Neuronal plasticity after spinal cord injury: identification of a gene cluster driving neurite outgrowth

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SPECIFIC AIMS

The first goal of our study was to identify a cluster of coregulated genes involved in neuronal and axonal plasticity in vivo after spinal cord injury (SCI). We used microarray analysis to identify a subset of genes involved in neuronal plasticity whose expression patterns were temporally coregulated and correlated to functional recovery after spinal cord injury. This cluster of genes may be involved in neurite outgrowth and regeneration after SCI.

A second aim was to characterize mRNA and protein expression at the single cell level in spinal cords. To identify expression of these transcripts and proteins at the single cell level in spinal cord, we performed 1) laser microdissection experiments in single neurons and in situ hybridization to detect mRNA expression of our transcripts and 2) immunofluorescence experiments with specific antibodies for our proteins of interest coincubated with neuronal, glial, and coregulated specific markers.

We also sought to verify whether overexpression of gene cluster members in neuronal cultures induces neurite outgrowth and if their effect is synergistic. We performed overexpression experiments using plasmid DNAs of our cluster genes in dorsal root ganglia neurons to verify whether their single and combined induction enhances neurite outgrowth.

PRINCIPAL FINDINGS

1. After experimentally induced trauma to the spinal cord in rats, we identified a cluster of genes/proteins

Included were neuritin, attractin, Mtap1a, and MOG (implicated in neuronal plasticity and myelination), which showed reduced expression at 24 h followed by coordinated recovery at 7–14 days after injury (Fig. 1), a period correlating with recovery of motor function.

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2. Both mRNAs and proteins were down-regulated at 24 h postinjury in neurons and axons in the injured group as opposed to shams. Recovering to normal levels by 14 day, these proteins were colocalized in neurons and were coexpressed in neurons and fibers that expressed the preregeneration marker GAP-43.

3. We demonstrated that coordinated expression of neuritin, membrane attractin, and MAP1A-LC2 are synergistic in promoting neurite outgrowth in DRG neurons (Fig. 2).

CONCLUSIONS AND SIGNIFICANCE

We believe that the systematic and coordinated use of microarray analysis with subsequent confirmation of protein behavior provides a useful and reliable methodology for identifying targets for therapeutic intervention aimed at enhancing regeneration. We speculate that the identified gene cluster may behave at the single cell level in a coordinated and sequential manner: neuritin triggers initial neurite development as well as axonal, dendritic, and synaptic plasticity; attractin helps track developing processes and may interact with Mtap1a, thus enhancing cytoskeleton rearrangements appropriate for neurite extension, while MOG may provide support for subsequent myelination.

![Figure 2](image2.png)

**Figure 2.** Overexpression of neuritin, membrane attractin, and MAP 1a LC2 in DRG neurons promotes neurite outgrowth. Bar graphs show neurite length expressed as percentage of control in DRG cells after transfection measuring the length of the longest neurite per cell (T test, **P < 0.01, ***P < 0.001).