Title:

Alzheimer's disease pathogenesis is dependent on neuronal receptor PTPσ

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Note: as there are 2 authors whose initials are YS, the last author is noted as "Y Shen".

Abstract:

β-amyloid accumulation and Tau aggregation are hallmarks of Alzheimer's disease, yet their underlying molecular mechanisms remain obscure, hindering therapeutic advances. Here we report that neuronal receptor PTPσ mediates both β-amyloid and Tau pathogenesis in two mouse models. In the brain, PTPσ binds to β-amyloid precursor protein (APP). Depletion of PTPσ reduces the affinity between APP and β-secretase, diminishing APP proteolytic products by β- and γ-cleavage without affecting other major substrates of the secretases, suggesting a specificity of β-amyloidogenic regulation. In human APP transgenic mice during aging, the progression of β-amyloidosis, Tau aggregation, neuroinflammation, synaptic loss, as well as behavioral deficits, all show unambiguous dependency on the expression of PTPσ. Additionally, the aggregates of endogenous Tau are found in a distribution pattern similar to that of early stage neurofibrillary tangles in Alzheimer brains. Together, these findings unveil a gatekeeping role of PTPσ upstream of the degenerative pathogenesis, indicating a potential for this neuronal receptor as a drug target for Alzheimer's disease.

Main Text:

A definitive pathological hallmark of Alzheimer's disease (AD) is the progressive aggregation of β-amyloid (Aβ) peptides in the brain, a process also known as β-amyloidosis, which is often accompanied by neuroinflammation and formation of neurofibrillary tangles containing Tau, a microtubule binding protein.

Although the etiological mechanisms of AD have been an ongoing debate, concrete evidence from human genetic studies showed that overproduction of Aβ due to gene mutations inevitably inflicts cascades of cytotoxic events, ultimately leading to neurodegeneration and decay of brain functions. Accumulation of Aβ peptides, especially in their soluble forms, is therefore recognized as a key culprit in the development of AD [1]. In the brain, Aβ peptides mainly derive from sequential cleavage of neuronal amyloid precursor protein (APP) by the β- and γ-secretases. However, despite decades of research, molecular regulation of the amyloidogenic secretase activities remains poorly understood, hindering the design of therapeutics to specifically target the APP amyloidogenic pathway.

Pharmacological inhibition of the β- and γ-secretase activities, although effective in suppressing Aβ production, interferes with physiological function of the secretases on their other substrates. Such intervention strategies therefore are often innately associated with untoward side effects, which have led to several failed clinical trials in the past [2-4]. To date, no therapeutic regimen is available to prevent the onset of AD or curtail its progression.

Here we report our findings that identify neuronal receptor PTPσ (protein tyrosine phosphatase sigma) as a potential molecular target to curb Aβ pathogenesis. Genetic

depletion of PTPσ lowers β-secretase affinity to APP and suppresses Aβ accumulation in a specific manner that does not generically inhibit β- and γ-secretase activities.

Besides Aβ, Tau is another biomarker that has been intensively studied in AD. Cognitive decline in patients often correlates better with Tau pathology than with Aβ burden [5,6]. Overwhelming evidence also substantiated that malfunction of Tau contributes to synaptic loss and neuronal deterioration [7]. However, what triggers the pathological changes of Tau in AD remains a mystery. Whether neurotoxic Aβ can lead to Tau pathology *in vivo* has been a debate since quintessential neurofibrillary Tau tangles have not been reported in any of the APP transgenic mouse models, even in those with severe cerebral β-amyloidosis. Here we show that during the process of aging, Tau aggregates form in the brains of two APP transgenic mouse models with a similar distribution pattern as seen in postmortem AD brains, suggesting that Tau misfolding can develop as an event downstream from the expression of amyloidogenic APP transgenes. Genetic depletion of PTPσ, which suppresses Aβ accumulation, also inhibits the aggregation of Tau.

In the two mouse models we studied, a spectrum of AD neuropathologies and behavioral deficits all demonstrate a clear dependency on PTPσ, indicating that this neuronal receptor is a pivotal upstream player in AD pathogenesis.

PTPσ is an APP binding partner in the brain.

Previously identified as a neuronal receptor of extracellular proteoglycans [8-10], PTPσ is expressed throughout the adult nervous system, most predominantly in the hippocampus [11,12], one of earliest affected brain regions in AD. Using

immunohistochemistry and confocal imaging, we found that PTPσ and APP (the precursor of Aβ) colocalize in hippocampal pyramidal neurons of adult rat brains, most intensively in the initial segments of apical dendrites, and in the perinuclear and axonal regions with a punctate pattern (Fig. 1a-f). To assess whether this colocalization reflects a binding interaction between these two molecules, we tested their co-immunoprecipitation from brain homogenates. In brains of rats and mice with different genetic background, using various antibodies of APP and PTPσ, we consistently detected a fraction of PTPσ that co-immunoprecipitates with APP, providing evidence of a molecular complex between these two transmembrane proteins (Fig. 1h, i; Extended Data Fig. 1).

