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A synthetic amino acid substitution of Tyr10 in Aβ peptide sequence yields a dominant negative variant in amyloidogenesis

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Summary

Alzheimer's disease (AD) is the most common cause of dementia in elderly people, and age is the major nongenetic risk factor for sporadic AD. A hallmark of AD is the accumulation of amyloid in the brain, which is composed mainly of the amyloid beta-peptide (Aβ) in the form of oligomers and fibrils. However, how aging induces Aβ aggregation is not yet fully determined. Some residues in the Aβ sequence seem to promote Aβ-induced toxicity in association with age-dependent risk factors for AD, such as (i) increased GM1 brain membrane content, (ii) altered lipid domain in brain membrane, (iii) oxidative stress. However, the role of Aβ sequence in promoting aggregation following interaction with the plasma membrane is not yet demonstrated. As Tyr10 is implicated in the induction of oxidative stress and stabilization of Aβ aggregation, we substituted Tyr 10 with a synthetic amino acid that abolishes Aβ-induced oxidative stress and shows an accelerated interaction with GM1. This variant peptide shows impaired aggregation properties and increased affinity for GM1. It has a dominant negative effect on amyloidogenesis *in vitro, in cellulo*, and in isolated synaptosomes. The present study shed new light in the understanding of Aβ-membrane interactions in Aβ-induced neurotoxicity. It demonstrates the relevance of Aβ sequence in (i) Aβ-membrane interaction, underlining the role of age-dependent enhanced GM1 content in promoting Aβ aggregation, (ii) Aβ aggregation, and (iii) Aβ-induced oxidative stress. Our results open the way for the design of peptides aimed to inhibit Aβ aggregation and neurotoxicity.

Key words: aggregation; Alzheimer; amyloid; GM1; oxidative stress; rafts.

Introduction

Alzheimer's disease (AD) represents the most common form of dementia in the elderly and is strongly linked to age. Late onset AD represents the majority of AD cases, while early onset variants of familial AD emerge only in a small fraction (< 5%) (Kern & Behl, 2009). A hallmark of AD is the accumulation of amyloid deposits in the brain, which are mainly composed by the amyloid beta-peptide (Aβ) in the form of oligomers and fibrils (Hardy & Selkoe, 2002). Aβ is produced by sequential cleavage of the amyloid precursor protein (APP) by β- and γ-secretases, which leads to the formation of 39- to 43-residue-long peptides. Aβ1–42 shows enhanced misfolding and aggregation propensity, resulting in increased neurotoxic effects in AD pathogenesis (Selkoe, 2001). Several studies indicate that soluble Aβ oligomers, rather than fibrils, play a major role in AD pathogenesis (Kirkitadze *et al.*, 2002). Dimers and trimers of Aβ seem to be the most toxic forms (Lesné *et al.*, 2008; Shankar *et al.*, 2008). However, the molecular mechanisms underlying the assembly of Aβ species are not yet fully determined and it remains to be elucidated how soluble Aβ begins to assemble and deposit into the brain. One still open question is the identification of the specific recognition elements that mediate Aβ oligomerization. Moreover, the mechanisms through which Aβ oligomers trigger neurotoxicity are not clear yet. Several evidences indicate that specific amino acids in the Aβ sequence play a key role in neurotoxicity as well as in the Aβ aggregation process. Notably, age is the major nongenetic risk factor for sporadic AD progression. However, it is not yet fully elucidated how soluble Aβ starts to assemble and deposit in the brain in an age-dependent manner and how this initiates any neurotoxic effects. Rodent Aβ shows impaired aggregation (Dyrks *et al.*, 1992, 1993). We may hypothesize that the Aβ sequence of primates possesses one or more amino acids that induce Aβ aggregation in age-dependent manner. Rodent Aβ presents three amino acid substitutions: Arg3Gly, Tyr10Phe, and His13Arg (Dyrks *et al.*, 1992). *In vitro*, Aβ has been shown to bind to various glycosphingolipids, especially gangliosides such as GM1 (Ariga *et al.*, 2001). GM1 acts as seed inducing Aβ aggregation (Hayashi *et al.*, 2004; Yamamoto *et al.*, 2010). GM1 level increases with age and is significantly increased in amyloid-positive synaptosomes compared with amyloid-free synaptosomes extracted from AD brains (Yamamoto *et al.*, 2004, 2008). In addition, the high-density GM1 clustering in synaptosomes is age dependent (Yamamoto *et al.*, 2004, 2008). Age-dependent accumulation of Aβ associated to GM1 is also observed in the brain of aged cynomolgus monkeys (Kimura & Yanagisawa, 2007). GM1 is a component of lipid rafts, which are membrane micro-domains enriched in cholesterol and glycosphingolipids, in which particular molecules are concentrated and participate in membrane-mediated signaling events (Fantini & Yahi, 2010; Simons & Gerl, 2010). The distribution of raft components such as phospholipids and cholesterol in brain mem-

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branes may be altered by aging and is associated with the pathophysiology of AD (Wood *et al.*, 2002).

To analyze the role of Aβ sequence in Aβ aggregation at the cell surface and in Aβ-induced oxidative stress, we decided to investigate the properties of an Aβ peptide variant withTyr10 substituted by the nonproteinogenic amino acid para-amino-phenylalanine (Fig. 1A). Tyr10 is also required for Cu^{2+}catalyzed Aβ aggregation (Dyrks *et al.*, 1993; Barnham *et al.*, 2004), dityrosine cross-linking (Smith *et al.*, 2007), hydrogen peroxide formation, and the subsequent neurotoxicity (Barnham *et al.*, 2004). Oxidative stress is linked to aging and plays a key role in AD pathogenesis and Aβ aggregation (Recuero *et al.*, 2009). Although several amyloidogenic proteins contain a Tyr residue potentially involved in the interaction with membrane sphingolipids and lipid rafts (Fantini & Yahi, 2010). It has been recently shown that substitution of Tyr10 with Phe does not affect the interaction of Aβ5–16 with GM1 *in vitro* (Fantini & Yahi, 2011). For this reason, we substituted Aβ1–42 Tyr10 with the synthetic amino acid para-amino-phenylalanine. The side chain of this amino acid has an amino group, which may enhance the interaction of Aβ with GM1. This variant Aβ1–42 shows increased binding to GM1 *in vitro* compared with wild-type (wt) Aβ and has impaired aggregation capability *in vitro* and *in cellulo* as well as with brain synaptosomes. Variant Aβ partially inhibits the binding of wt Aβ to the plasma membrane of differentiated SHSY-5Y cells and affects the aggregation of wt Aβ *in vitro* and *in cellulo*. Moreover, variant Aβ fails to induce Cu^{2+}-dependent oxidative stress and inhibits Cu^{2+}-induced neurotoxicity of wt Aβ. The present study sheds new light on the understanding of Aβ-membrane interactions in

Aβ-induced neurotoxicity. It demonstrates the relevance of Aβ sequence in (i) Aβ-membrane interaction, (ii) Aβ aggregation, (iii) and Aβ-induced oxidative stress. Furthermore, our results open the way for the design of peptides aimed to inhibit Aβ aggregation and neurotoxicity.

Results

Tyr10paraNH2Phe variant Aβ affects the interaction of wt Aβ with SHSY-5Y cells

We studied the interaction of wt and variant Aβ with GM1 using the Langmuir monolayer technique (Yahi *et al.*, 2010). Monolayers of GM1 purified from bovine brain were prepared at the air–water interface on a

