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3-*O*-Sulfation of Heparan Sulfate Enhances Tau Interaction and Cellular Uptake

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Abstract: *Prion-like transcellular spreading of tau in Alzheimer's Disease (AD) is mediated by tau binding to cell surface heparan sulfate (HS). However, the structural determinants for tau–HS interaction are not well understood. Microarray and SPR assays of structurally defined HS oligosaccharides show that a rare 3-O-sulfation (3-O-S) of HS significantly enhances tau binding. In Hs3st1$^{-/-}$ (HS 3-O-sulfotransferase-1 knockout) cells, reduced 3-O-S levels of HS diminished both cell surface binding and internalization of tau. In a cell culture, the addition of a 3-O-S HS 12-mer reduced both tau cell surface binding and cellular uptake. NMR titrations mapped 3-O-S binding sites to the microtubule binding repeat 2 (R2) and proline-rich region 2 (PRR2) of tau. Tau is only the seventh protein currently known to recognize HS 3-O-sulfation. Our work demonstrates that this rare 3-O-sulfation enhances tau–HS binding and likely the transcellular spread of tau, providing a novel target for disease-modifying treatment of AD and other tauopathies.*

Introduction

The pathology of Alzheimer's disease (AD) is characterized by amyloid plaques and neurofibrillary tangles (NFTs). NFTs are composed of the microtubule-associated protein tau (MAPT), whose normal functions include bundling and stabilizing microtubules (MTs) in neurons. Continued failure of anti-amyloid compounds in clinical trials has shifted the focus of AD research towards tau. In AD, tau becomes hyperphosphorylated and dissociates from microtubules, and aggregates to form NFTs. In contrast with amyloid plaques, tau pathology correlates well with cognitive decline in AD.[1] Recently, mounting evidence from cell culture,[2,3] animal models,[4–6] and human pathology[7] has established that tau spread through neural networks in an orderly and "prion-like" manner, mediated by transcellular movement of tau[8–10] (Figure 1A). Because NFTs directly correlate to cognitive deficits, inhibiting the prion-like spread of tau is likely a viable strategy to slow down cognitive decline and the progression of AD in patients. Thus, there is a pressing need to understand the molecular mechanisms of NFT spread.

A key step in tau transcellular movement is tau binding to heparan sulfate proteoglycans (HSPGs)[11–14] on the cell surface (Figure 1B), followed by the endocytosis of tau. HSPGs are HS glycosaminoglycan (GAG) chains covalently linked to a protein core. HS is a linear, polyanionic GAG composed of disaccharide repeats of uronic acid (glucuronic acid or iduronic) and glucosamine with sulfation substitution on the 3-OH, 6-OH and -NH of the glucosamine residue, and the 2-OH of the uronic acid residue (Figure 1C). While electrostatic interactions are the major driving forces, in many cases specific sulfation patterns are required for the recognition of HS by its binding partners.[15,16] Sulfation at the 3-*O* position is relatively rare compared to other modifications, with only six proteins reported to rely on the 3-*O*-sulfation for binding.[17–19] In humans, 3-O-sulfation of HS is catalyzed by seven isoforms of 3-*O*-sulfotransferase (*HS3ST*): *HS3ST1*, *HS3ST2*, *HS3ST3A*, *HS3ST3B*, *HS3ST4*, *HS3ST5*, and *HS3ST6*. Among these isoforms, *HS3ST1*, *HS3ST2*, and *HS3ST5* are only expressed in the brain,[20] with increased *Hs3st2* and *Hs3st4* levels in the hippocampus of AD patients.[21] Importantly, genome-wide genetic association (GWAS) studies have implicated *HS3ST1* in AD.[22,23] Moreover, a recent study showed that HS containing GAGs isolated from brains of AD patients exhibit enhanced tau binding, further suggesting the involvement of 3-*O*-sulfation in AD. However, how 3-*O*-sulfation contributes to AD remains unclear.

Here, utilizing a structurally defined HS oligosaccharide microarray, surface plasmon resonance (SPR), nuclear magnetic resonance spectroscopy (NMR), and cellular binding and uptake assays, we report for the first time that the rare 3-*O*-S is a crucial determinant in the tau–HS interaction and cellular uptake of tau. Our results provide molecular details of the link between 3-*O*-sulfation of HS and AD, pointing towards novel strategies for tau-targeted AD therapy.

Results and Discussion

3-O-S Enhances tau Binding to HS in Glycan Array Analysis

Previous interaction studies of tau/glycan have relied on heparin as a substitute for HS, but important structural and functional differences exist between heparin and HS. In this