Genetic depletion of PTPσ reduces β-amyloidogenic products of APP.

The molecular interaction between PTPσ and APP prompted us to investigate whether PTPσ plays a role in amyloidogenic processing of APP. In neurons, APP is mainly processed through alternative cleavage by either α- or β-secretase. These secretases release the N-terminal portion of APP from its membrane-tethering C-terminal fragment (CTFα or CTFβ, respectively), which can be further processed by the γ-secretase [13,14]. Sequential cleavage of APP by the β- and γ-secretases is regarded as amyloidogenic processing since it produces Aβ peptides [15]. When overproduced, the Aβ peptides can form soluble oligomers that trigger ramification of cytotoxic cascades, whereas progressive aggregation of Aβ eventually results in the formation of senile plaques in the brains of AD patients (Fig. 2a). To test the effect of PTPσ in this amyloidogenic processing, we analyzed the levels of APP β- and γ-cleavage products in mouse brains with or without PTPσ.

Western blot analysis with protein extracts from mouse brains showed that genetic depletion of PTPσ does not affect the expression level of full length APP (Fig. 2b; Extended Data Fig 2a). However, an antibody against the C-terminus of APP detects a band at a molecular weight consistent with CTFβ, which is reduced in PTPσ-deficient mice as compared to their age- sex-matched wild type littermates (Fig. 2b). Additionally, in two AD mouse models expressing human APP genes with amyloidogenic mutations [16,17], we observed a similar decrease of an APP CTF upon PTPσ depletion (Fig. 2b; Extended Data Fig. 2b). The TgAPP-SwDI and TgAPP-SwInd mice, each expressing a human APP transgene harboring the Swedish mutation near the β-cleavage site, were crossed with the PTPσ line to generate offsprings that are heterozygous for their respective APP transgene, with or without PTPσ. Because the Swedish mutation carried by these APP transgenes is prone to β-cleavage, the predominant form of APP CTF in these transgenic mice is predicted to be CTFβ. Thus, the reduction of APP CTF in PTPσ-deficient APP transgenic mice may indicate a regulatory role of PTPσ on CTFβ level. However, since the APP C-terminal antibody used in these experiments can recognize both CTFα and CTFβ, as well as the phosphorylated species of these CTFs (longer exposure of western blots showed multiple CTF bands), judging the identity of the reduced CTF simply by its molecular weight may be inadequate. We therefore performed CTFβ immunopurification with subsequent western blot detection, using an antibody that recognizes CTFβ but not CTFα (Fig. 2c, d; Extended Data Fig. 2c, d). With this definitive method, we confirmed that PTPσ depletion decreases the level of CTFβ originated from both mouse endogenous and human transgenic APP.

Because CTFβ is an intermediate proteolytic product between β- and γ-cleavage, its decreased steady state level could result from either reduced production by β-cleavage or increased degradation by subsequent γ-secretase cleavage (Fig. 2a). To distinguish between these two possibilities, we measured the level of Aβ peptides, which are downstream products from CTFβ degradation by γ-cleavage. Using ELISA assays with brain homogenates from the TgAPP-SwDI mice, we found that PTPσ depletion decreases the levels of Aβ peptides to a similar degree as that of CTFβ (Fig. 2e, f). Consistently, as Aβ peptides gradually aggregate into plaques during aging of the transgenic mice, we observed a substantial decrease of cerebral Aβ deposition in APP transgenic PTPσ-deficient mice as compared to the age-matched APP transgenic littermates expressing wild type PTPσ (Fig. 2g, h; Extended Data Fig. 2e, f). Thus, the concurrent decrease of β- and γ-cleavage products argues against an increased γ-secretase activity, but instead suggests a reduced β-secretase cleavage of APP, which suppresses not only the level of CTFβ but also downstream Aβ production in PTPσ-deficient brains.

Curtailed progression of β-amyloidosis in the absence of PTPσ.

Progressive cerebral Aβ aggregation (β-amyloidosis) is regarded as a benchmark of AD progression. To investigate the effects of PTPσ on this pathological development, we monitored Aβ deposits in the brains of 9-month old (mid-aged) and 16-month old (aged) TgAPP-SwDI mice. At 9 months of age, Aβ deposits are found predominantly in the hippocampus, especially in the hilus of the dentate gyrus (DG) (Fig. 2g, h). By 16 months, the pathology spreads massively throughout the entire brain. The propagation of

Aβ deposition, however, is curbed by genetic depletion of PTPσ, as quantified using the DG hilus as a representative area (Fig. 2i). Between the ages of 9 and 16 months, the Aβ burden is more than doubled in TgAPP-SwDI mice expressing wild type PTPσ [APP-SwDI(+)PTPσ(+/+)], but only shows marginal increase in the transgenic mice lacking functional PTPσ [APP-SwDI(+)PTPσ(-/-)]. Meanwhile, the Aβ loads measured in 9-month old APP-SwDI(+)PTPσ(+/+) mice are similar to those of 16-month old APP-SwDI(+)PTPσ(-/-) mice (p=0.95), indicating a restraint of disease progression by PTPσ depletion (Fig. 2i).

