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Research Report

Neurogenic potential of progenitor cells isolated from postmortem human Parkinsonian brains

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ABSTRACT

The success of cellular therapies for Parkinson's disease (PD) will depend not only on a conducive growth environment *in vivo*, but also on the *ex vivo* amplification and targeted neural differentiation of stem/progenitor cells. Here, we demonstrate the *in vitro* proliferative and differentiation potential of stem/progenitor cells, adult human neural progenitor cells ("AHNPs") isolated from idiopathic PD postmortem tissue samples and, to a lesser extent, discarded deep brain stimulation electrodes. We demonstrate that these AHNPs can be isolated from numerous structures (*e.g.* substantia nigra, "SN") and are able to differentiate into both glia and neurons, but only under particular growth conditions including co-culturing with embryonic stem cell-derived neural precursors ("ESNPs"); this suggests that PD multipotent neural stem/progenitor cells do reside within the SN and other areas, but by themselves appear to lack key factors required for neuronal differentiation. AHNPs engraft following *ex vivo* expansion and transplantation into the rodent brain, demonstrating their regenerative potential. Our data demonstrate the presence and capacity of endogenous stem/progenitor cells in the PD brain.

1. Introduction

Current pharmacological and surgical treatments for PD have been successful in addressing several motor symptoms, but to date have not been shown to be capable of modifying disease, or address levodopa resistance associated symptoms. Cell transplantation therapies have also faced numerous obstacles including ethical, safety and technical issues. It has been hypothesized that novel cell-based therapies aimed at stimulation of endogenous dopamine production within the brain may provide a more physiological and more elegant way to overcome the cardinal symptoms of PD (Arias-Carrion et al., 2007; Baquet et al., 2005; Geraerts et al., 2007; Preynat-Seauve et al., 2009).

While the existence of stem cells in the adult brain is widely accepted in the scientific community, the magnitude, pathogenetic relevance and restorative potential of neurogenesis in the diseased brain is far less understood. The number of studies on these processes in the adult human brain is

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limited. While studies have suggested the presence of precursor cells within the adult rodent substantia nigra (SN) (Lie et al., 2002), in general there is a lack of evidence for adult human neural progenitor cells (AHNPs) (Walton et al., 2006) in the Parkinsonian brain, including the SN (Srivastava et al., 2008). It also remains uncertain as to whether AHNPs possess the capacity to produce dopaminergic neurons (DA neurons) within the PD brain (Geraerts et al., 2007; Storch et al., 2004). In this study, we isolated and cultured AHNPs from the SN and three other regions of postmortem PD brains. PD AHNP primary cell lines were found to differentiate into neurons and astrocytes when cultured under specific growth conditions. We conclude that adult stem/progenitor cells exist in the SN and other regions within the PD brain, and that these cells represent a potentially valuable source for bioassays aimed at both understanding disease course, and screening for potential cellular and pharmacologic therapies.

2. Results

2.1. *Isolation and culture of AHNPs from PD brain*

Compared to other non-PD AHNP cultures (surgical resections for temporal lobe epilepsy, n=5), PD AHNPs in primary culture exhibited a lower adherence to a poly-ornithine-coated substrate with more than 50% of cells detaching after a few days (data not shown). The surviving neural progenitor cells formed a confluent monolayer after 4–7 weeks (Fig. 1A). AHNP cultures appeared to represent a stable expanding population in two or three passages (Figs. 1B,C). Figs. 1D,E document phenotypic changes that occurred within this cell population during expansion, including the appearance of many cells with neuronal morphology. Larger numbers of round and flat cells were observed in SN AHNP primary cultures (Fig. 1F), while more spindle-shaped and bipolar cells were present in cultures from other brain regions (Fig. 1G), but all of the cells became morphologically uniform after two passages. Compared to epilepsy cultures, PD cells in primary culture grew significantly slower ($p<0.05$) (Fig. 1K). PD AHNPs were obtained from tissue samples derived from cortex (CX), hippocampus (HC), subventricular zone (SVZ), and SN (see Table 1), and could be expanded for more than 50 population doublings (Fig. 1L). Under neurosphere (Laywell et al., 1999) conditions, cells from all regions also grew as spheres (Fig. 1H). However, spheres decreased in size over 6 passages, when sphere cultures were aborted (Fig. 1I).