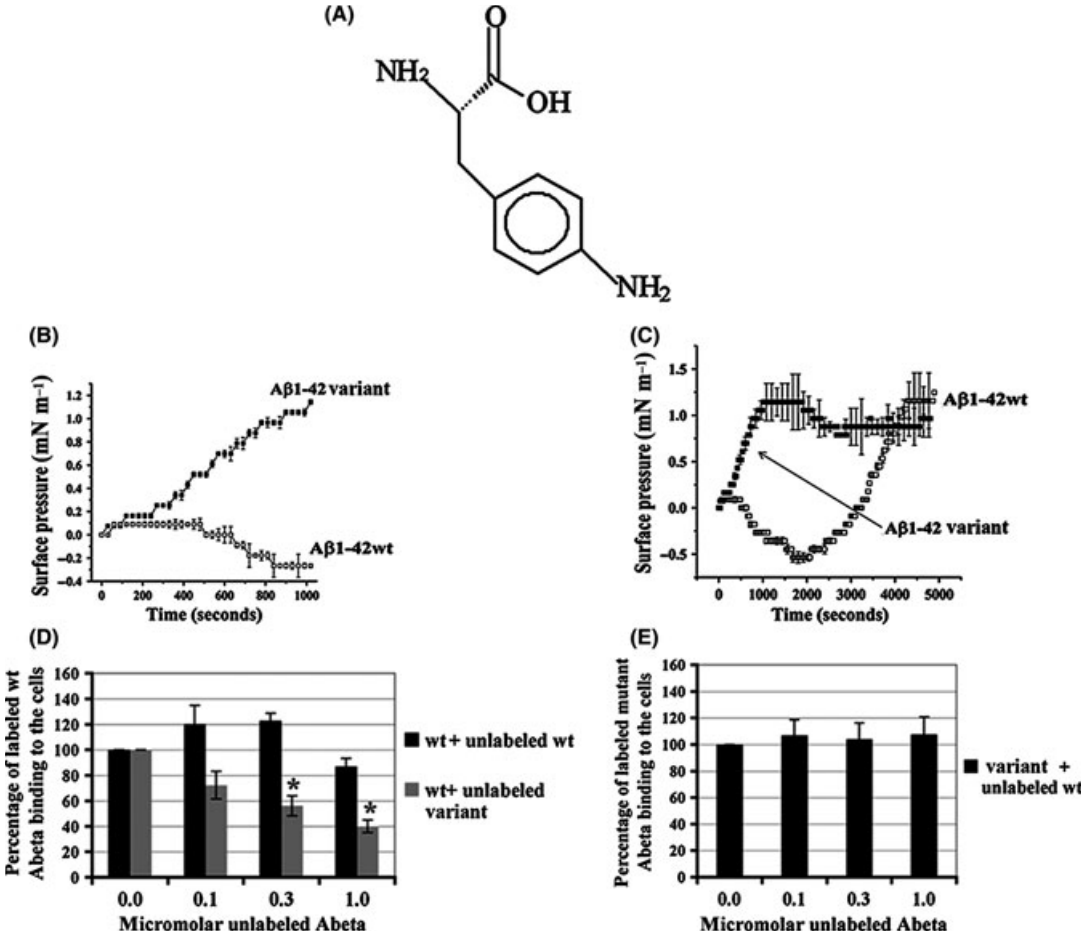

Fig. 1 Variant Aβ shows higher affinity for GM1 and the cell membrane compared with wt Aβ. (A) Lewis structure of the nonproteinogenic amino acid para-amino-phenylalanine, which was replaced by Tyr10 in Aβ. (B,C) Kinetics of aggregation of wt and variant Aβ into a monolayer of natural GM1. (B). Time-dependent surface pressure during the initial phase of the interaction (0–1000 s) between a monolayer of GM1 (initial pressure of 16 mN m^{-1}) and each peptide (wt or variant) injected in the aqueous subphase. (C) Represents the complete interaction (0–5000 s). The results shown are the mean ± SD of three independent experiments. (D) Analysis of tetramethylrhodamine-5-(and 6)-isothiocyanate (TRITC)-wt Aβ (0.3 μM) binding to SHSY-5Y cells both in the presence and absence of increasing concentration either unlabelled wt or variant Aβ. (E) Analysis of TRITC-variant Aβ (0.3 μM) binding to SHSY-5Y cells both in the presence and absence of increasing concentration unlabelled wt Aβ. (D,E) These experiments represent the average of three independent experiments performed in triplicate (n = 9, *P < 0.001 vs. the control TRITC-Aβ alone).

sub phase of ultra-pure water. The peptide was injected to the subphase, and the surface pressure was continuously measured with a microtensiometer (Yahi *et al.*, 2010). Variant Aβ peptide interacted more rapidly than wt Aβ, with no lag phase (Fig. 1B,C). After 5000 s of incubation, both Aβ peptides induced a similar increase in the surface pressure (Fig. 1C). Next, we investigated whether variant Aβ affects the interaction of wt Aβ with SHSY-5Y cells. Wt Aβ (0.3 μM) labeled with tetramethylrhodamine-5-(and 6)-isothiocyanate (TRITC) was incubated for 1 h at 37 °C with the cells alone or in combination with either unlabelled wt or variant Aβ. Addition of unlabelled wt Aβ reduces the binding of labeled wt Aβ only at 1 μM concentration (Fig. 1D). Variant Aβ squelched the binding of labeled wt Aβ in a dose-dependent manner (Fig. 1D). Similar results were observed when we added 1 μM TRITC-labeled Aβ and increasing concentration of unlabelled variant Aβ (Fig. S1). Unlabelled wt Aβ did not affect the binding of TRITC-variant Aβ at any concentration used (Fig. 1E). These experiments indicate that variant Aβ binds tighter to the membrane of SHSY-5Y cells than wt Aβ does, probably through interaction with GM1.

Tyr10paraNH2Phe variant Aβ peptide has a dominant negative effect on amyloidogenesis *in vitro*

We incubated differentiated SHSY-5Y cells 6 h with 3 μM dimers–trimers of TRITC-wt and/or fluorescein isothiocyanate (FITC)-variant Aβ (see Fig. S2). TRITC-wt Aβ aggregated, while FITC-variant showed a uniform staining (Fig. 2A, top). When TRITC-wt Aβ and FITC-variant Aβ were added together, we detected a uniform staining for TRITC-wt Aβ that partially co-localized with FITC-variant Aβ (Fig. 2A, bottom). These data are suggesting that the variant can inhibit the aggregation of wt Aβ. To investigate this hypothesis, we analyzed the aggregation rate of wt and variant Aβ alone or in combination *in vitro* by thioflavin T (ThT) measurements (Perrone *et al.*, 2010b). As control, we also analyzed the combination of wt and scrambled Aβ (1:1 ratio). Variant Aβ did not show an increase in ThT fluorescence compared with wt Aβ, suggesting that the aggregation capability of variant Aβ is impaired (Fig. 2B). The mixture of wt and variant Aβ (1:1 ratio) also showed diminished ThT fluorescence, suggesting that variant Aβ also affects the aggregation of wt Aβ after 1 week of incubation time (Fig. 2B). The addition of scrambled Aβ resulted in slower aggregation kinetics compared with TRITC-wt Aβ alone, but did not inhibit the aggregation of wt Aβ (Fig. 2B). Thus, the dominant negative effect of variant Aβ is specific. These results were supported by experiments using TRITC-labeled peptides. TRITC-variant Aβ was again unable to induce aggregation compared with TRITC-wt Aβ (Fig. 2C). After 1 week of incubation, there was not any significant difference between TRITC-wt Aβ and the mixture TRITC-scrambled/TRITC-wt Aβ (Fig. 2C), demonstrating that TRITC does not affect Aβ aggregation (Hu *et al.*, 2009).

The dominant negative effect on amyloidogenesis *in vitro* of variant Aβ was confirmed by agarose gel electrophoresis (Taneja *et al.*, 2007) and by dynamic light-scattering analysis of Aβ particle sizes during aggregation (Schlenzig *et al.*, 2009) (Figs S2A,B and S3).

We next analyzed the seeding capability of wt and variant Aβ *in vitro* by ThT measurements. Wt Aβ (50 μM) was incubated alone or in the presence of either wt or variant Aβ seeds (5 μM). Wt and variant Aβ seeds, obtained after 3 days incubation at room temperature, were sonicated and then added to freshly prepared wt Aβ for various time. Wt Aβ bound to wt Aβ, resulting in acceleration of aggregation, but not to variant Aβ seeds (Fig. 2D). These data are suggesting that variant Aβ containing seeds are unable to accelerate wt Aβ aggregation.

Ganglioside GM1 is a component of the membrane microdomains defined as lipid rafts (Sbai *et al.*, 2010). It has been demonstrated that exogenously added Aβ1–42 colocalizes with lipid rafts at the cell surface and that Aβ-lipid rafts association increases with the time of Aβ incubation with the cells (Williamson *et al.*, 2008). We incubated the cells with 3 μM wt and variant Aβ alone or in combination (wt/variant = 1:1 ratio) for 16 h and analyzed their lipid raft association by TX-100 extraction at 4 °C (Sbai *et al.*, 2010). TX-100 insoluble pellets (corresponding to lipid-raft-associated proteins) and precipitated soluble fractions were dissolved in 6 M urea, to measure the resistance of amyloid to a denaturing agent that fragments larger oligomers. We observed the presence of wt Aβ oligomers in both soluble and insoluble fraction (Fig. 2E) including bands around 72 and 135 kDa. The labeling of the 10 kDa band corresponding to a dimer was highly decreased in the soluble fraction for the wt Aβ. Variant Aβ showed oligomers of shorter size in both soluble and insoluble fraction and the presence of dimers in both fractions compared wt Aβ (Fig. 2E). By comparison with other gels showing that the dimer species of variant aggregates in multimers when incubated with cell (Fig. 4C and results not shown), these data demonstrate that variant Aβ formed oligomers more sensible to a denaturing agent. When wt and variant Aβ were added together (1:1 ratio), we found a decrease in high molecular oligomers by comparison with the wt Aβ. Moreover, as for variant Aβ alone, the dimers were detected in the soluble fraction in the mixture of wt/variant Aβ (Fig. 2E). These data are suggesting that the presence of variant Aβ together with wt Aβ resulted in the formation of unstable oligomers/aggregates, which are less resistant to urea treatment compared with wt Aβ alone *in cellulo*.

