In vivo tracking of ferritin over-expressing human neural progenitor cells in the rat central nervous system

Ksenija Bernau[1], Christina Lewis[2], Anna Petelinsek[3], Masatoshi Suzuki[4], Mary Elizabeth Meyerand[1,2], and Clive Svendsen[5]

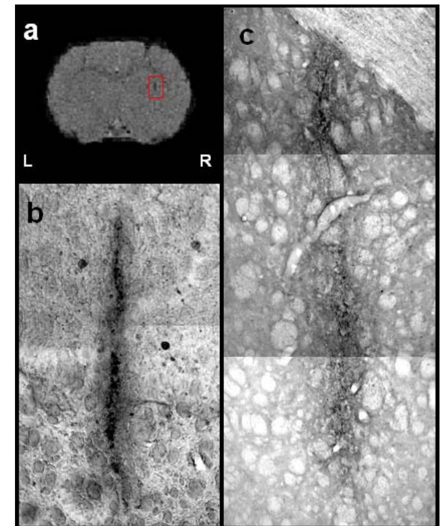
[1]*Biomedical Engineering, University of Wisconsin-Madison, Madison, WI, United States,* [2]*Medical Physics, University of Wisconsin-Madison, Madison, Wisconsin, United States,* [3]*Biochemistry, University of Wisconsin-Madison, Madison, WI, United States,* [4]*Comparative Biosciences, University of Wisconsin-Madison, Madison, WI, United States,* [5]*Regenerative Medicine Institute, Cedars-Sinai Medical Center, Los Angeles, CA, United States*

Target Audience: Cellular imaging scientists, stem cell biologists.

Purpose: In the development of stem cell therapies for neurodegenerative disease, the ability to visualize and track fates of engrafted cells *in vivo* is an essential tool[1]. A method to track cell migration and survival will reduce the number of subjects required for these studies and resources needed for histological analysis. It could further lead to an essential tool for monitoring cellular therapy in humans. In the last decade, our group has studied possible applications of human neural progenitor cells (hNPCs) to slow disease progression in rodent models of neurodegenerative disease[2]. This study aims to develop a method for imaging hNPCs in the rodent central nervous system (CNS) with MRI. Stable expression of the iron storage protein ferritin, which binds endogenous iron, holds potential to provide specific information about cell location and survival, leave cell dynamics unaffected, and be translatable into human subjects[3].

Methods: Stable ferritin expression was achieved through lentiviral infection of hNPCs. Verification of protein expression and unchanged cell differentiation and proliferation compared to wild-type cells was performed *in vitro* with bromodeoxyuridine (BrdU) labeling and immunocytochemistry. Ferritin over-expressing hNPCs (6×10^5 cells total) were transplanted into the right striatum of nine adult Sprague-Dawley rats, and varying quantities of negative control hNPCs were transplanted contralaterally in each subject. Three studies each involved a group of three subjects. Study durations were ten weeks, two weeks, and twelve weeks, respectively. *In vivo* imaging was performed at two or more time points for each study on a Varian 4.7T preclinical scanner. Sessions included a T2* weighted gradient echo pulse sequence; TR=500ms, TE=12ms, flip angle=20°, NEX=4, FOV=40mm, matrix size=256×256, 15×0.36mm slices without spacing. A multi-echo sequence was also collected at each session, with TR=500ms, TE=3.28ms, TE2=3.93ms, echoes=8, flip angle=20°, NEX=4, FOV=40mm, matrix size=256×256, 10×0.36mm slices without spacing. Magnitude and phase images as well as B_0 field maps were examined for each subject and time point. Histological analysis included a human cytoplasm stain to evaluate cell survival, immunostaining for ferritin expression, and a Prussian blue stain for iron accumulation.

Results: Ferritin over-expressing cells were detected in the right striatum at all imaging time points with maximum study duration of twelve weeks. Hypointense regions were also observed in the left striatum for eight out of nine subjects. These eight subjects corresponded to those that had bleeds on the left side during cell engraftment surgery. B_0 field maps did not differentiate between mechanisms of contrast. No appreciable signal change was observed over time. Histological analysis on the two available studies revealed that 77% of ferritin-positive sections corresponded to MRI contrast in the correlating image slice, but just 39% of regions of hypointense T2* contrast could be attributed to ferritin over-expressing cells. Iron accumulation was observed in 81% of ferritin over-expressing cell regions and 53% of negative control cell injection regions.



(a) Region of hypointense contrast in the striatum on T2* weighted gradient echo scan corresponds with (b) iron accumulation indicated by Prussian blue stain and (c) ferritin overexpression indicated by ferritin immunostain.

Discussion: Although cells over-expressing ferritin can be detected using MRI, the contrast provided may be non-specific. In this study, contrast correlating to ferritin cell location could not be distinguished from that caused by inflammation, hemorrhage, or cells which are not modified to over-express ferritin. Our approach holds promise in that cells expressing ferritin accumulate iron and provide hypointense contrast in the majority of cases, but the source of signal in the negative control case must be determined to increase specificity of signal.

Conclusion: Lentivirally induced ferritin over-expression provides stable long-term hNPC detectability after injection in the rodent striatum. However, the signal provided by negative control cells calls for further investigation and remedy. Further work involves development of acquisition and post-processing strategies, increasing levels of ferritin expression and iron accumulation efficiency, and application into different cell lines and systems.

References: 1. Wang P and Moore A. Molecular imaging of stem cell transplantation for neurodegenerative disease. Curr Pharm Design. 2012;18:4426-4440. 2. Suzuki M, McHugh J, Tork C, Shelley B, Klein SM, Aebischer P, Svendsen CN. GDNF secreting human neural progenitor cells protect dying motor neurons, but not their projection to muscle, in a rat model of familial ALS. PLoS ONE. 2007;2(8):e689. 3. Genove G, DeMarco U, Xu H, Goins WF, Ahrens ET. A new transgene reporter for *in vivo* magnetic resonance imaging. Nat Med. 2005;4(11):450-454.