Production of seedable Amyloid-β peptides in model of prion diseases upon PrPSc-induced PDK1 overactivation

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The presence of amyloid beta (Aβ) plaques in the brain of some individuals with Creutzfeldt-Jakob or Gertsmann-Straussler-Scheinker diseases suggests that pathogenic prions (PrPSc) would have stimulated the production and deposition of Aβ peptides. We here show in prion-infected neurons and mice that deregulation of the PDK1-TACE α-secretase pathway reduces the Amyloid Precursor Protein (APP) α-cleavage in favor of APP β-processing, leading to Aβ40/42 accumulation. Aβ predominates as monomers, but is also found as trimers and tetramers. Prion-induced Aβ peptides do not affect prion replication and infectivity, but display seedable properties as they can deposit in the mouse brain only when seeds of Aβ trimers are co-transmitted with PrPSc. Importantly, brain Aβ deposition accelerates death of prion-infected mice. Our data stress that PrPSc, through deregulation of the PDK1-TACE-APP pathway, provokes the accumulation of Aβ, a prerequisite for the onset of an Aβ seeds-induced Aβ pathology within a prion-infectious context.
Prion diseases, such as Creutzfeldt-Jakob (CJDs) and Gertmann-Strassler-Scheinker (GSS) diseases in humans, are neurodegenerative disorders with sporadic, genetic or iatrogenic origins. These pathologies are characterized by the accumulation in the central nervous system of an abnormally folded and toxic protein, the scrapie protein—PrP^Sc, the trans-conformational isofrom of the cellular prion protein PrP^C (ref. 1). Beyond PrP^Sc–induced neuropathological lesions, the occurrence of Alzheimer-like pathology was reported in the brain of elderly CJD or GSS patients2–7, as well as young individual with iatrogenic CJD (iCJD)8. Colocalization of β-amyloid (Aβ) with PrP^Sc in the same amyloid plaques9 suggests potential interrelationships between prion pathogenesis and production and/or deposition of Aβ. Accordingly, prion infection with the 139 A scapie strain of transgenic mice with Alzheimer-like pathology (Tg2576) enhances the cortical accumulation of fibrillar and aggregative Aβ42 (ref. 10). Clinical examination of the brain of iCJD patients contaminated with PrP^Sc-containing human growth hormone (hGH) or graft-with prion-infected dura mater showed Aβ deposits in the brain parenchyma as well as a cerebral amyloid angiopathy (CAA)11–13. However, the mechanisms whereby prion infection would connect to the rise and deposition of Aβ in prion diseases remain elusive. The accumulation of toxic Aβ peptides concomitantly with PrP^Sc also asks the question about the role exerted by Aβ on prion pathogenesis.

Normal PrP^C has been involved in the control of cellular Aβ production. PrP^C is a GlycosylPhosphatidylInositol (GPI)-anchored protein tethered to the outer leaflet of the plasma membrane, where its behaves as a signaling receptor or co-receptor14–16 or as a scaffolding protein regulating the assembly and functionality of diverse signaling membrane effectors17. Although controversial18, PrP^C may limit the production of Aβ by inhibiting β-secretase (BACE1)-mediated cleavage of the amyloid precursor protein (APP)19. PrPnull-mice display higher levels of brain Aβ than their PrP^C expressing counterparts and productions of Aβ40 and Aβ42 by neuroblastoma cells are cancelled upon PrP^C overexpression19. Alternatively, PrP^C–dependent activation of the α-secretase TACE (ADAM17)20 may limit Aβ production. Physiologically TACE contributes to the regulated cleavage of APP into the protective soluble fragment sAPPα, which precludes the production of Aβ peptides by the β-secretase pathway21,22.

We previously evidenced that deregulation of the PrP^C-TACE α-secretase signaling pathway plays a critical role in prion neuropathogenesis3,24. PrP^Sc induces (i) an overactivation of the 3-phosphoinositide-dependent kinase 1 (PDK1), and (ii) a downstream internalization of TACE. This internalization decreases by more than 80% the TACE shedding activity towards PrP^C, then amplifying PrP^Sc conversion into PrP^Sc (ref. 23). Whether PrP^Sc would also reduce APP α-cleavage by TACE, thus favoring the β-amyloidogenic pathway through corruption of the PrP^C-PDK1-TACE pathway, has never been investigated.

Combining in vitro and in vivo approaches, we here show that PrP^Sc–induced overactivation of PDK1 and downregulation of TACE activity shifts APP towards its β-processing and Aβ40/42 overproduction. Aβ40 and Aβ42 accumulate mainly as monomers, but trimers and tetramers of Aβ40/42 are also produced within a prion-infectious context. To address the role exerted by the overproduced Aβ on prion pathogenesis, we built an Aβ-free prion-infected cell system by chronically silencing the expression of APP in 1C11 neuronal stem cells25 (referred to as APPnull, 1C11 cells) prior to prion infection. The absence of Aβ does not alter prion replication in prion-infected APPnull,1C11 cells, and cell-based PrP^Sc inocula Aβ-free or not display similar prion infectivities when injected to C57Bl/6 J mice. We further show that PrP^Sc promotes a similar rise of Aβ monomers in the CSF of C57Bl/6 J mice whatever the presence of Aβ in PrP^Sc inocula. Prion infection of C57Bl/6 J mice also triggers the formation of Aβ trimers and tetramers, but the relative proportion of those Aβ oligomers varies according to the presence or not of Aβ species in PrP^Sc inocula. With the help of transgenic APP23 mice, we also provide evidence for brain deposition of PrP^Sc–induced Aβ, only when seeds of Aβ trimers are co-transmitted with PrP^Sc, whereas Aβ-free PrP^Sc inocula fail to induce any Aβ deposition. Importantly, prion infection combined to brain Aβ deposition reduces the survival time of prion-infected APP23 mice without modifying prion replication, indicating the contribution of amyloid deposition to the death of prion-infected mice.

Results

PrP^Sc corruption of the PDK1-TACE pathway triggers Aβ rise. Exploiting the 1C11 neuronal stem cell line25 that supports prion replication26, we measured through liquid chromatography-tandem mass spectrometry (LC-MS/MS) analyses accumulation of soluble Aβ40 and Aβ42 peptides in the surrounding milieu of 1C11-derived serotoninergic neuronal cells chronically infected by the Fukuoka-1 prion strain (Fk-1C115-HT cells) (Fig. 1a–b). We then assessed whether this rise of Aβ in the culture medium of Fk-1C115-HT cells would relate to a PDK1 overactivity, down-stream TACE internalization23 and subsequent reduction of APP α-cleavage. Pharmacological inhibition of PDK1 by BX912 (1 µM, 1 h) counteracted the prion-induced increase in Aβ40, Aβ42, and sAPPβ levels in the culture medium of Fk-1C115-HT cells and favored an enhanced production of the neuroprotective sAPPα fragment (Fig. 1a–d). The rescue of APP α-processing in prion-infected neuronal cells upon PDK1 inhibition depended on the restoration of TACE activity, since TACE inhibition with TAPI-2 (100 µM, 1 h) abrogated the decrease of Aβ40/42 and sAPPβ and the reciprocal increase of sAPPα induced by BX912 (Fig. 1a–d).