Decreased BACE1-APP affinity in PTPσ-deficient brains.

Consistent with these observations that suggest a facilitating role of PTPσ in APP β-cleavage, our data further reveal that PTPσ depletion weakens the interaction of APP with BACE1, the β-secretase in the brain. To test the *in vivo* affinity between BACE1 and APP, we performed co-immunoprecipitation of the enzyme and substrate from mouse brain homogenates in buffers with serially increased detergent stringency. Whereas BACE1-APP association is nearly equal in wild type and PTPσ-deficient brains under mild buffer conditions, increasing detergent stringency in the buffer unveils that the molecular complex is more vulnerable to dissociation in brains without PTPσ (Fig. 3). Thus a lower BACE1-APP affinity in PTPσ-deficient brains may likely be an underlying mechanism for the decreased levels of CTFβ and its derivative Aβ.

Although it cannot be ruled out that some alternative uncharacterized pathway may contribute to the parallel decrease of CTFβ and Aβ in PTPσ-deficient brains, these

data consistently support the notion that PTPσ regulates APP amyloidogenic processing, likely via facilitation of BACE1 activity on APP, the initial process of Aβ production.

The specificity of β-amyloidogenic regulation by PTPσ.

The constraining effect of PTPσ on APP amyloidogenic products led us to further question whether this observation reflects a specific regulation of APP metabolism, or alternatively, a general modulation on the β- and γ-secretases. We first assessed the expression level of these secretases in mouse brains with or without PTPσ, and found no change for BACE1 or the essential subunits of γ-secretase (Fig. 4a, b). Additionally, we tested whether PTPσ broadly modulates β- and γ-secretase activities, by examining the proteolytic processing of their other substrates. Besides APP, Neuregulin1 (NRG1) [18-20] and Notch [21-23] are the major *in vivo* substrates of BACE1 and γ-secretase, respectively. Neither BACE1 cleavage of NRG1 nor γ-secretase cleavage of Notch is affected by PTPσ deficiency (Fig. 4c, d). Taken together, these data rule out a generic modulation of β- and γ-secretases, but rather suggest a specificity of APP amyloidogenic regulation by PTPσ.

PTPσ depletion relieves neuroinflammation and synaptic impairment in APP transgenic mice.

Substantial evidence from earlier studies has established that overproduction of Aβ in the brain elicits multiplex downstream pathological events, including chronic inflammatory responses of the glia, such as persistent astrogliosis. The reactive

(inflammatory) glia would then crosstalk with neurons, evoking a vicious feedback loop that amplifies neurodegeneration during disease progression [24-26].

The TgAPP-SwDI model is one of the earliest to develop neurodegenerative pathologies and behavioral deficits among many existing AD mouse models [16]. We therefore chose these mice to further examine the role of PTPσ in AD pathologies downstream of neurotoxic Aβ.

The APP-SwDI(+)PTPσ(+/+) mice, which express the TgAPP-SwDI transgene and wild type PTPσ, have developed severe neuroinflammation in the brain by the age of 9 months, as measured by the level of GFAP (glial fibrillary acidic protein), a marker of astrogliosis (Extended Data Fig. 3). In the DG hilus, for example, GFAP expression level in the APP-SwDI(+)PTPσ(+/+) mice is more than tenfold compared to that in age-matched non-transgenic littermates [APP-SwDI(-)PTPσ(+/+)] (Extended Data Fig. 3b). PTPσ deficiency, however, effectively attenuates astrogliosis induced by the amyloidogenic transgene. In the APP-SwDI(+)PTPσ(-/-) brains, depletion of PTPσ restores GFAP expression back to a level close to that of non-transgenic wild type littermates (Extended Data Fig. 3b).

Among all brain regions, the most affected by the expression of TgAPP-SwDI transgene appears to be the hilus of the DG, where Aβ deposition and astrogliosis are both found to be the most severe (Fig. 2g, h; Extended Data Fig. 3). We therefore questioned whether the pathologies in this area have an impact on the mossy fiber axons of DG pyramidal neurons, which project through the hilus into the CA3 region, where they synapse with the CA3 dendrites. Upon examining the presynaptic markers in CA3 mossy fiber terminal zone, we found decreased levels of Synaptophysin and Synapsin-1

in the APP-SwDI(+)PTPσ(+/+) mice, comparing to their age-matched non-transgenic littermates (Extended Data Fig. 4, data not shown for Synapsin-1). Such synaptic impairment, evidently resulting from the expression of the APP transgene and possibly the overproduction of Aβ, is reversed by genetic depletion of PTPσ in the APP-SwDI(+)PTPσ(-/-) mice (Extended Data Fig. 4).

