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The current state of play in human neural stem cell models – what we have learnt from the rodent

Oikari LE, Griffiths LR, Haupt LM*

Genomics Research Centre

Institute of Health and Biomedical Innovation

Queensland University of Technology

Musk Avenue, Kelvin Grove, Brisbane, Queensland 4059, Australia

*Corresponding author:

Dr Larisa M. Haupt, Institute of Health and Biomedical Innovation, Queensland University of Technology, 60 Musk Ave, Kelvin Grove QLD 4059 Australia

E-mail: larisa.haupt@qut.edu.au, Phone: +617 3138 0801, Fax: +617 3138 6039

Abstract

Rodent (mouse and rat) models have been crucial in developing our understanding of human neurogenesis and neural stem cell (NSC) biology. The study of neurogenesis in rodents has allowed us to begin to understand adult human neurogenesis and in particular, protocols established for isolation and *in vitro* propagation of rodent NSCs have successfully been applied to the expansion of human NSCs. Furthermore, rodent models have played a central role in studying NSC function *in vivo* and in the development of NSC transplantation strategies for cell therapy applications. Rodents and humans share many similarities in the process of neurogenesis and NSC biology however distinct species differences are important considerations for the development of more efficient human NSC therapeutic applications. Here we review the important contributions rodent studies have had to our understanding of human neurogenesis and to the development of *in vitro* and *in vivo* NSC research. Species differences will be discussed to identify key areas in need of further development for human NSC therapy applications.

Keywords: neural stem cell, neurogenesis, rodent, human, cell therapy

Introduction

Neural stem cells (NSCs) are multipotent stem cells of the central nervous system (CNS) responsible for neurogenesis - the production of functional neurons throughout life. NSCs have the ability to self-renew, giving rise to neurons, astrocytes and oligodendrocytes through asymmetric division and due to their multilineage potential, NSCs hold tremendous potential for the regenerative treatment of CNS injuries [1]. Neurogenesis is conserved amongst mammalian species with the current understanding of human neurogenesis and NSC biology largely reliant upon studies on rodent mouse and rat model organisms. Additionally, these models have played a central role in the development of NSC isolation and *in vitro* expansion protocols and in the development of *in vivo* NSC transplantation strategies. In order to advance from rodent studies to successful human therapeutic applications, an understanding of the specific differences between rodent and human cells is crucial. This review will summarise the importance rodent models have had on understanding human neurogenesis, NSC biology and developing NSC transplantation methods. An insight into model and species differences will be given and future considerations on extrapolating rodent data into humans will be discussed.

Adult rodent and human neurogenesis – historical perspective, biological overview and species comparison

Adult neurogenesis is a relatively recently accepted occurrence, with neurogenesis previously thought to occur only in the developing brain. Our understanding of adult neurogenesis began in the 1960s with ground breaking work by Altman & Das, who demonstrated the presence of neurogenesis in the postnatal rat brain [2]. This work was initially controversial until further work by Kaplan and colleagues in the 1970s and 1980s demonstrated that new neurons were indeed produced in the adult rat and mouse brain, confirming the findings of Altman and Das [3, 4]. Following this, after elaborate research into adult neurogenesis in rodents, a breakthrough in understanding human neurogenesis occurred in the late 1990s, when Eriksson and colleagues demonstrated the presence of dividing and differentiating cells in the adult human brain. Neurogenesis was detected in a 72-year-old subject,

demonstrating the persistence of neurogenesis throughout life and giving a new understanding of nervous system biology [5].

Adult neurogenesis is now commonly accepted and the biology of rodent neurogenesis is well understood. In the adult rodent brain two major neurogenic areas have been identified: i) the subventricular zone (SVZ) lining the walls of the lateral ventricles, and ii) the subgranular zone (SGZ) of the dentate gyrus (DG) in the hippocampus; both regions contain NSCs that generate new neurons [6]. NSCs in the SVZ and the SGZ give rise to neuroblasts that migrate to their target sites where they mature into neurons. SVZ NSCs generate neuroblasts that migrate a long distance in chain formation through the rostral migratory stream (RMS) to the olfactory bulb (OB) giving rise to OB neurons [7]. Neuroblasts generated from the SGZ NSCs migrate only locally maturing into hippocampal dentate granule cell neurons [6]. Interestingly, NSCs from both the SVZ and SGZ have been shown to resemble astrocytes [8, 9].