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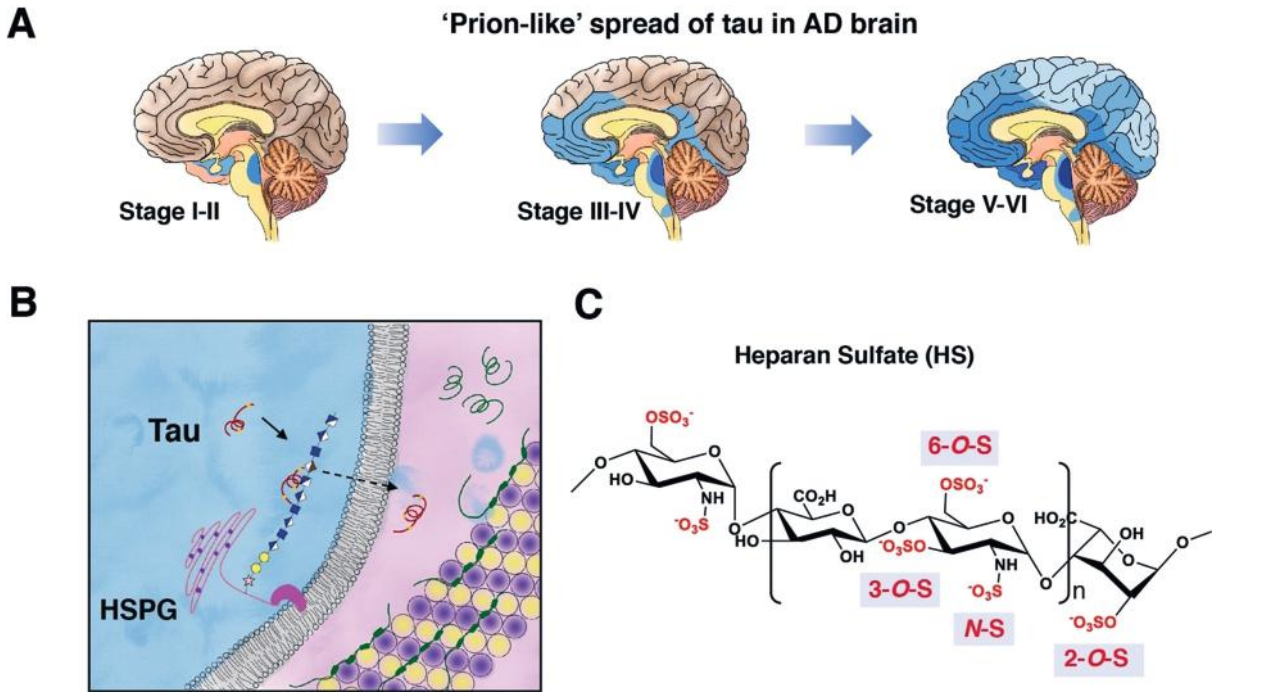

Figure 1. Cellular uptake of tau is mediated by HSPGs on cell surface. A) Prion-like spread of tau pathology (represented by blue color) in AD brain. B) Uptake of tau is mediated by the binding to heparan sulfate proteoglycans (HSPGs). Microtubules are represented by a tube composed of α- and β-tubulins (yellow and purple). C) Primary structure and sulfation pattern of heparan sulfate.

study, the tau/glycan interaction is examined using HS. Structurally defined HS oligosaccharides were synthesized by a chemoenzymatic method as previously described,[18,24] and were then immobilized on a microarray chip, creating the low-molecular-weight HS (LMHS) array. Full-length tau binding (or lack thereof) to the HS array was visualized by fluorescently labeled tau remaining on the chip after incubation and washing. As shown in Figure 2A, high fluorescence intensity was observed for a HS heptasaccharide (7-mer) on spot 4 (oligo-4), and three HS dodecasaccharides (12-mers) in spots 18,19, and 20 (oligo-18, oligo-19, and oligo-20). Remarkably, oligo-4, which only differs from oligo-5 by a single additional 3-*O*-sulfo group, exhibits about a 10-fold higher fluorescence intensity than oligo-5, indicating that the presence of 3-*O*-S increases the binding of the tau protein. The significance of 3-*O*-S is also underscored from the binding of tau to longer oligosaccharides as demonstrated by the microarray analysis. The HS 12-mers oligo-18 and oligo-19, containing two and one 3-*O*-S, respectively, displayed higher binding to tau compared to oligo-20, a HS 12-mer lacking 3-*O*-S. Oligo-21, which is not sulfated, exhibited negligible fluorescence.

3-O-S Promotes Inhibition of tau–HS Interaction by Oligosaccharides as Demonstrated by SPR Analysis

Binding kinetics and affinity between HS and tau have not been measured before. Here, HS from three different sources, porcine brain, porcine spine, and porcine intestine, were prepared, biotinylated, and immobilized on a SA sensor chip for binding studies using full-length tau. Brain, spinal, and intestinal HS exhibited similar binding patterns to tau, with a binding affinity (K_D) of 0.02 μM (Figure 2B; see Figure S3 in the Supporting Information), showing similar behavior in tau interaction with HS from these three different sources. The more accessible porcine intestinal HS was then used to further characterize the role of 3-*O*-sulfation in tau–HS binding, which likely resembles endogenous HS from brain tissues. Three synthesized HS 12-mers, oligo-19, oligo-20, and oligo-21 (the oligosaccharides on spots 19, 20, and 21 of the LMHS array, for chemical structure see Figure S1B) were tested by a solution/surface competition SPR assay (Figure 2C) to examine their ability to the inhibit tau–HS interaction. Full-length tau protein was individually premixed with each of three HS 12-mers and then flowed over a chip with surface-immobilized HS. The tau protein binding to the 12-mer in solution diminishes its interaction with the HS immobilized on the chip surface (Figure 2C). With increasing 12-mer solution concentrations, less and less binding to the surface was detected. An IC_{50} value of 0.9 μM and 4.9 μM for the inhibition of tau–HS interactions were obtained for oligo-19 and oligo-20, respectively (Figures 2D and E). The observed lower IC_{50} value for oligo-19 is consistent with the stronger binding of tau to oligo-19 in the HS microarray analysis. This approximate fivefold lower IC_{50} value indicates oligo-19 is much more effective in the inhibition of the tau–HS interaction. In contrast, oligo-21 showed very little inhibition of the tau–HS interaction, with an IC_{50} value higher than 700 μM (Figure 2F), which is also consistent with the negligible fluorescence signal for oligo-21 in the LMHS array. The significantly lower IC_{50} value of oligo-19 compared with that