Interestingly, we noticed that the APP-SwDI(+)PTPσ(-/-) mice sometimes express higher levels of presynaptic markers in the CA3 terminal zone than their age-matched non-transgenic wild type littermates (Extended Data Fig. 4g). This observation, although not statistically significant in our quantification analysis, may suggest an additional synaptic effect of PTPσ that is independent of the APP transgene, as observed in previous studies [27].

Tau pathology in aging AD mouse brains is dependent on PTPσ.

Neurofibrillary tangles composed of hyperphosphorylated and aggregated Tau are commonly found in AD brains. These tangles tend to develop in a hierarchical pattern, appearing first in the entorhinal cortex before spreading to other brain regions [5,6]. The precise mechanism of tangle formation, however, is poorly understood. The fact that Tau tangles and Aβ deposits can be found in separate locations in postmortem brains has led to the question of whether Tau pathology in AD is independent of Aβ accumulation [5,6]. Additionally, despite severe cerebral β-amyloidosis in many APP transgenic mouse models, Tau tangles have not been reported, further questioning the relationship between Aβ and Tau pathologies *in vivo*.

Nonetheless, a few studies did show non-tangle like assemblies of Tau in dystrophic neurites surrounding Aβ plaques in APP transgenic mouse lines [28-30], arguing that Aβ can be a causal factor for Tau dysregulation, despite that the precise nature of Tau pathologies may be different between human and mouse. In our histological analysis using an antibody against the proline-rich domain of Tau, we observed Tau aggregation in the brains of both TgAPP-SwDI and TgAPP-SwInd mice during the course of aging (around 9 months for the both the APP-SwDI(+)PTPσ(+/+) mice and 15 months for the APP-SwInd(+)PTPσ(+/+) mice) (Fig. 5; Extended Data Fig. 5). Such aggregation is not seen in aged-matched non-transgenic littermates (Fig. 5h), suggesting that it is a pathological event downstream from the expression of amyloidogenic APP transgenes, possibly a result of Aβ cytotoxicity. Genetic depletion of PTPσ, which diminishes Aβ levels, suppresses Tau aggregation in both TgAPP-SwDI and TgAPP-SwInd mice (Fig. 5; Extended Data Fig. 5).

Upon closer examination, the Tau aggregates are often found in punctate shapes, likely in debris from degenerated cell bodies and neurites, scattered in areas free of nuclear staining (Extended Data Fig. 6a-f). Rarely, a few are in fibrillary structures, probably in degenerated cells before disassembling (Extended Data Extended Data Fig. 6g, h). In both TgAPP-SwDI and TgAPP-SwInd mice, the Tau aggregates are found predominantly in the molecular layer of the piriform and entorhinal cortices, and occasionally in the hippocampal region (Fig. 5; Extended Data Fig. 5), reminiscent of the early stage tangle locations in AD brains [31]. To confirm these findings, we used an additional antibody recognizing the C-terminus of Tau and detected the same morphologies (Extended Data Fig. 6i) and distribution pattern (Fig. 5a).

Consistent with the findings in postmortem AD brains, the distribution pattern of Tau aggregates in the TgAPP-SwDI brain does not correlate with that of Aβ deposition, which is pronounced in the hippocampus yet barely detectable in the piriform or entorhinal cortex at the age of 9 months (Fig. 2g, h). Given that the causation of Tau pathology in these mice is possibly related to the overproduced Aβ, the segregation of Aβ and Tau depositions in different brain regions may indicate that the cytotoxicity originates from soluble Aβ instead of the deposited amyloid. It is also evident that neurons in different brain regions are not equally vulnerable to developing Tau pathology.

We next examined whether the expression of APP transgenes or genetic depletion of PTPσ regulates Tau aggregation by changing its expression level and/or phosphorylation status. Western blot analysis of brain homogenates showed that Tau protein expression is not affected by the APP transgenes or PTPσ (Extended Data Fig. 7), suggesting that the aggregation may be due to local misfolding of Tau rather than an overexpression of this protein. Moreover, the TgAPP-SwDI or TgAPP-SwInd transgene, which apparently causes Tau aggregation, does not enhance the phosphorylation of Tau residues including Serine191, Therionine194, and Therionine220 (data not shown), whose homologues in human Tau (Serine202, Therionine205, and Therionine231) are typically hyperphosphorylated in neurofibrillary tangles. These findings are consistent with a recent quantitative study showing similar post-translational modifications in wild type and TgAPP-SwInd mice [32]. Furthermore, unlike previously reported [28,29], we could not detect these phosphorylated residues in the Tau aggregates, suggesting that the epitopes are either missing (residues not phosphorylated or cleaved off) or embedded

inside the misfolding. Given the complexity of Tau post-translational modification, one cannot rule out that the aggregation may be mediated by some unidentified modification(s) of Tau. It is also possible that other factors, such as molecules that bind to Tau, may precipitate the aggregation.

