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Session 2 "Biosynthesis, Structure, and Degradation-2"

Functions of Chondroitin Sulfate/Dermatan Sulfate in the Brain Development: Critical Roles of E and/or iE Disaccharide Units Recognized by a Specific Antibody

Kazuyuki Sugahara

Proteoglycan Signaling and Therapeutics, Faculty of Advanced Life Science, Graduate School of Life Science, Hokkaido University, Japan



1971 – BA in Pharm. Sci.,
Fac. of Pharm. Sci., Kyoto
Univ.

1973 – MS in Biochem.,
Fac. of Pharm. Sci., Kyoto
Univ.

1976 – Ph. D in Biochem.,
Fac. of Pharm. Sci., Kyoto

Univ.

1976-1982 – Research Associate, Dept of Pediatrics
and Biochem, Univ. of Chicago

1982-1990 – Instructor, Dept of Biol. Chem., Fac. of
Pharm. Sci., Kyoto Univ.

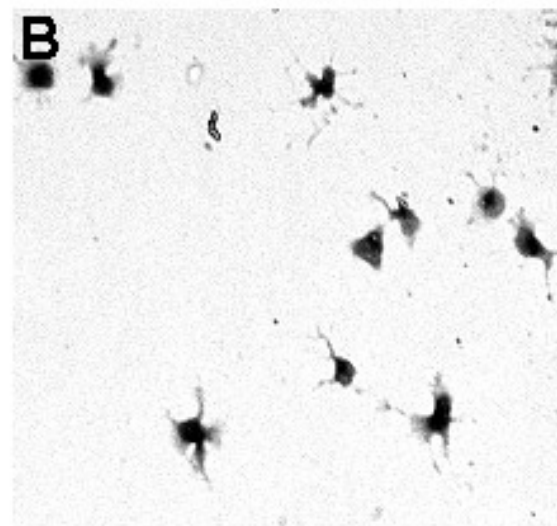
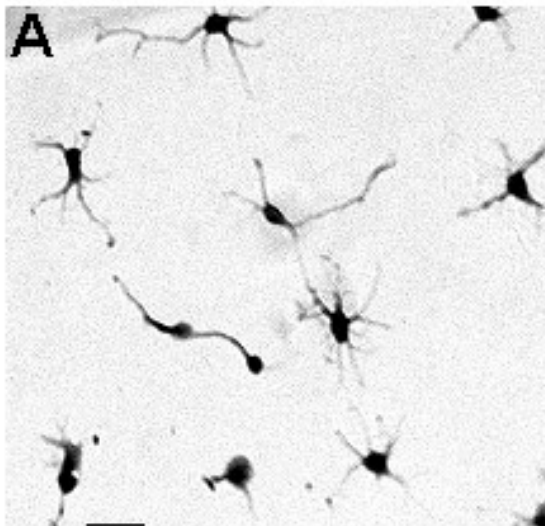
1990-1993 – Associate Professor, Dept of Physiol.
Chem., Kobe Women's Coll. of Pharmacy

1993-1994 – Professor, Dept of Biochem., Kobe
Women's Coll. of Pharmacy

1994-2006 – Professor, Dept of Biochem, Kobe
Pharmaceutical Univ.

2006 – Professor, Graduate School of Life Sci., Hok-
kaido Univ.

Figure: Inhibitory effects of a single chain antibody GD3G7 on the neurite outgrowth of hippocampal neurons cultured on CS-E-coated substratum. Hippocampal neurons cultured on the CS-E-coated substratum in the absence (A) or presence (B) of the single chain antibody GD3G7.



Chondroitin sulfate (CS) and dermatan sulfate (DS) chains have been implicated in the processes of neural development in the brain such as neuronal adhesion, migration and neurite formation. Variation in the sulfation of CS/DS chains regulates the central nervous system's development. Previously, we have demonstrated that CS/DS chains can act as neuritogenic molecules. In this study, we focused on the functional characterization of developmentally regulated brain CS/DS chains detected by a single chain antibody, GD3G7, produced by the phage display technique. *In situ* hybridization for the expression of *N*-acetylgalactosamine-4-sulfate 6-*O*-sulfotransferase (GalNAc4S-6ST) in the postnatal mouse brain, which is involved in the biosynthesis of CS/DS-E, showed a widespread expression of the transcript in the developing brain except at postnatal day 7 (P7), where strong expression was observed in the external granule cell layer in the cerebellum. The expression in the cerebellum switched from the external layer to the internal granule cell layer with development. Evaluation of the specificity of GD3G7 towards various glycosaminoglycan preparations showed

that this antibody specifically reacted with squid CS-E [rich in the GlcUA β 1 \rightarrow 3GalNAc(4,6-*O*-sulfate) disaccharide unit E], hagfish CS-H [rich in the IdoUA α 1 \rightarrow 3GalNAc(4,6-*O*-sulfate) unit iE] and shark skin DS (rich in both E and iE units). Interestingly, iE units produced by GalNAc4S-6ST from iA units, contained in pig skin DS, were also recognized specifically by GD3G7, confirming the specificity to both E and iE units. Immunohistochemical localization of GD3G7 in the mouse brain showed that the epitope was specifically expressed in the cerebellum, hippocampus and olfactory bulb. GD3G7 suppressed the growth of neurites in embryonic hippocampal neurons mediated by CS-E, suggesting that the epitope is embedded in the neurite outgrowth-promoting motif of CS-E. In addition, a CS-E deca-saccharide fraction was found to be the critical minimal structure needed for recognition by GD3G7. Four discrete deca-saccharide epitopic sequences were identified. The antibody GD3G7 has broad applications in investigations of CS/DS chains during the central nervous system's development and under various pathological conditions.

Keywords : monoclonal antibody, phage display antibody, chondroitin sulfate, dermatan sulfate, brain development, neurite, oligosaccharides