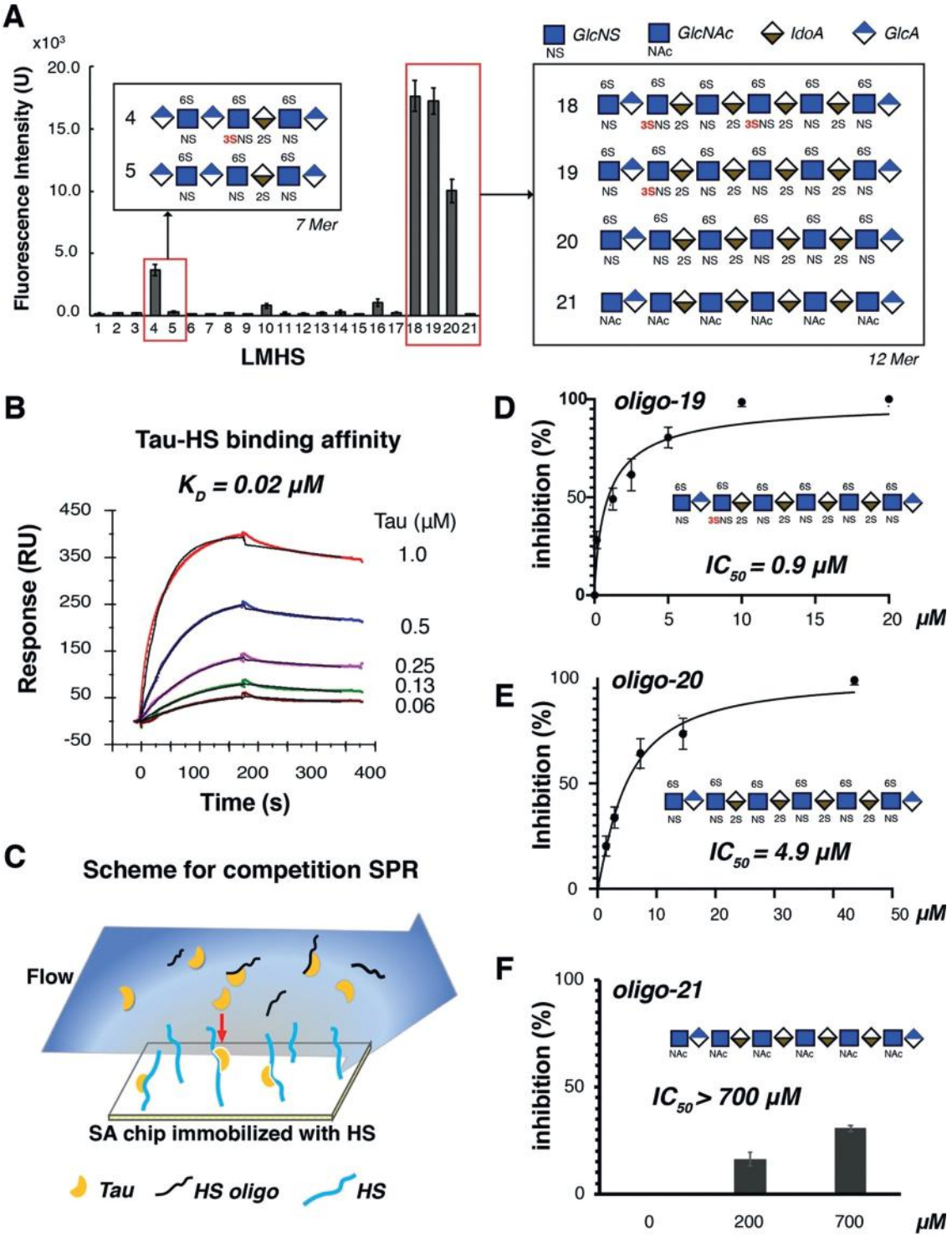

Figure 2. Low-molecular-weight heparan sulfate (LMHS) array and SPR assays show the crucial role of 3-*O*-sulfation (3-*O*-S) in tau binding. A) 3-*O*-S enhances tau binding to HS in LMHS analysis. Fluorescence intensity on each spot of the array was shown in a bar graph, with the monosaccharide composition/sulfation pattern drawn for the HS oligosaccharides with high fluorescence intensity (tau binding). Complete results of the LMHS array can be found in Figures S1 and S2. B) Binding affinity of the full-length tau–HS interaction was measured to be 0.02 μM by SPR binding kinetic assay for the first time. The association and dissociation curve of different tau concentrations were fitted (black line) by a 1:1 Langmuir kinetics model in Bio-evaluation. HS from three sources (porcine brain, porcine spine and porcine intestine) were tested (see Figure S3) and only porcine intestinal HS binding is shown here. C) Scheme for Competition SPR. D) Oligo-19 inhibits tau–HS binding with an IC_{50} value of 0.9 μM. E) Oligo-20 inhibits tau–HS binding with an IC_{50} value of 4.9 μM. F) Oligo-21 does not inhibits tau–HS binding, with an IC_{50} higher than 700 μM.

of oligo-20 and the lack of inhibition by oligo-21 demonstrates that sulfation is required for the ability of the HS 12-mer to inhibit tau–HS binding, and that 3-*O*-S greatly enhances this inhibition.

