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In vivo tracking of human neural progenitor cells in the rat brain using bioluminescence imaging

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HIGHLIGHTS

- We track Luc2 human neural progenitor cells (hNPC^{Luc2}) *via* bioluminescence imaging.
- hNPC^{Luc2} can be visualized in rat striatum up to twelve weeks.
- This method distinguishes dead *versus* live hNPC^{Luc2} *in vivo* in rat striatum.
- Region of interest-based image analysis reveals hNPC^{Luc2} contralateral migration.

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ABSTRACT

Background: Stem cell therapies appear promising for treating certain neurodegenerative disorders and molecular imaging methods that track these cells *in vivo* could answer some key questions regarding their survival and migration. Bioluminescence imaging (BLI), which relies on luciferase expression in these cells, has been used for this purpose due to its high sensitivity.
New method: In this study, we employ BLI to track luciferase-expressing human neural progenitor cells (hNPC^{Luc2}) in the rat striatum long-term.
Results: We show that hNPC^{Luc2} are detectable in the rat striatum. Furthermore, we demonstrate that using this tracking method, surviving grafts can be detected *in vivo* for up to 12 weeks, while those that were rejected do not produce bioluminescence signal. We also demonstrate the ability to discern hNPC^{Luc2} contralateral migration.
Comparison with existing methods: Some of the advantages of BLI compared to other imaging methods used to track progenitor/stem cells include its sensitivity and specificity, low background signal and ability to

Abbreviations: BLI, bioluminescence imaging; hNPC^{Luc2}, stable luciferase-expressing human neural progenitor cells; CNS, central nervous system; WT, wild type; HD, Huntington's disease; PD, Parkinson's disease; GDNF, glial cell line-derived neurotrophic factor; hNSC, human neural stem cells; DMEM, Dulbecco's modified Eagle medium; PSA, penicillin/streptomycin/amphotericin; EGF, epidermal growth factor; FGF-2, fibroblast growth factor-2; LIF, leukemia inhibitory factor; CMV, cytomegalovirus; hNPC-Luc2, transiently luciferase-expressing human neural progenitor cells; p-HEMA, polyhydroxyethylmethacrylate; IVIS, In Vivo Imaging System; SIN-W-PGK, self-inactivating lentiviral vector with posttranscriptional cis-acting regulatory elements of woodchuck hepatitis virus and mouse phosphoglycerate kinase 1 promoter; hNPC^{WT}, wild type human neural progenitor cells; PGK, phosphoglycerate kinase; PFA, paraformaldehyde; PBS, phosphate buffered saline; NDS, normal donkey serum; BSA, bovine albumin serum; GFAP, glial fibrillary acidic protein; BrdU, bromodeoxyuridine; TBST, Tris-buffered saline with 0.5% Tween 20; QA, quinolinic acid; TA, tibialis anterior; BVC, bupivacaine; IP, intraperitoneal; ROI, region of interest; RS, rostral; C, caudal; R, right; L, left; hCyto, human cytoplasmic marker; ANOVA, analysis of variance; SEM, standard error of the mean; RLU, relative light units; PGK, phosphoglycerase kinase; MRI, magnetic resonance imaging.

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distinguish surviving grafts from rejected ones over the long term while the blood–brain barrier remains intact.
Conclusions: These new findings may be useful in future preclinical applications developing cell-based treatments for neurodegenerative disorders.

1. Introduction

Stem cell therapies have emerged as promising treatment methods for a number of neurodegenerative diseases, as these cells are capable of surviving, migrating and integrating into the central nervous system (CNS). Neural stem and progenitor cells, distinct in their ability to differentiate into cells of the neural lineage, are especially well-suited for cell-based treatment of neurodegenerative disorders. Utilizing post-mortem histological analyses, these cells have been shown to survive over 7 months in the CNS of wild type (WT) rats and have been used in several preclinical studies of disorders such as Huntington's disease (HD) and Parkinson's disease (PD) (Lindvall et al., 2012; Gowing et al., 2013). Specifically, fetal brain-derived human neural progenitor cells (hNPC) have been transplanted in the striatum of a HD-induced rat model, demonstrating their ability to migrate, protect the striatum, and initiate recovery (McBride et al., 2004). Furthermore, protection of injured dopaminergic neurons has been demonstrated following striatal transplantations of hNPC overexpressing glial cell line-derived neurotrophic factor (GDNF) in animals experiencing PD-like partial striatal lesions (Behrstock et al., 2006). Finally, a study in PD symptomatic non-human primates showed human neural stem cell (hNSC) survival and migration as well as animals' functional improvement following cell transplants (Redmond et al., 2007). Overall, neural progenitor/stem cells appear to be a promising tool for therapy of neurodegenerative diseases.

