Heparan sulfate proteoglycans as possible diagnostic molecular tools with therapeutic potential in Alzheimer´s disease

Iván Fernández-Vega*, Laura Lorente-Gea, Carla Martín, Luis M. Quirós

Importance of dementia and Alzheimer's disease (AD): On Earth, there is a "country" that we call *Dementia* with around 50 million inhabitants and an estimated global economic cost of US $1 trillion (Wimo et al., 2017). AD may contribute to 60–70% of cases, with other major forms of dementia being vascular dementia, dementia with Lewy bodies and a group of diseases that contribute to frontotemporal dementia. However, the boundaries between different forms of dementia are indistinct and mixed forms often co-exist, which are only confirmed after postmortem neuropathological studies (Fernandez-Vega et al., 2015).

Alzheimer's disease is a debilitating neurodegenerative disorder with three cardinal histopathologic changes: beta-amyloid protein (Aβ) deposits in the form of neuritic plaques (NPs), cerebral amyloid angiopathy, which presents as microvascular plaques of Aβ and neurofibrillary tangles (NFTs). Tau protein becomes hyperphosphorylated and is therefore unable to bind to microtubules, which results in the production of paired helical filaments, a building unit of NFTs. After more than a century since the first diagnosis of AD was made, there is still no treatment available to cure AD or to alter the progressive deterioration of patients. The current diagnosis of AD is based principally on the assessment of a patient's cognitive state and behavior, supported by molecular tools to quantify biomarkers (Aβ peptides, neurogranin, polymorphic aggregated Aβ species, Tau aggregates, hyperphosphorylated Tau, amyloid precursor protein, and monocyte chemoattractant protein-1) in blood and cerebrospinal fluid. However, despite the great amount of research effort and resources focused on the development of molecular tools and techniques for the identification and quantification of new biomarkers, this has resulted in few meaningful changes in routine clinical diagnosis or treatment in the last few decades. As such, it seems time to change the research focus towards new possible therapeutic targets and molecular pathways such as heparan sulfate proteoglycans (HSPGs) and their catalytic enzyme heparanase (HPSE).

Heparan sulfate proteoglycans: The proteoglycans are a family of complex macromolecules consisting of a protein core to which several heparan sulfate (HS) glycosaminoglycan (GAG) chains are covalently attached. The functions of HSPGs ultimately rely on the fine structure of these chains. The cell surface HSPGs are membrane-spanning syndecans (SDCs) and lipid-anchored glypicans (GPCs) and the matrix-associated HSPGs are agrin (AGRN), collagen type XVIII (COL18A1) and perlecan (PRCAN), and they are also present in the secretory vesicles, i.e., serglycin (SRGN). The binding sites for many different ligands, such as growth factors, enzymes, cytokines, chemokines, and extracellular matrix, are defined by specific sets of variably modified disaccharides. Because of these interactions, HS is also involved in many physiological functions and pathologies including amyloid diseases, inflammation, infectious diseases, cancer and neurodegeneration (García et al., 2014; Vega IF et al., 2014).

Previous studies have found the variable expression of four distinct classes of PGs and GAGs associated with NFTs, NPs and cerebral amyloid angiopathy. Keratan sulfate proteoglycans, dermatan sulfate proteoglycans, and chondroitin sulfate are found only in the periphery of senile plaques (non-specific to AD), whereas HSPGs are associated with all three types of AD lesion, suggesting that this latter class has an important role in the formation and persistence of lesions (van Horssen et al., 2003).

Heparan sulfate proteoglycans and heparaneses in AD: Substantial scientific evidence exists that highlights the important roles played by HSPGs in the etiology of AD. HSPGs have been co-localized with both hyperphosphorylated Tau in NFTs and Aβ in NPs. Furthermore, previous work has suggested that HSPGs may promote Aβ or Tau fibrillization and described HS-Aβ interaction as mutually protective, i.e., Aβ is protected from protease degradation and HS is protected from the action of HPSE, an endo-D-glucuronidase that cleaves specific linkages in the structure of HS (van Horssen et al., 2003). Other published data have pointed out that the HS-Aβ interaction contributes to every stage of pathogenesis in AD, including production, clearance, accumulation, aggregation, and the toxic action of Aβ (van Horssen et al., 2002; Cui et al., 2013), while work over the last decade indicates that Tau can propagate aggregates between cells in a prion-like manner mediated by HSPGs (Holmes and Diamond, 2014).