Although the underlying mechanism is still unclear, our finding of Tau pathology in these mice establishes a causal link between the expression of amyloidogenic APP transgenes and a dysregulation of Tau assembly. Our data also suggest a possibility that PTPσ depletion may suppress tau aggregation by reducing amyloidogenic products of APP.

Malfunction of Tau is broadly recognized as a neurodegenerative marker since it indicates microtubule deterioration [7]. The constraining effect on Tau aggregation by genetic depletion of PTPσ thus provides additional evidence for the role of this receptor as a pivotal regulator of neuronal integrity.

PTPσ deficiency rescues behavioral deficits in AD mouse models.

We next assessed whether the alleviation of neuropathologies by PTPσ depletion is accompanied with a rescue from AD relevant behavioral deficits. The most common symptoms of AD include short-term memory loss and apathy among the earliest, followed by spatial disorientation amid impairment of many cognitive functions as the dementia progresses. Using Y maze and novel object assays as surrogate models, we evaluated these cognitive and psychiatric features in the TgAPP-SwDI and TgAPP-SwInd mice.

The Y-maze assay, which allows mice to freely explore three identical arms, measures their short-term spatial memory. It is based on the natural tendency of mice to alternate arm exploration without repetitions. The performance is scored by the percentage of spontaneous alternations among total arm entries, and a higher score indicates better spatial navigation. Compared to the non-transgenic wild type mice within the colony, the APP-SwDI(+)PTPσ(+/+) mice show a clear deficit in their performance. Genetic depletion of PTPσ in the APP-SwDI(+)PTPσ(-/-) mice, however, unequivocally restores the cognitive performance back to the level of non-transgenic wild type mice (Fig. 6a, Extended Data Fig. 8).

Apathy, the most common neuropsychiatric symptom reported among individuals with AD, is characterized by a loss of motivation and diminished attention to novelty, and has been increasingly adopted into early diagnosis of preclinical and early prodromal AD [33-35]. Many patients in early stage AD lose attention to novel aspects of their environment despite their ability to identify novel stimuli, suggesting an underlying defect in the circuitry responsible for further processing of the novel information [33,34]. As a key feature of apathy, such deficits in attention to novelty can be accessed by the "curiosity figures task" or the "oddball task" in patients [33,34,36]. These visual-based novelty encoding tasks are very similar to the novel object assay for rodents, which measures the interest of animals in a novel object (NO) when they are exposed simultaneously to a prefamiliarized object (FO). We therefore used this assay to test the attention to novelty in the APP transgenic mice. When mice are pre-trained to recognize the FO, their attention to novelty is then measured by the discrimination index denoted as the ratio of NO exploration to total object exploration (NO+FO), or alternatively, by the ratio of NO

exploration to FO exploration. Whereas both ratios are commonly used, a combination of these assessments provides a more comprehensive evaluation of animal behavior. In this test, as indicated by both measurements, the expression of APP-SwDI transgene in the APP-SwDI(+)PTPσ(+/+) mice leads to a substantial decrease in NO exploration as compared to non-transgenic wild type mice (Fig. 6b, c; Extended Data Fig. 9). Judging by their NO/FO ratios, it is evident that both the transgenic and non-transgenic groups are able to recognize and differentiate between the two objects (Extended Data Fig. 9a, b). Thus, the reduced NO exploration by the APP-SwDI(+)PTPσ(+/+) mice may reflect a lack of interest in the NO or an inability to shift attention to the NO. Once again, this behavioral deficit is largely reversed by PTPσ deficiency in the APP-SwDI(+)PTPσ(-/-) mice (Fig. 6b, c; Extended Data Fig. 9), consistent with previous observation of increased NO preference in the absence of PTPσ [27].

To further verify the effects of PTPσ on these behavioral aspects, we additionally tested the TgAPP-SwInd mice in both assays and observed similar results, confirming an improvement on both short-term spatial memory and attention to novelty upon genetic depletion of PTPσ (Extended Data Fig. 10).

Discussion

Here we report that β-amyloidosis and several downstream disease features are dependent on PTPσ in two mouse models of genetically inherited AD. This form of AD develops inevitably in people who carry gene mutations that promote amyloidogenic processing of APP and overproduction of Aβ. Our data suggest that targeting PTPσ is a potential therapeutic approach that could overcome such dominant genetic driving forces

to curtail AD progression. The advantage of this targeting strategy is that it suppresses Aβ accumulation without broadly affecting other major substrates of the β- and γ-secretases, thus predicting a more promising translational potential as compared to those in clinical trials that generically inhibit the secretases.

PTPσ was previously characterized as a neuronal receptor of the chondroitin sulfate- and heparan sulfate-proteoglycans (CSPGs and HSPGs) [8,9]. In response to these two classes of extracellular ligands, PTPσ functions as a "molecular switch" by regulating neuronal behavior in opposite manners [10]. Our finding of a pivotal role for the proteoglycan sensor PTPσ in AD pathogenesis may therefore implicate an involvement of the perineuronal matrix in AD etiology.

