pcDNA3 bearing ADAM9 cDNA were analysed for their ADAM9 contents by Western blot as described in Materials and methods. (B) Three different clones showing reduced ADAM9 expression were further tested for their JMV2770-hydrolysing activities and compared with mock cells as described above. The values represent the BB3103-sensitive activities normalized according to the protein concentrations in each cell types and are means ± SEM of three independent experiments.

substrate was used following the sole transfection of ADAM10 cDNA in ADAM10 knock-out cells, no additional activity was observed in the conditioned medium compared to non-transfected cells [4]. By opposition, the same experiment performed using JMV2770 as a substrate showed that the single complementation with ADAM10 was sufficient to detect endogenous ADAM9 shedding cleavage (Fig. 8C). One possible explanation could be that JMV2770 displays a better affinity for shedded ADAM10 when compared to the commercial substrate.

Concerning the forthcoming perspectives relative to this assay, they could be of several natures. First, it would be now feasible to operate a high-throughput screening in order to identify putative unknown PrP^c-specific cleaving enzymes targeting the 106–126 domain. Indeed, both the overall N1-forming activity and the JMV2770-degrading enzyme detected on intact cells were only partially inhibited (about 50%) by various metalloprotease inhibitors (i.e. *o*-phenanthroline, BB3103, and TapI). Therefore, it is highly plausible that additional activities which do not belong to the metalloprotease class of enzymes could be involved in the metabolism of PrP^c at the 111/112 site.

In the field of Alzheimer's disease, most of the pharmacological strategies aimed at lowering the time course of the pathology focused on the rational design of molecules able to inhibit the Aβ-forming enzymes (i.e. β- and γ-secretases) [13]. Because notch and other proteins fulfilling essential physiological functions are also γ-secretase substrates, strategies aimed at activating the α-secretase pathway progressively emerged during the past years. Indeed, a recent study established that, *in vivo*, overexpression of ADAM10 in a mouse model of Alzheimer's disease led to increased sAPPα and reduced Aβ, while plaques and memory deficits were delayed [11]. The hypothesis that α-secretase up-regulation would not only lower Aβ production but also preclude

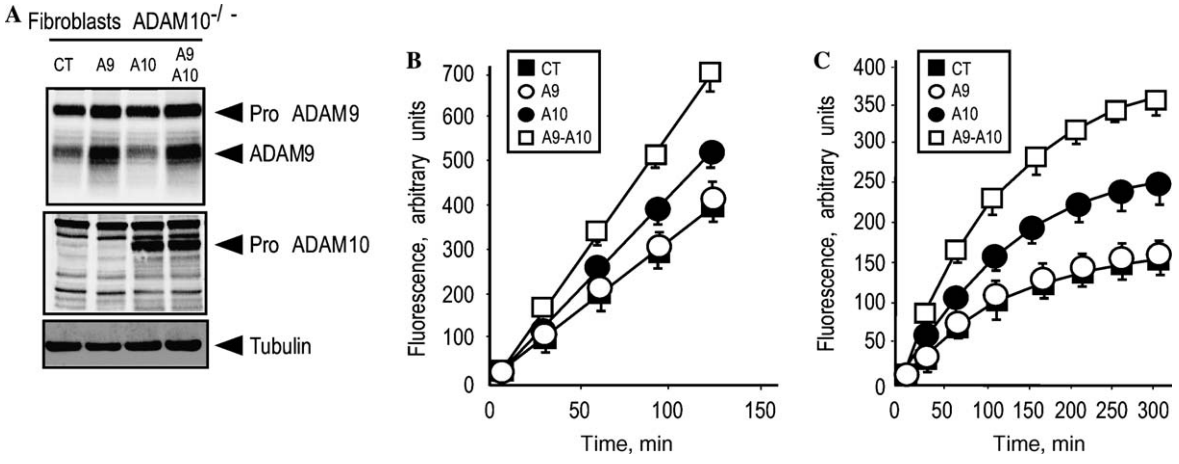

Fig. 8. ADAM9 potentiates ADAM10-mediated hydrolysis of JMV2770 on intact fibroblasts. (A) ADAM10 knock-out fibroblasts ($A10^{-/-}$) were transiently transfected with either pcDNA3 (CT) or with the indicated disintegrin cDNAs and transfection efficiencies were monitored 48 h later by Western blot. (B) Forty-eight hours after transfections, cells were incubated for 30 min with PBS containing (or not) the metalloprotease inhibitor BB3103 (10 μM), then JMV2770 was added at a final concentration of 10 μM. Media (100 μl) were collected at the indicated times and fluorescence was recorded. Values are means ± SEM of three independent determinations. (C) $ADAM10^{-/-}$ fibroblasts transiently transfected with the indicated cDNAs were allowed to secrete for 2 h at 37 °C in PBS containing or not the metalloprotease inhibitor BB3103 (10 μM), then media were taken out and 100 μl was incubated with JMV2770 (10 μM) in a 96-well plate. Values, normalized according to protein concentrations, represent the BB3103-sensitive activities and are means ± SEM of three independent determinations.

