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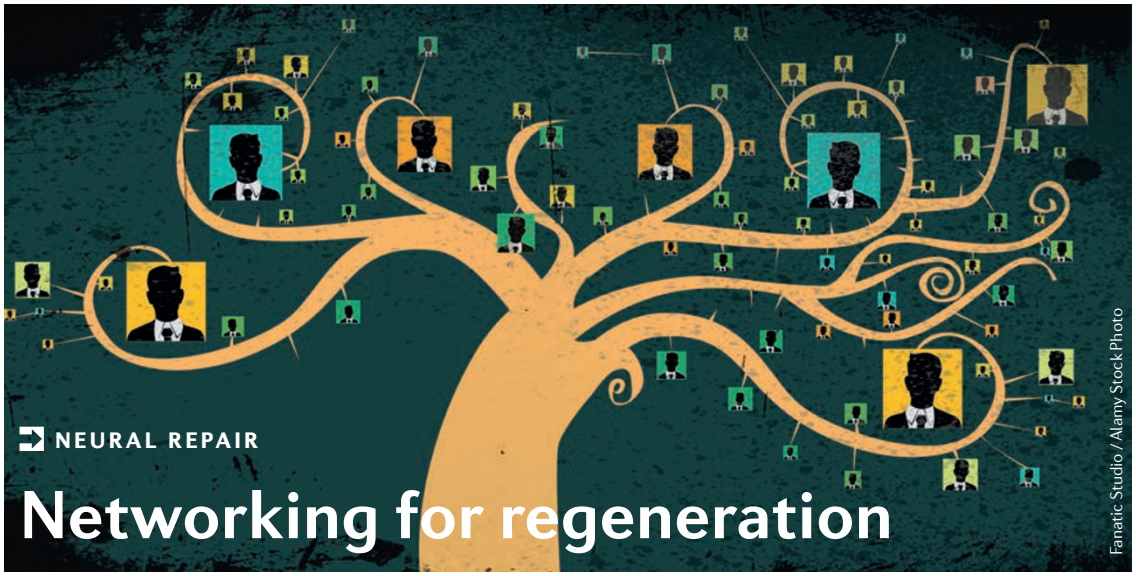

Peripheral nervous system (PNS) neurons have an enhanced regenerative capacity compared to their CNS counterparts, and it is thought that cell-intrinsic factors contribute to this. Indeed, it has been proposed that regeneration requires the coordinated transcriptional regulation of a large molecular network. In a collaborative effort that combines bioinformatic and experimental approaches with network analysis, Chandran *et al.* have now dissected the molecular 'growth program' that operates in PNS neurons after injury.

Previous studies have identified individual regeneration-associated genes (RAGs) that are regulated by injury. Here, the authors measured genome-wide changes in gene expression in dorsal root ganglion (DRG) neurons at several time points after PNS injury. By analysing combined datasets generated using different injury models in separate laboratories, they identified five 'co-expression modules', each consisting of genes that exhibit similar patterns of injury-induced changes in expression and that have related biological roles or are components of similar functional pathways. These co-expression relationships were replicated in separate microarray datasets relating to PNS injury, but not in those derived from CNS-injury models. Furthermore, the authors showed that a number of previously unknown candidate RAGs that were identified by their analysis could modulate neuronal outgrowth when overexpressed or knocked down in DRG neurons *in vitro*.

These findings suggest that PNS regrowth involves the synchronous regulation of expression of a defined set of genes. To determine the mechanisms responsible for this coordination, the authors examined transcription factor (TF) binding sites present in the putative RAGs. They identified 39 TFs whose binding sites were enriched in the promoters of genes within one or more RAG co-expression modules, including several that were previously linked to neuronal injury responses. Two of these TFs, activating transcription factor 3 (ATF3) and JUN, induced neurite outgrowth when overexpressed individually in DRG neurons *in vitro*, and synergistically enhanced outgrowth when overexpressed together.

Next, the authors sought to identify the protein-signalling pathways associated with the putative RAGs. Using existing independently generated interaction data, the authors constructed a protein–protein interaction (PPI) network. This network contained 280 core genes (or 'nodes') and was enriched for several signalling pathways proposed to be involved in neuronal regeneration. Many of the TFs whose binding sites were enriched in the RAG co-expression modules turned out to be highly connected (hub) nodes within the PPI network. The authors propose that these hub TFs may connect and coordinate different injury or growth-associated signalling pathways. Indeed, the authors' analysis of independently published data confirmed the co-expression of several hub TFs after PNS (but not CNS) injury.

These findings suggest that modulating the expression of genes within the PPI network in a coordinated manner might enhance neuronal regeneration. The authors used the expression profiles of the genes within the RAG modules to screen a database of drug-induced gene expression profiles for candidate small molecules that are likely to recapitulate the RAG expression profile. They showed that one candidate molecule identified by this approach, ambroxol, altered the expression of eight candidate genes (including five hub TFs) from the PPI network in DRG neurons. Furthermore, when administered to mice after a CNS (optic nerve) crush injury, there was a modest increase in axon regeneration in comparison to that in control mice.

This comprehensive analysis of the gene networks regulated during PNS axon regrowth suggests that manipulations designed to modulate the expression of core RAGs in a coordinated manner may represent a valid therapeutic strategy to induce regrowth in the injured CNS.

Katherine Whalley

> "these hub TFs may connect and coordinate different injury or growth-associated signalling pathways"

ORIGINAL ARTICLE Chandran, V. *et al.* A systems-level analysis of the peripheral nerve intrinsic axonal growth program. *Neuron* http://dx.doi.org/10.1016/j.neuron.2016.01.034 (2016)