Our results thus indicate that accumulation of Aβ peptides in prion-infected neuronal cells depends on PDK1 overactivity. Further supporting the view that prion infection engages APP towards the β-secretase pathway, we showed that the pharmacological inhibition of BACE1 by MK-8931 (1 µM, 1 h) reduced the Aβ40, Aβ42, and sAPPβ levels in the culture medium of Fk-1C115-HT cells with an opposite increase in the level of sAPPα (Supplementary Fig. 1). Despite a strong reduction (more than 80%) of plasma membrane TACE level in prion-infected neurons23, the increase in sAPPα level that typically accompanies BACE1 inhibition likely results from augmented bioavailability of APP for residual α-secretase activities27.

We next probed the oligomeric state of the Aβ peptides produced by prion-infected 1C115-HT cells through ion mobility coupled to electrospray ionization mass spectrometry (ESI-IM-MS)28. Only Aβ40 and Aβ42 monomers were present in the cell culture medium and lysates of uninfected 1C115-HT cells whereas in the cell culture medium of Fk-1C115-HT cells Aβ trimers representing ~2.5% of total Aβ (26.45 ± 2.35 pg ml⁻¹ of Aβ40 trimers—i.e., ~2.3% of total Aβ40, and 3.50 ± 0.45 pg ml⁻¹ of Aβ42 trimers—i.e., ~3.5% of total Aβ42) were measured. Aβ tetramers representing ~0.25% of total Aβ were also detected (2.35 ± 0.55 pg ml⁻¹ of Aβ40 tetramers—i.e., ~0.2% of total Aβ40, and 0.55 ± 0.16 pg ml⁻¹ of Aβ42 tetramers—i.e., ~0.5% of total Aβ42, close to the limit of quantification (0.35 pg ml⁻¹) of the ESI-IM-MS method used) (Fig. 1e–f). No other forms of soluble Aβ assemblies were evidenced in the culture medium of prion-infected cells, keeping in mind the lower sensitivity of the ESI-IM-MS approach to detect β-amyloid multimers with oligomeric orders higher than the tetramer (0.20–0.29 pg ml⁻¹ for orders 5–8 vs. 0.09–0.16 pg ml⁻¹ for orders 1–4, see Methods). In Fk-1C115-HT cell lysates, the vast majority of Aβ40 and Aβ42
displayed a monomeric structure with Aβ40 and Aβ42 trimers and tetramers representing less than 0.20% (42 ± 27 pg mg⁻¹ protein) over total Aβ species (24,405 ± 3800 pg mg⁻¹ protein). In Fk-1C115-HT cells BX912-mediated PDK1 inhibition, that lowered Aβ production (Fig. 1a–b), promoted a half to two-third reduction of Aβ40/42 trimers and rendered tetramers no longer detectable in the culture medium (Fig. 1e–f).
Because an increase of Aβ40/42 in the surrounding milieu of prion-infected neuronal cells might also mirror some dysregulation of Aβ degradation, we measured the Aβ40/42 clearance ratio (i.e., the production over degradation rates of both Aβ40 and Aβ42 peptides) in Fk-infected 1C115-HT cells. To this purpose, prion-infected neuronal cells were incubated with 3H-L-leucine for 6 h and the cell supernatants were collected over a 12 h time period. Aβ40 and Aβ42 were quantified through stable-isotope labeling tandem mass spectrometry29,30. We found that the Aβ40 and Aβ42 clearance ratios were increased by ∼1.5-fold in Fk-infected cells vs. uninfected ones (Fig. 1a–h), accounting for the accumulation of Aβ peptides in the culture medium of prion-infected cells (Fig. 1a–b). Exposure of Fk-1C115-HT neuronal cells to the PDK1 inhibitor BX912 (1 µM, 6 h) significantly reduced the impact of prion infection on the Aβ40 and Aβ42 clearance ratios (Fig. 1a–h).

These in vitro data thus show that PDK1 overactivation induced by prion infection provokes a deficit of the TACE APP α-processing and engages APP towards the β-secretase pathway, favoring the accumulation of Aβ40/42 monomers and multimers.

Similar infectivity of Aβ-free or Aβ-containing PrPSc inocula. We next wondered whether Aβ produced by prion-infected cells would influence prion infectivity. To address this issue, we built an Aβ-free cell system infected by prions exploiting 1C11 neuronal stem cells. Uninfected 1C11 cells were first silenced for the expression of APP using a stable siRNA-based strategy31. 1C11 cells stably repressed for APP expression (referred to as APPnull-1C11 cells) were then chronically infected by the Fukuoka-1 prion strain (referred to as APPnull-Fk-1C11 cells). We checked in APPnull-Fk-1C11 cells that Aβ40 and Aβ42 monomers, trimers, and tetramers were no longer detectable in both the culture medium and cell lysates as compared to Fk-1C11 cells by LC-MS/MS (Fig. 2a) and ESI-IM-MS (Fig. 2b) analyses.

![Fig. 2](image_url) APP silencing does not affect PrPSc replication in 1C11 cells. a Levels of Aβ40 and Aβ42 quantified by LC-MS/MS in the culture medium and lysates of Fukuoka-infected 1C11 cells expressing (Fk-1C11) or not (APPnull-Fk-1C11) the amyloid precursor protein APP. b ESI-IM-MS detection and quantification of Aβ40 and Aβ42 trimers and tetramers in the culture medium of Fk-1C11 and APPnull-Fk-1C11 cells. c ELISA-based quantification of proteinase K-resistant PrPSc in Fk-1C11 and APPnull-Fk-1C11 cells. Values are means ± sem of six independent experiments. n.d. not detected. Data were analyzed using the two-tail Student t-test. ** denotes p < 0.01. Source data are provided as a Source Data file.

Important, ELISA-based quantifications of proteinase K-resistant PrPSc did not significantly differ between Fk-1C11 and APPnull-Fk-1C11 cells (Fig. 2c).

We next injected into C57Bl/6J mice via the intracerebral route cell-based inocula either uninfected (1 × 10⁵ 1C11 cells) or PrPSc-infected Aβ-free (1 × 10⁵ APPnull-Fk-1C11 cells) or containing Aβ (1 × 10⁵ Fk-1C11 cells). Mice inoculated with control 1C11 cells (SHAM) remained healthy over more than 250 days post inoculation, while mice inoculated with Fk-1C11 or APPnull-Fk-1C11 cells both died at comparable times (168.1 ± 3.2 days and 171.8 ± 3.5 days, respectively, n = 10, Fig. 3a). Moreover, in mice injected with PrPSc inocula Aβ-free or not, the mean static rotord score dropped below 10 at 160 dpi, indicating similar impairments in motor coordination (Fig. 3b). Accordingly, at the end stage of prion disease highly comparable amounts of proteinase K-resistant PrPSc were measured in the brain of mice inoculated with either Fk-1C11 or APPnull-Fk-1C11 cells (Fig. 3c).

