Microscopy-based siRNA screen of microglia to identify neuroprotective drug targets

Veronika Neubrand, Mario Delgado

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

Neuroinflammation is a fundamental process contributing to the death of neurons in neurodegenerative diseases, such as Parkinson´s (PD) or Alzheimer´s disease (AD). During this process, activated microglia secrete cytotoxic substances which lead to neuronal death (1). 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. Our previous data (2) 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 (3). 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 (2). 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. Exploiting these anti-inflammatory properties of ASCs we set up a microscopy-based siRNA screen (Fig. 1C), identifying its hits by cell morphology (see Fig. 1A). In this light, we searched for molecules that inhibited the anti-inflammatory ASC-induced phenotype and thus are involved in the transition from neurotoxic microglia to neuroprotective ones. As changes in the cell shape are intrinsically related to changes of the cytoskeleton, we carried out the screen with the major cytoskeletal regulators. In addition, we included regulators of microglia-specific activation/inflammatory pathways as siRNA targets. Our project is the first siRNA screen performed in primary microglia and we have identified a list of molecules that are specifically implicated in the reversion from activated to neuroprotective microglia. Since our positive hits represent potential neuroprotective drug targets, the outcome of this screen opens up a variety of novel investigation lines and therapies in PD, AD or other neurodegenerative diseases.

Acknowledgement

This work was supported by a Michael J Fox Foundation research grant to VN and MD.

References

1. Block, Zecca, Hong (2007). Microglia-mediated neurotoxicity: uncovering the molecular mechanisms. Nat Rev Neurosci 8:57-69

2. Neubrand, Pedreño, Forte-Lago, Delgado, Gonzalez-Rey (2014). Mesenchymal stem cells induce the ramification of microglia via the small RhoGTPases Cdc42 and Rac1. Glia 62:1932-1942

3. Hanisch, Kettenmann (2007). Microglia: active sensor and versatile effector cells in the normal and pathologic brain. Nat Neurosci 10:1387-1394

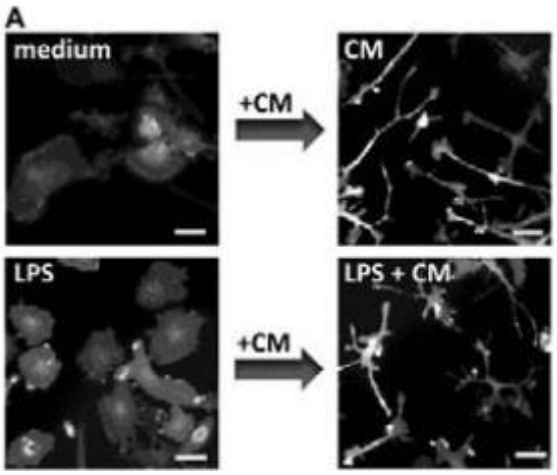
Figure legends

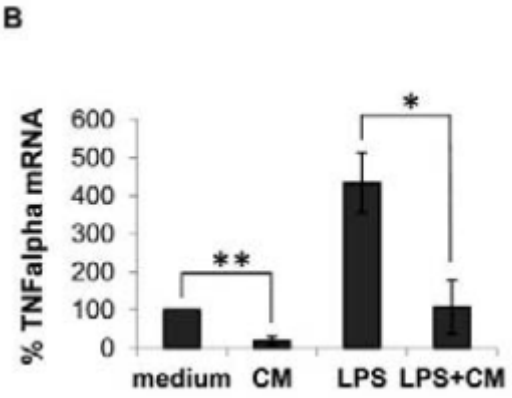
Figure 1A: Microglia underwent a dramatic cell shape change when treated with ASC CM, even in the presence of the inflammatory bacterial endotoxin lipopolysaccharide (LPS). Bars = 10 μm.

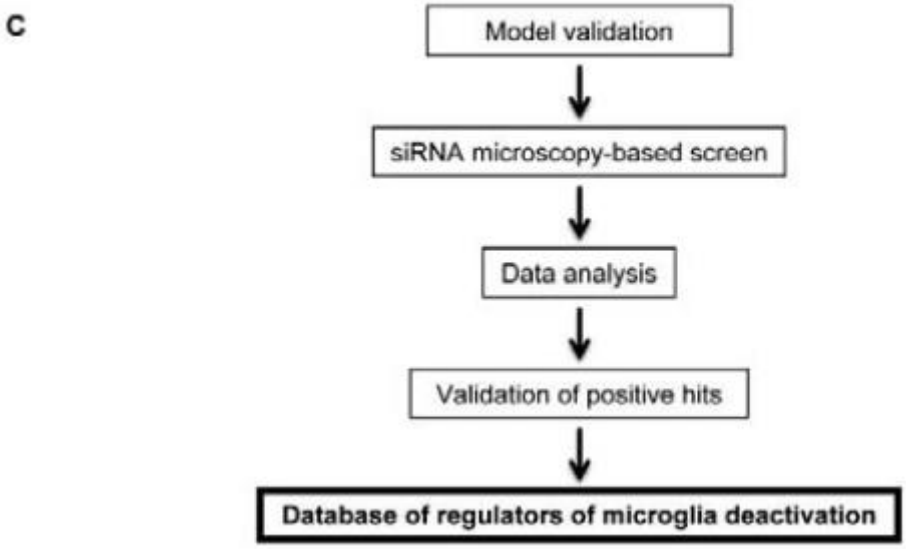
Figure 1B: Gene expression of the inflammatory cytokine TNFalpha was quantified by qRT-PCR. Mean +/-SEM. *$p<0.05$. **$p<0.001$.

Figure 1C: Flow-chart of the microscopy-based siRNA screen with the final objective in bold.

Figure 1


A
medium
+CM
CM
LPS
+CM
LPS + CM


B
% TNFalpha mRNA
600
500
400
300
200
100
0
**
*
medium
CM
LPS
LPS+CM


C
Model validation
siRNA microscopy-based screen
Data analysis
Validation of positive hits
Database of regulators of microglia deactivation