Hs3st Knockout Reduces tau Cell Surface Binding and Cellular Uptake

Based on the microarray and SPR data, we hypothesized that 3-*O*-S in HSPGs may play an important role in tau binding to the cell surface and its subsequent internalization. To test this hypothesis, we next carried out tau cell surface binding and cellular uptake assays using a pair of wild-type (WT) and *Hs3st1* knockout (*Hs3st1*$^{-/-}$) mouse lung endothelial cell (MLEC) lines. The selection of *Hs3st1* was based on the expression profiles of HS 3-*O*-sulfotransferases in primary mouse cerebral cortex neurons determined by RNA-seq, with the highest expression level observed for *Hs3st1* among all *Hs3st*s (see Figure S4). The *Hs3st1*$^{-/-}$ MLEC line was derived from the WT parent line using CRISPR-Cas9 gene-editing and expressed normal levels of NS, 6-*O*-S, and 2-*O*-S (see Figure S5A), but reduced level of 3-*O*-S (confirmed by significantly reduced cell surface binding to antithrombin III requiring a 3-*O*-S for binding, see Figure S5B).[25] Biotinylated tau was generated and incubated with cells, followed by washing and detection of surface-bound tau with streptavidin-HRP. Tau bound strongly to the surface of WT MLECs, while the binding was greatly diminished on the *Hs3st1*$^{-/-}$ MLECs surface, showing that 3-*O*-S strongly enhances HS binding of tau on the cell surface (Figure 3A). We next incubated both WT and *Hs3st1*$^{-/-}$ cells with Alexa488-labeled full-length tau (tau-Alexa) for 12 hours, followed by detection with both flow cytometry (Figure 3B) and confocal imagining (Figure 3C) to further investigate the effects of 3-*O*-S deletion on the cellular uptake of tau. Large amounts of tau were internalized into the WT MLECs, but internalization was greatly reduced in the *Hs3st1*$^{-/-}$ MLECs, indicating that 3-*O*-S indeed enhances HSPG-mediated tau internalization. Here, we demonstrate another role for cell-surface 3-*O*-S in tau pathology, in which it specifically recognizes extracellular tau and mediates efficient cellular uptake.

Oligosaccharides with 3-O-S Blocks tau Cell Surface Binding and Internalization

Interfering with the tau–HS interaction using heparin (HP, a highly sulfated analogue of HS) or its mimetics can block tau transcellular spreading in cell culture and animal models.[11] Designing glycan-based compounds to disrupt the tau–HS interface represents a novel strategy to develop effective therapeutics for tauopathy in AD. We asked whether 3-*O*-sulfated oligosaccharides could be more effective at blocking tau cell-surface binding and internalization than counterparts without 3-*O*-S. As expected, HP potently inhibits tau cell-surface binding and internalization (Figure 4). Oligo-19 and oligo-20, but not oligo-21, inhibit tau cell-surface binding and internalization with similar patterns as with HP. Compared with oligo-20, oligo-19 exhibits significantly greater inhibition of the cell surface binding and internalization of tau, underscoring the crucial role of 3-*O*-sulfation for effectively blocking the tau–HS interaction on the cell surface and tau internalization. The addition of 3-*O*-S modification may lead to more potent HS-based therapeutics for tauopathy.

3-O-S is Recognized by tau PRR2 and R2 Regions in NMR Titrations

We next determined which regions of tau are responsible for the recognition of 3-*O*-S in HS. The primary sequence of the longest tau isoform (441 residues) features the N-terminal projection region (N1 and N2), the proline-rich region (PRR1 and PRR2), and the microtubule binding region (MTBR) and the C-terminal region (Figure 5C). The MTBR includes four internal repeat motifs (R1–R4), which mediate tau interactions with MTs[26,27] and other proteins,[28] as well as tau aggregation.[3] We use full-length tau to map the binding sites of 3-*O*-S. Shorter and more accessible HS oligosaccharides, that is, oligo-4 (HS 7-mer with 3-*O*-S) and oligo-5 (HS 7-mer without 3-*O*-S), were used in the experiment. Oligo-4 and oligo-5 were individually added to ^{15}N-labeled tau and the refocused two-dimensional (2D) ^{1}H-^{15}N heteronuclear single quantum coherence (HSQC) NMR spectra of tau were recorded before (blue peaks in Figure 5A) and after the addition of the HS oligosaccharides (green and red peaks in Figure 5A). Significant chemical-shift perturbations (CSPs) in tau were observed upon addition of both oligo-4 (resonance in red) and oligo-5 (resonance in green; Figure 5). As expected, oligo-4 caused much larger CSPs than oligo-5 because of the stronger binding conferred by the 3-*O*-S modification. Several isolated peaks with large CSPs are magnified in Figure 5B. The CSP differences (ΔCSP) between CSPs due to oligo-4 and CSPs due to oligo-5 were plotted against the residue number (Figure 5C) to map the binding site of 3-*O*-S in tau (Figure 5C). Significant ΔCSPs were located at the PRR2 and R2 domains, in which residues V226, L243, and Q276 exhibit the largest ΔCSPs, indicating the interaction between 3-*O*-S and the PRR2 and R2 of tau. The hexapeptide ^{275}VQIINK^{280} in R2, which contributes to tau aggregation and MTs association, was previously identified as the main site of contact with HP.[29,30] PRR regions of tau are not only important for MTs binding,[31] but also hot spots for tau phosphorylation[32,33] and protein interactions.[34,35] The recognition of 3-*O*-S in HS by both PRR2 and R2 suggests HS interaction may modulate both tau aggregation and phosphorylation. The observed CSPs are small, partially because of the lower binding affinity of the HS 7-mer, and low ratio of HS oligos to tau (0.6) used in the NMR mapping experiment. Most ΔCSP values come from a simple increase in the scale of CSPs, not a change in the nature of the CSPs, suggesting 3-*O*-S enhances existing electrostatic interactions, for example, between numerous 6-*O*-S of HS and lysine and arginine side-chains of tau. The ΔCSP is smaller compared to the magnitude of florescence change in the LMHS microarray for oligo-4 and oligo-5. This smaller change is most likely due to an intrinsic difference between chip-based binding and

