ISOLATION, CHARACTERIZATION AND IN VITRO BEHAVIOR OF HUMAN FETAL SVZ DERIVED NEURAL PRECURSOR CELLS (NPCS)

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Introduction

Regeneration of tissues in human central nervous system (CNS) is very poor and cell damage can prolong the neurological deficit after CNS injury. For this rationale, considerable attention has focused to generate the large homogenous populations of neural precursor cells (NPCs) *in vitro* for investigating their possible applications in cell replacement therapy of the damaged CNS. Owing to this various significant developments and modifications have been made in neural stem cell research which is needed to be more specified and enrolled in clinical studies using advanced approaches. The present study was designed to identify the various cell population residing in human fetal sub ventricular zone (SVZ) derived cells. The study was also focused to know the *in vitro* behavior and characteristics of NPCs of different gestational age fetus's having long term proliferation and differentiation potential.

Methods

Human fetuses (gestation between 18-22 weeks) were used to isolate the SVZ derived cells. Different cell populations were identified by specific markers such as Nestin, Sox-2, Oct-4, Notch-2, ABCB1, ABCG2, ß-tubulin III, GFAP and O4 using immunocytochemical staining, flow cytometry and molecular analysis. NPCs were magnetically sorted using CD133 antibody and maintained in suspension culture for 21 days. The neurospheres after 2nd passage derived from CD133+ve cells were dissociated and induced to differentiate into their respective lineages under serum and retinoic acid induction medium.

Results

SVZ derived cells showed positivity for all selected markers except O4. The expression intensity of CD133 and Nestin were found high in 20 gestation period. FDA analysis showed >92% viability in CD133+ve cells. In suspension culture =100µm diameter neurospheres were formed after 14 days and able to survive for 21 days of initial plating.

Cells were subcultured for 6 passages and each passage showed high proliferation capability. After 2nd passage neurospheres derived cells showed high differentiation potential into their respective lineages; neurons, astrocytes and oligodendrocytes, under different culture conditions.

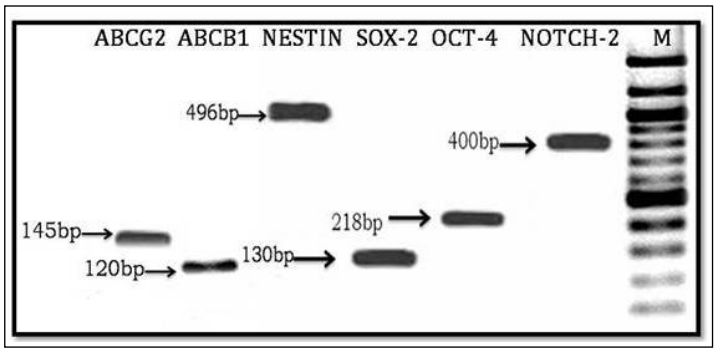

Figure 1 Representative gel image showing positive gene expression for NPCs specific markers

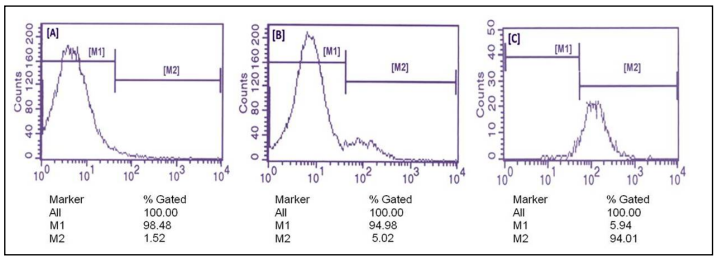

Figure 2 Percentage of CD133+ cells before and after MACS in 20wk gestation aged human fetal SVZ derived cells [A] Mouse IgG1-PE was used as isotype control for SVZ derived cells [B] Percentage of CD133+ cells before MACS (5.02%) [C] Percentage of CD133+ cells after MACS (94.01%)

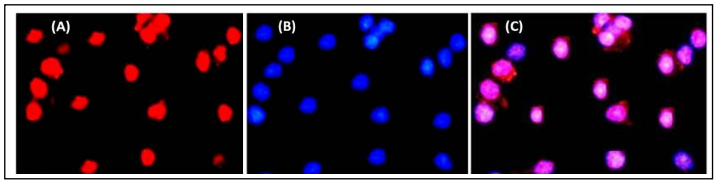

Figure 3 Immunocytochemical staining of CD133+ cells using anti -human nestin antibody. Almost all the CD133+ cells showed positivity for Nestin (Red). DAPI (Blue) was used as a counter dye to stain the cell nuclei (Magnification:40x)

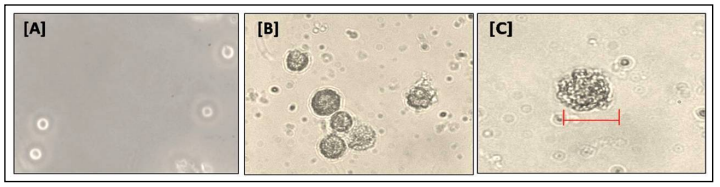

Figure 4 CD133+ sorted cells develop into neurospheres in suspension culture. Phase contrast image of growing spheres at (A) Day 1 (B) Day 14 and (C) day 21 (Magnification: 40x; Scale bar: 100µm)

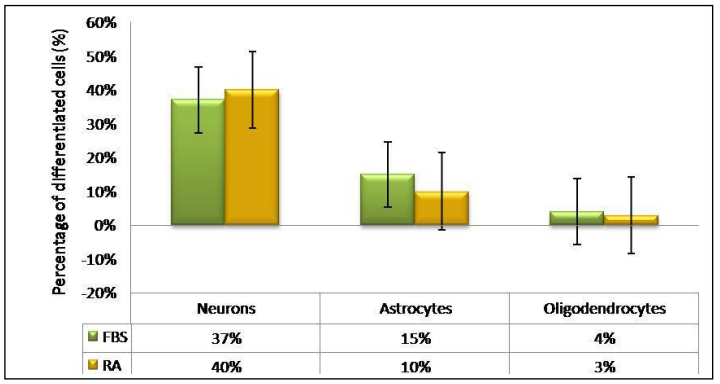

Figure 5 Average percentage of cell phenotypes differentiated from neurospheres developed by CD133+ sorted cells in two different culture conditions (Error bars are represented with standard error)

Conclusions

The present study revealed that key cell population in SVZ is NPCs though it also contains mature dedicated neuronal and astrocyte cells. CD133 may serve as more prominent marker to identify homogeneous NPCs population in SVZ tissue. High *in vitro* proliferation and differentiation potential of NPCs showed an option to resolve the paucity of NPCs availability and accessibility for their use in the treatment of CNS diseases.

Acknowledgement

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