We also generated cell cultures from DBS lead electrodes removed during lead replacement procedures (n=3). Primary cells were observed growing off of the electrodes within 72 h of culture initiation. These cells continued to grow for 3–5 weeks (Fig. 1J), but could not be expanded beyond 2 passages.

2.2. *Identification of AHNPs from the Parkinson's brain*

Immunocytochemistry was performed to identify the cultured PD cells as neural stem/progenitor cells. Most cells from all the brain regions sampled expressed neural stem cell markers nestin (Fig. 2A) and SOX2 (Brazel et al., 2005) (Figs. 2D, E), and the majority of the nestin/SOX2 positive cells were also immunoreactive for the radial glial marker vimentin (Fig. 2C) (Bramanti et al., 2010), and the glutamate transporter GLAST (glutamate astrocyte-specific transporter, Zecevic, 2004, or EAAT1; Fig. 2F). The GLAST labeling of all these PD cells was both nuclear and cytoplasmic, while concurrent immunolabeling studies showed that murine adult neural progenitor cells exhibited exclusive cytoplasmic localization (data not shown). Nuclear location of the glutamate astrocyte-specific transporter GLAST protein had been reported in human glioma cells (Ye et al., 1999), but their labeling pattern did not include a cytoplasmic component whereas the PD AHNP cells did exhibit light cytoplasmic in addition to strong nuclear labeling. The basis for this labeling pattern is not known, but it suggests the possibility that PD cells might have altered glutamate metabolism. We did not observe expression of CD133 (data not shown), which is thought to be predominantly expressed in S, G2 or M phase stem cells but downregulated in slow-cycling or dormant stem cells (Sun et al., 2009). These cells were also negative for multipotent mitotic glial markers A2B5 and NG2 (data not shown). No TuJ1 positive neurons or GFAP positive astrocytes were detected in any proliferating cell cultures after subculture. Though α-synuclein aggregates were observed in PD SN tissue from these patients, we did not detect α-synuclein in cultured AHNPs or their progeny (data not shown). We compared the proportion of nestin and SOX2 positive cells among populations from the four brain regions studied here, but did not find a significant difference (ANOVA, $p=0.47$ and 0.49, respectively) (Fig. 2M). We detected mRNA expression of progenitor markers in SN-derived cell cultures as well as in SVZ and SN tissue by RT-PCR. Both cells (Fig. 2N) and tissue (Fig. 2O) expressed nestin, SOX2, vimentin and GLAST, supportive of the existence of a progenitor cell pool in the PD SN. Interestingly, only GLAST mRNA showed a marked difference between SN and SVZ in tissue samples (Fig. 2N–O). Whether the weak expression of GLAST in SN is one of the pathological characteristics of PD will require further study.

2.3. *Limited potential of in vitro-expanded PD AHNPs*

To determine the neural lineage potential of the PD stem/progenitor cells, adherent AHNPs were induced to differentiate on poly-ornithine-coated glass coverslips with 3 types of differentiation media (DM). After 4–7 days culture in DM, in all the 3 groups of differentiation systems, approximately 50–60% of cells detached from the poly-ornithine-coated surface (Fig. 4A), and there were another 10–25% cells detached at later stages. Addition of 1% fetal bovine serum to the differentiation media only saved the later period of cell death (Fig. 4B), and in both conditions, immunocytochemical analysis on the surviving cells did not reveal any TuJ1 positive neurons or GFAP positive astrocytes. We detected only weak nestin and vimentin expression (Fig. 4C).

2.4. *In vitro evidence of improved neural differentiation of PD AHNPs following co-culture with rodent embryonic and adult neural precursor cells*

In contrast to isolated differentiation, AHNPs from PD patients' brain gave rise to both neurons and astrocytes when co-cultured