Finally, we showed that chronic intraperitoneal injection of the PDK1 inhibitor BX912 (5 mg per kg body weight per day; 0.25 µl h⁻¹) starting at 130 days post infection delayed mortality (Fig. 3a), reduced the motor deficits associated with prion infection (Fig. 3b) and decreased the PrPSc level (Fig. 3c) with the same magnitude in mice infected with PrPSc inocula Aβ-free or not. This indicates that the deregulation of PDK1 activity induced by prion infection is not influenced by Aβ species co-transmitted with PrPSc.

As a whole, these in vitro and in vivo data provide evidence that prion replication and infectivity are insensitive to the presence of Aβ in the inocula.

PrPSc increases CSF Aβ independently from Aβ co-transmission. We further exploited PrPSc inocula Aβ-free or not to address whether Aβ would fuel its own production when co-inoculated with PrPSc in mice. To this end, we measured at

Fig. 2 APP silencing does not affect PrPSc replication in 1C11 cells. a Levels of Aβ40 and Aβ42 quantified by LC-MS/MS in the culture medium and lysates of Fukuoka-infected 1C11 cells expressing (Fk-1C11) or not (APPnull-Fk-1C11) the amyloid precursor protein APP. b ESI-IM-MS detection and quantification of Aβ40 and Aβ42 trimers and tetramers in the culture medium of Fk-1C11 and APPnull-Fk-1C11 cells. c ELISA-based quantification of proteinase K-resistant PrPSc in Fk-1C11 and APPnull-Fk-1C11 cells. Values are means ± sem of six independent experiments. n.d. not detected. Data were analyzed using the two-tail Student t-test. ** denotes p < 0.01. Source data are provided as a Source Data file.
inoculated with uninfected 1C11 cells. Source data are provided as a Source Data file.

160 dpi, when prion-infected mice start developing clinical signs, products of APP β-processing in the CSF of C57Bl/6 J mice inoculated intracerebrally with either Fk-1C11 or APPnull-Fk-1C11 cells.

As compared to mice injected with uninfected 1C11 cells, LC-MS/MS analyses showed highly similar increases in the levels of Aβ40, Aβ42 and sAPPβ in the CSF of mice inoculated with either Fk-1C11 cells (1.6-fold for Aβ40, 1.8-fold for Aβ42, and 2-fold for sAPPβ) or APPnull-Fk-1C11 cells (1.6-fold for Aβ40, 1.7-fold for Aβ42, and 2-fold for sAPPβ) (Fig. 4a–c). Since no change in prion infectivity was evidenced between PrPSc inocula Aβ-free or not (Fig. 3a–c), we concluded that prion infection is sufficient to promote CSF accumulation of Aβ and sAPPβ, independently from the presence of any Aβ species in the inocula.

In the 160 dpi CSF of mice inoculated with Fk-1C11 cells, ESI-IM-MS analyses revealed the presence of Aβ40 and Aβ42 trimers (150.0 ± 3.7 and 33.0 ± 3.4 pg ml⁻¹, respectively) and tetromers (11.2 ± 2.2 and 11.5 ± 1.3 pg ml⁻¹, respectively) (Fig. 4e–f). Strikingly, in the 160 dpi CSF of mice inoculated with APPnull-Fk-1C11 cells, Aβ40 and Aβ42 tetromers (17.4 ± 5.3 and 23.8 ± 1.2 pg ml⁻¹, respectively) predominated, while Aβ40 and Aβ42 trimers (14.4 ± 2.2 and 2.8 ± 1.6 pg ml⁻¹, respectively) were ∼10 times less abundant than in the CSF of mice inoculated with Fk-1C11 cells (Fig. 4e–f). Since Aβ trimers and tetromers were detected only in the CSF of mice infected with PrPSc inocula Aβ-free or not, we concluded that prion infection is sufficient to promote the formation of Aβ multimers in vivo.

As for prion-infected 1C11⁵-HT cells, we recorded the PDK1-dependent imbalance of Aβ clearance ratios in the brain of mice inoculated with Fk-1C11 cells (Fig. 4g–h). Infection of mice with Fk-1C11 cells promoted a 1.8-fold and 1.5-fold increase of the CSF Aβ40 and Aβ42 production over degradation ratios, respectively, which were reversed upon chronic intraperitoneal injection of the PDK1 inhibitor BX912 (Fig. 4g–h). This indicates that in vivo PDK1 overactivation induced by prion infection directs APP towards the β-cleavage pathway. Accordingly, as compared to untreated infected mice, mice inoculated with either Fk-1C11 or APPnull-Fk-1C11 cells and infused with the PDK1 inhibitor exhibited at 160 dpi similar reductions in CSF Aβ40/42 and sAPPβ (Fig. 4a–c) and increases in CSF sAPPα (Fig. 4d) levels. In agreement with the BX912-mediated decreases in Aβ40/42, PDK1 inhibition in prion-infected mice also decreased CSF amounts of both Aβ40/42 trimers and tetrmers (Fig. 4e–f).

These in vivo data thus show that the rise in mouse CSF Aβ40/42 and sAPPβ levels upon inoculation of APP expressing- or APPnull-Fk-1C11 cells depends on PrPSc transmission only. The accumulation of Aβ40/42 monomers and the formation of Aβ40/42 multimers within a prion-infectious context originate from PrPSc-induced PDK1 overactivation. However, since the proportion of Aβ40/42 trimers and tetrmers varies in the CSF of prion-infected mice depending on the presence or absence of Aβ species in PrPSc inocula, this suggests that, in prion diseases, co-transmitted Aβ would influence the equilibrium between Aβ multimers generated upon prion infection.

Aβ trimers boost the PrPSc-induced Aβ multimers production. Among Aβ multimers, Aβ trimers self-propagate, display strong aggregative properties, and emerge as the elementary building blocks for the formation of supra-ordered Aβ multimers. To assess whether Aβ trimers present in PrPSc inocula would affect the production of Aβ40/42 multimers by prion-infected neurons, serotonic 1C11⁵-HT neuronal cells were infected with PrPSc homogenates prepared from 1 × 10⁶ Fk-1C11 or APPnull-Fk-1C11 cells supplemented or not with a mixture of synthetic human Aβ40 (25 pg ml⁻¹) and Aβ42 (2.5 pg ml⁻¹) trimers. The
Fig. 4 Deregulation of the PDK1-TACE axis in prion-infected C57Bl/6 J mice causes CSF accumulation of Aβ monomers and multimers. Concentrations at 160 dpi of Aβ40 (a), Aβ42 (b), sAPPβ (c), sAPPα (d), and multimers of Aβ40 (e) and Aβ42 (f) in the CSF of C57Bl/6 J mice inoculated with uninfected 1C11, Fk-1C11 or APPnull-Fk-1C11 cells and infused or not with the PDK1 inhibitor BX912 (n = 6–10 depending on the group) deduced from LC-MS/MS (a–d) and ESI-IM-MS (e–f) analyses. g–h Aβ40 and Aβ42 clearance ratios measured in the CSF of mice inoculated with uninfected 1C11 or Fk-1C11 cells and infused or not with BX912 (n = 5). Values are means ± sem. n.d. not detected. Data in Fig. 4a–d were analyzed using the two way ANOVA test with Bonferroni post-test correction. Data in Fig. 4e–h were analyzed using the two-tail Student t test. * denotes p < 0.05, and ** p < 0.01. Source data are provided as a Source Data file.
concentrations of synthetic Aβ trimers added to PrPSc homogenates were chosen by similarity to the Aβ trimers measured in the supernatants of chronically infected Fk-1C11-5-HT cells (Fig. 1e–f). 1C11-5-HT cells were also exposed to uninfected 1 × 10^5 1C11 cell-derived homogenates supplemented or not with Aβ40/42 trimers in order to delineate the impact of Aβ trimers on basal neuronal Aβ production, independently from prion infection.