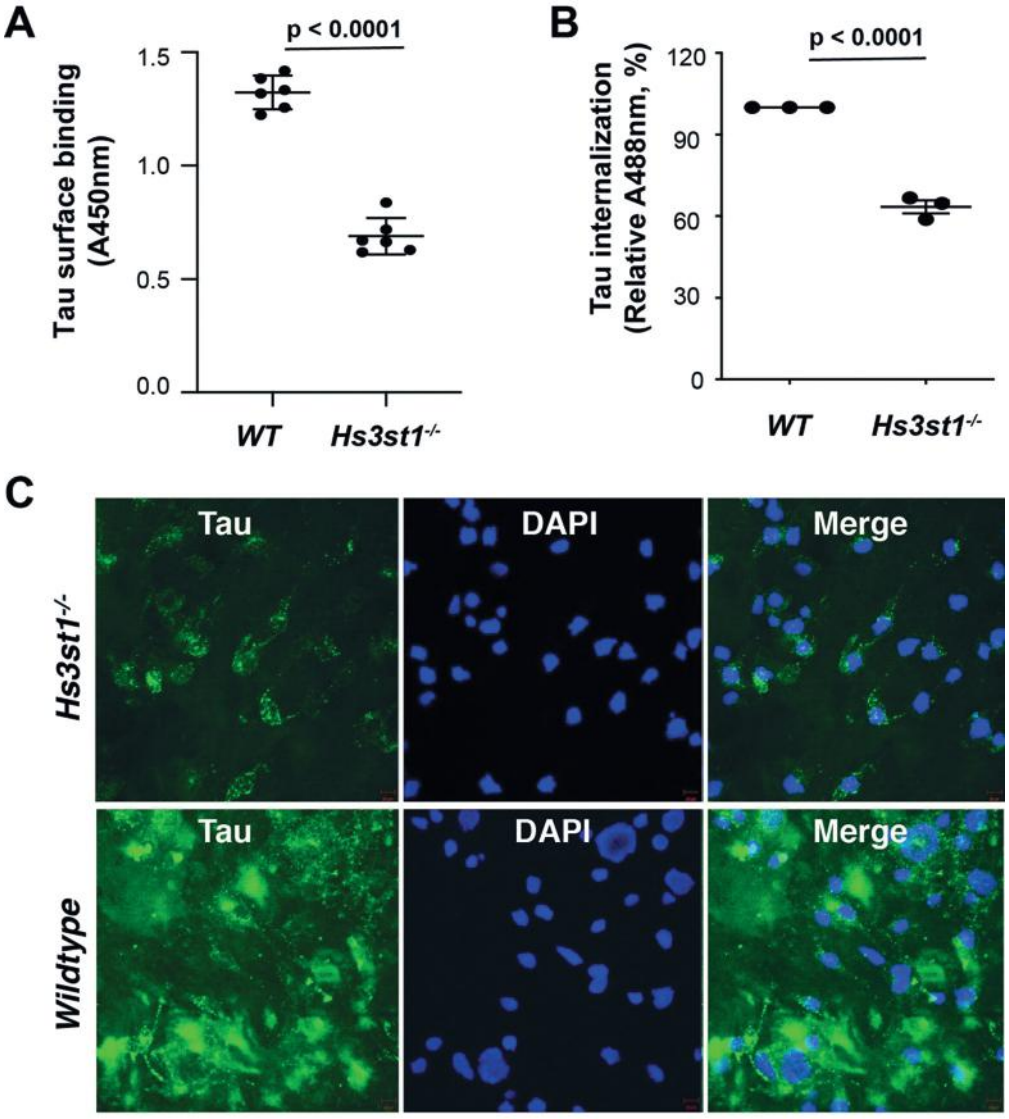

Figure 3. Deletion of *Hs3st1* diminishes tau cell surface binding and internalization. A) The *Hs3st1*$^{-/-}$ cells showed less (46.3% reduction) tau cell-surface binding, compared with WT. After fixing and incubating with biotinylated full-length tau (500 ng/mL, 100 µL/well) for 90 min at RT, the cell surface bound tau was measured after incubating with Streptavidin-HRP and color development. B) The *Hs3st1*$^{-/-}$ cells showed significantly less internalization of tau-Alexa (500 ng/mL) assessed by flow cytometry. C) The *Hs3st1*$^{-/-}$ cells showed significantly less internalization of tau-Alexa by confocal images. The cells in 12-well plate were incubating with tau-Alexa (2 µg/mL, 500 µL/well) at 37°C for 3 h. The data shown are representative of 2–4 independent experiments.

