IRON OXIDE INCORPORATION FOR CELL TRACKING DOES NOT PREVENT OSTEOGENIC, CHONDROGENIC OR ADIPOGENIC DIFFERENTIATION OF MESENCHYMAL STEM CELLS BUT DOES AFFECT EXTRACELLULAR MATRIX PATTERNS AND GENE EXPRESSION

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Background

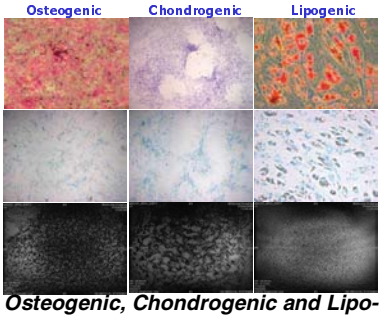
Knowledge about the fate of stem cells following implantation is of critical importance if the safety of stem cell therapy is to be verified. Recently, the ability to label stem cells with iron oxide nanoparticles and their subsequent visualization by magnetic resonance imaging (MRI) has been demonstrated. What is not clear, however, is the effect that this labeling has on the multipotent differentiation capacity of mesenchymal stem cells (MSCs). The aim of this study was to examine the osteogenic, chondrogenic and adipogenic differentiation potential of adult human MSCs when labeled with superparamagnetic iron oxide (SPIO) and to assess the long-term fate of labeled MSCs following seeding into a tissue engineered scaffold and in vivo implantation.

Material and Methods

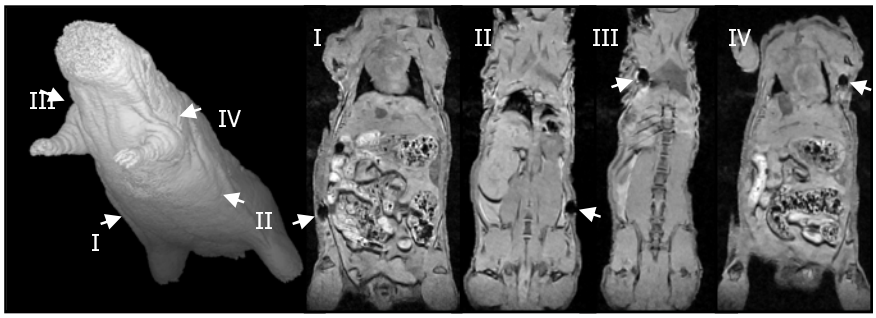
Adult human MSCs were labeled overnight with SPIO. Osteogenic, lipogenic and chondrogenic differentiation was induced. Cell cultures were scanned on a GE clinical 3.0T scanner using a 3D SPGR sequence (resolution 40x40x100 μm^3). Differentiation along the three different cell lineages was confirmed by histological staining and RT-PCR analysis. Labeled cells were also seeded onto collagen glycosaminoglycan (Collagen GAG) scaffolds for 21 days and then implanted subcutaneously into nude mice for a further 28 days (n=4). Unlabelled cells served as controls (n=5). Following the in vivo period, mice were imaged using a 3T clinical MRI scanner. Scaffolds were subsequently retrieved for histological analysis.

Results

Osteogenesis, lipogenesis, and chondrogenesis by MSCs were demonstrated in SPIO labeled cells by histological staining for mineralization, lipid vacuoles, and proteoglycans, respectively. Upon closer inspection, an altered matrix deposition was observed in chondrogenically treated cells following labeling with SPIO. Quantitative analysis of the expression of relevant genes revealed a subtle but significant change in the expression profile of these markers.



Osteogenic, Chondrogenic and Lipogenic differentiation of SPIO-labeled MSCs as assessed by histology, Prussian Blue staining and MRI (from top to bottom).



In vivo MR images of SPIO-labeled MSCs following seeding on collagen GAG scaffolds, long-term in vitro culture, and subcutaneous implantation.

Following in vivo implantation of labeled MSCs on scaffolds, long-term retention of SPIO (at least 7 weeks) by labeled MSCs was demonstrated. However, SPIO-labeled MSCs did exhibit profoundly altered morphologies and extracellular matrices compared to unlabeled MSC's in vivo.

Conclusions

MSCs retain their multi-lineage differentiation potential following labeling with SPIO and retain label during differentiation allowing visualization by MRI. However, labeling with SPIO does alter the molecular properties of the different lineages, potentially resulting in an altered phenotype, depending on environmental factors.