siRNA screen of microglia to identify neuroprotective drug targets in Parkinson´s disease

Mario Delgado, Marta Pedreño, Elena González-Rey, Veronika E. Neubrand

IPBLN - CSIC, Cell Biology and Immunology, Armilla - Granada, Spain

Question: Neuroinflammation is a fundamental process contributing to the death of dopaminergic neurons in Parkinson´s Disease (PD). During this process, activated microglia secrete cytotoxic substances which lead to neuronal death. Therefore, we are looking for the molecular mechanism that reverses the inflammatory activation of microglia, since this knowledge would be essential to protect from neurodegeneration.

Methods and Results: Very interestingly our previous data (Neubrand et al., 2014) indicate that adipose derived mesenchymal stem cells (ASCs) exert important anti-inflammatory actions on microglia. We observed that microglia exposed to ASCs or their secreted factors (conditioned medium, CM) underwent a dramatic cell shape change into a highly elongated morphology (Fig 1A), similar to the phenotype of microglia observed in a healthy brain. The elongation induced by ASCs was associated with a decrease of the pro-inflammatory cytokine TNFalpha (Fig 1B) as well as with an upregulation of neurotrophic factors. Thus, ASC stimulated microglia represent an ideal tool to study the intracellular events necessary for the transition from inflammatory activated to non-inflammatory neuroprotective microglia. In this way we have already identified the small RhoGTPases Rac1 and Cdc42, which are important regulators of the actin cytoskeleton, as essential molecules in this transition (Fig 1C).

Since these molecules represent possible drug targets to induce the reversion of neurotoxic microglia to neuroprotective ones, we are currently performing an siRNA screen to identify the molecular players of this ASC-induced reversion. Because this transition is easily detectable by light microscopy (see Figs 1A and C) and changes in the cell shape are intrinsically related to changes of the cytoskeleton, we are carrying out a microscopy-based screen of the major cytoskeletal regulators. In addition, we are including in the screen the regulators of microglia-specific activation/inflammatory pathways as siRNA targets.

Conclusion: Our project is the first siRNA screen performed in primary microglia and we aim to identify a list of molecules that are specifically implicated in the reversion from activated to neuroprotective microglia. Since positive hits would represent potential neuroprotective drug targets, the outcome of this screen opens up a variety of novel investigation lines and therapies in PD or other neurodegenerative diseases.

Figure legend

Figure 1A: Microglia underwent a dramatic cell shape change when treated with ASC CM, even in the presence of the inflammatory bacterial endotoxin lipopolysaccharide (LPS). Bars = 10 μm.

Figure 1B: Gene expression of the inflammatory cytokine TNFalpha was quantified by qRT-PCR. Mean +/-SEM. $*p<0.05$. $**p<0.001$.

Figure 1C: GFP as negative control and the dominant negative (DN) mutants of the small RhoGTPases Rac1 and Cdc42 were transfected into microglia and quantified with the form factor (right panel). Bars = 10 μm. Mean +/-SEM. $*p<0.05$. $**p<0.001$.

Figure 1