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Grant Title: Regulation of amyloid clearance by heparan sulfate remodeling

Abstract:
Protein aggregation diseases represent one of the most compelling research subjects both in protein chemistry and molecular medicine. Amyloid known as tissue deposits of protein aggregates causes protein aggregation diseases. Amyloid in tissues is cleared by innate immune phagocytes such as microglia in the brain and macrophages in the peripheral tissues. Conversely, impairment of the cellular clearance leads to excess of deposits and disease progression. Alzheimer's disease (AD) is a brain-type protein aggregation disease. A multitude of studies have shown that heparan sulfate (HS) interacts with aggregation-prone peptides, including Amyloid ß (Aß) and tau in AD, and coexists as a non-protein component in deposits of protein aggregates in AD brains (1). HS in amyloid deposits is involved in inhibition of cellular clearance of amyloid by forming a protective shield around them. We previously showed that S-domains, highly sulfated domains of HS composed of IdoA2S-GlcNS6S units, are accumulated in tissue amyloid of model mice and patients with AD. We also showed that removal of the sulfate group at position 6 of S-domains by Sulf2, an extracellular sulfatase, facilitated microglial clearance of amyloid *ex vivo*. The aim of this study is to evaluate mitigation effects of Sulf2 on AD brain pathogenesis *in vivo.*

Sulf2 conditional transgenic mouse models that allow cell type-specific expression of the human Sulf2 gene were generated. These mice were bred with an activated microglia-specific Cre-driver and then crossbred with J20 AD model mice (2). We currently analyze the degree of amyloid deposits and AD pathogenesis in the brain of these mice with age. Microglial clearance of Aß amyloid may be facilitated by the Sulf2 action in AD brain. On the other hand, we utilized chemically synthesized 6-*O*-phosphorylated HS oligosaccharide derivatives to analyze possible roles of C-6 sulfate groups of the S-domains in Aβ aggregation (3). We tested them for assays of Aβ fibril formation. Compounds 30 and 38 showed inhibitory effects on Aβ fibril formation in the presence of heparin, a structural analogue of HS S-domains, as measured by the ThT fluorescence intensities and studied by atomic force microscopy (3). Upon aggregation the transition of an unstructured state to a β-sheet structure can create sulfated GAG-binding sites. Compounds 30 and 38 may bind to these conformers and interfere with the subsequent elongation process. These small sulfated and phosphorylated HS glycans may be utilized in vivo with AD models in future challenge. The transgenic model mice would also aide to investigate potential roles of post-translational modifications of Sulf2 (4) in its biological functions *in vivo*. The Sulf2 project will be extended to study prion-like behavior of p53 aggregates (5).

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