Identification of the neurogenic areas in the rodent brain led to the discovery of neurogenic areas in the adult human brain. Following the demonstration of adult human hippocampal neurogenesis, comparison of the expression of neurogenesis-associated markers between the human and rat hippocampus revealed the same markers were expressed in the two species [5]. The presence of proliferating cells in the adult human SVZ was demonstrated several years later and as in rodents, these cells also resembled astrocytes [10]. The adult human RMS was not initially identified, requiring further research leading to its characterisation. The human RMS differs structurally from the rodent RMS, however as with the rodent, the human RMS also contains migrating neuroblasts [11]. Continued investigations have shown that, as with the rodent RMS, neuroblasts in the human fetal RMS form chains, however, due to the small number of migratory neuroblasts, no evidence of chain migration in the adult human RMS has been observed [12]. In addition, to date, there is currently no evidence of neuroblasts in the adult human OB, thus the fate of human SVZ neuroblasts remains unknown [12]. A summary comparing the milestones and advances of adult neurogenesis research in rodent and human models is presented in figure 1.

Neurogenesis is conserved amongst mammalian species, supported by similar neurogenesis-associated marker expression between rodents and humans. Interestingly, the spatial as well as temporal expression of these markers is similar between the two models [13]. A common characteristic to both rodent and human neurogenesis is the decrease with age, however the relative decrease is higher in rodents than humans [13, 14]. Other reported differences between rodent and human neurogenesis include hippocampal neuron turnover, i.e. the number of new neurons added to the DG to replace missing neurons, which has reported to be higher in humans than in rodents [14]. In addition, a distinct and unique attribute of the human SVZ is a structure termed the astrocyte ribbon, which has not been observed in any other species [10]. In addition, lack of chain migration in the adult human RMS demonstrates that human neurogenesis contains unique features that differ from the rodent [12]. An overview of differences between adult rodent and human neurogenesis is presented in table 1.

Rodent and human neural stem cell models – isolation and in vitro expansion

The understanding that neurogenesis persists in the adult mammalian brain lead to the search of common precursor cells of neurons and glial cells in the CNS. This was achieved in the early 1990s when neural precursor cells with multilineage differentiation capacity were isolated from adult mouse and rat brains [15, 16]. Subsequent identification and isolation of human NSCs was achieved in the late 1990s, a significant breakthrough in human NSC research [17]. The discovery of NSCs resulted in the establishment of rodent and human NSC *in vitro* isolation and culturing protocols and today multiple rodent and human NSC lines are routinely expanded and commercially available. The culture conditions for the *in vitro* propagation of both rodent and human NSCs are similar, using serum-free conditions along with the presence of key growth factors, EGF and FGF-2, to maintain cells in an undifferentiated state [18].

The two main methods of expanding NSCs in culture are the neurosphere assay and the adherent monolayer culturing system. The neurosphere assay developed by Reynolds and Weiss in 1992, was the initial assay established for the isolation and *in vitro* propagation of NSCs from the rodent brain [16]. A neurosphere is a free-floating

cluster of cells formed by cells composed of NSCs, progenitor cells and differentiating cells. During primary neurosphere culture, using serum-free culture conditions in the presence of EGF and FGF-2, the majority of the cells die, leaving the NSCs, which are responsive to these culture conditions [19]. The primary neurosphere can then be dissociated to form secondary spheres, which can be further propagated or induced to differentiate toward the neural lineages [19]. The formation of secondary spheres and their subsequent differentiation represents the self-renewal and multipotent differentiation capacity of NSCs [19]. The neurosphere assay is well established and has been successfully applied to the isolation and propagation of NSCs from the fetal and adult human brain [17, 20].

Adherent monolayer culturing of NSCs offers an alternative to the neurosphere assay, which is challenged by high cell heterogeneity and the low number of NSCs within a neurosphere [21]. In adherent long-term culturing, the presence of both EGF and FGF-2 is required to support symmetrical division of NSCs and to maintain their multilineage differentiation potential [21]. Protocols for the stable adherent culture of rodent and human NSCs are now well established with differentiation of adherent NSC cultures achieved through plating of the cells on extracellular matrix substances such as laminin [21]. An advantage of these methods includes maintaining high levels of homogeneity, reduced spontaneous differentiation and the potential to expand the cells to over 40 passages [18]. Adherent NSC cultures can also be derived from embryonic stem cells (ESCs) through exposure to defined culture conditions to induce neural differentiation [22]. ESCs are pluripotent with a high expansive potential and differentiation of NSCs from human ESCs provides an advantageous means to derive human NSCs [22]. Advances in NSC isolation and the development of *in vitro* culturing methods are illustrated in figure 1.

