Characterization of leptomeningeal-derived oligodendrocytes and their regenerative potential in a rat model of spinal cord injury

V. Berton[1], A. Braga[1], F.J. Rodríguez[2], P.H. González-Sánchez[2], G. Malpeli[3], A. Pino[1], M. Krampera[4], F. Bifari[3], I. Decimo[1], G. Fumagalli[1]

[1]Dept. of Public Health and Community Medicine, Section of Pharmacology, University of Verona, Italy
[2]Group of Molecular Neurology, Hospital Nacional de Parapléjicos, Toledo, Spain
[3]Dept. of Pathology, Section of Pathological Anatomy, University of Verona, Verona, Italy
[4]Dept. of Clinical and Experimental Medicine, Stem Cell Research Laboratory, Section of Hematology, University of Verona, Italy

In most diseases of the spinal cord, demyelination plays an important role in both generation and progression of the neurodegenerative lesion; in addition, endogenous oligodendrocytes regeneration is apparently insufficient for repair. Transplantation of oligodendrocytes represents a promising avenue for treatment of demyelinating disorders, although clinical application is hindered by the lack of adequate cell sources. Our group has described a population of neural stem/progenitor cells that resides in the adult leptomeninges and can be cultured in vitro maintaining their undifferentiated state; due to their superficial anatomical localization and their ability to differentiate into mature neural phenotypes in vitro, leptomeningeal stem cells (LeSCs) represent a promising source of cells in the settings of autologous cell transplantation therapy. In this work we aim to test LeSCs regenerative potential in a contusive spinal cord injury rat model.

We here report the development of a multi-step protocol to differentiate LeSCs into mature oligodendrocytes with high efficiency: starting from neurospheres generated by a small biopsy (approximately 1 cm in length) of adult rat spinal cord meninges and through sequential culture with three different media, we generated a mean number of 287.6 transplantable oligodendrocytes from each single LeSC at the beginning of the protocol. To characterize the cells during the differentiation protocol, we performed morphological and protein expression analysis by using immunofluorescence confocal microscopy and gene expression analysis by qRT-PCR. We show that with this protocol LeSCs gradually differentiate and acquire the typical oligodendroglial morphology by increasing the expression of the myelin-specific genes Cnp, Mag, Mbp, Mog and Plp1.

We then assessed the regenerative and reparative potential of LeSCs-derived oligodendrocytes in a contusive model of spinal cord lesion by intraparenchymal injection of $6x10^5$ eGFP-labelled cells 6 days after the injury. Evaluation of functional recovery with the Basso, Beattie and Bresnahan (BBB) rating scale and subscale and Catwalk gait analysis at different timepoints and up to 56 days after cells transplantation, evidenced a statistically significant enhancement in locomotor recovery in animals injected with LeSCs-derived oligodendrocytes. In order to establish the mechanisms through which transplanted cells might have contributed to a better functional recovery, we analysed the localization and phenotype of $eGFP^+$ cells in spinal cord samples of 2 cm of length centred on the site of lesion and injection. Histological analysis revealed that transplanted cells had migrated rostrally and caudally to the injection point 2 months after the injection. Preliminary data show that most of the transplanted cells don't express the oligodendroglial markers NG2, O4, GalC or MBP. Histological analysis is currently proceeding and is extending to the potential role of LeSCs-derived oligodendrocytes in modulation of the glial scar reaction.

In conclusion, we have established an efficient and reproducible method to generate large numbers of oligodendrocytes from a small meningeal biopsy; in addition, the evidence of a correlation between LeSCs-derived oligodendrocytes transplantation and improvement of functional recovery in a rat model of contusive spinal cord injury, suggest that leptomeningeal cells could play a role in regenerative therapies of demyelinating disorders.

This study was supported by International Foundation for Research in Paraplegia & CH.