Exploring neural differentiation of adipose stem cells for therapeutic use

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Introduction

Spinal cord injuries (SCI) are one of the most complex injuries to treat in the medical area. Due to the complexity of the spinal cord, the treatments are mostly palliative, used to prevent the progression of the injury, handle spasticity, deafferentation pain syndromes and dysautonomia[1]. Herein, we propose a cell-based approach, taking benefit of particular characteristics of adipose derived stem/stromal cells (hASC). When compared to other stem cell sources, hASC present key advantages such as their immuno-modulatory properties, low immunogenicity, high cell yield per gram of processed tissue, and, their neural differentiation potential, which have been described[2]. In this project, we aimed to explore and validate procedures to differentiate hASC into cells of the neural lineage, through the use of xeno-free and therapeutic grade reagents along the entire cell manufacturing process.

Materials and Methods

Human ASC (hASC xeno-free, irisbiosciences, Portugal) were cultured in xeno-free media as control, or in neural induction media (Cell Therapy Systems, Life Technologies). Three culturing phases were tested: i) Expansion, for cell proliferation up to p2-3; ii) Pre-Induction, where cells were stimulated with 20ng/mL bFGF and 20ng/mL EGF and iii) Differentiation, where cells were cultured 10 days, triggered by brain derived neurotrophic factor (BDNF, 10ng/mL). Additionally, cells were cultured in presence or absence of poly-l-lysin/laminin coated surfaces. After pre-induction and differentiation phases, gene expression of neural markers were determined by qRT-PCR: bIII tubulin and neurofilament (NEFL) for early and mature neural differentiation, respectively; myelin basic protein (MBP) as an oligodendrocytic marker, and glial fibrillary acidic protein (GFAP) as astrocytic marker.

Results

When compared to expansion media, cells cultured in neural induction media proliferated less, and formed so-called neuro-spheres in non-adherent plates. Yet, cells that remained in monolayer, demonstrated higher gene expression of neural markers. This was also observed for cells cultured in coated surfaces. At end of culture, NEFL was the mostly expressed marker, followed by MBP (Fig.1)

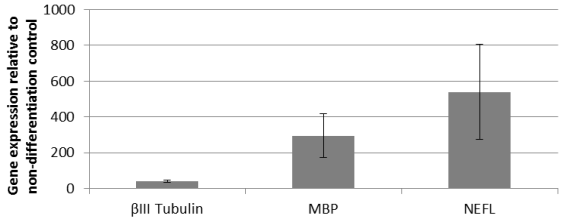

Fig. 1 Gene expression of neural markers in differentiated cells relative to non-differentiated .

Discussion and Conclusions

With the developed procedure, yet to be validated, high up-regulation of neural lineage genes were determined after differentiation, relatively to non-differentiation control: 10-40x of βIII tubulin neuron marker; 60-540x of NEFL neuron marker and 7-295x of MBP oligo-dendrocyte marker. No GFAP expression was detected suggesting that the conditions tested do not promote establishment of astrocytic cells. This data suggest that the tested protocol might become suitable for production of cells useful for SCI repair therapies.

References

1. Silva, N.A., et al., Prog. Neurobiol. 10.1016/ j.pneurobio.2013.11.002, 2013; **2.** Gimble J, Guilak F. Cytotherapy. 5(5):362-9, 2013.

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