ELISA-based measurements of PrPSc levels revealed that human Aβ40/42 trimers did not promote nor amplify PrPSc conversion into PrPSc. Proteinase K-resistant PrPSc was not detected in 1C11-5-HT cells exposed for 10 days to uninfected 1C11 cell homogenates supplemented with Aβ trimers (Fig. 5a) and the amount of PrPSc in 1C11-5-HT cells infected with Fk-1C11 or APPnull-Fk-1C11 cell homogenates supplemented or not with Aβ trimers remained unchanged (Fig. 5a).

As concerns LC-MS/MS quantifications, addition of human Aβ trimers to uninfected 1C11 cell homogenates did not change the basal productions of Aβ40 (Fig. 5b) and Aβ42 (Fig. 5c) monomers by 1C11-5-HT neuronal cells, indicating that Aβ trimers are unable to induce the production of Aβ40/42 monomers within a non-infectious context. Prion infection of 1C11-5-HT cells with Fk-1C11 or APPnull-Fk-1C11 cell homogenates enriched or not with Aβ trimers triggered equivalent rises in Aβ40 (6-fold) and Aβ42 (2.5-fold) cell supernatant levels (Fig. 5b–c), showing that Aβ trimers present in the inocula do not interfere with the prion-stimulated production of Aβ40/42 monomers by neuronal cells.

As expected, Aβ40/42 trimers were detected by ESI-IM-MS in the supernatant of 1C11-5-HT cells exposed to uninfected 1C11 cell homogenates supplemented with Aβ trimers (Fig. 5d–e). We however found lower concentrations of Aβ trimers (7.1 ± 0.3 and 1.6 ± 0.2 pg ml^-1 for Aβ40 and Aβ42, respectively) than those introduced with the inocula (25 pg ml^-1 for Aβ40 and 2.5 pg ml^-1 for Aβ42), suggesting a partial degradation of human Aβ40 and Aβ42 trimers after 10 days incubation with 1C11-5-HT neuronal cells. Infection of 1C11-5-HT cells with PrPSc homogenates derived from either Fk-1C11 or APPnull-Fk-1C11 cells promoted comparable accumulations of Aβ40 (25.4 ± 3.7 pg ml^-1 for Fk-1C11 vs. 22.5 ± 3.3 pg ml^-1 for APPnull-Fk-1C11) and Aβ42 (3.5 ± 0.2 pg ml^-1 for Fk-1C11 vs. 2.8 ± 0.2 pg ml^-1 for APPnull-Fk-1C11) multimers in the surrounding milieu of prion-infected 1C11-5-HT cells (Fig. 5d–e). This was ~50% amplified upon supplementation of PrPSc inocula with human Aβ trimers (73.4 ± 10.5 and 7.0 ± 0.8 pg ml^-1 for Aβ40 and Aβ42 multimers, respectively) (Fig. 5d–e) and exceeded the sum of the Aβ multimers concentrations produced upon prion infection plus Aβ trimers introduced in PrPSc homogenates.

Again these in vitro data support the view that prion infection of neurons drives the production of Aβ40/42 monomers and multimers independently from the presence of Aβ in PrPSc inocula. Although exogenous Aβ trimers have no impact on the production of Aβ40/42 monomers, Aβ trimers, likely acting as Aβ seeds, can boost the formation of Aβ multimers within a prion-infectious context.

Aβ seeds transmitted with PrPSc promote brain Aβ deposition. The next question was to probe whether Aβ monomers and multimers generated upon prion infection would deposit and...
form Aβ plaques in the brain of prion-infected mice. As the presence of Aβ trimers in PrPSc inocula influences the amount of Aβ multimers generated upon prion infection (Fig. 4e–f, Fig. 5d–e), we also assessed whether Aβ deposition would quantitatively vary according to the load of seeds of Aβ trimers co-transmitted with PrPSc by exploiting PrPSc inocula derived from Fk-1C11 and APPnull–1C11 cells supplemented or not with synthetic human Aβ trimers.

Because C57Bl/6J mice do not deposit Aβ with age or when infused with Aβ-containing brain extracts,36 we used APP23 transgenic mice17-39 as an experimental paradigm to probe the amyloidogenic properties of PrPSc inocula. Three-month-old female APP23 transgenic mice were thus intracerebrally injected with PrPSc inocula containing murine Aβ (2.5 × 10^5 Fk-1C11 cells) or not (2.5 × 10^5 APPnull–Fk-1C11 cells) enriched or not with synthetic human Aβ trimers (25 pg ml⁻¹ Aβ40 plus 2.5 pg ml⁻¹ Aβ42, n = 10 for each group). The capacity of synthetic Aβ trimers to promote Aβ deposition independently from prion infection was also challenged in APP23 mice injected with homogenates of 2.5 × 10^5 murine 1C11 cells supplemented with human Aβ trimers. Amyloid deposition in the mouse brain was followed by positron emission tomography (PET) imaging after [¹¹C]-Pittsburgh compound B (PiB) injection23,40 from one to eight months post inoculation (mpi).

Mice injected with Fk-1C11 cells-derived inocula started depositing Aβ at 3 mpi and the number of mice with amyloid deposition progressively increased till 7 mpi (Fig. 6a, open triangles; Supplementary Table 1). Mice injected with APPnull–Fk-1C11 cells-derived inocula were not positive for Aβ deposits till 6 mpi (Fig. 6a, open squares; Supplementary Table 1). This suggests that PrPSc does not display β-amyloidosis activity and that deposition of Aβ in APP23 mice injected with Fk-1C11 cells-derived inocula has been promoted by Aβ seeds co-transmitted with PrPSc. Accordingly, we showed that (i) supplementation of APPnull–Fk-1C11 cells-derived inocula with synthetic human Aβ trimers induced brain Aβ deposition as soon as 4 mpi (Fig. 6a, close squares; Supplementary Table 1), and (ii) enrichment of Fk-1C11 cells-derived inocula with synthetic human Aβ trimers accelerated Aβ deposition (start at 2 mpi) and enhanced the number of mice with amyloid deposition (Fig. 6a, close triangles; Supplementary Table 1). This supports the view that the Aβ trimers of murine or human origin present in PrPSc inocula play the role of Aβ seeds responsible for amyloid deposition in prion-infected mice. None of the mice injected with homogenates of non-infectious 1C11 cells supplemented with synthetic human Aβ trimers deposited Aβ within a 8 month time-frame (Supplementary Table 1), indicating that, independently from prion infection, Aβ trimers fail to promote Aβ deposition.

