

Abstract Presentation

***In vitro* characterization of third trimester human amniotic fluid cells and their use in a mouse model of neurodegenerative disease**

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Since its devastating consequences, spinal cord injury (SCI) has polarized the efforts of many research groups all around the world. Pharmacological approaches aimed at preventing secondary degeneration in spinal cord injury have been recently flanked by cellular methods and stem cells have been studied as a new tool for spinal cord therapy (Reier PJ, *NeuroRx* 1:424-451, 2004; Schultz SS. *Curr Drug Targets* 6:63-73, 2005). Latest evidences indicate that stem cells could be a good tool to reduce secondary degeneration in spinal cord injury.

Multiple cell types derived from the developing fetus compose the cellular compartment of the amniotic fluid. For instance, epithelioid cells derive from fetal skin and urinary tract, amniotic fluid specific cells come from fetal membranes and trophoblast and fibroblastic cells derive from fibrous connective tissue and dermal fibroblasts (Gosden CM, *British Medical Bulletin* 39(4):348, 1983).

These cells are able to differentiate in adipogenic, osteogenic, myogenic, endothelial, neurogenic and hepatic lineages,, indicating their multipotency (De Coppi P et al, *Nature Biotechnology* 25(1):100, 2007).

The main goal of this study was the characterization of the cells from the third trimester amniotic fluid obtained from programmed caesarean births and test their therapeutic potential in a mouse model of SCI. Different populations of adherent cells were isolated from eleven human amniotic fluids and they were characterized for *in vitro* proliferation and differentiation potential. The antigenic profile was performed either by immunocytochemistry and citofluorimetric analysis. In particular, four cultures were deeply investigated and, by immunohistochemical analysis, all of them showed the expression of neural markers such as nestin, β tubulin III and GFAP. After citofluorimetric analysis, the samples showed a noticeable expression of adult mesenchymal markers (CD146+, CD73+, CD105+, CD90+) directed to the muscle-neural lineage (CD146+, NG2+, CD56+) (#3.5, #3.6 and #9.1); one of them also expressed CD117 (#3.6); culture #1.1, instead, showed a mesenchymal phenotype directed to the perivascular lineage (CD146+, CD90+, CD73+). From morphological point of view we were able to identify a new sub population of small cell spindle like shaped which were highly representative in #9.1 culture. We decided to use four populations (#3.6, #3.5, #1.1, #9.1) to transplant spinal cord injured mice. One week after transplantation immunosuppressed animals were intravenously injected with cells or PBS (controls) and motor recovery of the transplanted animals was studied for other 28 days by open field analysis (Basso DM et al, *J Neurotrauma* 23:635, 2006). The animals transplanted with culture #3.5, #3.6 showed a significant motor recovery than animals treated with PBS only; animals transplanted with cultures #1.1 and #9.1, instead, didn't show any significant enhanced performance than PBS treated animals. We tried then to investigate the reasons of these different results and, after histological analysis, we noticed that cultures #3.6 and #3.5 (the "therapeutic" lines) induced a better preservation of the myelin in the ventral white matter within the lesion site than PBS animals. Moreover, in these animals we could appreciate an increased angiogenesis in the peri-injured area, one month after lesion, in transplanted animals compared to the controls. These could be good data to investigate.