pure solution-phase binding, because the local concentrations of oligos immobilized on the chip may be abnormally high at the chip/solution interface. The association between the 7-mer and tau is likely in fast exchange on the NMR time scale, based on the lack of line broadening and small value of CSP observed in the NMR titration. A full titration of 3-*O*-S sulfated oligosaccharides was not carried out because of the limited supply of pure oligo-19 and oligo-20, and the inability of 7-mers to saturate tau binding sites with its lower affinity. Because of the complicated nature of binding between two highly dynamic molecules, more structural studies are needed for the interaction between tau and a high-affinity 3-*O*-sulfated oligosaccharide.

Growing evidence has established that tau propagates in a "prion-like" manner.[36,37] While the mechanisms underlying the transcellular spread of tau are not completely understood, a required step in this process is tau binding to HSPGs on the recipient cell surface.[11] HS interactions with proteins are mainly driven by electrostatic forces between positively charged side-chains on proteins and negatively charged sulfo groups on HS.[38] Although charge-based association is relatively nonspecific, many HS-binding proteins require specific sulfation patterns in the glycan, for example, the heparin–antithrombin III (ATIII) interaction requiring a pentasaccharide sequence with a 3-*O*-sulfo group in its central residue. In contrast to the less stringent requirements for sulfation patterns reported for α-synuclein and Aβ binding to

