Identification of molecules that promote re-growth of injured nerve fibers by laser microdissection of *in vivo* regenerating spinal neurons

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A novel combination of cutting-edge strategies was used to identify molecules that promote re-growth of injured CNS nerve fibers. First, we induced injured spinal cord neurons to regenerate nerve fibers by implantation of a growth-permissive bridge (made of Schwann cells) into the completely transected spinal cord of anesthetized adult rats. Next, we used a “laser capture microscope” to dissect thousands of individual spinal neurons that regenerated nerve fibers into this transplant. We also dissected a similar number of non-regenerating spinal neurons. We then used “GeneChip technology” to determine what molecules these regenerating neurons were making relative to the non-regenerating neurons: we identified 552 such molecules. In separate follow-on experiments, to identify which of these 552 molecules might be potent therapies for increasing nerve fiber re-growth, we increased the level of each of these molecules individually in injured neurons grown in miniature Petri dishes. To simulate the environment of the injured spinal cord (which inhibits nerve fiber growth), we pre-coated these Petri dishes with growth inhibitory molecules including “chondroitin sulphate proteoglycans” and “myelin-associated glycoprotein”. Using this model system, we have discovered a novel RAG which, when its levels are increased in neurons, resulted in lengthy nerve fibers even in the presence of different growth-inhibitory molecules. We have also discovered other RAGs which, when their levels are reduced in neuronal-like cells, also resulted in lengthy nerve fibers. Together, these are new, potential targets for promoting axon regeneration after spinal cord injury.

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