

ADULT NEURAL STEM CELLS (ANSCs) AS A TOOL TO STUDY THE MECHANISMS LEADING TO NEURODEGENERATION IN VPS-54 MUTANT MICE

Botti Francesca¹, Ottolina Arianna², Marcuzzo Stefania¹, Mennini Tiziana³, Parati Eugenio², Tonini Marcello¹, Curti Daniela¹

¹Dept. Cell. & Molec. Physiological and Pharmacological Sci., University of Pavia, Pavia;

²Inst. Neurology "G. Besta", Dept. Neuropharmacology, Milan; ³Lab. Receptor Pharmacology, "M. Negri" Inst. for Pharmacological Research, Milan, Italy

Background: Mice bearing a spontaneous recessive mutation of the gene encoding for the membrane-trafficking protein Vps54 (wobbler mice), develop progressive motor dysfunction (1) with loss of motor neurons (MNs) and astrogliosis (particularly in the cervical tract of the spinal cord). Wobbler astrogliosis is characterised by profound metabolic and morphological changes of astrocytes both in vivo and in primary cultures detectable as strong immunoreactivity to glial fibrillary acidic protein (GFAP), paucity of cell-cell contacts, thick, short processes (2).

Objectives: 1) to compare the competence of neural stem cells (ANSCs) obtained from the subventricular zone (SVZ) of adult wobbler (wr) and disease free (ctr) mice in different culture conditions; 2) to investigate if differentiated astrocytes display features of the disease.

Materials and Methods: ANSCs were isolated from the SVZ of 5 week-old mice. Multipotentiality was assessed with DMEM/F12 without growth factors (standard conditions), with Neurobasal medium + BDNF and with Neurobasal medium conditioned by purified primary motoneuron cultures. Neural phenotypes were identified using antibodies specific for neurons (β -tubulin III), oligodendrocytes (O4) and astrocytes (GFAP). Differentiated astrocytes were processed for immunocytochemistry to detect markers of the intermediate filaments (IF) nestin and vimentin.

Results: in all cultural condition used, the majority of differentiated ANSCs were astrocytes. However, a significantly higher number of β -tubulin III⁺ cells ($p < 5 \times 10^{-7}$) were observed in the wr cultures in comparison with ctr ones. A subpopulation of astrocytes strongly immunoreactive to GFAP was much more represented ($p < 0.0003$) in the wr cultures. Furthermore, wr astrocytes displayed hypertrophic soma with thick and short processes.

Conclusions: the multipotentiality of wr ANSCs is preserved. However, the proliferative capacity is reduced, neurogenesis is increased and the astrocytes differentiated from the wobbler ANSCs display alterations resembling those observed in primary cultures of adult wr astrocytes. In reactive astrocytes the production of the intermediate filament (IF) nestin is resumed and the expression of the IFs vimentin and GFAP is up-regulated; therefore, we are currently analysing the expression of these markers in ctr and wr astrocyte cultures in basal conditions and after treatment with the bacterial endotoxin lipopolysaccharide (LPS).

References:

- (1) Schmitt-John T., Drepper C., Musmann A., Hahn P., Kuhlmann M., Thiel C., Hafner M., Lengeling A., Heimann P., Jones J.M., Meisler M.H., Jockusch H. (2005). *Nat. Genet.* 37: 1213-1215.
- (2) Hantaz-Ambroise D., Ait Ikhlef C.J., Parmentier C., Leclerc P., Cambier D., Zadigue G., Rieger F. (2001). *Differentiation* 69:100-107.