We further provided evidence that the amount of brain Aβ deposits varied according to the load of Aβ trimers in PrPSc inocula: robust depositions of Aβ occurred in APP23 mice injected with Fk-1C11 cells-derived inocula enriched with synthetic human Aβ trimers (Fig. 6a, close triangles), while lower Aβ deposits were recorded in mice inoculated with homogenates containing low levels of Aβ multimers, i.e., either Fk-1C11 cells-derived homogenates (Fig. 6a, open triangles) or APPnull–Fk-1C11–derived homogenates supplemented with synthetic human Aβ trimers (Fig. 6a, close squares). Corroborating the quantitative PET results, post-mortem histological analyses revealed that Aβ deposition in the brain of APP23 mice injected with Fk-1C11 cells-derived inocula supplemented with synthetic Aβ trimers was more substantial than in the brain of prion-infected APP23 mice that did not receive Aβ trimers (Supplementary Fig. 2A). We further showed that brain Aβ deposition in prion-infected APP23 mice injected or not with synthetic human Aβ trimers occurred mainly in the cortex, and to a lesser extent in the thalamus, the hippocampus, the striatum, and the cerebellum (Supplementary Fig. 2B). This likely reflects the cortico-tropism of the Fukuoka-1 strain41 and thereby the strongest incidence of prion infection on the production and deposition of Aβ in this brain area.

Importantly, we found that the survival of prion-infected APP23 mice depended on the amount of Aβ seeds co-transmitted with PrPSc. While APP23 mice infected with Fk-1C11-derived inocula (low level of Aβ seeds) died at >217 dpi (Fig. 6a, open triangles; Supplementary Table 1), APP23 mice infected with Fk-1C11-derived inocula supplemented with synthetic Aβ trimers (high level of Aβ seeds) displayed a reduced survival time (180.3 ± 0.4 dpi) (Fig. 6a, close triangles; Supplementary Table 1). Enrichment of APPnull–Fk-1C11 inocula with Aβ trimers provoked the death of two mice out of ten (Fig. 6a, close squares; Supplementary Table 1), while none of the mice injected with prion inocula free of Aβ seeds died before they were sacrificed at 8 mpi (Fig. 6a, open squares; Supplementary Table 1). This suggests that brain Aβ deposition induced by Aβ trimers present in the inocula tends to accelerate the death of prion-infected mice. Since brain levels of PrPSc were not statistically different between each prion-infected mice group at the end stage of the disease or when mice were sacrificed at 8 mpi (Fig. 6b), we concluded that the death of prion-infected APP23 mice does not relate to an over-accumulation of PrPSc but is rather influenced by the induced Aβ pathology, whose severity depends on the dose of transmitted Aβ seeds.

These data thus indicate that prion infection generates conditions that permit brain Aβ deposition catalyzed by seeds of Aβ trimers co-transmitted with PrPSc. We showed that Aβ deposition induced by Aβ seeds in prion-infected APP23 mice appears to depend on PDK1, whose overactivation was strictly related to prion infection, but not to Aβ trimers (Fig. 6c). Infusion of the PDK1 inhibitor BX912 (5 mg per kg body weight per day; 0.25 µl h⁻¹, starting at 1 mpi) prevented Aβ deposition in APP23 mice injected with Fk-1C11 cells inocula (Fig. 6a, open circles; Supplementary Table 2) or delayed Aβ deposition in mice inoculated with Fk-1C11 cells- or APPnull–Fk-1C11 cells-derived homogenates supplemented with synthetic human Aβ trimers (Fig. 6a, close circles and close diamonds; Supplementary Table 2). BX912 infusion also significantly reduced PrPSc levels (Fig. 6b), and thereby increased the survival time of all APP23 mice groups infected with prions (Fig. 6a, Supplementary Table 2). We thus concluded that the PDK1-dependent deregulation of APP α-processing upon prion infection fuels the production of Aβ40/42 to a certain threshold that allows cerebral β-amyloidosis induced by the transmitted exogenous Aβ seeds.

Discussion

Our findings indicate that PrPSc-induced PDK1 overactivity promotes the production of Aβ40 and Aβ42 monomers and multimers both in vitro and in vivo. Whatever their structure, the Aβ peptides generated upon prion infection do not impact on PrPSc replication nor take part to prion infectivity. We monitor that the Aβ peptides generated upon prion infection deposit in mouse brain only when PrPSc and seeds of Aβ are co-inoculated to mice. Importantly, prion infection combined to brain Aβ plaque deposition accelerates the death of prion-infected mice.

Corruption of PrPSc signaling function by PrPSc is at the root of PDK1 overactivity via post-translational mechanisms. By stimulating the Src kinase-P13K pathway, prion infection contributes to a rise in PDK1 activity23. This cascade promotes PDK1 translocation from the cytoplasm to the plasma membrane, docking PDK1 to PiP3 and inducing Src-mediated PDK1 phosphorylation at Tyr9 and Thr376 (ref. 42). This gain of PDK1 activity within a
prion-infectious context also depends on the upstream over-
activation of the ROCK kinase that interacts with PDK1 and
phosphorylates PDK1 at Ser160 (ref. 24). In prion-infected neu-
rons, over-activated PDK1 then provokes the internalization of
α-secretase TACE in caveolin-1-enriched vesicles, reducing
the TACE neuroprotective activity23,24. Internalization of TACE
decreases its cleavage activity towards TNFα receptors (that
accumulate at the plasma membrane and render diseased neurons
highly sensitive to TNFα inflammation) but also towards PrPSc
that amplifies the production of PrPSc (ref. 23). Our data provide
evidence that PDK1-induced TACE internalization in prion-
infected neurons or mice also attenuates the
α-cleavage of APP into the neuroprotective sAPPα fragment and favors the APP β-
processing into the neurotoxic Aβ40 and Aβ42 peptides. The
pharmacological inhibition of PDK1 is sufficient to rescue sAPPα
production to pre-infectious levels and to counteract Aβ40/42