Tyr10paraNH2Phe variant Aβ peptide has a dominant negative effect on amyloidogenesis *in cellulo*

We next investigated the uptake and the aggregation of wt and variant Aβ by a biotin internalization assay (Perrone *et al.*, 2005, 2008). Wt and variant Aβ were labeled with a cleavable biotin, which allows the isolation of internalized Aβ. Biotin labeling did not affect the aggregation of wt and variant Aβ (data not shown) (Saavedra *et al.*, 2007). Dimers and trimers of Aβ seem to produce the major toxic form (Lesné *et al.*, 2008; Shankar *et al.*, 2008). Following biotinylation, we separated dimers and trimers of variant and wt Aβ (Fig. S4A, input), which were added to the cells for 6 h at 37 °C in duplicate (3 μM concentration). We confirmed by agarose gel electrophoresis of biotinylated Aβ that only dimers and trimers were added to the cells without any contamination of preformed biotin-Aβ aggregates (Fig. 3A, input). Following incubation with the cells, one sample was treated with glutathione to cleave the biotin linked to Aβ at the cell surface, while only biotin-Aβ internalized was protected from the cleavage. One sample was not treated with glutathione to recover the total biotin-Aβ associated to the cells. Cells were lysed with RIPA buffer. Biotin-Aβ was detected by western blot analysis of total extracts using streptavidin-HRP. Both wt and variant Aβ oligomerized following incubation with the cells, forming tetramers and an oligomeric form of about 70–72 kDa, whereas only wt Aβ formed an oligomer of about 56 kDa after 6 h of incubation with the cells (Fig. S4A), which is considered a highly toxic form (Lesné *et al.*, 2006, 2008). In agreement with a previous report (Saavedra *et al.*, 2007), only trimers and tetramers of wt Aβ are internalized, while higher mass oligomeric species stay at the cell surface (Fig. S4A). On the contrary, the 70 kDa oligomeric species from variant Aβ was internalized and variant Aβ showed an increased uptake compared with wt Aβ (Fig. S4A,B).

We next investigated whether wt and variant Aβ formed amyloid following interaction with the cells, using BSB, a cell-permeable derivative of Congo red that detects the amyloid (Ye *et al.*, 2005). Dimers–trimers of TRITC-wt or TRITC-variant Aβ were incubated for 6 h at 37 °C with

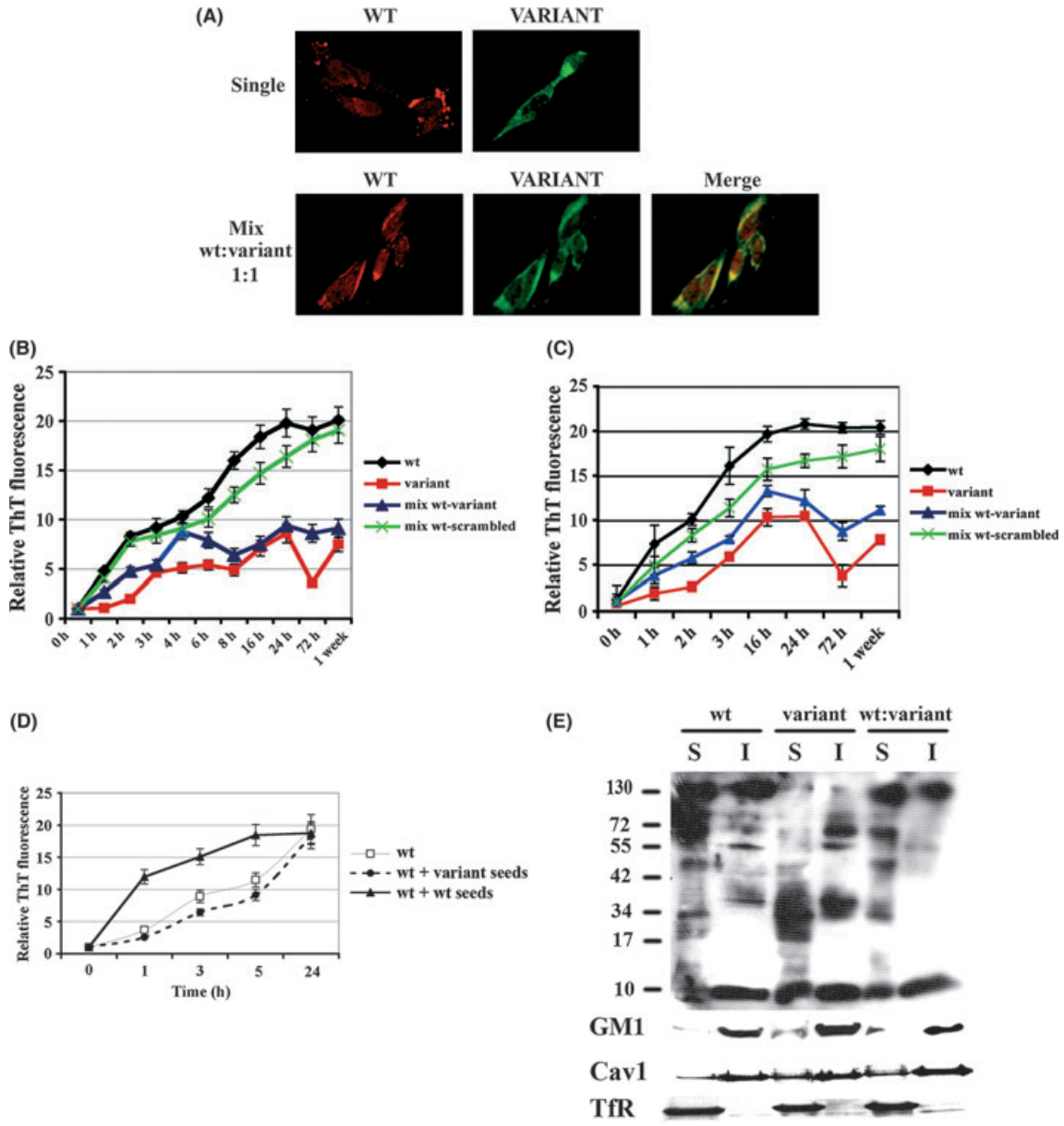

Fig. 2 Variant Aβ has a dominant negative effect on amyloidogenesis. (A) Immunofluorescence analysis of tetramethylrhodamine-5-(and 6)-isothiocyanate (TRITC)-wt and fluorescein isothiocyanate-variant Aβ incubated with SHSY-5Y cells alone (top) or in combination (bottom). The results shown are representative of three independent experiments. (B) *In vitro* analysis of variant (50 μM), wt Aβ (50 μM), and the mixtures wt/variant and wt/scrambled Aβ (50 μL in total) (1:1 ratio) aggregation kinetic by thioflavin T (ThT) binding. (C) *In vitro* analysis of the aggregation kinetics by ThT fluorescence of TRITC-variant (30 μM), TRITC-wt Aβ (30 μM), and the mixture TRITC-wt/TRITC-variant (30 μL in total, 1:1 ratio) or TRITC-wt/TRITC-scrambled (30 μL in total, 1:1 ratio). (D) *In vitro* analysis of wt Aβ (45 μM) aggregation kinetics by ThT binding both in the absence and presence of either wt or variant Aβ seeds (5 μM). (B,C,D) The results are the average of three independent experiments performed in triplicate (n = 9). (E) Lipid-raft association of wt and variant Aβ alone or in combination. Cell extracts were resolved on SDS-PAGE. Aβ was detected by Western blotting using monoclonal anti-Aβ antibody. Protein loading of the insoluble fraction was normalized by Western blotting using HRP-ChTB to detect GM1, anti polyclonal caveolin 1 (Cav1) for the insoluble fraction, and anti-transferrin receptor (TfR) for the soluble fraction antibodies. S = soluble fraction. I = insoluble fraction (rafts). The results shown are representative of three independent experiments.

SHSY-5Y cells. As previous reported (Saavedra *et al.*, 2007; Williamson *et al.*, 2008), TRITC-wt Aβ formed BSB-positive aggregates at the cell surface, whereas TRITC-variant Aβ showed a diffuse staining that was not labeled by BSB (Fig. S4C, top and middle), demonstrating that variant Aβ cannot form amyloid aggregates. We did not detect any signal by incubating the cells 6 h with TRITC alone together with BSB (Fig. S4C, bottom).