We have previously observed the overexpression of *HPSE* and its natural competitive inhibitor heparanase 2 (HPSE2) in neurodegenerated areas of AD brains, closely correlated with the Braak & Braak stage (Garcia et al., 2017). In our most recent published work, we focused on the analysis of the expression levels of the 17 genes that encode the core proteins of HSPGs at different stages of AD using RT-PCR, after which we localized certain significant proteins in the tissue using immunohistochemistry (Lorente-Gea et al., 2020). The most interesting finding was the consistent overexpression of *SDC4* found in every brain area in samples from all AD stages (mild, moderate and severe). Immunohistochemistry indicated that the presence of SDC4 was mainly related to Tau pathology and Aβ deposits in all three types of AD lesion. SDC4 was demonstrated to be involved in endocytosis but very little is known about the real function of SDC4 in AD. However, a recently published work by Marija Usenovic et al. using *in vitro* techniques with human IPSC neurons suggested SDC4's involvement in Tau cellular internalization, and they postulated the use of SDC4 as a novel therapeutic strategy to inhibit the spread of tau pathology (Usenovic et al., 2018). As part of our research, we also noted a significant overexpression of *SRGN* in almost all areas of AD brains and in all types of AD lesion. In this light, considering that SRGN is the only intracellular HSPG, this molecule could play a central role in AD stabilization and progression throughout the 3-O-sulfated domains in HS chains. In addition, we also described the presence in AD of other major alterations in expression levels of HSPGs such as *SDC1*, *GPC4*, *GPC6* and two "part-time" HSPGs (which can vary depending on source and growth conditions and may or may not have a GAG chain composed of HS), *CD44v3* and *Neuropilin* 1 (NRP1). These latter two molecules were also consistently underexpressed in every area from samples of mild AD (**Figure 1**). Regarding extracellular matrix HSPGs, most changes were observed in the limbic area, particularly the transentorhinal region and the hippocampus, as has previously been reported. AGRN and COL18A1 were also found to be involved in all three types of AD lesion. Although some articles have pointed to the presence of PRCAN in NPs, NFTs, and amyloid-laden vessels, others, including this study, have been unable to confirm these observations using monoclonal antibodies against the core protein of PRCAN. Overall, HSPG core proteins suffer precise alterations in their levels of expression (overexpression of *SDC4+SRGN* and underexpression of *CD44v3+NRP1*) that could be implemented as biomarkers to better diagnosis suspected cases of AD either on cerebrospinal fluid or blood samples. However, further studies should be performed to check the specificity of these alterations to AD and rule out their occurrence in other brain diseases as well. That said, these results may well point to a new research line in the search for an alternative therapeutic strategy against AD.

HSPGs and HPSEs play a crucial role in AD pathogenesis. While HPSE activity may help to halt the progression of the disease by breaking down Aβ-HS deposits or blocking Tau intracellular fibril formation and propagation, HPSE2 appears to act as an inhibitor of HPSE. This blocking of HPSE by HPSE2 may interfere with its hypothetic beneficial role in stopping AD pathogenesis (Lorente-Gea et al., 2017). In this scenario, it is possible that the lack of progress in AD investigation may be a consequence of the underestimation of the structural and molecular roles of HS molecules in AD pathogenesis. In this regard, considering that HS-Aβ interaction contributes to every stage of Aβ pathogenesis in AD, it seems reasonable to hypothesize that interfering in the HS-Aβ interaction could have multiple beneficial effects. In this vein, in our latest

