

T11-12B

Presentation on Friday July 17, 2015 at 3:48pm in room 'Poster area'
in the session Poster session II (even poster numbers), starting at 2:15pm

MYELINATING OLIGODENDROCYTES GENERATED BY DIRECT CELL REPROGRAMMING FROM ADULT RAT ADIPOSE TISSUE

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Obtaining oligodendroglial cells from dispensable tissues would be of great interest for autologous or immunocompatible cell replacement in demyelinating diseases as well as for studying myelin pathologies. Recently, two laboratories have simultaneously reported that mouse fibroblasts could be converted into oligodendroglial cells by direct reprogramming with transcription factors involved in oligodendrocyte development (Najm et al., Nat. Biotechnol. 31:426, 2013, Yang et al., Nat. Biotechnol. 31:434, 2013). We have lentivirally transfected combinations of tetracycline-inducible sox10 (S), olig2 (O), zfp536 (Z) and/or nkx6.1 (N) transgenes in adult rat adipose tissue-derived stromal cells (ADSCs) and checked for the generation of functional oligodendrocytes. Immunostaining with the O4 monoclonal, which binds to oligodendrocyte progenitor cell membranes, was used as initial marker of oligodendroglial reprogramming. The first signs of ADSC conversion into oligodendrocytic cells were observed with the S-O-Z combination by 6 weeks and the O4+ cell population kept rising the following months, eventually constituting more than 50% of cells. Increasing numbers of O1 (galactocerebroside), p75 (low-affinity NGF receptor), and GFAP-positive cells appeared in that period. Cells expressed also myelin-associated proteins like MBP, MAG, MOG and PLP1/DM20. Transduction with the S-O-N-Z transgene combination also produced oligodendrocyte progenitors but did not improve the result; S-O-N, O-Z or O transductions did not consistently produce oligodendrocytes in our hands. After 3 months of continuous expansion, the reprogrammed cells became independent of transgene activation by doxycycline. Antibiotic selection of transduced cells (with zeocin) did not show to be more efficient for reprogrammed cell purity and proliferation than unselected cultures. Different pre-induction treatments were tried in an attempt to improve the efficiency or speed of reprogramming: of these, treatment with Repsox followed by retinoic acid or adipocytic pre-differentiation, appeared to increase reprogramming consistency or to slightly accelerate the process. When seeded onto rat dorsal root ganglion neurons, transdifferentiated cells were observed to cover lengths of one or several axons with typical myelin-like appearance. We propose that functional oligodendrocytes can be efficiently generated from adult mesenchymal cells in the rat by direct cell reprogramming.

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Image

