Principal Investigator: Herbert M. Geller
Grant Title: Novel Glycosaminoglycan Binding to Type IIa Protein Phosphatase

**PROGRESS REPORT for Mizutani Foundation Research Grant**

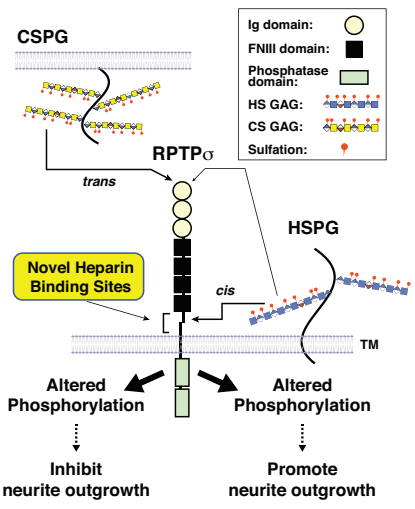
**Reference Number:** 17-0116
**Principal Investigator:** Herbert M. Geller
**Organization:** National Heart, Lung, and Blood Institute
**Period: From** April 2017 to March 2018
**Grant Title:** Novel Glycosaminoglycan Binding to Type IIa Protein Phosphatases


## Abstract

Emerging evidence suggests that proteoglycans are independent signaling molecules, binding directly to transmembrane receptors to exert distinct biological effects. In the nervous system, the glycosaminoglycan (GAG) chains of proteoglycans bind to members of the Type IIa receptor protein tyrosine phosphatases (RPTPσ/RPTPδ/LAR). RPTPσ is composed of two cytoplasmic phosphatase domains and extracellular immunoglobulin (Ig) domains followed by fibronectin type III (FNIII) repeats (Figure). Previous mutagenesis and structural studies have shown that a GAG-binding site for both heparan sulfate (HS) and chondroitin sulfate (CS) lies in the first Ig domain of RPTPσ and comprises an extended positively charged surface of basic residues (Lys- and Arg-loop). This has led to the conundrum that both HS and CS bind to the same site but produce opposite actions: HS promotes axonal growth and CS impedes the growth of axons.

We have investigated whether the complex formation between full length RPTPσ and GAGs impacts the receptor's enzymatic activity under physiological conditions. When 293 cells expressing wild-type RPTPσ were treated with heparin, a mimic of HS, the level of Tyr phosphorylation (pTyr) was transiently upregulated. Intriguingly, the increase in pTyr by heparin was found even after disruption of the Lys/Arg loop (ΔLys mutation) whereas CS-E-induced upregulation was substantially reduced by the mutation. Solid-phase binding assays with the extracellular domain (ECD) of RPTPσ showed the presence of multiple binding sites for heparin on RPTPσ. The fact that heparin binding upregulated pTyr independently of Lys/Arg loop binding site on RPTPσ led us to identify novel heparin binding sites on the receptor.

To further characterize the novel binding site(s), a "heparin protection assay" was performed to identify potential amino acids, followed by mutagenesis of these amino acids together with heparin affinity chromatography. This allowed the identification of 2 novel heparin-binding sites located in the juxtamembrane domain (Figure). Disruption of all 3 heparin-binding sites (one in the 1st Ig domain and two in the juxtamembrane domain) completely eliminated the binding to heparin.




One question that arises here is whether these novel heparin-binding sites are involved in the altered phosphorylation upon heparin binding. When the heparin-binding deficient mutant was expressed on 293 cells, we observed very high level of baseline

pTyr without heparin. Addition of heparin did not alter the level of phosphorylation. This implies that elimination of all heparin binding sites on RPTPσ caused the dysregulation of pTyr within the cells. Thus, we propose a model for differential interactions of HS and CS GAG chains with RPTPσ (Figure). HSPGs on the same cell surface interact in *cis* with RPTPσ through the novel binding sites and regulate the enzymatic activity of RPTPσ. When CSPGs are presented in *trans*, the binding is through the first Ig domain and triggers the changes in phosphorylation of distinct sets of proteins within the cells, resulting in the recognition of CS as inhibitory cues by RPTPσ. Our discovery of new heparin binding sites near the transmembrane domain of RP provides new insights into GAG signaling.