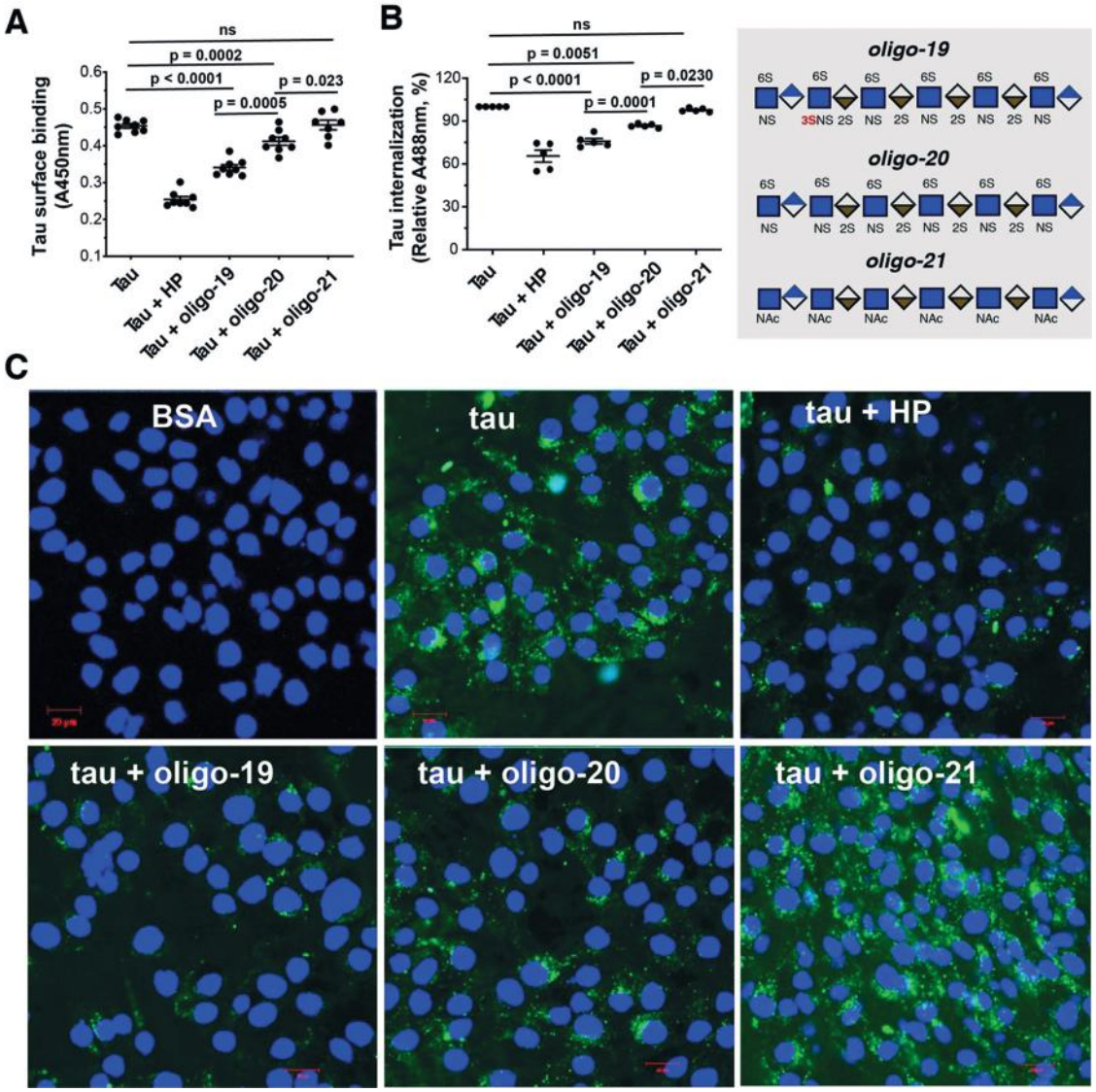

Figure 4. 3-*O*-S modification enhances the inhibitory potency of HS oligo on tau-cell interaction and tau cellular uptake. A) HP, oligo-19, and oligo-20 inhibit tau cell surface binding by 46.3%, 28.0% and 13.0%, respectively. After fixing and incubating with biotinylated tau (500 ng/mL, 100 µL/well) without or with HP (50 ng), HS oligos (25 ng) for 90 mins at RT, the cell surface bound tau was measured after incubating with Streptavidin-HRP and color development. Oligo-19 has a stronger inhibitory potency than oligo-20. Oligo-21 has no inhibition. B) HP, oligo-19, and oligo-20 inhibit tau-Alexa (500 ng/mL) internalization assessed by flow cytometry. C) HP, oligo-19, and oligo-20 inhibit tau internalization assessed by confocal images. The cells were incubated with tau-Alexa (2 µg/mL, 500 µL/well) without or with HP (10 µg/mL), HS oligo (2.5 µg/mL) at 37°C for 3 h. Oligo-19 has a stronger inhibitory potency than olig-20. Olig-21 has no inhibition. The data shown are representative of 2–4 independent experiments.

HS,[14] tau requires more specific sulfate moieties.[13,29,14] In previous work, we were the first to report that 6-*O*-S, but not 2-*O*-S, is required for tau binding, using structurally heterogeneous polysaccharides.[29]

Here, we demonstrate that the 3-*O*-sulfation strongly enhances the tau–HS interaction and cellular uptake of tau, using LMHS microarray, SPR, cellular binding and uptake assays, and NMR studies. The structurally defined HS 7-mer and 12-mer, with an additional 3-*O*-S, exhibited significantly stronger binding to tau in the LMHS array (Figure 2). This stronger binding was then confirmed in SPR competition assays showing that an HS 12-mer with one additional 3-*O*-S (oligo-19) inhibits tau–HS interaction with about a fivefold lower IC_{50} value than the same HS 12-mer without 3-*O*-S (oligo-20; Figure 2). The reduced cell-surface binding and internalization of tau in *Hs3st1*$^{-/-}$ cells indicates that 3-*O*-sulfation significantly enhances the cellular uptake of tau (Figure 3). These data conclusively demonstrate that 3-*O*-S modification plays a crucial role in the tau–HS interaction and tau cellular uptake. Our data provide a mechanistic rationale for the recent observation that the expression of *Hs3st2* and *Hs3st4* is elevated in AD brain and that HS containing GAGs isolated from AD brain exhibit enhanced tau binding.[21]

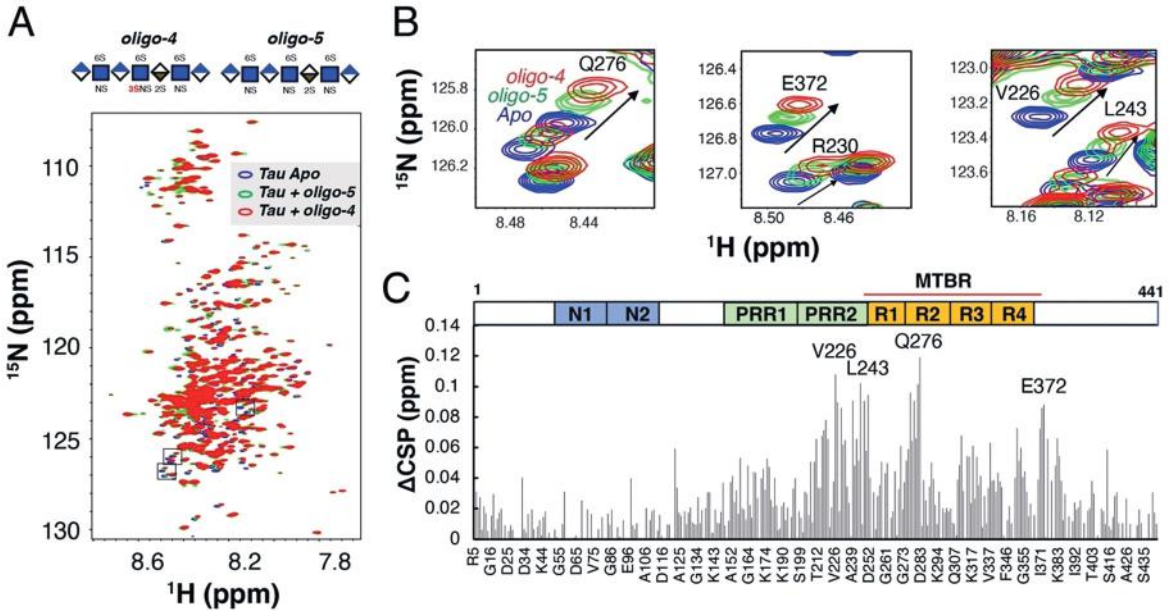

Figure 5. Chemical-shift perturbation difference (ΔCSP) reveals specific interactions between 3-*O*-S and PRR2 and R2 domain of full-length tau. A) Overlay of ^{1}H-^{15}N HSQC spectra of full-length tau before (blue) and after 1:0.6 molar ratio addition of HS 7-mer oligo-5 (green) and HS 7-mer oligo-4 (red). B) Magnified NMR spectra of residues with biggest CSPs. C) CSP differences (ΔCSP) reveals specific interaction between 3-*O*-S and tau PRR2 and R2 domain. Construct of tau is shown above the figure. PRR = proline-rich region, MTBR = microtubule binding region.