We analyzed by the biotin-based endocytosis assay the uptake of wt and variant Aβ alone or in combination following 24 h of incubation, which corresponds to the formation of wt large aggregates *in vitro*. Separation on agarose gel of total extracts demonstrated that wt Aβ formed large aggregates and oligomers of higher molecular weight compared with variant Aβ (Fig. 3A). Wt Aβ aggregates and high-size oligomers are not internalized (Fig. 3A). We observed the presence of internalized oligomers of wt Aβ (Fig. 3A). Variant Aβ did not form large aggregates, while only oligomers that were fully internalized (Fig. 3A,B). In agreement with the data obtained *in vitro*, we did not observe the formation of large

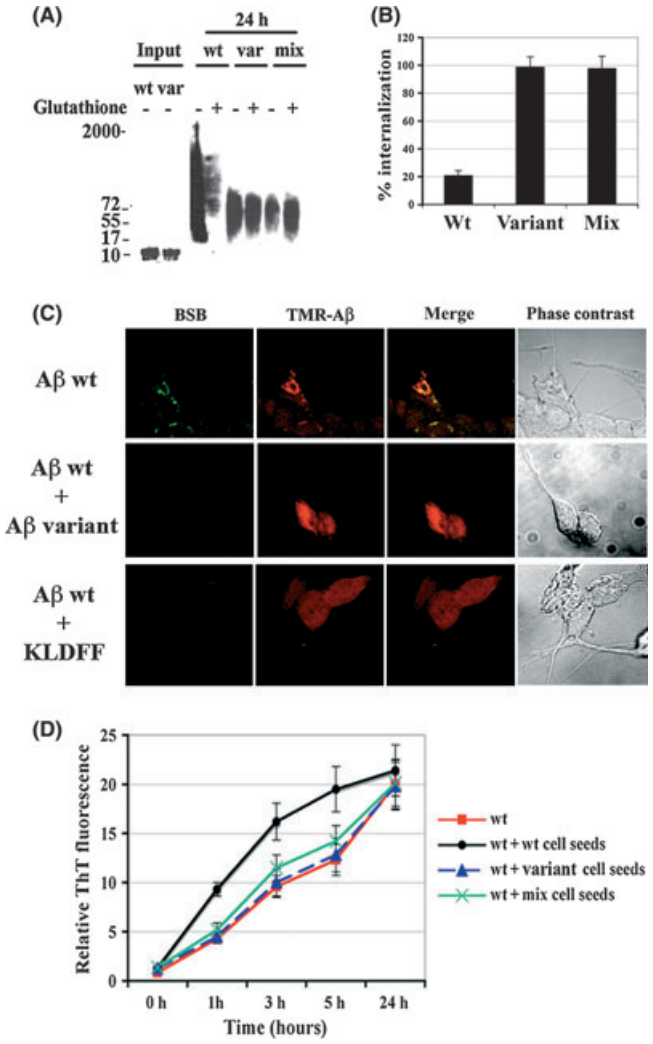

Fig. 3 Variant Aβ has a dominant negative effect on amyloidogenesis. (A) Dimers–trimers of biotinylated wt and biotinylated variant Aβ were incubated alone or in combination (3 μM) with the cells for 24 h at 37 °C. The uptake of wt and variant Aβ was analyzed by the endocytosis assay. Biotinilated Aβ is detected with streptavidin-HRP. Wt = wild-type Aβ; var = variant Aβ; mix = mixture of wt/variant Aβ (1:1 ratio). (B) quantification of the experiments shown in A. The data are the average of three independent experiments. (C) Dimers–trimers of tetramethylrhodamine-5-(and 6)-isothiocyanate (TRITC)-wt Aβ alone or together with either TRITC-variant Aβ or the β sheet breaker KLDFF was incubated with the cells for 24 h at 37 °C. The formation of aggregates was determined by adding BSB 20 min before fixation of the cells. The immunofluorescence was analyzed by confocal microscopy. (D) *In vitro* analysis of wt Aβ (45 μM) aggregation kinetics by thioflavin T (ThT) binding both in the absence and presence of seeds derived from extracts of cells treated with either wt, or variant, or the mixture of wt and variant (1:1 ratio) Aβ. (A,C) The results shown are representative of three independent experiments.

aggregates when wt Aβ was added to the cells together with variant Aβ (1:1 ratio) (Fig. 3A). The mixture of wt and variant Aβ resulted in the formation of oligomers of lower molecular weight that were fully internalized (Fig. 3A,B). Immunofluorescence analysis confirmed that incubation of the cells for 24 h with dimers–trimers of TRITC-wt Aβ resulted in the formation of Aβ aggregates BSB positive (Fig. 3C). Incubation of the cells for 24 h with TRITC-wt Aβ together with TRITC-variant Aβ (1:1 ratio) showed uniform TRITC staining and no labeling by BSB, demonstrating that variant Aβ inhibited the aggregation of wt Aβ (Fig. 3C). We obtained equal inhibition of wt Aβ aggregation by incubating the cells for 24 h with TRITC-wt Aβ together with a well-characterized beta sheet breaker peptide (sequence KLDFF) (Permanne *et al.*, 2002) (wt Aβ:KLDFF ratio was 1:10) (Fig. 3C). To verify the specificity of the dominant negative effect of variant Aβ, we incubated TRITC-wt with TRITC-scrambled Aβ (1:1 ratio) with SHSY-5Y cells for 24 h. TRITC-scrambled Aβ alone did not form any aggregates following staining with BSB and was not able to inhibit the formation of BSB-positive aggregates when incubated together with TRITC-wt Aβ (Fig. S5). Thus, the dominant negative effect in amyloidogenesis of variant Aβ is specific. We also observed that GM1 staining is beaded when SHSY-5Y cells are incubated with wt Aβ (Fig. S5), suggesting that the aggregation of wt Aβ at the cell surface in living cells may affect the integrity of the plasma membrane. This effect is not produced by variant Aβ. We hypothesize that variant Aβ does not affect the membrane integrity because it does not aggregate at the cell surface, whereas it is fully internalized.

We next investigated whether variant Aβ affects the seeding capability of wt Aβ aggregates formed *in cellulo*. Cells loaded for 24 h with wt Aβ and variant Aβ alone or in combination were sonicated with SDS, and diluted homogenates were added to 30 μM Aβ1–42 and the *in vitro* aggregation kinetics were investigated by ThT emission measurement and compared with the *in vitro* aggregation kinetics of Aβ1–42 alone (Fig. 3D). According with a previous report (Hu *et al.*, 2009), the extract of cells loaded with wt Aβ seeded the aggregation of wt Aβ *in vitro* (Fig. 3D). On the contrary, extracts from cells loaded with variant Aβ or wt and variant Aβ in combination (1:1 ratio) were unable to accelerate the aggregation of wt Aβ *in vitro* (Fig. 3D). Extracts from cells untreated with any Aβ did not seed the aggregation of wt Aβ *in vitro* (data not shown).

The capability to interact with the cell membrane of both wt and variant Aβ correlates with the GM1 cellular content

It has been demonstrated that the interaction with the cell surface and the uptake of Aβ are cell specific (Hu *et al.*, 2009). We investigated whether the specificity of Aβ interaction with cells correlates with variations of GM1 content between different cell lines. As it has been shown that in Madin-Darby canine kidney cells (MDCK) only few cells express GM1 and expose it on the apical surface (Chen *et al.*, 2008), we compared the association to the cell membrane of TRITC-variant and TRITC-wt Aβ in SHSY-5Y and MDCK cells. Immunofluorescence and confocal analysis demonstrate that all SHSY-5Y cells expose GM1 at the cell surface, which correlates with TRITC-wt Aβ aggregation spots (Fig. 4A). We also found that about 50% of wt Aβ colocalizes with GM1 in SHSY-5Y, and variant Aβ shows a significant higher colocalization with GM1 compared with wt and scrambled Aβ (Fig. S6B). On the contrary, only few MDCK cells express and expose GM1 at the surface (Fig. 4B). Following 24 h incubation of TRITC-Aβ, only TRITC-wt showed some interaction with MDCK cells, mostly in cells GM1 positive and did not form large aggregates (Fig. 4B). We did not detect TRITC-variant Aβ interacting with MDCK cells by confocal analysis (Fig. 4B). We may hypothesize that in MDCK cells, wt Aβ interacts with surface receptors some of them localized in GM1-labeled domains, while variant Aβ is not recognized by these receptors. Western blotting of total extracts following 10 h of incubation with SHSY-5Y cells showed that both variant and wt Aβ formed oligomers. Only wt Aβ showed the 72- and the 56-kDa oligomers (Fig. 4C). In MDCK cells, we observed oligomers of shorter size indicating a diminished aggregation of wt Aβ (Fig. 4C). Only a weak staining were detected when variant Aβ was incubated with MDCK cells (Fig. 4C), suggesting that the interaction of variant Aβ with the low amount of GM1 in MDCK

