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FINAL NARRATIVE REPORT

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SPINAL CORD RESEARCH

Original aims of the project

(1) To investigate whether Ca^{2+} signaling through calcineurin phosphatase (CaN) and protein phosphatase 1 (PP1) mediates inhibitory actions of MAG, NogoA, and OMgp of CNS axons.

(2) To test that inhibitor-1 is the key regulator for PP1 activity underlying myelin inhibition of axons and is phosphorylated and dephosphorylated by PKA and CaN, respectively.

(3) To achieve several training goals during the fulfillment of the project, including

a) To get familiar with the whole process in a research cycle, from proposal preparation, reasonable fund allocation to individual tasks, making progress report, making adjustment to original aims, data collection, manuscript writing and paper submission, to project conclusion.

b) To develop the skills and habits required for preparing competitive research project grants in the future, those include extensive reading, critical thinking, routine discussion with advisor and colleagues, to name just a few.

c) To enhance my knowledge in the area of spinal cord repair and axon regeneration & guidance in central nervous system.

d) To acquire new experimental skills and techniques, e.g. establishing cell culture models and biochemical assay for phosphatases' activities. These techniques will eventually be made routine in my current lab.

Project successes

(1) In *Xenopus* spinal neurons, we successfully found that the growth cone repulsion induced by MAG (one of the major inhibitory myelin components) involves PP1 activation, and the deprivation of endogenous I-1 blocked the effect. To our knowledge, this is the first report about the role of PP1 and I-1 in MAG signaling. Given the fact that I-1 is the link

growth cones when treated with myelin inhibitors (MAG).

(7) To pursue the second aim in our proposal, we successfully constructed several DNA vectors to express proteins exogenously in primary neuronal cultures and cell lines in both wild type and mutant forms, such as wild type I-1, DARPP-32 and their T35A mutants. RNA interference, through siRNAs or plasmid transfection, was successfully used to knock down protein expressions.

(8) During the fulfillment of the project, training goals were also achieved, such as knowledge buildup in the area of spinal cord repair and axon regeneration, and acquisition of new experimental skills and techniques.

Project challenges and Implications for future research

(1) Growth cones are tiny structures of neurons, which make the intracellular Ca^{2+} measurements hard to do comparing to regular cellular measurements. Numerous works has been done to optimize various factors in imaging in order to achieve maximal sensitivity. We're finally able to detect Ca^{2+} changes in growth cones, however, the magnitudes were small. Furthermore, analyses of these Ca^{2+} were difficult and time consuming because growth cones tended to change their morphology constantly and quickly. Now we are trying different imaging softwares to make analysis accurate and efficient.

(2) Although our data strongly suggest that inhibitor-1 (I-1), a key inhibitor of PP1, is robust to modulate myelin inhibitors-induced axonal guidance, their effects in axonal growth and growth cone collapse were not supported by our current data which derived from I-1 expression and knockdown experiments. One possible reason is that our Western blot results showed that both I-1 and DRAPP-32 are expressed in significant amount in rat cerebellar and DRG neurons, while our previous data suggested that I-1 is the predominant form in *Xenopus* spinal neurons (Han et al., 2007). Dopamine- and cAMP-regulated phosphoprotein of Mr 32 kDa (DARPP-32) and I-1 are the two best-characterized PKA-activated inhibitors of PP1 and co-expressed in many cell types. Importantly, I-1 and DARPP-32 exhibit extensive sequence homology within the N-terminal 40 amino acids, which include PP1 binding motif and PKA-phosphorylation

site, and are functionally complement to some degree. Now we are repeating some experiments in the absence of I-1 and DARPP-32 simultaneously.

Publication and Meeting Abstract

Han, J., L. Han, P. Tiwari, Z. Wen, and J.Q. Zheng. 2007. Spatial targeting of type II protein kinase A to filopodia mediates the regulation of growth cone guidance by cAMP. *J Cell Biol.* 176:101-11. (cover story)

Han J and J.Q. Zheng. 2008. A kinase anchoring proteins in growth cone spatial signaling. EMBO Workshop: Semaphorin Functions and Mechanisms of Action. Abbaye des Vaulx de Cernay, France.