PrP^{sc} replication due to the lack of PrP^{c} template makes this approach even more attractive. Indeed, the presence of PrP^{c} is an absolute requirement for PrP^{sc} to propagate since mice invalidated for PrP^{c} were shown to be totally resistant to prion diseases following scrapie inoculation [14]. In this context, our assay could help to make a step forward in the search for α-secretase-activating molecules.

In CJD brains, an additional breakdown takes place at the 90/91 peptide bond and leads to the formation of the N2 and C2 products [1,15]. This leftward shift preserves the integrity of the 106–126 sequence and likely promotes pathogenicity. Since the proteolytic activities responsible for this cleavage are presently unknown, efforts are currently conducted in our laboratory in order to set up a fluorimetric assay specific for the characterization of the ''pathological'' cleavage of PrP with the ultimate goal to develop specific, powerful, and bioavailable inhibitors.

Acknowledgments

We are grateful to Drs. C. Blobel, R. Black, Y. Zhang, B. De Strooper, P. Saftig, and C. Lunn for antibodies and cell lines. This work was supported by the Fondation pour la Recherche Médicale, URMA, APOPIS, and CNRS.

References

[1] S.G. Chen, D.B. Teplow, P. Pachi, J.K. Teller, P. Gambetti, L. Autilio-Gambetti, Truncated forms of the human prion protein in normal brain and in prion diseases, J. Biol. Chem. 270 (1995) 19173–19180.

[2] G. Forloni, N. Angeretti, R. Chiesa, E. Monzani, M. Salmona, O. Bugiani, et al., Nature 362 (1993) 543–546.

[3] B. Vincent, E. Paitel, P. Saftig, Y. Frobert, D. Hartmann, B. De Strooper, et al., The disintegrins ADAM10 and TACE contribute to the constitutive and phorbol ester-regulated normal cleavage of the cellular prion protein, J. Biol. Chem. 276 (2001) 37743–37746.

[4] M. Alfa Cissé, C. Sunyach, S. Lefranc-Jullien, R. Postina, B. Vincent, F. Checler, The disintegrin ADAM9 indirectly contributes to the physiological processing of cellular prion by modulating ADAM10 activity, J. Biol. Chem. 280 (2005) 40624–40631.

[5] D. Andrau, C. Dumanchin-Njock, E. Ayral, J. Vizzavona, M. Farzan, M. Boisbrun, et al., BACE1 and BACE2-expressing human cells: characterization of β-amyloid precursor protein-derived catabolites, design of a novel fluorimetric assay, and identification of new in vitro inhibitors, J. Biol. Chem. 278 (2003) 25859–25866.

[6] B. Vincent, E. Paitel, Y. Frobert, S. Lehmann, J. Grassi, F. Checler, Phorbol ester-regulated cleavage of normal prion protein in HEK293 human cells and murine neurons, J. Biol. Chem. 275 (2000) 35612–35616.

[7] M.M. Bradford, A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein–dye binding, Anal. Biochem. 72 (1976) 248–259.

[8] P.J. Middelhoven, A. Ager, D. Roos, A.J. Verhoeven, Involvement of a metalloprotease in the shedding of human neutrophil FcγRIIIB, FEBS Lett. 414 (1997) 14–18.

[9] R.A. Black, C.T. Rauch, C.J. Kozlosky, J. Peschon, J.L. Slack, M.F. Wolfson, B.J. Castner, K.L. Stocking, P. Reddy, S. Srinivasan, N. Nelson, N. Boiani, K.A. Schosley, M. Gerhart, R. Davis, J.N. Fitzner, R.S. Johnson, R.S. Paxton, C.J. March, D.P. Ceretti, A metalloproteinase disintegrin that releases tumour necrosis factor-α from cells, Nature 385 (1997) 729–733.

[10] I. Laffont-Proust, B.A. Faucheux, R. Hässig, V. Sazdovitch, S. Simon, J. Grassi, et al., FEBS Lett. 579 (2005) 6333–6337.

[11] R. Postina, A. Schroeder, I. Dewachter, J. Bohl, U. Schmitt, E. Kojro, et al., J. Clin. Invest. 113 (2004) 1456–1464.

[12] S.S. Sisodia, β-Amyloid precursor protein cleavage by a membrane-bound protease, Proc. Natl. Acad. Sci. USA 89 (1992) 6075–6079.

[13] H. Büeler, A. Aguzzi, A. Sailer, R.A. Greiner, P. Autenried, M. Aguet, et al., Cell 73 (1993) 1339–1347.

[14] A. Aguzzi, C. Haass, Games played by rogue proteins in prion disorders and Alzheimer's disease, Science 302 (2003) 814–818.

[15] F. Checler, B. Vincent, Alzheimer's and prion diseases: distinct pathologies, common proteolytic denominators, Trends Neurosci. 25 (2002) 616–620.