More than 95% of AD cases are sporadic, which are not genetically inherited but likely result from insults to the brain that occurred earlier in life. AD risk factors, such as traumatic brain injury and cerebral ischemia [37-40], have been shown to induce overproduction of Aβ in both human and rodents [41-45], and speed up progression of this dementia in animal models [46-48]. However, what promotes the amyloidogenic processing of APP in these cases is still a missing piece of the puzzle in understanding the AD-causing effects of these notorious risk factors.

Coincidently, both traumatic brain injury and cerebral ischemia cause pronounced remodeling of the perineuronal microenvironment at lesion sites, marked by increased expression of CSPGs [49-52], a major component of the perineuronal net that is upregulated during neuroinflammation and glial scar formation [53-55]. In the brains of AD patients, CSPGs were found associated with Aβ depositions, further suggesting an uncanny involvement of these proteoglycans in AD development [56]. On the other hand, analogues

of heparan sulfate (HS, carbohydrate side chains of HSPGs that bind to PTPσ) were shown to inhibit BACE1 activity, suggesting their function in preventing Aβ overproduction [57]. After cerebral ischemia, however, the expression of Heparanase, an enzyme that degrades HS, was found markedly increased [58]. Collectively, these findings suggest a disrupted molecular balance between CSPGs and HSPGs in brains after lesion, which may ignite insidious signaling cascades preceding the onset of AD.

We hence speculate upon a mechanism for further study, whereby chronic CSPG upregulation or HSPG degradation in lesioned brains may sustain aberrant signaling through their neuronal sensor PTPσ, leading to biased processing of APP and a neurotoxic "Aβ cascade". As such, altered signaling from PTPσ after traumatic brain injury and ischemic stroke may explain how these risk factors can trigger subsequent onset of AD. Restoring the integrity of brain microenvironment therefore could be essential in preventing AD for the population at risk.

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Supplementary Materials:

Materials and Methods:

Mouse lines: Mice were maintained under standard conditions approved by the Institutional Animal Care and Use Committee. Wild type and PTPσ-deficient mice of Balb/c background were provided by Dr. Michel L. Tremblay [59]. Homozygous TgAPP-SwDI mice, C57BL/6-Tg(Thy1-APPSwDutIowa)BWevn/Mmjax, stock number 007027, were from the Jackson Laboratory. These mice express human APP transgene harboring Swedish, Dutch, and Iowa mutations, and were bred with Balb/c mice heterozygous for the PTPσ gene to generate bigenic mice heterozygous for both TgAPP-SwDI and PTPσ genes, which are hybrids of 50% C57BL/6J and 50% Balb/c genetic background. These mice were further bred with Balb/c mice heterozygous for the PTPσ gene. The offspring from this mating are used in experiments, which include littermates of the following genotypes: TgAPP-SwDI(+/-)PTPσ(+/+), mice heterozygous for TgAPP-SwDI transgene with wild type PTPσ; TgAPP-SwDI(+/-)PTPσ(-/-), mice heterozygous for TgAPP-SwDI transgene with genetic depletion of PTPσ; TgAPP-SwDI(-/-)PTPσ(+/+), mice free of TgAPP-SwDI transgene with wild type PTPσ. Both TgAPP-SwDI(-/-)PTPσ(+/+) and Balb/c PTPσ(+/+) are wild type mice but with different genetic background. Heterozygous TgAPP-SwInd (J20) mice, 6.Cg-Tg(PDGFB-APPSwInd)20Lms/2Mmjax, were provided by Dr. Lennart Mucke. These mice express human APP transgene harboring Swedish and Indiana mutations, and were bred with the same strategy as described above to obtain mice with genotypes of TgAPP-SwInd (+/-)PTPσ(+/+) and TgAPP-SwInd (+/-)PTPσ(-/-).

Antibodies:

