In Vivo differentiation of Magnetically Labeled Mesenchymal Stem Cells into Hepatocytes for Cell Therapy to Repair Damaged Liver

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INTRODUCTION

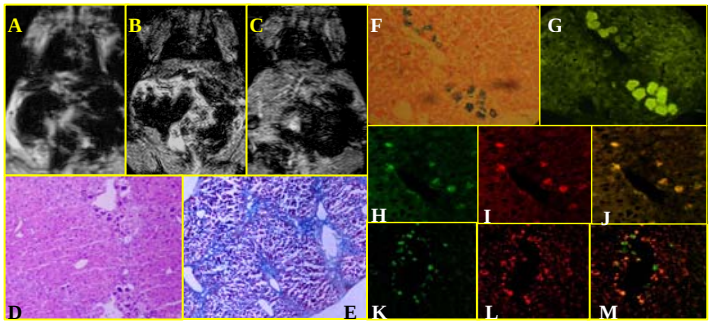
Ever since the transdifferentiation of bone marrow cells (BMCs) into hepatocytes was documented [1-2], BMC has been an attractive cell source in regenerative medicine. MR imaging appears most promising for dynamically monitoring in vivo cell migration after magnetically labeled cell transplantation [3]. It is not clear, however, whether systemically administered mesenchymal stem cells (MSCs) labeled with superparamagnetic iron oxide nanoparticles can also transdifferentiate into hepatocytes. We built a new in vivo model for monitoring the transdifferentiation of magnetically labeled green fluorescent protein (GFP) positive MSCs into albumin-positive hepatocytes, under the specific "niche" made by CCl4 induced persistent liver damage. We also tracked magnetically labeled MSCs by using magnetic resonance (MR) imaging in vivo in mice.

METHODS

MSCs were isolated from GFP transgenic C57BL/6 mice and labeled with home synthesized superparamagnetic particles Fe_2O_3-PLL [4]. The liver damage model was established by injection of 0.5 ml/kg of carbon tetrachloride (CCl_4) into the peritoneum of Balb/c nude mice twice a week for five weeks. One day after the second injection, cell transplantation was performed by injection of 1×10^6 MSC_S via the caudal tail vein. Thirty-two recipient nude mice were randomly divided into five groups, three with and two without liver damage. Liver damage groups included mice injected with magnetically labeled GFP-positive MSCs (group A, n=8), GFP-positive MSCs (group B, n=8), or saline alone (group C, n=8). Control groups included mice injected with magnetically labeled GFP-positive MSCs (group D, n=4) and mice without injection (group E, n=4). MR examinations were performed 24 hours and 4 weeks after cell injection in groups A, B, and C. Liver signal-to-noise ratios (SNRs) on T2*-weighted MR images were measured. In all groups, serum albumin levels were analyzed four weeks post-injection, and the mice were then sacrificed for histologic tissue examination. Hematoxylin-eosin staining and Masson staining were performed to confirm liver damage. To assess the injected cells, consecutive slides were analyzed about both GFP and iron expressing. Cells expressing GFP were analyzed by both fluorescent microscopy and immunofluorescence with anti-GFP antibody. Cells expressing iron were assessed by Prussian blue staining. To determine whether injected cells differentiated toward hepatocytes, fluorescence immunohistochemistry analysis was performed with anti-albumin antibody, and albumin-positive cells ratios in GFP-positive cells were calculated.

RESULTS

GFP-positive MSCs could be effectively labeled with approximately 95% efficiency. Migration of transplanted labeled cells to the liver was successfully documented with in vivo MR imaging. SNRs on T2*-weighted images decreased significantly in the liver 24 hours after injection of MSCs ($p < .05$) and returned to the level achieved without labeled cell injection in 4 weeks (Fig.A-C, N). For groups A-E, serum albumin levels (g/l) were 20.0 ± 6.7, 20.3 ± 9.2, 18.0 ± 2.2, 34.5 ± 5.8, and 31.5 ± 5.1, respectively. Serum albumin was lower for all liver damage groups than control non-damage groups ($p < .05$); it increased for both group A and B at 4 weeks after MSC transplantation but still insignificant compared to Group C ($p > .05$). Histologic HE and Masson staining confirmed the existence of liver damage and hepatic fibrosis in groups A, B and C (Fig.D-E). GFP-positive and Prussian blue staining MSCs were mainly distributed in the sinusoids of periportal areas and the foci of CCl4-induced liver damage. Positive Prussian blue staining cells were highly correlated with GFP-positive cells in group A (Fig.F-G). In group D, no iron-GFP-positive cells cound be found in liver. Albumin was expressed in 34 ± 6% and 35 ± 7% of GFP-positive cells in group A and B, respectively; there was no significant difference between the two groups (Fig.H-M).



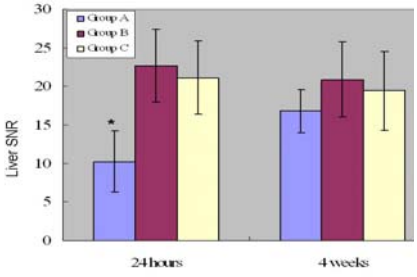


Fig.N: Graph compares liver SNRs among groups A, B and C. Injection of labeled cells caused substantial decline of SNRs at 24 hours after injection compared with SNR of control mice ($P < .05$). SNR still low at 4 weeks, but insignificant ($P > .05$).

Fig.A-C: T2*-weighted MR images of Balb/c mouse 24 hours (A), 4 weeks (B) after injection of 1×10^6 magnetically labeled GFP-positive MSCs, as well as one mouse at 24 hours after injected GFP-positive MSCs (C). Note the decrease and return of SI compared with SI in control mouse. **Fig.D-E:** Photomicrographs show obvious liver injury in mice. D: Hematoxylin-eosin stain, magnification, ×100. Masson staining (E) confirmed the existence of collagenous fibers (blue) in hepatic fibrosis (magnification ×40). **Fig.F-G:** Comparison of Prussian blue staining (F) and fluorescent microscopy (G). All injected cells are both iron-positive and GFP-positive in Group A. **Fig.H-J:** Expression of GFP in liver at 4 weeks after transplantation of GFP-positive cells (red, GFP in immunofluorescence with anti-GFP antibody; green, GFP under fluorescent microscopy; and yellow, GFP both in immunofluorescence and fluorescent microscopy) (magnification ×200). **Fig.K-M:** Albumin expression at 4 weeks after transplantation of GFP-positive MSCs. Double fluorescent staining (red, albumin; green, GFP; and yellow, albumin and GFP) of liver at 4 weeks after cells transplantation. Some cells (34 ± 6% and 35 ± 7% in groups A and B, respectively, 5 high-power fields per section) are both albumin-positive and GFP positive (magnification, ×100).

CONCLUSION

GFP-positive bone MSCs could be efficiently labeled with Fe_2O_3-PLL, and the relocation of the labeled cells to mouse livers after transplantation could be depicted at in vivo MR imaging. The magnetically labeling technique does not interfere with the process of differentiation and amending function of MSCs in vivo and corresponds well with GFP labeling. Both magnetically labeled and unlabeled MSCs have the potential to differentiate into hepatocytes and to perform cell therapy on repair damaged liver under special niche.

REFERENCES :(1) Okamoto R et al. *Nat Med* 2002; 8: 1011–7 (2) Korbling M et al. *N Engl J Med* 2002; 346:738–46 (3) Bulte JW et al. *Magn Reson Med* 2003; 50: 201-5 (4) Ju S et al. *Radiology* 2007; 245:206-15