Soutenance

THESE

Vendredi 30 Novembre 2018 à 14h

Salle des séminaires

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Characterisation of semaphorin 3A-chondroitin sulphate interaction in the central nervous system

Thèse de Doctorat de l'Université de Grenoble

Perineuronal nets (PNNs) are the key regulators of neuronal plasticity and regeneration in the mature central nervous system (CNS). They are a unique and highly organised extracellular matrix (ECM) structure, found around sub-population of neurons, composed mainly of chondroitin sulphate proteoglycan (CSPG). Chondroitin sulphate (CS) is a linear polysaccharide belonging to glycosaminoglycans (GAGs) family. The sulphation pattern defines different types of CS, which interact with different signalling proteins including those regulating axonal outgrowth and guidance such as semaphorin 3A (Sema3A). Sema3A is a secreted chemorepulsive protein found accumulated in the PNNs through its interaction with CS. This process is believed to potentiate Sema3A signalling through plexin A1 (PlxnA1) and Neuropilin 1 (Nrp1) and regulate plasticity and regeneration. The aim of the thesis project is to characterise the interface of Sema3A- CS interaction.

For this purpose, Sema3A is expressed in eukaryote cells and purified. Interestingly, two major forms were obtained: a full length Sema3A (90 kDa) which remains attached to the cell surface GAGs and a truncated form without the C-ter part (65 kDa) which is released to the culture medium. With the use of surface plasmon resonance (SPR), we observed that full length Sema3A binds selectively to CS-E (4,6-di-sulphated chondroitin) and heparan sulphate with a high affinity (KD in the sub pM range), while the truncated Sema3A does not bind to any GAG. Four putative GAG binding sequences were identified in the C-ter of Sema3A and mutated using site directed mutagenesis. SPR analysis then revealed that two out of these four sites are required for the binding to CS-E. The importance of these GAG-binding sequences in inhibition of neurites outgrowth of dorsal root ganglion neurons in culture was also reported, indicating thus the importance of GAG-binding in Sema3A signalling. In parallel, the minimal required sequence of Sema3A-binding of CS-E was determined as being a tetrasaccharide. The Sema3A-CS interface was thus characterized. Furthermore, quartz crystal microbalance with dissipation monitoring analysis suggested that Sema3A could crosslink GAG chains. This suggests Sema3A could be involved in stabilising the PNN network and induces mechanical changes on neuronal surface.

The detail characterization of Sema3A-CS interaction may enable the design of new strategies aiming at enhancing plasticity and regeneration for neurodegenerative diseases or spinal cord injury.