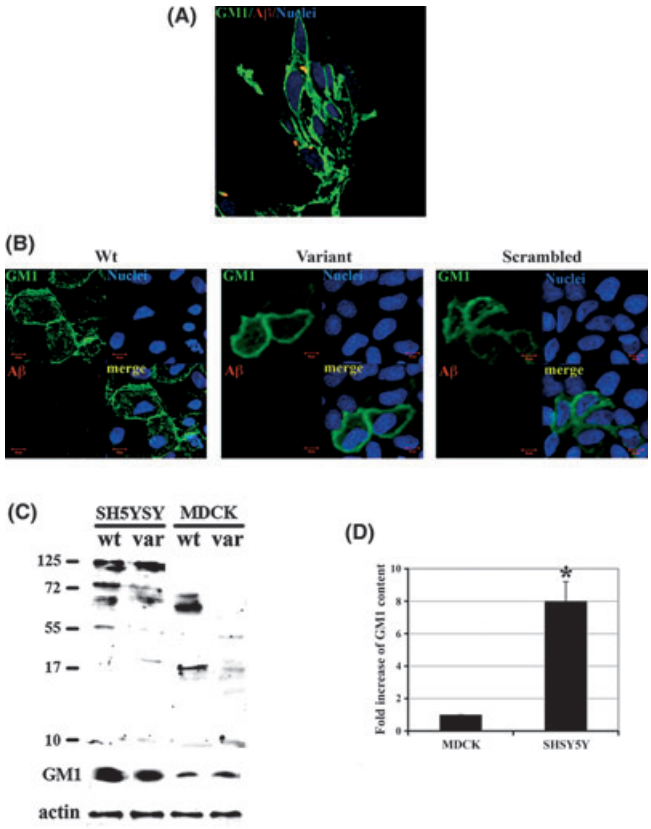

Fig. 4 Wt and variant Aβ show strongly diminished binding to Madin-Darby canine kidney (MDCK) cells. (A) SHSY-5Y cells incubated for 24 h at 37 °C with dimers–trimers of tetramethylrhodamine-5-(and 6)-isothiocyanate (TRITC)-wt Aβ (3 μM) (in red). The presence of GM1 at the cell surface was detected using fluorescein isothiocyanate (FITC)-ChTB (in green). Nuclei are labeled with Hoecst (in blue). The immunofluorescence was analyzed by confocal microscopy. (B) MDCK cells. Dimers–trimers of TRITC-wt, TRITC-variant and TRITC-scrambled Aβ (3 μM) (in red) were incubated with the cells for 24 h at 37 °C. The presence of GM1 at the cell surface was detected using FITC-ChTB (in green). Nuclei are labeled with Hoecst (in blue). The immunofluorescence was analyzed by confocal microscopy. (C) Dimers–trimers of wt or variant Aβ were incubated with MDCK cells for 10 h at 37 °C. Aβ oligomerization was analyzed by Western blotting using anti-Aβ antibody. Wt = wild-type Aβ; var = variant Aβ. (C,D) GM1 content was detected by Western blotting using HRP-ChTB. Loading control was performed using an anti-actin antibody. (D) Quantification of GM1 content in MDCK cells and average of three independent experiments (*$P < 0.001$). The results shown are representative of three independent experiments.

cells is below the limit of the detection systems we used. The decreased interaction of both wt and variant Aβ with MDCK cells parallels the diminished content of GM1 in these cells compared with SHSY-5Y cells (Fig. 4C,D). Our results in MDCK cells are in agreement with previous results, showing an absence of Aβ uptake in human embryonic kidney cells (HEK293) and confirm the cell specificity of Aβ interaction.

Aging enhances GM1 content in synaptosomes, leading to faster wt Aβ aggregation that is inhibited by variant Aβ

Previous studies demonstrated that age induces enhanced GM1 content in synaptosomes, leading to enhanced Aβ1–40 aggregation analyzed by ThT measurements (Yamamoto *et al.*, 2008). We confirmed that synaptosomes derived from 2-year-old rats exhibit a higher GM1 content (factor 3) compared with synaptosomes from 4-week rats (Fig. 5A,B). We investigated the aggregation kinetics between 1 and 24 h of wt and variant Aβ (30 μM) in the presence of synaptosomes extracted from either young (4 weeks) or old (2 years) rats. Synaptosomes were obtained as previously described (Lévêque *et al.*, 2000). Incubation of wt Aβ with synaptosomes from 2-year-old rats accelerated Aβ aggregation compared with incubation of wt Aβ with synaptosomes from 4-week-old rats (Fig. 5C, 1, 2, 3 h points in the time course). Variant Aβ showed a significant lower ThT value compared with wt Aβ starting from 1 h incubation and in the subsequent points of the time course (Fig. 5C). The mixture scrambled/wt Aβ (1:1 ratio) incubated with synaptosomes derived from old rats showed a delayed increment in ThT value compared with variant/wt Aβ (Fig. 5D). Thus, variant Aβ acted as a dominant negative in amyloidogenesis also when coincubated with the wt Aβ in the presence of synaptosomes.

We next incubated wt and variant Aβ 2 h in the presence or absence of synaptosomes prepared from either young or old rats. Western blotting analysis revealed that variant Aβ did not oligomerize in any fraction, while wt Aβ showed the formation of a multimer of 17 kDa and a more diffuse band around 70 kDa in the presence of synaptosomes from old rats (Fig. 5E).

Synaptosomes were incubated for 6 h with dimers–trimers of either TRITC-wt Aβ or TRITC-variant Aβ on coverslips (coated with polylysine) in DMEM containing 2% serum and analyzed by confocal analysis. Synaptosomes were labeled with FITC-ChTB. TRITC-wt and TRITC-variant Aβ bind synaptosomes membranes derived from both young and old rats, with TRITC-wt Aβ showing the formation of clusters-like structures (Fig. S7A). Incubation of synaptosomes from old rats with wt Aβ produced clusters-like structures of higher size compared with the structures obtained by incubating TRITC-variant Aβ with synaptosomes from old rats (see scale bar in Fig. S8). A magnification of the confocal analysis demonstrated that in the presence of synaptosomes from old rats, TRITC-wt Aβ also formed some clusters-like structures of higher size with a fibril-like shape, which were absent in synaptosomes from young rats (Fig. S8). Dimers–trimers of either TRITC-wt or TRITC-variant Aβ were also incubated in DMEM containing 2% serum on coverslips coated with polylysine in a cell- and synaptosome-free system. We did not detect any aggregates until 7 days of incubation, and the size of particles was at least one order of magnitude lower compared with the structures detected when TRITC-wt and TRITC-variant Aβ are incubated with the synaptosomes (Fig. S7B). These data confirm that synaptosomes seed the aggregation of wt Aβ and have some effect also on variant Aβ, even if at lesser extent compared with wt Aβ.

Tyr10paraNH2Phe variant Aβ peptide does not induce oxidative stress

Tyrosine 10 coordinates redox-active transition metals such as copper, leading to the generation of reactive oxygen species (ROS) and the substitution Y10A in Aβ posses reduced capability in producing ROS in the presence of Cu^{2+} and ascorbate (Barnham *et al.*, 2004). We demonstrated that Cu-Aβ in the presence of ascorbate forms extracellular ROS, which leads to the subsequent intracellular production of ROS inducing cell death (Perrone *et al.*, 2010b). We analyzed the intracellular ROS production induced by incubating the cells for 1 h with wt and variant Aβ alone or in combination both in the presence and absence of Cu^{2+} and ascorbate (Fig. 6A). Wt Aβ-induced intracellular ROS formation in the presence of Cu^{2+} and ascorbate and to a lesser extent in the absence of Cu^{2+} and ascorbate (Fig. 6A). The variant Aβ did not induce ROS formation both in the presence and the absence of Cu^{2+} and ascorbate (Fig. 6A). We observed a sensible reduction in intracellular ROS formation when wt and