Comparison of rodent and human NSCs and their therapeutic potential

Rodent and human NSCs share many common characteristics including the use of similar culture conditions for *in vitro* propagation and the expression of markers used for lineage identification and characterisation. Upon withdrawal of growth factors, both rodent and human NSCs can be induced to differentiate toward the three neural lineages: neurons, astrocytes and oligodendrocytes [18]. The self-renewal and

multilineage differentiation abilities of both rodent and human NSCs are characterised during *in vitro* expansion through the expression of markers, many of which are common between rodents and humans [23]. Commonly utilised NSC characterisation markers are summarised in table 2.

Several studies undertaking direct comparisons have reported distinct differences between rodent and human NSCs. These include differences in surface marker expression including the detection of several surface markers in human cells not expressed in rodent cells [24]. Rodent and human NSCs have been shown to respond differently in culture to cytokines and growth factors suggesting regulation by different signalling environments [25]. In addition, distinct differences in neural lineage differentiation and the rate of cell growth have been demonstrated between rodent and human NSCs. [26]. Interestingly rodent NSCs have been shown to express higher levels of telomerase and exhibit longer telomeres than human NSCs [27]. Differences in sensitivity to neurotoxins and chemicals affecting proliferation and apoptosis between rodent and human NSCs have also been reported and furthermore, human cells show a higher level of variation in neurite outgrowth from culture to culture than rodent cells [28, 29]. These observations highlight important differences that need to be acknowledged and considered when expanding these cells and developing models of repair.

The self-renewal and multilineage differentiation ability of NSCs make them a promising cell therapy tool for the regenerative treatment of CNS damage and due to the establishment of rodent and human NSC isolation and *in vitro* expansion protocols, our understanding of NSC biology has vastly broadened. Importantly, rodent models of neurodegenerative disease and brain injury have contributed to the development of transplantation strategies for *in vivo* applications. NSC transplantation experiments initially conducted through rodent-rodent studies, successfully demonstrated that isolation, *in vitro* expansion and subsequent transplantation produced viable cells that survived and differentiated in the host [30]. These promising results inspired the testing of human NSC transplantation in rodent models. Interestingly, despite the acknowledged differences in brain structure, human NSCs survived, migrated and differentiated in the host rodent brain [31].

With rodents accepting human cells with a limited immune response upon transplantation [32], the use of rodent models have enabled testing of modified, or “primed” human NSCs for the development of more efficient human NSC transplantation strategies [33]. Ultimately, human NSC research is aimed at the development of successful transplantation strategies of human cells into human hosts for the treatment of neurodegenerative disease or brain injury. To date, this has included the use of cells from several sources including induced pluripotent stem cells, mesenchymal stem cells as well as human fetal and adult NSCs (reviewed in [34, 35]). However, human NSC therapy remains challenging. The use of fetal human cells raises ethical issues and adult cell therapy is challenged with difficulties in harvesting cells, limited expansive potential of adult cells as well as developing methods for the successful transplantation into human hosts [34]. Thus, the need for further development of improved human NSC transplantation strategies remains an important area of investigation by researchers and clinicians alike.

Rodent models and human cells - toward developing improved strategies of human NSC transplantation

Rodents have played a major role in NSC research and specifically, in the application of NSCs for the treatment of CNS damage. The benefit of using rodent NSCs include the ease of deriving and establishing *in vitro* expansion models of these cells, enabling the examination of adult NSCs, with adult human NSCs challenging to harvest. In addition, rodent models have enabled the study of NSC function *in vivo* and the development of NSC transplantation methods. As such, much of the knowledge obtained from rodents has been successfully applied to human NSC studies. However, in order to develop improved human NSC transplantation strategies, the clear similarities and differences between the two models is an important consideration when extrapolating rodent NSC data into the human system.

Studies in rodent models that have been translated to human studies have been based on the identified similarities between rodent and human NSCs in terms of their expansion and growth characteristics and marker expression within their localised microenvironment. However, reported differences including surface markers as well

responsiveness to signalling molecules suggests rodent and human NSCs are regulated through different mechanisms and different signalling events or pathways [24, 25]. Reported differences in sensitivity of the two cell populations upon exposure to exogenous chemicals [28, 29] highlight distinct species differences and suggest direct comparisons of these models are not always applicable. As an example, NSC transplantation experiments of rodent and human NSCs into rodent hosts have been successful, with survival, migration and differentiation of transplanted NSCs observed [30-33]. However, the central difficulty in applying rodent protocols to human cells lies in the acknowledged structural complexity of the human brain and associated biological differences in rodent and human neurogenesis. In particular, observed *in vitro* differences between rodent and human NSCs indicate the two cell types do not follow the same pattern of growth and migration upon transplantation. Furthermore, the significantly larger size of the human brain places an additional load in terms of number of transplanted NSCs in order to improve the likelihood of success, as they will likely face challenges during migration to the target site. A summary of the comparison between rodent and human NSC models, outlining similarities and differences is presented in figure 2.

