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FINAL REPORT

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3. Grant Title:	**Roles of ephrin-A5 in spinal cord development**
4. Grant Number:	05B-013-SCR1
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1. Original Aims of the Project:

Our long-term objective is to identify axon guidance cues that contribute to the construction of functional spinal cord neural circuits. We hypothesize that ephrin-A5 expressed in the dorsal gray matter functions to prevent ventral spinal cord axons from projecting to the dorsal gray matter, and thus playing critical roles in the organization of the spinal cord circuits. We have proposed experiments with the following specific aims: (1) to examine expression of ephrin-A5 and EphA5 during critical time periods when spinal cord axons are being organized into the peripheral axon tracks, away from the gray matter; (2) to study effects of disruption of ephrin-A5 and EphA5 interaction on neural architecture of the spinal cord in vitro and analyze effects of inactivation of ephrin-A5 and EphA5 genes in knockout mice.

2. Project Successes:

Elucidation of spatial and temporal patterns of Ephrin-A5 and EphA5 expression in the developing spinal cord. We have completed the studies of the analysis of ephrin-A5 and EphA5 expression during spinal cord development. Ephrin-A5 expression was assessed using two different affinity probes, EphA5-Fc, and EphA5-AP, which bind to the ligand. Ephrin-A5 knockout mouse spinal cord sections were used as controls. EphA5 expression was analyzed using beta-galactosidase knockin mice, and affinity ligand probe binding using ephrin-A5-AP. 7 different developmental stages (E11, E13, E15, E17, P0, P7 and 1 month) were analyzed. We conclude that EphA5 is expressed in the ventral spinal cord, while ephrin-A5 is located in the dorsolateral regions of the spinal cord throughout development. These results show that EphA5 and ephrin-A5 are expressed over broad developmental stages and may play important roles in establishing the dorsoventral organization of the spinal cord. These studies have now been published (Washburn et al., 2007).

Signal transduction pathways that mediate ephrin-A5 biological function. We have expanded the original aim and studied signal transduction mechanisms underlying ephrin-A5 function. These studies were performed using hippocampal neurons because of the need of a more uniform population and large amount of material for biochemical analysis. We have shown previously that the ventral spinal cord neurons and the hippocampal neurons respond to ephrin-A5 in a similar fashion. Thus, information obtained from the hippocampal neurons should be applicable to the spinal cord neurons.

In this analysis, effects of Eph receptor activation by ephrin-A5 on several important signal transduction pathways are examined. In addition, the roles of these pathways in ephrin-A5-induced growth cone collapse were assessed with a combination of biochemical analyses, pharmacological inhibition, and overexpression of dominant-negative and constitutively active mutants. These analyses showed that ephrin-A5 inhibits Erk activity while activates c-Jun N-terminal kinase. However, regulation of these two pathways is not required for ephrin-A5-induced growth cone collapse in hippocampal neurons. Artificial Erk activation by expression of constitutively active Mek1 and B-Raf failed to block ephrin-A5 effects on growth cones, and inhibitors of the

Erk pathway also failed to inhibit collapse by ephrin-A5. Inhibition of JNK had no effects on ephrin-A5-induced growth cone collapse either. In addition, inhibitors to PKA and PI3-K showed no effects on ephrin-A5-induced growth cone collapse. However, pharmacological blockade of phosphotyrosine phosphatase activity, the Src family kinases, cGMP-dependent protein kinase, and myosin light chain kinase significantly inhibited ephrin-A5-induced growth cone collapse. These observations indicate that only a subset of signal transduction pathways is required for ephrin-A5-induced growth cone collapse. These results have now been summarized in a recent publication (Yue et al., 2008).

Furthermore, the funding contributed to two other related studies, which have been submitted for publication (Hu et al., 2008; Shi et al., 2008).

3. Project Challenges:

Although we completed studies of the temporal and spatial expression of both EphA5 and ephrin-A5 during spinal cord development and elucidated key signal transduction pathway mechanisms, we failed to detect consistent spinal cord-related motor deficiencies. One possible explanation is that other ephrins and Eph receptors can compensate for loss of function of ephrin-A5 or EphA5 in regulating spinal cord neural circuitry. It is known that EphA3 and EphA4 are also expressed during spinal cord development, and they may compensate for the loss of EphA5 receptor function. Ephrin-A5 and EphA5 may also have other functions. For example, mediating formation of sensory circuits in the spinal cord. Little is known in this area.

4. Implications for future research and/or clinical treatment:

The high levels of expression of ephrin-A5 and EphA5 suggest critical roles in spinal cord development. Future research needs to take into consideration other Eph receptors and ligands that are also expressed during spinal cord development. The implication of participation of multiple ephrins in spinal cord function suggest an approach that blocks function of all ephrins may be necessary for therapeutic interventions.

Along this line of reasoning, our signal transduction pathway analysis provides several downstream pathways that can serve as therapeutic targets. These include the Src kinases, cGMP-dependent kinase, and myosin light chain kinase. Since redundant Eph receptors are likely to use the same downstream signling pathways, targeting the these pathways may overcome the difficulty of molecular redundancy and enhance therapeutic efficacy.

5. Plans to continue this research, including applications submitted to other sources for ongoing support:

Grants to NIH and NSF to further study roles of Eph/ephrins are being planned.

6. List of publications:
Published papers:

Washburn CP, Cooper MA, Zhou R. Expression of the tyrosine kinase receptor EphA5 and its ligand ephrin-A5 during mouse spinal cord development. Neurosci Bull. 2007 Sep;23(5):249-55.

Yue X, Dreyfus C, Kong TA, Zhou R. A subset of signal transduction pathways is required for hippocampal growth cone collapse induced by ephrin-A5. Dev Neurobiol. 2008 Jun 18;68(10):1269-1286.

Submitted:

Hu T, Shi G, Larose L, Rivera GM, Mayer BJ, Zhou R. Regulation of process retraction and cell migrtion by EphA3 is mediated by the adaptor protein Nck1. Submitted to Biochemistry.

Shi G, Yue G, Sheng M, Chen S, and Zhou R. EphA3 function is regulated by multiple phosphotyrosine residues. Submitted to Journal of Biological Chemistry.

Copies of published papers and manuscripts attached: 4.