While delaying neural degeneration with neural progenitor/stem cells seems possible, one of the major roadblocks in therapeutic efforts arises from the inability to monitor cell fate *in vivo*. In animal studies, cell survival and migration can be assessed using post-mortem histological analyses (Tang et al., 2003; Behrstock et al., 2008; Riley et al., 2009). However, a method of *in vivo* noninvasive, longitudinal cell tracking in clinical settings would be invaluable, allowing scientists to understand cell dynamics in single subjects as well as cohorts and adapt progenitor/stem cell therapies for further studies.

Several molecular imaging techniques are used for non-invasive stem cell tracking *in vivo* (Gera et al., 2010). In order for cells to be efficiently detected, they must first be distinguished from surrounding tissues. Additionally, the ideal imaging modality must be sensitive enough to detect the appropriate cell number required for treatment and have sufficient resolution to identify their location and migration over time. Furthermore, to achieve meaningful information from a cellular imaging modality, cell signal must also be reflective of survival/viability. Currently, no one imaging technique has been shown to successfully address all of these important issues.

Bioluminescence imaging (BLI) is an optical imaging technique that relies on light emission from the cells or tissues of interest. It has been explored for stem cell tracking because of its capability of detecting small populations of cells (Kim et al., 2006; Daadi et al., 2009). BLI exhibits low background signal due to emission of optical light without an external light source, as well as the lack of autobioluminesence in mammalian tissues. In order to be detected with BLI, stem cells must first be induced to express a luciferase protein. Among them, firefly luciferase was originally extracted from the North American firefly and then further engineered to be used for imaging purposes. For signal to be detected, stem cells must also be in the presence of ATP and O_2, which in concert with luciferase allow D-luciferin to be converted into oxyluciferin and light. Luciferase expression has been used for a variety of assays such as gene expression quantification (Lipshutz et al., 2000), tumor development tracking in rats (Kondo et al., 2009), and stem cell localization in mice (Bradbury et al., 2007), showing that BLI is valuable in determining cell viability and approximate location *in vivo*. Until this point, luciferase overexpression has not been explored for detection of slow proliferating progenitor cells in the rat brain, particularly in a structure as deep as the striatum. Concerns about bioluminescence signal penetrating rat's skull, brain tissue and hair have been some of the reservations of the scientific community.

In this study, for the first time, luciferase expression in hNPC was induced to assess and track cells *in vivo* in the rat striatum. We show that these cells can be visualized long-term *in vivo* and that their survival and location can be deduced from BL images. These methodological findings may be useful in future preclinical applications aimed at developing cell-based treatments for neurodegenerative disorders.

2. Methods

2.1. Cell culture

Human neural progenitor cells were isolated between 10 and 15 weeks gestation using the protocols set by the National Institutes of Health (NIH) and the local ethics committees at the University of Wisconsin, Madison and University of Freiburg, Germany. All of the work was approved by the Institutional Review Board. A previously described method was used to prepare human cortical neural progenitor cells, G010 line, from fetal brains and induce their optimal cell expansion (Svendsen et al., 1997). These cells were grown as neurospheres in basic medium containing Dulbecco's modified Eagle medium (DMEM, Sigma–Aldrich, St. Louis, MO) and Ham's F12 (Sigma–Aldrich) (7:3), and penicillin/streptomycin/amphotericin B (PSA, 1% v/v, Life Technologies, Carlsbad, CA), supplemented with B27 (2% v/v, Invitrogen), epidermal growth factor (EGF, 100 ng/ml, Millipore Corp., Billerica, MA,), fibroblast growth factor-2 (FGF-2, 20 ng/ml, WiCell Research Institute, Inc.) and heparin (5 μg/ml, Sigma–Aldrich). Neurospheres were passaged approximately every 14 days by chopping with McIlwain automated tissue chopper (Mickle Engineering, Gomshall, Surrey, UK) (Svendsen et al., 1998). After passage 10, the cells were switched to maintenance medium: basic medium supplemented with N2, EGF, leukemia inhibitory factor (LIF, 10 ng/ml, Millipore), FGF-2 and heparin, helping to increase the rate of expansion and permitting stable growth for another 20 to 30 passages.

2.2. Transient luciferase expression

Transient luciferase expression was established using the Lonza Nucleofection System (Lonza Group Ltd., Basel, Switzerland). Nucleofection is a non-viral method of transfection that employs both electroporation and lipofection in order to achieve high cDNA incorporation with minimal cell death. Luciferase cDNA, pF9A-Luc2 (Promega Corp., Madison, WI), was under the control of the cytomegalovirus (CMV) promoter. Briefly, hNPC were dissociated using a Trypsin solution (TrypLE, Invitrogen) and 5×10^6 cells