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Title: Functional synapse formation by heparan sulfate/autism-linked protein binding

Abstract

Heparan sulfate (HS) regulates diverse cell-surface signaling events and is critical for development of the nervous system. A growing body of evidence revealed genetic association between HS synthesis genes and autism, a heterogeneous cognitive syndrome mainly characterized by impaired social interaction. We have recently reported impairment in glutamatergic synapse activity and the full range of autism-like symptoms in the postnatal neuron-specific HS deficient mice (Irie *et al*., 2012). Although these findings strongly suggest physiological significance of HS in normal brain functioning and implication of its deficiency in autism, molecular mechanism underlying HS-mediated functional synapse development remains unclear. Recently, we made a substantial progress toward addressing this issue: neuroligins (NLGNs), autism-linked proteins, are associated with synaptic HS. In this study, we characterized the biochemical properties of interaction between heparin/HS and NLGNs, and determined physiological significance of HS in NLGN-mediated synapse development.

First, we characterized sulfation on HS involved in the binding to NLGNs. Binding assay was performed by co-precipitation between recombinant NLGN1 and gllypican-1 carrying HS modified with various sulfotransferases. HS modified with *6O*- and *3O*-sulfations exhibited significant binding, while *N*- and *2O*-sulfated HS was not bound to NLGN1. Among *3O*-sulfotransferases, HS3ST5 showed the highest activity for generation of HS binding to NLGN1. Since it was reported that *HS3ST5* gene is associated with autism (Wang *et al.,* 2009), *3O*-sulfation on HS may play an important role in the regulation of NLGN functions at synapses and cognitive function of brain.

For NLGN-mediated synapse development, primary hippocampal neurons were transfected with wild type or HS-binding mutant NLGN1, followed by immunostaining of glutamatergic synapse marker, AMPA-type glutamate receptors. As previously reported, overexpression of wild type NLGN1 in primary neurons promoted glutamatergic synapse development such as increased synaptic expression of AMPA glutamate receptors. However, NLGN1 with mutations at basic amino acids critical for binding to heparin/HS did not elevate synaptic expression of AMPA receptors, suggesting essential role of HS in NLGN-mediated glutamatergic synapse development. This study has provided the first evidence showing implication of HS-protein interaction in synaptic function and will offer a novel avenue for the possible therapeutic application.

Irie F, Badie-Mahdavi H, Yamaguchi Y (2012) "Autism-like socio-communicative deficits and stereotypies in mice lacking heparan sulfate" *Proc. Natl. Acad. Sci. USA* **109,** 5052-5056