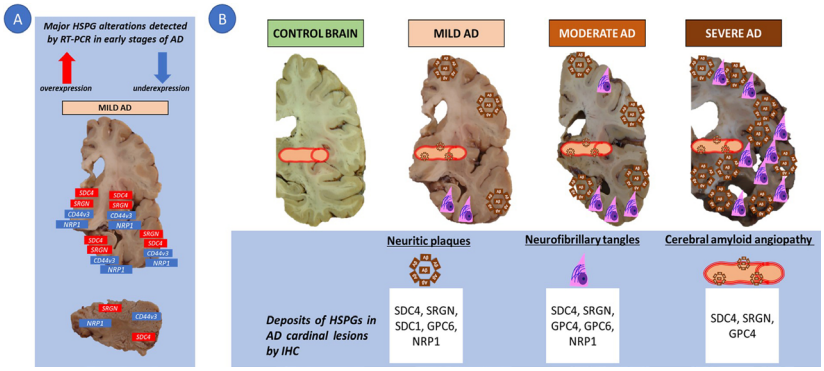

Figure 1 | **Schematic representation of the principle alterations of HSPG core proteins in AD.**
(A) Significant HSPG molecular modifications detected by RT-PCR in early stages of AD. (B) HSPG core proteins co-localized in neuritic plaques, neurofibrillary tangles and cerebral amyloid angiopathy. AD: Alzheimer's disease; GPC: glypican; HSPG: heparan sulfate proteoglycans; NRP1: neuropilin 1; RT-PCR: reverse transcription-polymerase chain reaction; SDC: syndecan; SRGN: serglycin.

work we were able to identify SDC4 and SRGN as the HSPGs molecules that were most often altered in every stage of AD. Although further research is needed in this field, the information above could lead to the development of novel insights into AD pathogenesis and may lead to the development of innovative therapeutic strategies.

For example, HS-protein interactions and specific motifs on HS chains are emerging as potential therapeutic targets in AD. To this end, we briefly describe here some of the most promising therapeutic findings using HSPGs against AD. In experiments using zebrafish, the role of HS has been targeted through two different approaches. HS antagonists (surfen and oxalyl surfen) have been identified as efficient therapeutic candidates for 3-O-sulfated HS and 6-O-sulfated HS motifs in tau pathology, known to be critical for tau seed internalization (Alavi Naini and Soussi-Yanicostas, 2018). On the other hand, targeting pathological tau proteins via immunotherapy also appears to be a promising strategy for the disease-modifying treatment of AD. To this effect, the administration of exogenous antibodies (passive immunotherapy) such as DC8E8 against the microtubule binding domain containing DC8E8 epitopes and HSPGs on the neuron surface, has shown in animals to block cell-to-cell propagation of tau by preventing the neuronal internalization of extracellular tau, (Weisova et al., 2019). Specific clinical trials are ongoing using both these approaches.

As outlined above, HSPG and HPSEs play significant and multifactorial roles in the pathogenesis of AD. Additional studies, however, remain to be performed to further clarify the roles of SDC4, SRGN and many other HSPGs involved in AD pathology. Although specific HSPGs have been immunohistochemically localized in the principle histological lesions of AD, their biochemical isolation from these structures, which would allow for the identification of the number of GAG chains and the extent and position of GAG sulfation, has yet to be performed. This determination of the chemical structure of HSPG is a necessary step toward a complete understanding of the roles of HSPG in the etiology of AD. It may also facilitate the discovery of possible alterations in HSPG metabolism in AD and resolve apparent discrepancies in the immunohistochemical localization of HSPG core proteins in cardinal histopathological lesions in AD.

Conclusion: Specific alterations in HSPGs and HPSEs have been described in recent years that support the idea that these molecules play a crucial role in AD pathogenesis. However, a deeper understanding of the chemical structure of HSPGs would assist in the development of rational, targeted therapeutic strategies to combat AD, the most frequent neurodegenerative disease. Preliminary studies are currently ongoing in our laboratory concerning the biochemical determination of the chemical structure of HSPGs in order to better understand their roles in AD pathogenesis. In conclusion, we have shown briefly here several novel insights concerning HSPGs in AD pathogenesis that might lead to the development of additional diagnostic biomarkers and strengthen new innovative therapeutic strategies.

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