Fig. 6 Aβ seeds co-transmitted with PrPSc provoke brain Aβ deposition and decrease the survival of APP23 mice. a Time-dependent 11C-PIB uptake (% of the Injection Dose g⁻¹, upper panel) and survival curves (lower panel) of transgenic APP23 mice inoculated via the intracerebral route (i.c.) with 2.5 × 10⁵ Fk-1C11 or APPnull-Fk-1C11 cells-derived homogenates supplemented or not with synthetic Aβ trimers, and infused or not with the PDK1 inhibitor BX912 (n = 10 per group). mpi months post inoculation, dpi days post inoculation. Each number refers to one mouse in each group. b, c ELISA-based quantification of Proteinase K-resistant PrPSc amount (b) and PDK1 activity (c) in the brain of APP23 mice treated as in a at the end stage of prion disease or when sacrificed at 260 dpi. Values are means ± sem. Data in Fig. 6b–c were analyzed using the two way ANOVA test with Bonferroni post-test correction. *denotes p < 0.05, **p < 0.01, and ***p < 0.001 vs. APP23 mice inoculated with non-infectious 1C11 cells-derived homogenates supplemented or not with Aβ trimers. Source data are provided as a Source Data file.
accumulation. By showing a deficit of TACE-mediated APP α-processing within a prion-infectious context, our results extend the list of TACE substrates (whose products normally exert protective roles), which are no longer cleaved in prion-infected neurons. This may also concern the neurotrophin p75 receptor44, for which a TACE-mediated shedding defect likely contributes to neuronal damages induced by the prion peptide 106–126 (ref. 44).

Beyond an enhanced production of Aβ trimers, our data show that prion infection promotes the formation of Aβ40 and Aβ42 trimers and tetramers. In the cell culture medium of prion-infected neurons or in the CSF of prion-infected C57Bl/6J mice Aβ multimers represent ~2.75% of Aβ monomers and depend on the upstream production of Aβ monomers. The rescue of APP α-processing upon PDK1 inhibition and the subsequent down-regulation of APP β-processing reduce the level of Aβ multimers, reflecting a dynamic association equilibrium between Aβ species as observed in primary cultures of neurons from transgenic mice with Alzheimer-like pathology45.

We found that Aβ peptides produced by prion-infected cells have no impact on prion replication and infectivity. This conclusion arose from our strategy, which consisted in silencing APP expression in 1C11 neuronal cells prior to prion infection. APPnull-1C11 cells no longer produce the Aβ40/42 peptides, but remain permissive to prion infection by the Fukuoka-1 strain. Importantly, the PrPSc level does not vary significantly between Fk-1C11 and APPnull-Fk-1C11 cells, clearly indicating that the Aβ produced upon prion infection do not contribute to PrPSc conversion into PrPSc. According to the concept that PrPSc transconformational changes depend on accessory proteins, i.e., protein(s) X46–48, the invariance of PrPSc levels between prion-infected APPnull- and APP expressing 1C11 cells supports the view that neither APP nor its cleavage products are one of those proteins X facilitating PrPSc replication. With the help of Fk-infected APPnull-1C11 cells, we further established that the infectivities of prion inocula do not depend on the presence of Aβ. Intracerebral injections of 1C11 cells-derived PrPSc inocula Aβ-free or not in C57Bl/6J mice indeed cause similar motor impairments, similar kinetics of a prion disease onset, and highly comparable accumulation of PrPSc in the brain. This latter point contrasts with the observation by Sarell et al. that synthetic Aβ40/42 trimers die before those injected with PrPSc only. As brain PrPSc levels are highly comparable between these two mice groups, an early death likely relates to the accumulation of Aβ multimers and deposition of Aβ in the brain of prion-infected animals. Severe cerebrovascular amyloid deposition, also called cerebral amyloid angiopathy (CAA), that is associated with marked Aβ deposition in capillaries, small arteries and arterioles, and vasculopathies, leads to hemorrhages and ischemic brain lesions (for review, see ref. 56, and references therein). The accelerated death of prion-infected mice with Aβ deposition might be due to the occurrence of mixed pathologies, that is prion disease and CAA11. In any case, inhibiting PDK1 counteracts the toxicity of both prion and transmitted Aβ seeds as BX912 infusion in prion-infected APP23 mice not only reduces the load of PrPSc, but also the brain deposition of Aβ, which thus reinforces the critical role of PDK1 in amyloid-based neurodegenerative diseases23,24,57,58.

Methods

Chemicals. Dibutyryl cyclic AMP (dbcAMP), cyclohexane carboxylic acid (CCA) were purchased from Sigma-Aldrich (St. Louis, MO, USA). The PDK1 inhibitor BX912 was from Axon MedChem BV (Groningen, The Netherlands). The TACE inhibitor, TNF-α processing inhibitor-2 (TAPI-2), was purchased from Peptides International (Louisville, KY, USA). The BACE1 inhibitor MK-8931 (Verubecestat) was from Abcam (Cambridge, UK).

Preparation of synthetic human Aβ40/42 trimers. Human Aβ40 and Aβ42 trimers were synthesized and purified in the Roche AG Chemistry Department by site-exclusion chromatography, reversed phase high-performance liquid chromatography, and cross-linking44.

Cell culture, APP silencing, and prion infection. 1C11 precursor cells were established in the laboratory after stable transfection of mouse embryonal carcinoma F9 cells59 with the recombinant pK4 plasmid60,61. 1C11 cells were grown in DMEM supplemented with 10% fetal calf serum and induced to differentiate along the serotonergic (1C115-HT) pathway25. Briefly, on addition of dbcAMP (1 mM) and CCA (0.05%), almost 100% of 1C11 cells acquire within 4 days a complete serotonergic (1C115-HT) differentiation. To constitutively repress APP in 1C11 precursor cells, an shRNA coding sequence encompassing codons 498–511 was introduced in the pTer plasmid62 between the bglII and HindIII sites in the luciferase gene. The shRNA was cloned upstream of the cytomegalovirus (CMV) promoter and brought flanked with oligonucleotides 5'-GGdAdG-3' and 5'-UUGGCCAAGACAUCGUC-GGdAdG-3' (ref. 62), and inserted into the pTer vector. The correct expression of APP shRNA was confirmed by Western blot analysis (data not shown) and by real-time PCR. As shown in Fig. 5A, the APP expression in 1C11 cells transfected with 2 μg of pTer-shRNA/APP plasmid31 and clones silenced for APP expression were selected according to a reduction of APP level of more than 95% (referrerd to as APPnull-1C11 cells). 1C11, Aβ trimers deposit Aβ, while none of the mice only injected with the same dose of Aβ trimers display Aβ deposition, indicating that PrPSc and seeds of Aβ trimers cooperate for amyloid deposition. It is likely that PrPSc-stimulated production of Aβ monomers and multimers to a certain threshold generates favorable conditions for Aβ deposition induced by exogenous seeds of Aβ trimers. We also show that the amount of Aβ seeds co-injected with PrPSc determines the intensity of Aβ deposition: higher is the quantity of Aβ trimers in PrPSc inocula, higher and/or faster is Aβ deposition. This may reside in the capacity of seeds of Aβ trimers to amplify the PrPSc-dependent production of seedable Aβ multimers, to self-replicate and to aggregate. Our overall data would thus explain, at least in part, why Aβ deposition is not systematically associated with CJD cases of different etiologies in several age-matched patient cohort studies51. They would also account for the presence of Aβ plaques and Aβ pathology in the brain of patients who developed a tauopathy, Creutzfeldt-Jakob disease after injection of human growth hormone (hGH) contaminated with pathogenic prions62, or after neurosurgical grafting of dura mater13. Demonstration has recently been made that PrPSc-contaminated hGH52,53 as well as engrafted dura mater13,54,55 were positive for Aβ seeds.

