Regulation of neuronal functions by interaction of tenascin-R with its neural receptors

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Regulation of Neuronal Functions by Interaction of Tenascin-R with Its Neural Receptors

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1. Summary

The growth of axons is dependent upon its microenvironment in which is present the balance between both effects of the promotion and inhibitory molecules. Tenascin-R is a brain-derived inhibitory molecule which is composed of modules of repeated units including epidermal growth factor-like (EGF-L) repeats, fibronectin type III (FN) homologous repeats, and a fibrinogen-like domain (FG). My thesis work is focused on the research of neuronal functions of the interaction of tenascin-R with its receptors or associated proteins in order to better understand axonogenesis and neuronal regeneration.

As a first step, my research was focused on identifying distinct domains of tenascin-R using recombinant fragments that may related to neuronal cell functions. The results indicated that 1) neurites and growth cones are strongly repelled from areas coated with fragments of EGF-L domains, and the FG domain, which can be recognized by monoclonal antibodies 620 and 619, and those effects can be blocked by these two antibodies, respectively; 2) EGF-L prevents neurite outgrowth altogether. Polyclonal antibodies against tenascin-R and F3, and H7, a PKC inhibitor can block this effect; 3) F3-transfected CHO cells are repelled by tenascin-R and EGF-L when coated as substrates, which also can be blocked by tenascin-R and F3 polyclonal antibodies, and staurosprin, a PKC inhibitor. 4) The binding site of F3 is localized to EGF-L; 5) Morphological polarization of hippocampal neurons is exclusively associated with FG; 6) Other domains of TN-R also show repulsive activity for growth cones, but not stronger than EGF-L and FG. These results further suggest the existence of multiple neuronal tenascin-R receptors which influence the response of neurons to their extracellular matrix environments.

Recently, I tried to isolate new receptors or associated proteins of tenascin-R from brain, using affinity chromatography made with diferent tenascin-R domains. From these approaches, two molecules were isolated: 1) XL1, a 190 kDa tenascin-R binding protein with the properties of promoting neurite outgrowth, was isolated from the soluble part of brain; 2) Xprocan, a receptor protein tyrosine phosphatase ζ/β related to phosphacan, a chondroitin sulfate proteoglycan, with the properties of blocking the inhibitory effects of tenascin-R on neurons, isolated from mouse brain membranes. Xprocan is developmentally colocalized with tenascin-R in CNS. Interestingly, the results of hippocampal neuron culture demonstrated that Xprocan alone prevents neurite outgrowth, but the complex of Xprocan and tenascin-R promotes neurite outgrowth. These results further suggest that tenascin-R, the inhibitory molecule with mosaic arrangement provides a framework for multiple recognition sites that are able to confer positive and negative modes of interaction with other recognition molecules. These

interactions between the molecules in the complex may provide a microenvironment for determining the promotion or inhibition of neurite outgrowth.

More recently, another interesting finding from my research is the axonal defasciculation involved by the interaction between tenascin-R and F3. Polyclonal antibodies against tenascin-R and F3, 620 monoclonal antibody and polyclonal antibodies against the EGF-L domains of tenascin-R, antisense oligonucleotide of tenascin-R, and staurosprine can induce neurite fasciculation from cerebellar microexplant culture, which can be blocked by using PMA or TPA, PKC activators. This is the first report about defasciculation induced by the growth inhibitory molecule, tenascin-R and its receptor, F3.

Very recently, MAG was found to be bound to tenascin-R by overlay assays and ELISA, and to block repulsive effects of tenascin-R on growth cones. Interestingly, MAG alone has an inhibitory effect on neurite outgrowth, but the complex of MAG and tenascin-R promotes neurite outgrowth on the E17 to E18 hippocampal neuron culture. This complex also repels both cell bodies and growth cones from both P0 and P14 DRG neurons. Furthermore, tenascin-R induces the repulsion of MAG-transfected CHO cells, which suggest that MAG has the potential to transduce signals from tenascin-R to the interior of the cell. These observations indicate that MAG is a functional tenascin-R associated protein and their interactions exert either positive or negative influences on the different neuronal cell types during development of the CNS.