ABSTRACT SUMMARY

A novel methodology utilizing combined imaging techniques (micro-CT) and cellular contrast nanotechnology agents was developed, in order to image and track Mesenchymal Stem cells (MSCs) within the brain. The new technique was utilized in cell therapy application for depression disorders. Our results provide evidence that this methodology enables detection and migration tracking of MSCs after inoculation in a rat model of depression. We demonstrated the therapeutic impact of MSCs on the depressive-like behavior displayed by the rat model.

INTRODUCTION

Depression is a highly prevalent and disabling psychiatric condition associated with significant morbidity and a lifetime prevalence approaching 17%. Unfortunately, despite substantial advancement in psychopharmacology and psychotherapy over the last decades, these therapies are far from ideal and the response rates hover at about 65%, with even worse full remission rates (1). Mesenchymal stem cells (MSCs) are Mesoderm derived cells that reside in adult bone marrow or adipose tissues that can differentiate to generate cells such as fat, bone and cartilage. Recent reports have demonstrated that these cells have also the potential to differentiate into cells with neural phenotypes (2). MSCs are regarded as potential candidates for treating a variety of neurological disorders due to their ability to secrete vital soluble factors, promote neurogenesis and migrate to sites of injury (3). Monitoring the location, distribution and long-term engraftment of administered cells is critical for demonstrating, cell therapy success. The challenge of long-term tracking of small number of cells in the brain, within a single live animal, has not yet been addressed. Computed tomography (CT) is among the most convenient imaging/diagnostic tools today that enables differentiation between tissues according to different degrees of X-ray attenuation. Gold Nanoparticles (GNPs) are characterized by high density and biocompatibility, potentiating them to become the next generation of CT contrast agents for biological applications (4). Therefore, we will develop a novel methodology utilizing combined imaging techniques (micro-CT) and GNPs-labeled MSCs, in order to track MSCs in-vivo and ex-vivo in animal models of depression disorders.

EXPERIMENTAL METHODS

GNPs were prepared using sodium citrate, according to the known methodology described by Enüstun and Turkevich (5). Particles were then characterized using DLS (Dynamic Light Scattering), TEM (transmission electron microscopy) and UV-Vis (Ultraviolet-visible spectroscopy). MSCs were isolated from adult human subcutaneous fat tissue. Cells were then differentiated into cells that express NSC phenotypes. Cellular uptake of the particles by the stem cells was quantified using Flame atomic absorption. An MTT growth array was conducted to assess whether the incorporated gold affected the metabolism, viability and proliferation rate of the cells. 250*10^3 cells were loaded MSC’s in vivo on a rat model. GNPs loaded MSCs engrafted rats (n=5) at specific time points post injections, and ex-vivo. A battery of behavioral tests was conducted before and three weeks post cells injection.

RESULTS AND DISCUSSION

We have successfully synthesized ~20 nm glucose coated GNPs. Shape and uniformity was measured using transmission electron microscopy (TEM) (Fig 1A) and UV-Vis spectroscopy (Fig 1B). Successful coating of the GNPs was demonstrated using UV-Vis spectra, showing a clear broadening of the peak of the SPR after each coating layer.

Fig 1a: 20nm GNPs TEM image 1b. Ultraviolet-visible spectroscopy of the bare GNPs, MDDA coated GNPs and Glucose-MDDA coated GNPs (GF2-GNP).

We have been able to track and image the GNPs loaded MSC’s in-vivo on a rat model. GNPs loaded MSCs were implanted into four FSL rats. CT scans were conducted in-vivo on GNPs-MSCs engrafted rats at
specific time points post injections. Figure 2 represents volume rendering in vivo CT scans of the brain obtained 1 month post injections. One rat was injected with GNPs loaded MSC’s (Fig. 2A) and as a control experiments, a rat was injected only with GNPs (without MSC’s) (Fig. 2B). As expected, there is a clear difference between the spatial distributions and volume of the GNPs in both images. The GNPs in the control rat are more scattered and widely spread across the brain (ventricles on both hemispheres) compared to the GNP-MSC engrafted rat, where they are denser and confined to more specific sub regions of the brain. Further ex vivo CT analysis (fig. 2C-2D) confirmed the localization and cell homing obtained from the in vivo CT results. MSCs engrafted at the dentate gyrus of the hippocampus.

The actual MSCs based cell therapy was tested on 25 FSL rats. The rats underwent baseline measurement of their depressive state in the Forced Swim Test. All rats underwent stereotoxic surgery and received 2x105 control MSCs or differentiated MSCs (to NSC like cells) from adipose unilaterally into the lateral ventricles. After three weeks, rats were subjected to additional behavioral tests, to check attenuation of the depressive-like behavior. Rats receiving differentiated MSCs showed a significant decrease in immobility time during the swim test compared to control (p < 0.01). Fold change differences from baseline were tested using one way ANOVA (One-way analysis of variance) and a Bonferroni's multiple comparison post-test comparing all pairs of columns. A significant difference in fold change from baseline was found in differentiated MSCs compared to control (p < 0.01), as well as in differentiated MSCs compared to undifferentiated MSCs (p < 0.05) (Fig 3).

CONCLUSION
This work demonstrates a novel method for stem cells tracking. We were able to trace cells within the brain for a long period of time. This method has the potential to reveal insight regarding the mechanisms underlying the success or failure of cell therapy, which is currently poorly understood. Our research suggests a new cell therapy approach for treating Neuropsychiatric disorders. We detected reduced depressive-like behavior after MSCs treatment. Recent evidence linked impaired neurogenesis in the hippocampus with depression; we detected the cells engraftment after a month in this area. We anticipate that this research will lay the groundwork for further fundamental research regarding MSCs cell therapy for multiple neuropsychiatric and neurologic disorders and help to accomplish the great challenge of tracking small number of cells in the brain.

REFERENCES