Primary Antibodies	Application	Clone #	Catalog #	Supplier
Mouse anti-Actin	WB	AC-40	A4700	Sigma-Aldrich
Rabbit anti-APH1	WB		PA5-20318	Thermo Scientific
Rabbit anti-APP C-term	WB, IP, IHC	Y188	NB110-55461	Novus Biologicals
Mouse anti-murine Aβ, 1-16	WB, IP	M3.2	805701	Biolegend
Mouse anti-human Aβ, 1-16	WB, IP, IHC, ELISA	6E10	803001	Biolegend
Mosue anti-Aβ, 17-24	WB, IHC	4G8	SIG-39220	Biolegend
Mouse HRP-conjugated anti-Aβ 1-40	ELISA	11A50-B10	SIG-39146	Biolegend
Mouse HRP-conjugated anti-Aβ 1-42	ELISA	12F4	805507	Biolegend
Rabbit anti-BACE1 C-Term, B690	WB		PRB-617C	Covance
Guinea Pig anti-BACE1 C-Term	IP		840201	Biolegend
Chiken anti-GFAP	IHC		ab4674	Abcam
Rabbit anti-Neuregulin	WB		sc-348	Santa Cruz Biotechnology
Rabbit anti-Nicastrin	WB		5665	Cell Signaling
Rabbit anti-Notch NICD (val1744)	WB		4147	Cell Signaling
Rabbit anti-Notch (C-20)	WB		sc-6014R	Santa Cruz Biotechnology
Rabbit anti-PEN2	WB		8598	Cell Signaling
Rabbit anti-Presenilin 1/2 NTF	WB		840201	Abcam
Rabbit anti-Presenilin 1 CTF	WB		5643	Cell Signaling
Rabbit anti-Presenilin 2 CTF	WB		9979	Cell Signaling
Mouse anti-PTPσ ICD	WB, IHC	17G7.2	MM-002-P	Medimabs
Mouse anti-PTPσ ECD	WB		ab55640	Abcam
Rabbit anti-Synaptophysin	IHC		AB9272	Millipore
Mouse anti-Tau	WB, IHC	Tau-5	MAB361	Millipore
Mouse anti-Tau	IHC	Tau-46	4019	Cell Signaling
Secondary and Tertiary Antibodies	**Application**	**Clone #**	**Catalog #**	**Supplier**
Goat anti-mouse IgG HRP-conjugated	WB		7076S	Cell Signaling
Goat anti-rabbit IgG HRP-conjugated	WB		7074S	Cell Signaling
Goat anti-mouse IgG Alexa488	IHC		A-11001	Invitrogen
Donkey anti-goat IgG Alexa488	IHC		A-11055	Invitrogen
Chicken anti-rabbit IgG CF568	IHC		SAB4600426	Sigma-Aldrich
Donkey anti-chicken IgG Cy3	IHC		703-165-155	JacksonImmunoResearch

Immunohistochemistry: Adult rat and mice were perfused intracardially with fresh made 4% paraformaldehyde in cold phosphate-buffered saline (PBS). The brains were collected and post-fixed for 2 days at 4 °C. Paraffin embedded sections of 10 μM thickness were collected for immunostaining. The sections were deparaffinized and sequentially rehydrated. Antigen retrieval was performed at 100 °C in Tris-EDTA buffer (pH 9.0) for 50 min. Sections were subsequently washed with distilled water and PBS, incubated at room temperature for 1 hour in blocking buffer (PBS, with 5% normal donkey serum, 5% normal goat serum, and 0.2% Triton X-100). Primary antibody

incubation was performed in a humidified chamber at 4°C overnight. After 3 washes in PBS with 0.2% Triton X-100, the sections were then incubated with a mixture of secondary and tertiary antibodies at room temperature for 2 hours. All antibodies were diluted in blocking buffer with concentrations recommended by the manufacturers. Mouse primary antibodies were detected by goat anti-mouse Alexa488 together with donkey anti-goat Alexa488 antibodies; rabbit primary antibodies were detected by chicken anti-rabbit CF568 and donkey anti-chicken Cy3 antibodies; chicken antibody was detected with donkey anti-chicken Cy3 antibody. Sections stained with only secondary and tertiary antibodies (without primary antibodies) were used as negative controls. At last, DAPI (Invitrogen, 300 nM) was applied on sections for nuclear staining. Sections were washed 5 times before mounted in Fluoromount (SouthernBiotech).

Wide field and confocal images were captured using Zeiss Axio Imager M2 and LSM780, respectively. Images are quantified using the Zen 2 Pro software and ImageJ.

Protein extraction, immunoprecipitation, and western blot analysis: For the co-immunoprecipitation of APP and PTPσ, RIPA buffer was used (50 mM Tris-HCl, pH 8.0, 1 mM EDTA, 150 mM NaCl, 1% NP40, 0.1% SDS, 0.5% sodium deoxycholate). For the co-immunoprecipitation of APP and BACE1, NP40 buffer was used (50 mM Tris-HCl, pH 8.0, 1 mM EDTA, 150 mM NaCl, 1% NP40) without or with SDS at concentration of 0.1%, 0.3%, and 0.4%. For total protein extraction and immunopurification of CTFβ, SDS concentration in RIPA buffer was adjusted to 1% to ensure protein extraction from the lipid rafts. Mouse or rat forebrains were homogenized thoroughly on ice in homogenization buffers (as mention above) containing protease and

phosphatase inhibitors (Thermo Scientific). For each half of forebrain, buffer volume of at least 5 ml for mouse and 8 ml for rat was used to ensure sufficient detergent/tissue ratio. The homogenates were incubated at 4°C for 1 hour with gentle mixing, sonicated on ice for 2 minutes in a sonic dismembrator (Fisher Scientific Model 120, with pulses of 50% output, 1 second on and 1 second off), followed with another hour of gentle mixing at 4°C. All samples were used fresh without freezing and thawing.

