Tracking 4T1-piPSCs homing to primary and metastatic tumor with MRI

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INTRODUCTION: Stem cell-based cell-converting therapy is considered to be a future therapeutic strategy for cancer. In order to efficiently deliver cell-converting cancer therapy in a clinical setting, there is a need for imaging techniques that confirm the successful targeted delivery of therapeutics. MRI is a favorable tool to track cells in a typical clinical setting because of its high spatial resolution and excellent soft-tissue contrast. Mammary specific protein-induced pluripotent stem cells (piPSCs) demonstrate therapeutic potential in tumor therapy [1]. The QQ-protein delivery is a novel technique that can specifically deliver proteins into the targeted intracellular organelle of given cells [2]. We hypothesize that the QQ-protein delivery can be used to label cells with MRI-detectable proteins such as virtually "toxicity-free" ferritin. Our goal is to investigate the feasibility of using QQ-ferritin to label 4T1 (a malignant breast cancer cell line) derived piPSCs (4T1-piPSCs) and the feasibility of using MRI to track down the ferritin labeled 4T1-piPSCs homing to primary breast cancer as well as metastatic lesions in the mouse model.

MATERIALS AND METHODS: Ferritin was QQ-reagent modified for 4T1-piPSC-labeling. Cell viability was examined *in vitro* after ferritin labeling. The ferritin-labeled 4T1-piPSCs were injected into the #9 fat pad without tumors in the 4T1 breast cancer-bearing mice to study detection sensitivity by MRI. Dynamic MRI was performed before and after ferritin-labeled 4T1-piPSCs injection at the #9 fat pad of the 4T1 metastatic mice model with a 4T1 tumor in the #4 fat pad. All studies were performed using a 7T animal MRI system.

RESULTS: QQ-protein delivery could efficiently deliver ferritin into the 4T1-piPSCs and the labeled cells retained the ability for differentiation, proliferation, and cell viability. As few as 500 ferritin-labeled 4T1-piPSCs could be visualized by MRI *in vivo* after injections of ferritin-labeled 4T1-piPSCs. For the 4T1 metastatic mouse model, MRI showed a negative contrasting effect in both primary and metastatic breast tumors after the injection of ferritin-labeled 4T1-piPSCs and reached signal void in 8-20 hours, Histological results (Ferritin stain, Prussian Blue stain) confirmed that 4T1-piPSCs migrated to both primary and metastatic breast tumors (Figs 1 and 2).

DISCUSSION AND CONCLUSION: Local growth of malignant breast cancer is generally not considered life-threatening but metastatic lesions can be lethal. Currently, there is a poor survival rate for metastatic breast cancer patients which are due in part to the inability to deliver locally targeted therapeutic agents to kill metastatic cancer cells without harming normal cells. Major findings of this study are 1) the QQ technique can label cells with ferritin, which naturally occurs in the body, for MRI cell-tracking. 2) QQ-labeled ferritin does not affect cell viability, as expected since the Fe^{3+} stored in ferritin does not participate in the Fenton reaction and cells have their "nature"way of interacting with proteins, in contrast to commonly used MRI detectable nanoparticles. 3) 4T1-piPSCs displayed a tropism effect that allowed them to track down both primary and metastatic tumors and 4) ferritin-labeled 4T1-piPSC offers high sensitivity for MRI detection. To our knowledge, this is the first study to use MRI and piPSC techniques to detect metastatic lesions in a spontaneous metastatic breast cancer animal model. **In conclusion**, the preliminary study suggests that 4T1-piPSCs show great potential as a delivery vehicle for breast cancer therapies. MRI, when used in conjunction with ferritin-labeled 4T1-piPCSs, can evaluate and monitor these therapeutic techniques.

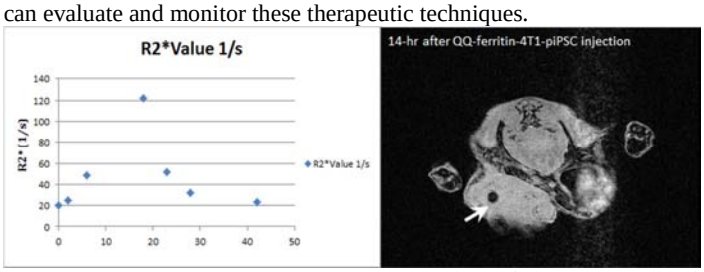

Figure 1. A typical case showing changes in R2* after the injection of ferritin-labeled 4T1-piPSCs at the left breast. The accumulation of ferritin-labeled 4T1-piPSCs in the primary tumor at the right breast reached the maximum around 18 hours in this case.

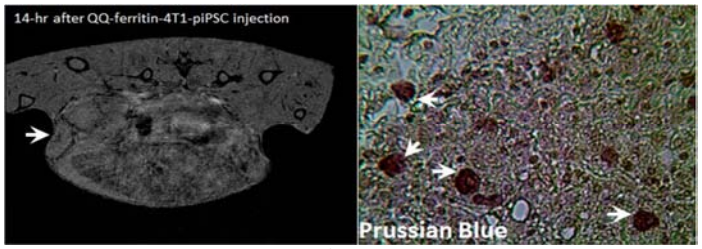

Figure 2. A typical case showing ferritin-labeled 4T1-piPSCs homing to the metastatic tumor 14 hours after ferritin-labeled 4T1-piPSC injection at the left breast. Prussian Blue-positive result was consistent with the MRI result.

Reference:

1. *Sharkis SJ, etc, "Pluripotent Stem Cell-Based Cancer Therapy: Promise and Challenges". Science Translational Medicine 2012, 4(127):127.*
2. Wang J, etc *"An in vivo protein delivery technology and applications". Patent under review 2012.*