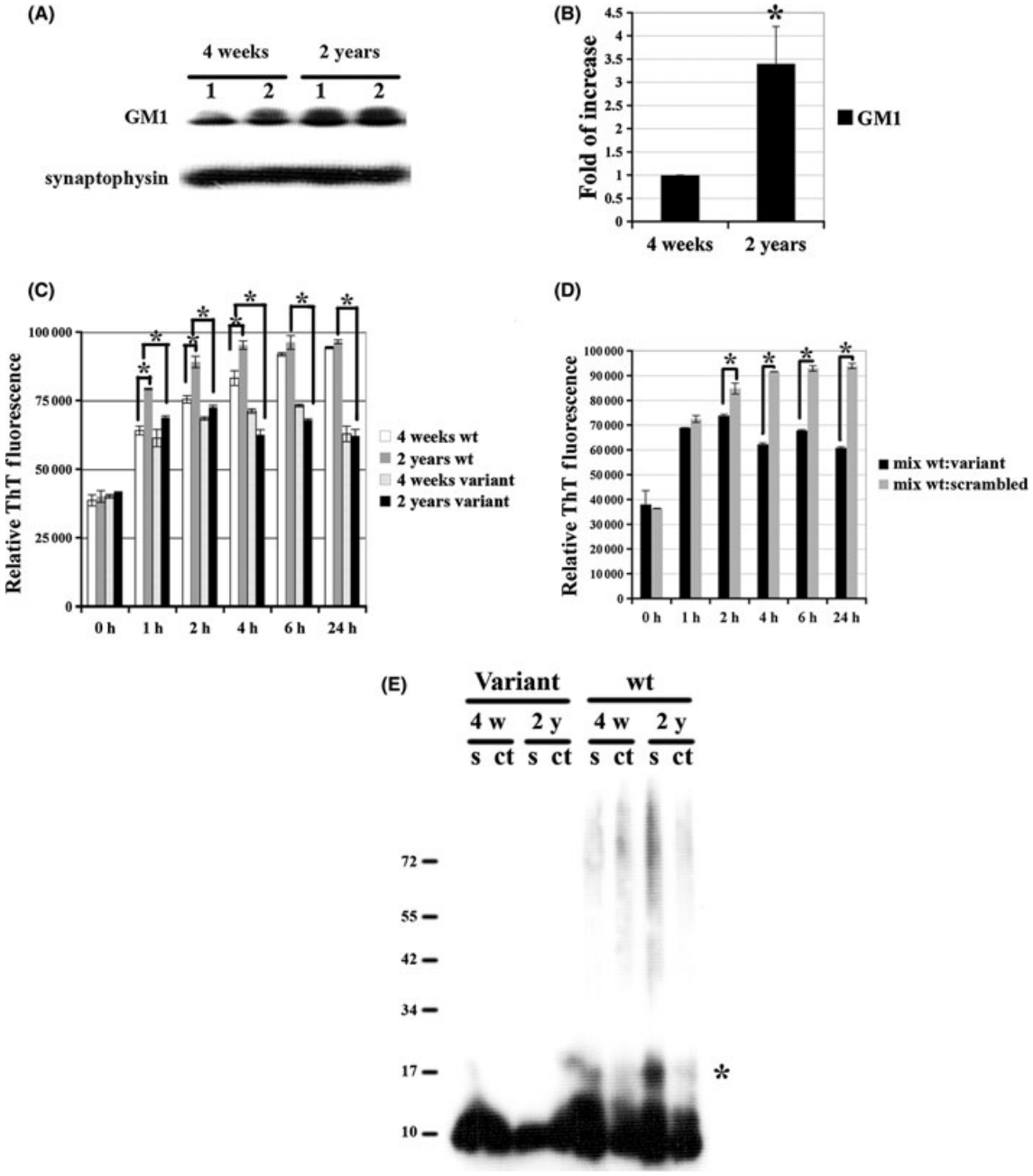

Fig. 5 Synaptosomes from old rats accelerate wt Aβ aggregation that is inhibited by coincubation with variant Aβ. (A) Western blotting analysis of GM1 content in the synaptosomes (5 μg) derived from the brain of either 4-week (two rats) or 2-year-old rats (two rats) using HRP-ChTB. Protein loading was normalized by Western blotting using an anti-synaptophysin antibody (representative of three independent experiment). (B) Quantification of GM1 content in synaptosomes from young and old rats (n = 3). *P < 0.05 (C). *In vitro* analysis of variant (30 μM) and wt Aβ (30 μM) aggregation kinetics by thioflavin T (ThT) binding in the presence of synaptosomes from either young (4 weeks) or old (2 years) rats (n = 8), *P < 0.001. (D) In *in vitro* analysis, the aggregation kinetics of the mixture wt/variant (1:1 ratio, final 30 μM) and wt/scrambled Aβ (1:1 ratio, final 30 μM) by ThT binding in the presence of synaptosomes from old (2 years) rats (n = 8), *P < 0.001. (E) Western blotting analysis, using a monoclonal anti-Aβ antibody, of variant and wt Aβ aggregates following 2 h of incubation in presence (S) or absence (Ct) of synaptosomes from either young (Y) or old (O) rats (representative of four experiment). *17 kDa oligomer.

variant Aβ are added together (1:1 ratio) both in the presence and the absence of Cu^{2+} and ascorbate (Fig. 6A).

To investigate Cu-Aβ-induced cell death, cells were incubated with wt and variant Aβ alone or in combination in the presence of Cu^{2+} and ascorbate for 24 h. Wt Aβ-induced cell death compared with control cells treated with Cu^{2+} and ascorbate (Fig. 6B), while variant Aβ did not and showed reduced cell toxicity compared with the control (Fig. 6B). Variant Aβ decreased wt Aβ-induced cell toxicity when the two peptides were added together (Fig. 6B).

Wt Aβ induced a slight oxidative stress also in the absence of Cu^{2+} and ascorbate, which is sensibly lower compared with the oxidative stress induced by wt Aβ in the presence of Cu^{2+} and ascorbate (Fig. 6A). It has

