PROJECT 1

DoS: Roberto Furlan

Title: Microglial microvesicles as therapeutic vector for neuroinflammation

Curriculum: Neuroscience and Experimental Neurology

Link to OSR/UniSR personal page:

Project description (*Number of characters*, *including spaces*: 2.000 - 3.000):

Microglia is considered a privileged and difficult target for the progressive, more degenerative phases of multiple sclerosis. The inability to target efficiently microglia is considered one of the reasons of the therapeutic failure of several drugs tested in progressive MS. Recent evidence suggest that microglia communicates through the release of microvesicles to neighboring cells to modulate their phenotype and function. Further, it has been recently shown that peripheral injection of engineered microvesicles can specifically target neural cells. We have evidence that microglia-derived microvesicles are preferentially up-taken by other microglia or by neurons. Thus, microglial microvesicles appear a promising drug-delivery tool to specifically target microglia and neurons with anti-inflammatory or neuro-protective molecules. To develop microglial microvesicles as a CNS-specific drug-delivery tool we will have taken advantage of a human microglial cell line that we have recently characterized for its ability to polarize towards different functional phenotypes (M1 or M2), and to release microvesicles. Our final aim was to generate a cell line able to release microglial microvesicles able to target microglia and neurons when injected in vivo, and loaded with anti-inflammatory or neuroprotective molecules. We have a long-standing experience with the gene transfer of IL-4 as an anti-inflammatory and neuroprotective molecule in neuroinflammation. We obtained stable cell lines expressing IL4 and releasing IL4-containing microvesicles, others expressing GFP and CRE recombinase, and we obtained cell lines expressing both IL4 and rabies virus glycoprotein and the eat-me signal MFGE8. We verified, at the molecular and protein level, that IL4-containing microvesicles are uptaken by microglial recipient cells and are able to induce expression of M2 anti-inflammatory markers. We characterized this uptake with a number of in vitro experiments showing that the signal is delivered at least by both protein and mRNA, but possibly also miRNA. We have performed in vivo delivery of luciferase and CRE-containing micerovesicles in the CNS of recipient C57BL/6 mice detecting them mainly in ependymal and leptomeningeal cells. We delivered IL-4 containing microvesicles to EAE mice obtaining a surprising long-lasting protection from disease, associated with the induction in the brain of several anti-inflammatory molecules. The aims of the proposed project are:

- Dissect what is the main component of the engineered microvesicles cargo to exert the therapeutic effect and the mechanisms of uptake
- Understand the detailed mechanism of action of engineered microvesicles to estimate duration of the effect
- Explore the potential of microvesicles to deliver different molecular types of cargo
- Validate engineered microvesicles as a new drug delivery tool for neurological pathologies

Skills to be acquired by the student:

The successful candidate will have to develop skills in cell biology, cellular imaging, including the use of optical tweezers to study microvesicles-cell interactions, molecular biology, handling

and microsurgery of experimental animals, neuropathology and cell immunology. This is, technically, a demanding project. All protocols are already set, and there is no need to develop new techniques with the exception of the use of optical tweezers, where the candidate will be required to attend a collaborating laboratory to import the needed protocols.

References (max. 3)

Giacomo Casella, Federico Colombo, Annamaria Finardi, Gerard Ill-Raga, Erica Butti, Paola Podini, Antonello Spinelli, Luca Muzio, Gianvito Martino, Giancarlo Comi and Roberto Furlan. Intrathecal IL-4-containing microvesicles inhibit the development of neuroinflammation. In preparation.

Verderio C, Muzio L, Turola E, Bergami A, Novellino L, Ruffini F, Riganti L, Corradini I, Francolini M, Garzetti L, Maiorino C, Servida F, Vercelli A, Dalla Libera D, Martinelli V, Comi G, Martino G, Matteoli M, Furlan R. Myeloid microvesicles are a marker and therapeutic target for neuroinflammation. Ann. Neurol. 2012 72:610-624

Casella G, Garzetti L, Gatta AT, Finardi A, Maiorino C, Ruffini F, Martino G, Muzio L, Furlan R. IL4 induces IL6 producing M2 macrophages associated to inhibition of neuroinflammation in vitro and in vivo. J Neuroinflamm. 2016 13:139.