Most importantly, APP23 mice co-inoculated with PrPSc and synthetic Aβ trimers die before those injected with PrPSc only. As brain PrPSc levels are highly comparable between these two mice groups, an early death likely relates to the accumulation of Aβ multimers and deposition of Aβ in the brain of prion-infected animals. Severe cerebrovascular amyloid deposition, also called cerebral amyloid angiopathy (CAA), that is associated with marked Aβ deposition in capillaries, small arteries and arterioles, and vasculopathies, leads to hemorrhages and ischemic brain lesions (for review, see ref. 56, and references therein). The accelerated death of prion-infected mice with Aβ deposition might be due to the occurrence of mixed pathologies, that is prion disease and CAA11. In any case, inhibiting PDK1 counteracts the toxicity of both prion and transmitted Aβ seeds as BX912 infusion in prion-infected APP23 mice not only reduces the load of PrPSc, but also the brain deposition of Aβ, which thus reinforces the critical role of PDK1 in amyloid-based neurodegenerative diseases23,24,57,58.
APP<sup>1–111</sup>, or 1C11<sup>5–HT</sup>, cells were chronically infected by the mouse-adapted Fukuoka (Fk) prion strain<sup>49</sup>.

**Cell extract preparation.** Cells were washed in PBS/Ca<sup>2+</sup>/Mg<sup>2+</sup> and incubated for 30 min at 4 °C in lysis buffer (50 mM Tris-HCl pH 7.4, 150 mM NaCl, 5 mM EDTA, 1% Triton X-100, protease and phosphatase inhibitors (Roche)). After centrifugation of the lysate (14,000 × g, 15 min), the protein concentration in the supernatant was measured with the bicinchoninic acid method (Pierce, Rockford, IL, USA).

sAPP<sub>a</sub>, sAPP<sub>P</sub>, APP<sub>40</sub>, and APP<sub>42</sub> quantification. The detection and quantification of different APP cleavage products (sAPP<sub>a</sub>, sAPP<sub>P</sub>, APP<sub>40</sub>, and APP<sub>42</sub>) in the culture medium and lysates of prion-infected cells as well as in the CSF of prion-infected mice were achieved by a stable-isotope dilution methodology in combination with LC-MS/MS<sup>48</sup>.

Detection and quantification of Aβ multimers were performed by ESI-IM-MS<sup>38</sup>. Approximately 50 µl of cell culture supernatant or CSF were added to 950 µl of methanol and acetic acid (1%) solution maintained at 37 °C for 30 min and then centrifuged for 1 h at 1500 × g. The supernatant was subjected to analysis by LC-MS/MS without further sample preparation. Electrospray Ionization Mass Spectrometry (ESI-MS) coupled with Traveling Wave Ion Mobility Spectrometry (TWIMS) experiments were performed with a Synapt G2 HDMS mass spectrometer (Waters) equipped with a Z-spray ESI source. Capillary voltage of 2.0 kV, sampling cone voltage of 50 V, extraction cone voltage of 4.0 V, source temperature of 100 °C, desolvation temperature of 200 °C, trap and transfer collision energy of 10 eV were used as parameters for ESI. Although no signal was detected after 300 ms, data were acquired over the m/z range of 500–2000 for 2 min. Helium flow rate of 30 ml min<sup>−1</sup> and nitrogen flow rate of 25 ml min<sup>−1</sup> were used for TWIMS experiments, with 250 m s<sup>−1</sup> for wave velocity and 8.0 V for wave height. Calibration of the experimental arrival time to determine collision cross section (Ω<sub>i</sub>) values was performed following previously reported methods<sup>48</sup> using ubiquitin and cytochrome c as calibrants. The raw data were processed using Mass Lynx v4.1 software (Waters). The reported data are the average of three independent experiments. The ESI-IM-MS data were assigned to specific Aβ oligomers on the following basis: (i) First, ion mobility is often described as proportional to collision cross-section-to-charge ratio (Ω<sub>z</sub>/m). Any charge state (z) in the mass spectrum could correspond to several species (e.g., M + z, D + 2z, Tr + 3z, Te + 4z; with M = monomers, D = dimers, Tr = trimers, and Te = tetramers). These species have the same m/z value and are therefore indistinguishable in the mass spectrum. They can be distinguished by IM because their Ω<sub>z</sub> differ. For example, the Te charge would be theoretically four times that of M; however, the Ω<sub>z</sub>Te would be smaller than four times the Ω<sub>z</sub>M because of the favorable interactions between the monomers and higher order species. Thus Ω<sub>z</sub>Te < 4 × Ω<sub>z</sub>Tr/3 < Ω<sub>z</sub>2Tr < 12M/Ω<sub>z</sub>, i.e., the species that have smallest drift times were initially assigned to those corresponding to the largest oligomer. For the APP<sub>40</sub> spectra at m/z 2165, four contributions were detected in the drift time domain of 4.1, 5.1, 7.7, and 12.8 ms. These peaks were initially assigned to the highest and lowest oligomer order, respectively. (ii) Second, we assigned a given oligomer order on the basis of the 13C isotope distribution associated with each of the peaks separated in the mobility dimension<sup>49</sup>. Applying this consideration to the m/z 2-m/z peak of APP<sub>40</sub> observed at m/z 2165, the 13C isotope distribution associated with the mobility peaks was consistent with charges +2 and +4, respectively, and therefore were assigned to M + 2 and D + 4, respectively. The resolution of the 13C isotope distribution for the mobility peaks observed at 5.1 and 4.1 ms was not sufficient for their assignment. In this case, the charge envelope in the 2D ESI-IM-MS spectrum was assigned a given oligomer order on the basis of the 13C isotope distribution associated with each of the peaks separated in the mobility dimension<sup>49</sup>. Applying this consideration to the m/z 1732 showed two contributions in the drift time domain (4.4 and 7.4 ms), and the 13C isotope distribution associated with these two mobility peaks was consistent with +5 charges, so they were assigned to compact and extended forms of the Aβ<sub>1–42</sub> oligomer. The m/z 1732 APP<sub>40</sub> complexes were observed by ESI-IM-MS as follows: 0.09 pg ml<sup>−1</sup> for APP<sub>40</sub>/APP<sub>42</sub> monomers, 0.10 pg ml<sup>−1</sup> for dimers, 0.12 pg ml<sup>−1</sup> for trimers, 0.16 pg ml<sup>−1</sup> for tetramers, 0.20 pg ml<sup>−1</sup> for pentamers, 0.25 pg ml<sup>−1</sup> for hexamers, 0.28 pg ml<sup>−1</sup> for heptamers and 0.29 pg ml<sup>−1</sup> for octamers.