For co-immunoprecipitation and immunopurification, the homogenates were then centrifuged at 85,000 x g for 1 hour at 4°C and the supernatants were collected. Protein concentration was measured using BCA Protein Assay Kit (Thermo Scientific). 0.5 mg total proteins of brain homogenates were incubated with 5 μg of designated antibody and 30 μl of Protein-A sepharose beads (50% slurry, Roche), in a total volume of 1 ml adjusted with RIPA buffer. Samples were gently mixed at 4°C overnight. Subsequently, the beads were washed 5 times with cold immunoprecipitation buffer. Samples were then incubated in Laemmli buffer with 100 mM of DTT at 75°C for 20 minutes and subjected to western blot analysis.

For analysis of protein expression level, the homogenates were centrifuged at 23,000 x g for 30 min at 4°C and the supernatants were collected. Protein concentration was measured using BCA Protein Assay Kit (Thermo Scientific). 30 μg of total proteins were subjected to western blot analysis.

Electrophoresis of protein samples was conducted using 4-12% Bis-Tris Bolt Plus Gels, with either MOPS or MES buffer and Novex Sharp Pre-stained Protein Standard (all from Invitrogen). Proteins were transferred to nitrocellulose membrane (0.2 μm pore size, Bio-Rad) and blotted with selected antibodies (see table above) at concentrations

suggested by the manufacturers. Primary antibodies were diluted in SuperBlock TBS Blocking Buffer (Thermo Scientific) and incubated with the nitrocellulose membranes at 4°C overnight; secondary antibodies were diluted in PBS with 5% nonfat milk and 0.2% Tween20 and incubated at room temperature for 2 hours. Membranes were washes 4 times in PBS with 0.2% Tween20 between primary and secondary antibodies and before chemiluminescent detection with SuperSignal West Pico Chemiluminescent Substrate (Thermo Scientific).

Western blot band intensity was quantified by densitometry.

Aβ ELISA assays: Mouse forebrains were thoroughly homogenized in tissue homogenization buffer (2 mM Tris pH 7.4, 250 mM sucrose, 0.5 mM EDTA, 0.5 mM EGTA) containing protease inhibitor cocktail (Roche), followed by centrifugation at 135,000 x g (33,500 RPM with SW50.1 rotor) for 1 hour at 4°C. Proteins in the pellets were extracted with formic acid (FA) and centrifuged at 109,000 x g (30,100 RPM with SW50.1 rotor) for 1 hour at 4°C. The supernatants were collected and diluted 1:20 in neutralization buffer (1 M Tris base, 0.5 M Na_2HPO_4, 0.05% NaN_3) and subsequently 1:3 in ELISA buffer (PBS with 0.05% Tween-20, 1% BSA, and 1 mM AEBSF). Diluted samples were loaded onto ELISA plates pre-coated with 6E10 antibody (Biolegend) to capture Aβ peptides. Serial dilutions of synthesized human Aβ 1-40 or 1-42 (American Peptide) were loaded to determine a standard curve. Aβ was detected using an HRP labeled antibody for either Aβ 1-40 or 1-42 (see table above). ELISA was developed using TMB substrate (Thermo Scientific) and reaction was stopped with 1N HCl. Plates

were read at 450nm and concentrations of Aβ in samples were determined using the standard curve.

Behavior assays: The Y-maze assay: Mice were placed in the center of the Y-maze and allowed to move freely through each arm. Their exploratory activities were recorded for 5 minutes. An arm entry is defined as when all four limbs are within the arm. For each mouse, the number of triads is counted as "spontaneous alternation", which was then divided by the number of total arm entries, yielding a percentage score. The novel object test: On day 1, mice were exposed to empty cages (45 cm x 24 cm x 22 cm) with blackened walls to allow exploration and habituation to the arena. During day 2 to day 4, mice were returned to the same cage with two identical objects placed at an equal distance. On each day mice were returned to the cage at approximately the same time during the day and allowed to explore for 10 minutes. Cages and objects were cleaned with 70% ethanol between each animal. Subsequently, 2 hours after the familiarization session on day 4, mice were put back to the same cage where one of the familiar objects (randomly chosen) was replaced with a novel object, and allowed to explore for 5 minutes. Mice were scored using Observer software (Noldus) on their time duration and visiting frequency exploring either object. Object exploration was defined as facing the object and actively sniffing or touching the object, whereas any climbing behavior was not scored. The discrimination indexes reflecting interest in the novel object is denoted as either the ratio of novel object exploration to total object exploration (NO/NO+FO) or the ratio of novel object exploration to familiar object exploration (NO/FO). All tests and data analyses were conducted in a double-blinded manner.

Statistics: 2-tailed Student's *t* test was used for two-group comparison. Relationship between two variables (SDS concentration and APP-BACE1 association, as in Fig 3) was analyzed using linear regression. All error bars show standard error of the means (SEM).