DOI 10.7603/s40855-014-0002-6

PROGRESS IN STEM CELL 2014, 1(1):03-06

PROGRESS IN STEM CELL
An Open Access Journal

Available online at http://www.springer.com/globalsciencejournals
BioMedPress (BMP)
Journal homepage: http://www.cellstemcell.org
http://www.biomedpress.org/psc

Commentary

Using Defined Neural Cell Populations as a Possible Solution for Challenges in Neural Stem Cell Therapy

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ARTICLE INFO

Article history:
Received 03 September 2014
Accepted 15 September 2014
Published 20 September 2014

ABSTRACT

Neural stem cells exist in the mammalian nervous system. Despite extensive research to improve methods for isolation, propagation and differentiation of these cells, the clinical application of the *in vitro* expanded neural stem cells has remained challenging. Specifically, these challenges include heterogeneity of neural stem cell progeny, limited neuronal cell yield both in number and phenotype, paucity of oligodendroglial cells, and predominant astroglial differentiation *in vitro* and after transplantation. Moreover, uncontrolled proliferation and tumorigenicity of the undifferentiated progeny possibly limit the clinical application of neural stem cells. Here, we propose using defined neural cell populations as a main solution.

Keywords:
Neural stem cell; Differentiation; Defined cell population; Cell therapy.

Introduction

The ground-breaking discovery of neural stem cells (NSCs) in adult central nervous system (CNS) (Reynolds and Weiss, 1992) has led to a promising avenue of research for cell therapy in devastating neurological diseases. Neural stem cells mainly reside in the subventricular areas of the CNS along the ventricular neuraxis (Golmohammadi et al., 2008). These cells are capable of long-term self-renewal, unlimited cell divisions,

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Published online: 06 October 2014

and production of a large number of progeny. Although our understanding of the biology and physiology of NSCs has significantly increased, still we are far away from safe, universally-accepted and standardized approaches for clinical application of neural stem cells. In this short commentary I will focus on some of the main problems associated with therapeutic application of NSCs and propose the generation of defined neural cell populations as a main solution for a successful stem cell therapy regimen.

Neural stem cells are a heterogeneous cell population

Neural stem and progenitor cells are commonly isolated and propagated from adult and fetal neural tissue using the neurosphere assay (NSA) (Azari et al., 2010; Azari et al., 2011b; Reynolds and Weiss, 1992). Cytomorphological analysis of neurosphere-derived cells reveals that these cells are very heterogeneous in their phenotype, size, granularity, cytoplasmic content and are in different phases of the cell cycle (Bez et al., 2003). Subsequently, *in vitro* differentiation of NSC progeny gives rise to many different cells including neuronal and glial progenitor cells, and also undifferentiated *bona fide* NSCs. This is even more problematic when the undifferentiated NSC progeny are directly implanted into various diseased environments with no control over NSC fate decisions while particular neural cell types are needed (Hofstetter et al., 2005).

Limited neuronal cell yield, in number and phenotype upon neural stem cell differentiation

For therapeutic applications, increasing neuronal yield of NSCs and generating a variety of different neuronal phenotypes is important. For instance, while culturing NSCs at low levels of oxygen can increase neuronal differentiation (Panchision, 2009; Ross et al., 2012), overexpressing particular transcription factors such as Nurr1, Fezf2 can change the ultimate fate of the resulting neurons (Tan et al., 2011; Zuccotti et al., 2014). Despite successful increases in both neuronal cell yield and derivation of the needed neuronal phenotypes employing the above-mentioned protocols, the resulting cells are still contaminated with un-desirable NSC progeny and do not serve as a safe and efficient cell source for clinical applications.

Neural stem cells predominantly differentiate into astroglial cells *in-vitro* and upon transplantation into target CNS tissue

The main goals of cell therapy for CNS injuries are to support the injured cells, replace the lost cells and re-establish the disrupted circuitries in order to restore the lost function. Toward these ends, proportionate differentiation and synergistic action of all three NSC progeny, namely the neurons, astrocytes and oligodendroglial cells is needed. However, the majority of NSC progeny differentiate into glial fibrillary acidic protein (GFAP) expressing astrocytes both *in vitro* and upon transplantation. Moreover, astroglial differentiation is more pronounced when the undifferentiated NSCs are implanted directly into a lesioned CNS environment (Karimi-Abdolrezaee et al., 2010). Astrocytic differentiation of implanted NSCs can cause many undesirable side effects including pain and hypersensitivity (Hofstetter et al., 2005).

Uncontrolled proliferation and tumor formation of neural stem cells upon transplantation

Usually the animal studies of NSC transplantation are short-term studies and do not focus on the long-term proliferative potential of these cells after implantation. However, some long term studies showed that NSCs could actively proliferate even six months after implantation despite the hostile environment of the diseased CNS tissue, so that the number of donor cells remains the same or even higher than the number of cells that were initially transplanted (Yan et al., 2007). Furthermore, some reports indicate that implantation of undifferentiated human NSCs can cause tumors (Amariglio et al., 2009). Therefore, implementing strategies to minimize the risk of tumor formation by transplanted NSC progeny is critical.

Defined neural cell populations, a main possible solution

Highly purified neural cell populations, i.e., dopaminergic, GABAergic, glutamatergic, noradrenergic neuronal cells, oligodenroglial and astroglial progenitor cells can be yielded from a renewable source of NSCs. This enables us to study the effects that each particular neural cell or combination of different neural cell types at pre-defined ratios has on the disease progress, and to understand the underlying mechanisms. This eventually can lead to formulating the best neural cell type combinations and dosing strategies for different diseases depending on the stage and the nature of the disease to be treated. To benefit from the NSC therapy for different CNS diseases, using defined neural cell populations also lowers the risk of post-transplantation complications.

To this end, we have recently established protocols for the differentiation and subsequent purification of neuronal progenitors from fetal mouse NSC progeny. Using these strategies, we can successfully generate nearly 100% homogeneous immature neuronal cells that are able to differentiate into fully functional mature neurons both *in vitro* and upon transplantation into the CNS, showing no active sign of uncontrolled proliferation and tumor formation (Azari et al., 2011a; Azari et al., 2014). Application of the same strategy to human fetal NSCs is also very promising and we can generate human neurons in large-scale and near to 100% homogeneity (unpublished data).

Conclusion

NSCs hold great promise in the treatment of CNS diseases, as they are capable of generating all three main cell populations of the CNS tissue. Advancement in technologies and development of new methodologies for consistent large-scale generation of defined neural cell populations will pave the way for successful and safe therapeutic application of these cells in the treatment of many devastating neurological diseases in the near future.

Abbreviations

CNS, central nervous system; NSC, neural stem cell

Competing interests

The author declares that he has no competing interests.

Acknowledgements

This work was supported by the office of the vice chancellor for research in Shiraz University of Medical Sciences, Shiraz, Iran. The author would like to thank Mr. Jeff M. Fortin and Mrs. Sharareh Sharififar for their critiques on the manuscript.

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Cite this article as:
Azari, H. (2014). Using Defined Neural Cell Populations as a Possible Solution for Challenges in Neural Stem Cell Therapy. *Progress In Stem Cell, 1*(1):03-06.
doi:http://dx.doi.org/10.15419/psc.v1i1.47