DIFFERENTIATION OF RAT FULL TERM AMNIOTIC FLUID STEM CELLS INTO DOPAMINERGIC NEURONS PHENOTYPE VIA ADHERENT AND NON-ADHERENT NEURAL INDUCTION PROTOCOLS

Siti Nurusaadah Hamzah, Sharmili Vidyadaran, Norshariza Nordin

Genetic and Regenerative Medicine Research Centre, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia

**Article Info**

Published: 1st December, 2014

*Corresponding author email: shariza@upm.edu.my

**Abstract**

Recent studies have demonstrated the ability of midterm human amniotic fluid cells to generate dopaminergic (DA) neurons where none using amniotic fluid cells from full term pregnancies has been published. We have established rat AFS cell lines from full term pregnancies, where characteristics of highly potent cells were observed with their ability to differentiate into derivatives representing the three primary germ layers including neural cells. Here we aimed to explore the differentiation potential of our newly established rat full term AFS cells to differentiate into DA neurons phenotype *in vitro*. Neural-like cells expressing mature and DA neurons phenotype were successfully derived using two established protocols of adherent and non-adherent neural induction assays. These findings have provided us an insight into the use of full term AFS cells as a valuable tool in cell replacement therapy for neurodegenerative diseases such as Parkinson’s disease (PD).

**Keywords**

Amniotic fluid stem cells, Neural differentiation, Dopaminergic neurons

**1.0 Introduction**

One of the central pathophysiology of PD is the progressive degeneration of midbrain DA neurons leading to shortage of the neurotransmitter dopamine which is crucial in motor control. Dopamine replacement drug treatments can provide symptomatic relief but fail to slow the disease progression and develop side effects over time. Therefore, cell replacement approach is seen as particular importance as it may tackle the main cause by transplanting new dopamine-producing neurons in the brain of PD patients. Various types of stem cells are being extensively explored to generate DA neurons *in vitro*. Among others, our established rat full term AFS cell lines have demonstrated the ability to differentiate into different types of cells and, with less ethical and safety issues, they hold great potential for clinical application (1). A number of protocols can be used to differentiate stem cells into neurons including adherent monoculture (2) and the formation of non-adherent embryoid bodies (EB) followed by the addition of retinoic acid (RA) (3). This study aimed to observe the differentiation potential of rat full term AFS cells into DA neurons using two established neural induction protocols; adherent monoculture and non-adherent assays (4-/4+ protocol).

**2.0 Materials and Methods**

**2.1 AFS cell culture**

AFS cells were maintained in embryonic stem cell medium (ESM) in the presence of leukemia inhibitory factor (LIF) to maintain their undifferentiated state.

**2.2 Neural differentiation protocols**

a. **Adherent monoculture.** Undifferentiated AFS cells were directed to differentiate into neural lineage in the neural selective medium N2B27 for 8 days.

b. **4-/4+ protocol.** AFS cells were seeded into non-adherent bacterial-grade Petri dishes in ESM without LIF to
induce spontaneous multicellular aggregates, EBs. The EBs were let to mature for 4 days prior to induction towards neural lineage for another 4 days in 10 µM RA. The EBs were then dissociated and replated in the N2B27 medium for another 8 days (3).

2.3 Analyses

The efficiency of neural induction by both methods was assessed by immunocytochemistry (ICC) for qualitative analysis and fluorescence-activated cell sorting (FACS) for quantitative analysis using neural-associated markers; microtubule-associated protein 2 (MAP2) and tyrosine hydroxylase (TH).

3.0 Results

Neuron-like cells derived from both techniques expressed mature (MAP2) and DA (TH) neuronal markers by ICC and FACS (Figure 1C-D). However, more prominent neural-like extensions and higher percentage of TH/MAP2 were observed in neural differentiation by 4-/4+ protocol (95.6%) compared to the adherent monoculture technique (81.4%).

4.0 Discussion and Conclusion

Adherent monoculture provides a simpler and truly directed approach for neural differentiation of stem cells (2). On the other hand, EBs mimic the in-vivo developmental scenario with morphology less structured than a normal embryo and the addition of RA activates the pathway into neural lineage (3). This might explain the higher expression of TH by our AFS cell-derived neurons generated by the 4-/4+ protocol. Nevertheless, our AFS cells have managed to differentiate into TH positive neurons indicating DA phenotype without addition of specific patterning factors for both established methods. This clearly suggests the potential use of full term AFS cells as an alternative source for in-vitro production of DA neurons in the quest for PD therapy.

Acknowledgement

This research was funded by Research University Grant Scheme (RUGS); project no. 04-02-12-1758-RU. And Special Graduate Research Allowance (SGRA), UPM.

References