Feridex Labeling of Human Mesenchymal Stem Cells Inhibits in Vitro Chondrogenesis

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Introduction

Culture-expanded bone-marrow-derived mesenchymal stem cells (MSCs) are a promising source of multipotential cells for treatment of injury and disease conditions, ranging from cardiac ischemia to spinal cord injury. Under the right circumstances, MSCs may "home" to injuries, engraft and differentiate (1). Design and improvement of MSC therapies will be aided by the development of sensitive, noninvasive, and nondestructive techniques of tracking MSCs following implantation or infusion. MR tracking of magnetically labeled MSCs may fulfill this requirement. For intracellular magnetic labeling, several groups (2-5) have used Feridex following coating with a transfection agent (such as poly-L-lysine (PLL)). For *in vivo* MR studies of implanted Feridex-labeled MSCs, it is important to determine the effects of Feridex treatment on the proliferation and differentiation of MSCs. We show here that Feridex does not affect proliferation, adipogenesis, or osteogenesis. Surprisingly, however, Feridex-treated cells were markedly diminished in their ability to undergo chondrogenesis *in vitro*.

Materials and Methods

Human MSC cultures were established from anonymous adult donors. Iliac crest marrow aspirates were used to initiate adherent cultures. For magnetic labeling, MSCs from three donors were labeled for 24 h with 25 μg Fe/ml Feridex and 375 ng/ml PLL (2) or with PLL only as control. For in vitro studies of adipogenic and osteogenic differentiation, confluent (labeled) monolayers of hMSCs grown in DMEM with 10% fetal bovine serum were trypsinized and replated. Adipogenesis was determined by neutral lipid accumulation while osteogenesis was quantitatively measured by calcium deposition. For the *in vitro* chondrogenic assay, cells were gently centrifuged to form a pelleted micromass, which was subsequently cultured in a serum-free media. Micromass culture ended after 21 days, at which time sections were stained with Safranin O or immunostained with antibody against Type II collagen (Col II) to reveal the presence of macromolecules diagnostic of chondrogenic differentiation (6). *In vivo* mesenchymal differentiation of cells was evaluated by adsorbing MSCs to hydroxyapatite-tricalcium phosphate (HA-TCP) cubes (3 mm to a side), then implanting cubes subcutaneously in an immunocompromised mouse host. After 6 weeks, thin sections of cubes were stained and visually evaluated for bone and cartilage on a five-point scale (7). *In vitro* and *in vivo*, Feridex-containing cells were identified after fixation by staining with Prussian Blue (PB) stain.

Results

The *in vitro* proliferation of MSCs appeared to be unaffected by PLL-Feridex treatment (94% viability unlabeled vs 92% viability labeled cells, as assessed by Trypan Blue staining). PLL-Feridex-labeled MSCs underwent *in vitro* adipogenic and osteogenic differentiation as efficiently as unlabeled cells. However, PLL-Feridex labeling interfered with chondrogenesis in both donors studied. PLL-Feridex labeled cultures showed no evidence of chondrogenesis, in contrast to Feridex-free controls (Fig. 1). The sensitivity of this inhibition was evaluated by halving the Feridex concentration. MSCs from donor 2 but not donor 1 were able to undergo limited chondrogenesis in this condition. Chondrogenesis was unaffected by treatment of MSCs with PLL alone. We next mixed equal numbers of Feridex-labeled and unlabeled MSCs together. For donor 1, chondrogenesis was much reduced, while for donor 2 it was only modestly affected (Fig. 2). Surprisingly, Feridex-labeled and -unlabeled cells segregated into distinct "patches;" and only the areas without PB staining accumulated Col II (Fig. 2). When adsorbed to HA-TCP cubes and implanted subcutaneously, these Feridex-labeled and unlabeled MSCs produced modest amounts of osteogenic material, scoring "1" on a scale of 0 to 4 (7). The extent of *in vivo* osteogenic differentiation was unaffected by labeling cells with Feridex prior to implantation.

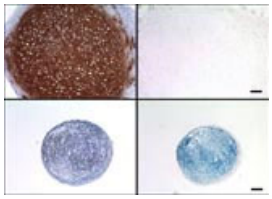

Figure 1

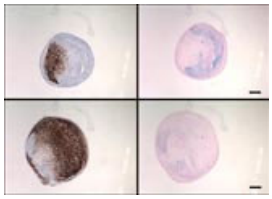

Figure 2

Figure 1: Donor 1 MSCs unexposed to Feridex generate large amounts of Col II-rich extracellular matrix in 21 days of micromass culture (top left, brown), and are unstained by PB (top right). When Feridex labeled prior to the onset of chondrogenesis, pellets fail to expand in size and lack Col II (bottom left). PB staining reveals Feridex-containing cells throughout pellet (bottom right).

Figure 2: Partial inhibition of chondrogenesis in pellets made from 1:1 mixtures of Feridex-labeled and -unlabeled MSCs. For donor 1 (top) and donor 2 (bottom), Col II staining (left) is restricted to parts of the section unstained by PB (right). The self-segregation of Feridex- labeled and unlabeled cells is evident in each case. Bars, 250 μm.

Conclusion

At appropriate concentrations, Feridex labeling did not affect proliferation, osteogenesis, or adipogenesis in MSC cultures, and is thus a suitable labeling agent for therapies reliant on these processes. Unexpectedly, we found that Feridex can block chondrogenesis. The extent of the reduction in differentiation shown by donor 1 suggested that Feridex might actively interfere with intracellular signaling processes required to drive mesenchymal condensations to a chondrogenic fate. Reducing the Feridex labeling concentration by 50% alleviated this problem for donor 2 but not donor 1, though at the expense of PB staining intensity, and thus possibly MRI detection sensitivity. The nature of Feridex' interference with this mesenchymal pathway of chondrogenic differentiation is unknown.

References

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