To date, tau is only the seventh protein shown to specifically recognize 3-*O*-S in HS.[39] The Heparin–ATIII interaction has been the prime example of the specific interaction mediated by 3-*O*-S. Interestingly, 3-*O*-S also facilitates cellular entry of the Herpes simplex virus (HSV-1), which has been linked to AD.[40–42] 3-*O*-S enhances the HS interaction with viral envelope glycoprotein D (gD).[43,44] Thus, both Herpes virus and tau entry into a cell are enhanced by the 3-*O*-S functional group, raising the possibility of mechanistic crosstalk between the spread of tau pathology and Herpes infection in the brains of AD patients.

By establishing the critical role of the rare 3-*O*-S HS modification in tau–HS interactions, we provide one of the most important insights for developing HS-based therapies against the spread of tauopathy: to efficiently inhibit cellular uptake of tau, a 3-*O*-sulfo group is required. In this work, efficient inhibition of the tau–HS interaction has been achieved with a HS 12-mer containing 3-*O*-S (oligo-19), with an IC_{50} value of 0.9 μM, in a SPR competition assay (Figure 2D). Significant inhibition of cellular binding and uptake of tau was also observed (Figure 4) by the same oligo-19. Based on these data, we propose that the 3-*O*-S and tau interface represents a novel target for disease-modifying therapy to block tau transcellular propagation in AD. HS-based therapeutics targeting the tau/HS interface have the additional challenge of crossing the blood brain barrier (BBB) because of their high hydrophilicity. Very recently, highly sulfated HS oligosaccharides were reported to penetrate the hippocampal BBB in a murine sepsis model.[45] This report suggests that HS oligosaccharides or analogues may be able to target the AD hippocampus, which also has increased BBB permeability. Prodrugs may be another viable approach to enhance BBB permeability, wherein a more hydrophobic precursor molecule is processed to an active compound after crossing BBB.

As 3-*O*-sulfotransferases are overexpressed in the AD brain,[21] inhibiting the expression or activity of 3-*O*-sulfotransferases may represent another avenue for inhibiting the propagation of NFT pathology. Indiscriminate inhibition of *Hs3st* may result in significant side-effects because 3-*O*-sulfation is crucial for multiple physiological interactions, such as heparin interaction with ATIII in coagulation, and HS interaction with fibroblast growth factors (FGFs)[46] in cell growth and propagation. However, there are seven isoforms of human *Hs3st*, among which *Hs3st2* and *Hs3st4*[47,48] are specifically expressed in the brain. In addition, *Hs3st2* and *Hs3st4* are overexpressed in the brains of AD patients,[21,49] raising the possibility that they may be specifically targeted to avoid some nonspecific effects. Although reducing the 3-o-sulfation level will not completely inhibit tau uptake, a significant delay of the spread of tau pathology and dementia can still have strong impact in patients' quality of life and have important social and economic benefits. For example, it is estimated that delaying the onset of dementia by only five years can decrease the prevalence of AD and associated medical costs by about a whopping 40 %.[50]

NMR mapping shows 3-*O*-S (Figure 5C) preferably binds to the PRR2 and R2 domain of full-length tau, crucial regions for aggregation,[51] MTs association,[31,52] and interaction with heparin[29,53] and other proteins.[34,35] 6-*O*-S also binds to the R2 domain as previously studied. Taken together, we suspect there may be a synergistic effect between 3-*O*-S and 6-*O*-S that enhances the binding of HS to tau. Similarly, in the ATIII–heparin interaction both 3-*O*-S and 6-*O*-S modifications are critical for inducing the conformational change in ATIII[54] needed for anticoagulant activity of heparin. Unlike

ATIII, tau is an intrinsically disordered protein (IDP) without a fixed 3D structure, rendering it a more challenging system for conventional structural characterization. More work is needed to delineate the specific HS motifs (the combination of chain length, monosaccharide composition and precise sulfation pattern) required for tight binding to tau in the human brain and in AD, and to understand the structural basis of the specific interactions between 3-o-S and tau residues at atomic resolution.

In the brain, tau uptake is complicated and its mechanism depends on cell type. For example, a therapeutic tau-antibodies promoted tau uptake in microglial cells while blocking uptake in neurons.[55] In addition to HSPG-mediated micropinocytosis, the propagation of tau pathology likely involves other mechanisms such as exosome fusion,[56] receptor-mediated endocytosis,[57] phagocytosis,[58] and nanotubes.[59] More studies in CNS cell types such as neurons and glial cells are needed to further establish the role of 3-*O*-S in tau uptake in AD.

Conclusion

In summary, our results demonstrate the key role of 3-*O*-S in the tau–HS interaction and cellular uptake of tau, uncovering a unique structural requirement of HS recognition by tau. This work represents a major step forward in our understanding of the mechanism of the tau–HS interaction, with important implications for 3-*O*-S as a pharmacophore targeting the spread of tau pathology in the development of effective AD therapy.

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Conflict of interest

The authors declare no conflict of interest.

Keywords: Alzheimer's disease · cell surfaces · electrostatic interactions · heparan sulfate · proteins

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