Further comparative studies are required to gain a full and comprehensive understanding of the species differences between rodent and human NSCs and their therapeutic potential. Rodent models have been central to our current understanding of the *in vivo* molecular and cellular interactions of NSCs, but further study utilising human neural tissue is required to understand key aspects of human-specific nervous system biology. Through combining our knowledge of human *in vitro* NSC data with rodent *in vivo* studies, with an understanding of differences between rodent and human NSC regulatory factors, these models will provide data more readily applicable to human applications. Our ability to successfully target these similarities and differences can be applied to the development of more efficient human NSC therapeutic applications to establish strategies for the successful transplantation of human NSCs into a human host.

Conclusions

As rodent and human NSCs share similar *in vitro* culture conditions, protocols established for rodent NSCs have successfully been applied to the isolation and expansion of human NSCs. The establishment of rodent and human *in vitro* NSC culturing systems has enabled an understanding of NSC biology *in vitro* and the application of this knowledge to *in vivo* regenerative treatment models of CNS injuries. Although progress has been made with human NSC models, this research faces a number of challenges. Human NSCs, in particular adult NSCs are difficult to harvest and human NSC transplantation remains difficult. The development of improved *in vitro* models of human NSCs for therapeutic applications are needed with the use of these cells in combination with rodent models likely to provide insight into *in vivo* regulatory mechanisms. A thorough understanding of both the similarities and differences between rodent and human NSC models is required to ensure the efficacy of these models.

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Table legends

Table 1. The process of neurogenesis in rodents and humans can be distinguished by clear physiological and structural differences.

Table 2. A summary of the markers currently used for the characterisation of rodent and human neural stem cells. These markers include markers of “stemness” or multilineage potential as well as proliferation and the three major neural lineages.

Figure legends

Figure 1. A timeline illustrating advances in rodent and human adult neurogenesis and neural stem cell research. Rodent model systems (rat and mouse) have provided significant advances in adult neurogenesis research as well as in the identification, isolation and propagation of NSCs enabling the identification and use of human NSC models. The timeline demonstrates and compares the advances between rodent and human adult neurogenesis research along with the development of models of NSC isolation and expansion.

Figure 2. A schematic representation of similarities and differences between rodent and human neural stem cell *in vitro* models. A) The expansion of rodent and human NSCs share similarities with both rodent and human NSCs isolated from fetal and adult tissue and expanded in culture as neurospheres or as an adherent monolayer in the presence of growth factors EGF and FGF-2. Upon withdrawal of growth factors rodent and human NSCs can be induced to differentiate toward neurons, astrocytes or oligodendrocytes. B) A summary of previous direct comparative studies of rodent and human NSCs that have identified distinct differences between these *in vitro* models. Differences observed include characterisation markers, cell growth, lineage differentiation, cytokine and growth factor signalling, telomere length, telomerase expression and sensitivity to exogenous factors.

<i>Location</i>	<i>Differences</i>	<i>Ref.</i>
Hippocampus	<ul style="list-style-type: none"> • Neuron turnover higher in humans than rodents • Relative age-related decrease of neurogenesis higher in rodents than humans 	Spalding <i>et al.</i> [14] Spalding <i>et al.</i> [14]
SVZ	<ul style="list-style-type: none"> • Presence of astrocyte ribbon in humans • No chain migration in human RMS • No neuroblasts detected in human OB 	Sanai <i>et al.</i> [10] Wang <i>et al.</i> [12] Wang <i>et al.</i> [12]

<i>Neural Stem Cell</i>	<i>Proliferation</i>	<i>Neuronal</i>	<i>Astrocyte</i>	<i>Oligodendrocyte</i>
CD133 (Prominin-1)	BrdU	Dcx	GFAP	GalC
Nestin	Ki67	MAP2	S100B	O4
Sox1	Mcm2	NeuN		
Sox2	PCNA	Neurofilament		
Musashi 1		NSE		
Vimentin		PSA-NCAM		
		TuJ1 (β III-tubulin)		
Ref. [13,18,21,23]	[5,11-13]	[5,8,10,13,18,21]	[5,8,10,18,21]	[10,18,26]