Development of New, More Sensitive MR-Reporter Genes for Stem Cell Tracking and Gene Therapy Imaging

L. S. Politi[1], S. Pizzi[1], A. Cozzi[2], M. Amendola[3], A. Falini[1], L. Naldini[3], S. Levi[2], A. Biffi[3], and G. Scotti[1]

[1]Neuroradiology, San Raffaele Scientific Institute, Milan, Italy, [2]Iron Metabolism Unit, San Raffaele Scientific Institute, Milan, Italy, [3]HSR-TIGET, San Raffaele Scientific Institute, Milan, Italy

PURPOSE

MR-reporter genes might allow sustained and stable intracellular contrast accumulation for long-term stem cell (SC) tracking studies and gene therapy applications. However, the sensitivity of previously described MR-reporter genes, such as intracellular ferritin, is low. Thus, we are exploiting potentially MR-stronger reporters in combination with late generation lentivirial vectors (LV), allowing high efficiency SC transduction and robust, long-term expression in their progeny upon integration into the host genome.

METHOD AND MATERIALS

The following MR-reporter genes were compared: a) a mutated form of human ferritin L chain (mLfer) that aggregates and stores great iron quantities; b) wild type H and L human ferritin chains (Hfer and Lfer); c) the human tyrosinase (hTyr) that catalyzes two limiting reactions during melanin synthesis. LV carrying the cDNA of these MR-reporters downstream to the human phosphoglycerokinase promoter were produced. Further, we generated bidirectional LV, allowing efficient and simultaneous expression of a MR reporter gene and a conventional reporter for pathology (green fluorescent protein), or, alternatively, of Hfer and Lfer. 2 cell lines, murine and human hematopoietic and neural SC, were transduced with these LV. Proliferation, differentiation and apoptosis of cell lines and SC were assessed after transduction. MR imaging and relaxometry studies were performed on agarose phantoms and transplanted mice using a 3T human MR scanner. Further, LV carring DNA of all these MR reporter genes were injected in the hippocampus of nude mice.

RESULTS

Among the tested candidate genes our data indicate in mLfer the stronger reporter allowing sensitive and long-term MR detection of transduced cells. Indeed, after transduction, cells became detectable in T2, T2* and Susceptibility Weighted Images both in vitro and in vivo, concomitantly with the appearance of a positive staining for iron within cellular bodies. The R2 and corrected R2* values correlated with gene expression and number of examined cells. Viability, proliferation and multipotency of transduced cells were not affected, using as referral wild type H-ferritine. MR-signal change was also detected in the hippocampus of injected mice and correlated to histology and immunofluorescence. Also hTyr transduction and the simultaneous expression of H and Lfer allowed significant improvement in cell detection.

CONCLUSION

The tested genes are promising MR-reporters allowing sustained and sensitive detection of SC also at 3T.