**PrP<sup>Sc</sup> quantification.** The amounts of proteinase K-resistant PrP<sup>Sc</sup> in Fk-infected 1C11, 1C11<sup>5–HT</sup>, and APP<sup>1–111</sup>, 1C11<sup>5–HT</sup>, cells were chronically infected by the mouse-adapted Fukuoka (Fk) prion strain<sup>49</sup>.

**Ethics statement.** Adult C57BL/6 mice and transgenic APP<sub>P23</sub> mice were bred and underwent experiments in level-3 biological risk containment, respecting European guidelines for the care and ethical use of laboratory animals (Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes). Ten mice per group were inoculated intracerebrally with 20 µl of sample containing 1 x 10<sup>5</sup> cells for 8-week-old male C57Bl/6 mice; 2.5 x 10<sup>3</sup> cells for 3-month-old female APP<sub>P23</sub> mice<sup>69</sup>. Cells were submitted to three freeze-thaw cycles and suspensions were sonicated for 2 min (Cup-horn sonicator; Nanolab Inc, Waltham, MA, USA). CSF was collected from the cisterna magna under anesthesia with 3% isoflurane. All animal procedures were approved by the Animal Care and Use Committee at the University of Fribourg (Switzerland) and by the Comité Régional d’Ethique en Matière d’Exécuter Animalerie de Strasbourg (France) with number CEEA35 ref AL/01/01/13.

**Chronic intraperitoneal injection of BX912 into mice.** Mice were fasted overnight and received saline solution every 12 h before the experiment. They were then anesthetized with isoflurane and intraperitoneal injection was performed. The mice peak onto the peritoneum the polypeptide catheter of an osmotic pump (Alzet, Cupertino, CA, USA). BX912 (PDK1 inhibitor) or vehicle (1% DMSO in sterile normal saline buffer) was administered at a flow rate of 0.25 µl per h, which corresponded to 100 pg per mouse per day (5 mg kg<sup>−1</sup> per day). Pumps were replaced every 4 weeks.

**Behavioral testing.** Motor function in prion-infected mice was assessed by the static rod test<sup>60</sup>.

**Measurement of Aβ production over degradation ratios.** 1C11<sup>5–HT</sup> and their infected counterparts were incubated with 15<sup>3</sup>C<sub>6</sub>-leucine (98% 13<sup>C</sup>) in OptiMEM medium for 6 h. Pulse medium was then replaced by fresh OptiMEM and supernatants were collected over a 12 h time window. APP<sub>40</sub> and than APP<sub>40</sub> were serially immunoprecipitated from the samples using C-terminal specific antibodies (NDBio, 223 from Novus Biologicals and ARIN36343 from Antibodies online, Atlanta, GA, USA). Purified Aβ peptides were then digested with trypsin and 15<sup>C</sup>C<sub>6</sub>-leucine abundance in these trypsic fragments quantified using tandem mass spectrometry<sup>29,40</sup>. For in vivo measurement of Aβ clearance ratios, the same methodology was used except that 15<sup>C</sup>C<sub>6</sub>-leucine was delivered intravenously by a miniosmotic pump model 2002 (Alzet, Cupertino, CA).

**In vivo detection of amyloid deposition.** Amyloid deposition in transgenic APP<sub>P23</sub> mice were detected by PET imaging<sup>40</sup> using a microPET FOCUS F120 scanner (Siemens Medical Solutions, Bern, Switzerland). The Pittsburgh B compound was from Scintomics (Fuerstenfeldbruck, Germany) and used at a concentration of 1.5 mmol l<sup>−1</sup>. The threshold of PET positivity was considered as the limit of quantitation (LOQ) of PIB binding determined by the statistical methodology was used except that 15<sup>C</sup>C<sub>6</sub>-leucine was delivered intravenously by a miniosmotic pump model 2002 (Alzet, Cupertino, CA).

**Measurement of PKD1 activity.** PKD1 activity was measured in cell extracts using a fluorescently labeled PKD1 substrate (SFAM-ARKRERTYSFGHHA-COOH, Caliper Life Sciences, Hanover, MD, USA)<sup>24</sup>. The relative amounts of substrate peptide and product phospho-peptide were determined using a Caliper EZ-reader (Caliper Life Sciences, Hanover, MD, USA).

**Data analysis.** An analysis of variance of the cell/animal response group was performed using the Kaleidagraph software (Synergy Software, Reading, PA, USA) and the GraphPad Prism software (San Diego, CA, USA). Values are given as mean ± s.e.m. Significant responses (P < 0.05) and their corresponding P-values are provided in figure legends. Survival times were analyzed by Kaplan-Meier survival analysis using a log-rank test for curve comparisons.
Reporting summary
Further information on research design is available in the Nature Research Reporting Summary linked to this article.

Data availability
The data that support the findings of this study are available from the corresponding authors upon reasonable request. The source data underlying Figs. 1-6 are provided as a Source Data file.

Received: 5 November 2018 Accepted: 9 July 2019
Published online: 01 August 2019

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Acknowledgements
We thank V. Mutel, G. Zurcher, E. Borroni, Z. Lam, M. Bühler, N. Pierrot, and R. Hockmayer for providing skillful methodological assistance for all data acquisition and statistical analyses. We acknowledge M. Briley for helpful discussions and critical reading of the manuscript. This work was supported by the French Agence Nationale de la Recherche (TargetingPDK1inAD, n°ANR-16-CE16-0021–01), the European Joint Program on Neurodegenerative Diseases and Agence Nationale de la Recherche (PrP® & PDK1, n° ANR-14-JPCD-0003–01), the foundation Vaincre ALZHEIMER (grant n°F-R-15033) and INSERM. JE is funded by Domaine D’Intérêt Majeur - Cerveau& Pensée - Région Ile de France. ZAA is a post-doctoral fellow funded by the ANR PrP®&PDK1 European project and the ANR TargetingPDK1inAD program. VB is funded by the ANR PrP® & PDK1 European project.

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Conceived and designed the experiments: J.M.L., O.K. and B.S. Performed the experiments: J.E., V.B., Z.A.A., F.B.D., A.M.H., Y.B. and J.M.L. Analyzed the data: J.E., V.B., M.P., A.B., O.K., J.M.L. and B.S. Wrote the paper: M.P., A.B., O.K., J.M.L. and B.S.

Additional information
Supplementary Information accompanies this paper at https://doi.org/10.1038/s41467-019-11333-3.

Competing interests: J.M.L. has non-financial competing interests with Hoffmann La Roche Ltd laboratories. He acts as an expert witness for Hoffmann La Roche Ltd laboratories. This does not alter our adherence to all Nature Communications policies on sharing data and materials. The remaining authors declare no competing interests.

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Peer review information: Nature Communications thanks the anonymous reviewer(s) for their contribution to the peer review of this work. Peer reviewer reports are available.

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