RHO ISOFORM-SELECTIVE MODULATION OF AXON GROWTH

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Cytoskeleton activity regulates different aspects of neuron shape change and surface structure remodeling. Small GTPases of the rho family activate intracellular signaling networks leading to cytoskeleton dynamics. The usage of constitutive active (CA) and dominant negative (DN) isoforms showed a complex hierarchical interplay between the different rho GTPases. However, in neurons such a hierarchy is less clear, as shown by the opposite effects of Rac1 and CDC42 versus RhoA on neurite extension. Overall, little is known about the role of rho-dependent signaling events in the context of primary neuron axon growth and guidance.

To better clarify this issue, we investigated the role of CDC42, Rac1 on neuronal development by using well characterized L61 CA mutants, in which a second F37A or Y40C mutation was introduced to confer selectivity in downstream signaling delivery (Lamarche et al., 1996). To focus the analysis on primary neuronal cells, we adopted a Trojan approach by fusing the various rho mutants to a TAT trojan sequence.

The capability of TAT fusion proteins to rapidly and efficiently accumulate in primary neuron was tested with a TAT-GFP fusion protein. Following, we treated cortical neurons plated for 1 day with different concentrations of TAT-Rac1 and TAT-Cdc42 (CA, DN forms and double mutants L61F37A and L61Y40C), either alone or in combination. Upon fixing and staining, neurites were measured by semiautomatic software tracing and normalized to the data obtained in neurons treated with TAT-wild type proteins.

The Rac1-L61F37A, but not the Y40C, double mutant induced a 20% increase of axon length. Rac1 DN mutant was toxic after 24-48 hours of treatment, confirming a pro-survival role of Rac1, already described in literature. Rac-L61Y40C induced a reduction of axonal ramification. The Cdc42 L61F37A double mutant increased axonal branching, whereas the L61Y40C triggered a reduction of dendrite number and length. The DN form of Cdc42 gave a strong reduction of neurite growth along with reduction of cell viability. When we combined the different Rac1 and CDC42 mutants we confirmed that only Rac1 activation is responsible for the increase of axon length. Moreover, combining together Rac-L61F37A and Cdc42-L61Y40C or Rac-L61Y40C and Cdc42-L61F37A had no effect, suggesting that the pathways activated by the two different mutants counter react each other.

In conclusion our data strongly suggest a selective role of Rac1 and Cdc42 on axonal growth and branching, respectively. Further analysis of single and combined treatments on neuronal phenotype, together with biochemical analysis, will allow improving our knowledge on the complex interplay between Rac1 and Cdc42 in neuron development.