Imaging Structure and Function of Stem Cell Grafts in the Mouse Brain by combining 19F Magnetic Resonance Imaging with Bioluminescence Imaging

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Introduction: Stem cells are a promising candidate for new therapeutic approaches of acute and degenerative disorders of the brain. However, before stem cell therapy can be translated to the clinic the role and function of these cells in vivo needs to be understood in more detail in animal models. 1H Magnetic Resonance Imaging (MRI) of magnetically labeled cells has shown great potential to monitor their spatio-temporal dynamics after implantation [1]. More recently, labeling cells with 19F agents and subsequent tracking with 19F MRI has been shown [2]. 19F MRI benefits from a lack of background 19F in tissue, hence allowing unambiguous locating of the cells. Furthermore, the 19F image intensity is proportional to the amount of 19F, which allows quantification of the number of cells. However, MRI of prelabeled cells highlights only structural information on the cell graft. Here we present a novel combination of 19F MRI with bioluminescence imaging (BLI) to visualize stem cells in vivo. For BLI detection, murine neural progenitor cells derived from embryonic stem cells were stably transduced to express either a membrane bound form of the Gaussia luciferase (Gluc+) [3] or the Clickbeetle luciferase (CBluc+). These cells were labeled with a 19F agent for 19F MRI detection. As the luciferase gene is only expressed in viable cells, the BLI signal is a measure of cell graft vitality. We carefully assessed the impact of the 19F label and luciferase expression on cell function by comparing 19F labeled Gluc+, CBluc+, and wildtype (WT) cells with non-labeled controls. We show bimodal detection of CBluc+ cells after implantation into the mouse striatum in a proof-of-concept. The system presented here can flexibly be adapted to many preclinical models of stem cell-mediated regeneration. It will help to optimize these models in terms of cell distribution (19F MRI) and viability (BLI).

Material and Methods: For 19F MRI cells were incubated for 42 h with a commercially available 19F agent (CS1000, Celsense, Pittsburgh, USA) at a previously optimized dose (25 µl/ml). To assess the intracellular distribution, we used the same protocol with a fluorophore coupled 19F agent (CS1000 green). Cell viability was determined with the trypan blue exclusion assay directly after the labeling. Survival rate was assessed by counting the fraction of viable cells after the treatment with 19F agent with respect to unlabeled controls. Influence on the cell proliferation was assessed by seeding the labeled cells and subsequent cell countings up to 4 days. To prove BLI/19F MRI detection in vitro, cells were seeded in 96-well plates, Coelenterazine, Viviren, or luciferin (Nanolight, Pinetop, USA) were added as BL substrates directly before the measurement with the optical imaging system (Biospace lab., Paris, France). After BLI, cells were prepared for 19F MRI at an 11.7 T scanner (Bruker BioSpin, Ettlingen, Germany) with a homebuilt surface coil. The protocol consisted of a background 1H turbo spin echo (TSE) image (8 echoes, FOV=2.88x1.92 cm^2, TR/TE=2.2 s/42 ms, MTX=192x128, 8 slices, 0.5 mm sl. thickness, 4 averages). The coil was then tuned to the 19F frequency and an identical TSE was acquired with centric encoding (TE=10.5 ms), lower resolution (MTX=72x48, 1mm sl. thickness) and 256 averages. For an in vivo proof of principle NuNu mice were implanted with CS1000 labeled Gluc+ cells (n=3) or CBluc+ cells (n=1) with deposits of 300,000 cells either into the striatum (AP +0.5; L/R ±2.0; V -2.5) or s.c. into the neck. One day after surgery, animals were injected different concentrations of the BL substrate (i.v., i.c., i.a., or i.p.) and scanned with BLI. A few hours later, animals were anesthetized with Ketamine/Xylazine to avoid 19F background signal from fluorinated anesthesia gases and scanned with MRI, parameters identical to in vitro. MRI experimental time was ~1.5 h.