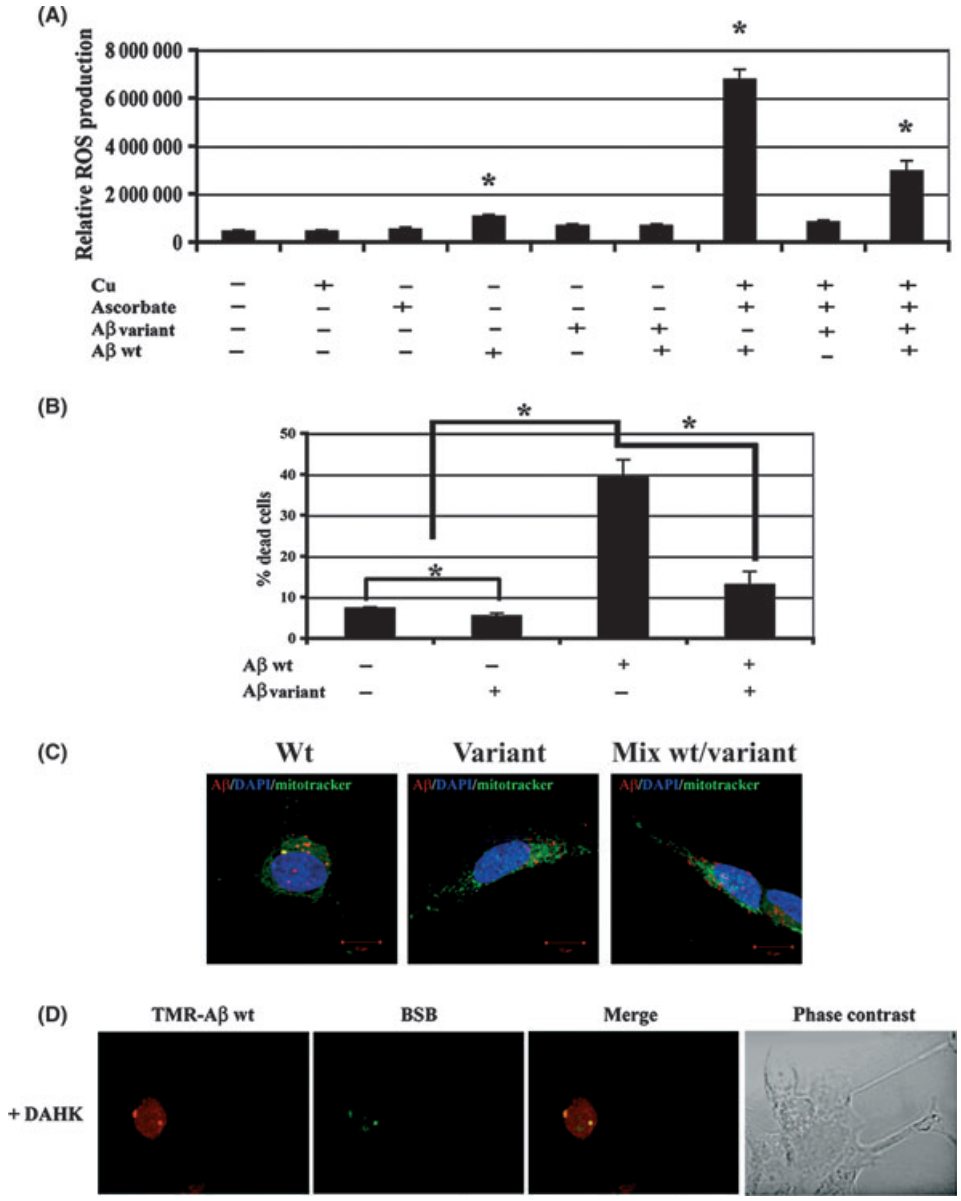

Fig. 6 Variant Aβ inhibits Cu-Aβ-dependent ROS formation and subsequent cell death. (A) Effect of Cu^{2+}–Aβ (wt, variant or the two peptides in combination) in the presence of ascorbate on intracellular early ROS production in SH-SY5Y cells measured using DCF fluorescence. These results are the average of two independent experiments performed in triplicate (n = 6, *P < 0.05 compared with the control untreated cells, #P < 0.05 compared with the control untreated cells) (B) Effect of Cu^{2+}–Aβ (wt, variant or the two peptides in combination) in the presence of ascorbate on cell death. These results are the average of three independent experiments performed in duplicate (n = 6, *P < 0.001 compared with the control). (C) Dimers–trimers of tetramethylrhodamine-5-(and 6)-isothiocyanate (TRITC)-wt, TRITC-variant Aβ (3 μM) and the mixture TRITC-wt/TRITC- variant Aβ (1:1 ratio) were incubated for 24 h at 37 °C with SHSY-5Y cells. Mitochondria are detected by adding fluorescein isothiocyanate-mitotracker in the cell medium before fixation. These data are representative of three independent experiments. (D) Dimers–trimers of TRITC-wt Aβ were incubated with the cells for 24 h at 37 °C both in the presence and the absence of the peptide DAHK (1:10 ratio), which inhibits the Cu^{2+}-Aβ interaction. The formation of aggregates was determined by adding BSB 20 min before fixation of the cells. The immunofluorescence was analyzed by confocal microscopy. The results shown are representative of three independent experiments.

been shown that wt Aβ can interact with surface receptors, be internalized and transported to the mitochondria, leading to oxidative stress and neuronal dysfunction (Takuma *et al.*, 2009). After 1 h of incubation of both wt and variant Aβ with SHSY-5Y cells, we did not detect any colocalization of both wt and variant Aβ with mitochondria (data not shown). After 24 h of incubation, we found that a fraction of internalized wt Aβ colocalized with the mitochondria, while variant Aβ did not colocalize with the mitochondria (Fig. 6C). Notably, the mixture wt/variant Aβ (1:1 ration) did not colocalize with the mitochondria (Fig. 6C). These data suggest that wt Aβ interaction with mitochondria may participate in inducing cell dysfunction and that variant Aβ is protective by inhibiting the targeting of wt Aβ to mitochondria.

The interaction of Aβ with $Cu^{2+}$and ascorbate and the subsequent ROS production lead to the formation of Aβ dityrosine that participate in Aβ aggregation (Barnham *et al.*, 2004; Smith *et al.*, 2007). We investigated whether inhibition of wt Aβ association with the trace of Cu^{2+} present in the cell media was capable to affect wt Aβ aggregation *in cellulo*. We previously showed that incubation of wt Aβ with the peptide DAHK

inhibits Cu^{2+}-dependent aggregation *in vitro* because of squelching Cu^{2+}-Aβ interaction (Perrone *et al.*, 2010b). SHSY-5Y cells were loaded with wt Aβ (3 μM) in the presence of 30 μM DAHK for 24 h and Aβ aggregation was investigated by immunofluorescence analysis following addition of BSB. This treatment only moderately affected the aggregation of wt Aβ, and we still observed the formation of BSB positive aggregates (Fig. 6D).

Discussion

Although the extracellular deposition of Aβ is an invariable pathological feature of AD, it is not yet fully elucidated how Aβ starts to aggregate in the brain in an age-dependent manner. Several studies indicate that the following age-dependent modifications participate in Aβ aggregation and toxicity: (i) high-density clustering of GM1 in synaptosomes (Hayashi *et al.*, 2004; Yamamoto *et al.*, 2004, 2008, 2010; Kimura & Yanagisawa, 2007); (ii) modifications of lipid domains in brain membranes (Wood *et al.*, 2002; Recuero *et al.*, 2009); (iii) oxidative stress (Recuero *et al.*, 2009; Perrone *et al.*, 2010b). Previous studies suggested that Tyr10 in Aβ is crucial for the production of extracellular ROS in the presence of the redox-active metal Cu^{2+} (Barnham *et al.*, 2004; Smith *et al.*, 2007). For this reason, we decided to design and to analyze the properties of an Aβ Tyr10 variant, which has modified capability to interact with GM1 and is unable to induce of ROS formation in the presence of Cu^{2+}. As it has been shown that Tyr10Phe substitution does not affect Aβ-GM1 interaction (Fantini & Yahi, 2011), we substituted Tyr10 with a synthetic amino acid (para-amino-phenylalanine), which resulted in an accelerated binding to GM1, which has been reported to be essential for Aβ seeding and aggregation *in cellulo* (Yamamoto *et al.*, 2010). The enhanced affinity of variant Aβ for GM1 is probably due to the amino group of para-amino-phenylalanine, which can interact with the glycone part of GM1 through hydrogen bonds. We also demonstrate that variant Aβ partially squelches the interaction of wt Aβ with the plasma membrane. Interestingly, the addition of an excess of unlabeled wt Aβ is completely inefficient in inhibiting the interaction of TRITC-variant Aβ with SHSY-5Y cells. On the contrary, the addition of an excess of unlabeled variant Aβ strongly diminished the interaction of TRITC-wt Aβ with SHSY-5Y cells, but does not fully block the interaction of TRITC-Aβ with the cells. These data suggest that variant Aβ may modify the uptake of wt Aβ in SHSY-5Y cells and that this effect is detectable only when wt Aβ is labeled with TRITC. Indeed, wt Aβ is fully internalized in SHSY-5Y only in the presence of variant Aβ. Both wt and variant Aβ interact not only with GM1 and lipid rafts, but also with membrane domains outside lipid rafts. We may hypothesize that wt and variant Aβ may also interact with surface receptors with distinct affinities. In agreement, wt Aβ is also known to interact with surface receptors (Diarra *et al.*, 2009; Nygaard & Strittmatter, 2009; Origlia *et al.*, 2009; Thathiah & De Strooper, 2009).

The major novelty described herein consists in demonstrating that variant Aβ acts as a dominant negative factor in amyloidogenesis both *in vitro*, *in cellulo*, and in isolated synaptosomes. It affects Aβ aggregation at the plasma membrane and Aβ-induced oxidative stress. At present, only one natural mutation with dominant negative effects in amyloidogenesis has been described (Di Fede *et al.*, 2009). Our results strongly support the hypothesis that the addition of an amyloidogenic protein to a more amyloidogenic protein inhibits amyloid formation. Indeed, there is evidence that murine APP/Aβ affects the aggregation of human Aβ because transgenic mice that carry human APP and lack the endogenous mice APP show enhanced Aβ deposition (Radde *et al.*, 2008). We demonstrate that variant Aβ possesses impaired aggregation capability and that it affects the aggregation of wt Aβ *in vitro*. From our results, we may hypothesize that variant Aβ acts as a dominant negative in amyloidogenesis via two additive mechanisms: by squelching the binding of wt Aβ with seeding domains at the cell surface and by destabilizing directly wt Aβ aggregation by interacting with wt Aβ.

The dominant negative effect in amyloidogenesis is specifically because of the amino-para-phenylalanine substitution at position 10 both *in vitro* and *in cellulo*. Scrambled Aβ only partially affects the aggregation property of wt Aβ *in vitro* by inducing slower aggregation of wt Aβ, but it is not capable to block wt Aβ aggregation following prolonged time of incubation. Furthermore, scrambled Aβ does not affect the aggregation of wt Aβ at the cell surface of SHSY-5Y cells.

In agreement with previous studies (Saavedra *et al.*, 2007; Matsuzaki, 2011), wt Aβ aggregates mostly at the cell surface. However, a study underlines the relevance of Aβ1–42 aggregation in an endocytic compartment (Hu *et al.*, 2009). The differences observed may be due to the cell treatment conditions. Indeed, Hu *et al.* (2009) analyzed Aβ1–42 uptake in the absence of serum, using lower Aβ concentration and longer incubation time.