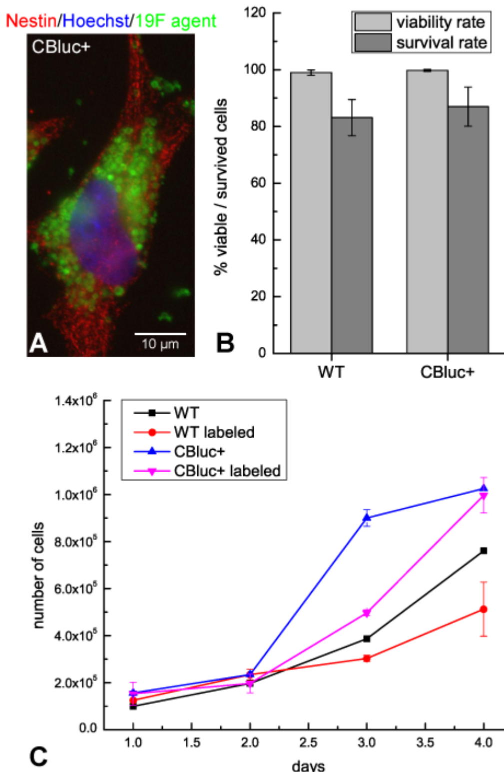

Figure 1 (A) Fluorescence microscopy of cells labeled with the CS1000 green revealed high intracellular uptake. (B) Viability and survival rates of the 19F labeled cells. There was no difference between CBluc+ and WT, however a small decrease of survival after 19F labeling. (C) CBluc+ cells proliferated faster than WT. For both WT and CBluc+ proliferation was slightly slowed due to 19F labeling.

Results: In vitro BLI showed a linear relationship between BLI signal and cell number for both luciferases with a higher signal for Gluc+ cells compared to the CBluc+. However, for none of the substrates or administration routes Gluc+ cell grafts were detectable in vivo. Therefore we continued all experiments with the CBluc+ cells only. Cells efficiently took up the 19F agent without help of transfection agents leading to ~1-3x10^{12} 19F spins/cell. The label was almost exclusively intracellular (Fig. 1 A). We saw only minor effects on viability or proliferation due to the labeling (Fig. 1 B, C). 19F labeled cells could be detected with high signal to noise ratio (SNR) in all implantation sites, BLI showed a very strong signal for the s.c. and for the CBluc+ striatal grafts whereas there was no signal from a WT control graft (Fig. 2).

Discussion: For the first time we have shown efficient 19F labeling of non-immortalized murine neural progenitor cells with minor effects on cell function. Furthermore, the novel bimodal detection of these cells with 19F MRI and BLI presented here will allow to (i) improve BLI 3D reconstruction methods, (ii) normalize and quantify BLI signal of grafts of different implantation depth due to high tissue penetration depth of MRI, (iii) control graft structure and viability (iv) monitor graft structure and cell differentiation state when the luciferase is set under a cell-specific promoter in future experiments.

Literature: [1] Kallur et al, EJN (2011) 34(3):382-93 [2] Ruiz-Cabello et al, MRM (2008) 60(6):1506-11 [3] Santos et al, NatMed (2009) 15(3):338-44

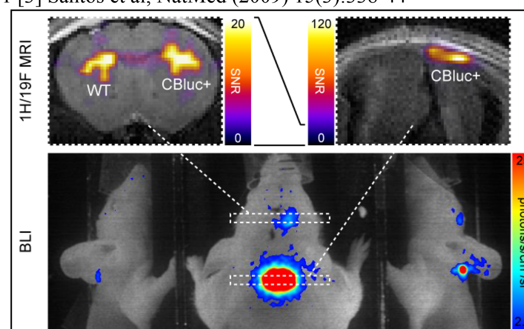

Figure 2 Top panel shows coronal 1H/19F MR images of a mouse with two CBluc+ and one WT implant, each 300,000 19F labeled cells. All grafts could be readily detected with lower SNR for the striatal grafts due to surface coil B1 profile. The transgenic cells in the right striatum and the neck generated light emission well above the background signal of WT cells on the left striatum (bottom panel), clearly to be depicted from top and the side views of two mirrors placed next to the animal.

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