In agreement with a recent study (Matsuzaki, 2011), at least 50% of wt Aβ colocalizes with GM1 in neuronal cells. Variant Aβ shows increased colocalization with GM1 compared with wt Aβ in SHSY-5Y cells. In agreement with a previous report (Hu *et al.*, 2009), wt Aβ interactions at the membrane are cell specific and are diminished with MDCK cells, which parallels a strongly decreased content of GM1 in these cells compared with SHSY-5Y cells. However, as variant Aβ shows a decreased affinity of MDCK cells compared with wt Aβ, we hypothesize that in these cells, Aβ mostly interact with surface receptors. The 56 kDa oligomer, which is considered to be the more toxic (Lesné *et al.*, 2006, 2008), is produced only by wt Aβ in SHSY-5Y cells as evident at early time in the aggregation kinetic *in cellulo*, while after 24 h, there appear several oligomers around these size and it is not possible to discriminate with the agarose gel separation the exact size of the oligomers. Variant Aβ is unable to form the 56-kDa oligomers at early time in the aggregation kinetic *in cellulo* and wt Aβ fails to produce it in MDCK cells, suggesting that the formation of the 56 kDa oligomers is cell specific and may correlate with the GM1 content in the cells. Indeed, a recent study underlines the role of gangliosides in the formation of Aβ toxic oligomers (Matsuzaki, 2011). Besides the 56-kDa oligomer, we also detected the formation of other oligomers that are specifically produced only by wt Aβ, suggesting that additional toxic oligomeric species may exist. A previous study demonstrated that synaptosomes from old mice enhance wt Aβ1–40 aggregation following 24 h of incubation (Yamamoto *et al.*, 2008). We observe the same effect at shorter times, probably because Aβ1–42 aggregates more rapidly than Aβ1–40. We confirmed that age-dependent enhanced GM1 content in synaptosomes accelerates wt Aβ aggregation. Notably, we show that the dominant negative effect of variant Aβ is more effective in the presence of synaptosomes from old rats, which display an increased GM1 content.

A previous study revealed the role of Tyr10 oxidation in Aβ aggregation (Barnham *et al.*, 2004). However, we found that blockade of wt Aβ-Cu^{2+} interaction and subsequent ROS formation only partially inhibits wt Aβ aggregation *in cellulo* in SHSY-5Y cells.

We confirmed that Tyr10 is important for ROS production in the presence of Cu^{2+}/ascorbate and that ROS production has an important function in Aβ toxicity. Wt Aβ induces intracellular ROS formation even in the absence of Cu^{2+}/ascorbate, while variant Aβ does not. It has been demonstrated that internalized wt Aβ can be targeted to the mitochondria, leading to mitochondrial damage that participates in neuronal death (Takuma *et al.*, 2009). We carried out experiments aimed to analyze Aβ-induced cellular oxidative stress by incubating the cells 1 h with Aβ both in the presence and absence of Cu^{2+}/ascorbate. We did not detect

any colocalization of wt Aβ with mitochondria before 24-h incubation of wt Aβ with SHSY-5Y cells. Thus, we can exclude that the intracellular ROS production is because of mitochondrial damage, while it may be due to the interaction of Aβ with surface receptors. Mitochondrial damage may be implicated in wt Aβ-induced cell death following 24 h of wt Aβ incubation with SHSY-5Y cells. Coincubation of variant Aβ with wt Aβ inhibits wt targeting to mitochondria, as well as it reduces SHSY-5Y death. The detailed analysis of wt Aβ intracellular targeting in the presence of variant Aβ and the effect on mitochondrial function is under investigation and will be part of another manuscript. We may speculate that variant Aβ impairs wt Aβ toxicity via multiple pathways: blocking extracellular ROS production, affecting wt Aβ aggregation and the formation of toxic oligomeric species, and by inhibiting the targeting of wt Aβ to mitochondria.

This study sheds new light on the role of Aβ interaction with GM1 and in Aβ-induced ROS formation. Moreover, it open the way for the design of variant peptides aimed to inhibit Aβ aggregation as a novel therapy for AD, having as defined target the age-dependent risk factors for AD: increased GM1 and oxidative stress.

Experimental procedures

Material

See Supporting information section.

Aβ preparation

Variant, scrambled, and wt Aβ1–42 were synthesized and purified by H. Marzaguil. The detailed protocol for Aβ preparation is described in the Supporting information section.

Aβ-GM1 interaction analysis *in vitro*

Surface pressure measurements revealing peptide-lipid interactions were studied as described by Yahi *et al.* (2010). The detailed protocol is described in the Supporting information section.

ThT fluorescence

The detailed protocol is described in the Supporting information.

Cell culture

The detailed protocol is described in the Supporting information section.

Competition assay in Aβ-cell interaction

The detailed protocol is described in the Supporting information section.

Endocytosis assay

Wt and variant Aβ were biotinylated with cleavable biotin as described earlier. The endocytosis assay was carried out as previously described (Perrone *et al.*, 2005, 2008). The detailed protocol is described in the Supporting information section.

Agarose gel electrophoresis

We performed the agarose gel electrophoresis as previously described (Taneja *et al.*, 2007). The detailed protocol is described in the Supporting information section.

Immunofluorescence analysis

TRITC-labeled wt, scrambled and variant Aβ were incubated with differentiated SHSY-5Y or MDCK cells at 37 °C at 3 μM final concentration. The detailed protocol is described in the Supporting information section.

Triton X-100 extraction of lipid-raft associated proteins

Cells were lysed at 4 °C in TX-100, and the soluble and insoluble fractions were obtained as previously reported (Sbai *et al.*, 2010).

Western blotting analysis of Aβ oligomerization in MDCK and SHSY-5Y cells

Wt and variant Aβ (3 μM) were added to the appropriate culture medium containing 2% FCS and incubated for 10 h at 37 °C. Cells were lysed in RIPA buffer, and an equal amount of protein was incubated 5 min in Laemmli (1X final) at room temperature. Proteins were separated on a gradient 4–20% SDS-PAGE. Proteins and GM1 were transferred on nylon membrane by semi-dry transfer system (Biorad Marnes-la-Coquette, France). Detection was performed as previously described (Perrone *et al.*, 2009, 2010a). The detailed protocol is described in the Supporting information section.

Analysis of the effect of synaptosomes on Aβ aggregation

All procedures were performed according to the French law on animal care guidelines. Animal Care Committee of University Aix-Marseille II approved protocols. Male Sprague-Dawley rats were maintained with normal food and water at libitum. We prepared the synaptosomes from the whole brain of two 4-week-old rats and two 2-year-old rats as previously described (Lévêque *et al.*, 2000). The detailed protocol is described in the Supporting information section.

Determination of intracellular ROS levels

This assay was performed as we previously described (Perrone *et al.*, 2010b). The detailed protocol is described in the Supporting information section.

Cell viability assay (MTT)

This assay was performed as we previously described (Perrone *et al.*, 2010b). The detailed protocol is described in the Supporting information section.

Dynamic light scattering

Dynamic light scattering was performed similar as already described (Funke *et al.*, 2010). The detailed protocol is described in the Supporting information section.

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Italy) for providing SHSY-5Y cells, Dr. Andre Lebivic (IBDML, CNRS, Marseille, France) for providing MDCK cells, and Dr. Lotfi Ferhat (NICN, UMR 6184, Marseille, France) for providing the anti-synaptophysin antibody. We thank also Dr. Michael Seagar (Neurobiologie des Canaux Ioniques :UMR 641, Marseille, France) for reading the manuscript and the helpful suggestions.

Author contributions

H. Mazarguil ideated the variant Aβ, synthesized the Aβ peptides, performed the quality assay for the Aβ peptide preparation, and participated in planning the experiments; C. Leveque and D. Bartnik performed experiments and participated in the discussion of the work and in writing the manuscript; T. Gouget performed experiments; J. Fantini performed experiments and participated in planning the experiments and writing the manuscript; M.A.B. Melone, S.A. Funke and D. Willbold participated in planning the experiments and writing the manuscript, L. Perrone performed experiments, planned and directed the experiments, and wrote the manuscript.

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Supporting Information

Additional supporting information may be found in the online version of this article:

Fig. S1 Analysis of TRITC-wt Aβ (1 μM) binding to SHSY5Y cells both in the presence and absence of increasing concentration unlabelled variant Aβ.

Fig. S2. Analysis of variant (50 μM) and wt Aβ (50 μM) aggregation kinetics *in vitro*.

Fig. S3 Particle size analysis of wt Aβ, variant Aβ and wt Aβ/variant Aβ mixtures analyzed by dynamic light scattering.

Fig. S4 Variant Aβ does not aggregate in cellulo.

Fig. S5 The dominant negative effect of variant Aβ is specific.

Fig. S6 Variant Aβ shows increased binding to GM1.

Fig. S7 Variant Aβ displays an evident diminished aggregation capability in the presence of synaptosmes.

Fig. S8 Variant Aβ displays an evident diminished aggregation capability in the presence of synaptosmes.

Data S1 Materials and methods.

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