Evaluation of Cell Therapy as a Treatment Approach for Osteogenesis Imperfecta

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Disclosures:

Introduction: Osteogenesis imperfecta (OI) is a genetic disorder caused by reduced or improper type I collagen production that results in bone fragility. Recent studies have been directed at silencing or correcting the mutation in bone marrow stromal cells (BMSCs) derived from patients with OI. Despite the success of these in vitro therapeutic approaches, further development of techniques for reintroducing engineered cells back into the patient is required. Previous studies involving transplantation of BMSCs in murine models of disease have shown that donor cells engraftment is low. The evidence for successful systemic transplantation is very limited, and in most studies it is not clear whether the short-term improvements are the result of the aggressive preparation of the host by radiation or pharmacological treatment. A recent report indicated that performing direct transplantation of cells that were extensively grown in vitro and then transduced with retroviruses was feasible. The main concerns with these studies are the heterogeneity of the cells used for transplantation and the inability to evaluate the proportion of the donor cells and their effectiveness in generating new healthy bone. We aimed to evaluate whether donor cells delivered locally into femurs were able to engraft, differentiate into osteoblasts, and contribute to formation of normal bone.

Methods: All animal procedures were IACUC approved by UConn Health Center. Cell culture: Donor BMSCs from mice harboring the bone-specific Col2.3GFP transgene were cultured in 5% O2 for 7 days prior to transplant to expand undifferentiated mesenchymal progenitors (Col2.3GFPneg at transplantation). Transplantation: The osteogenesis imperfecta murine (OIM) model was utilized as the recipient. Mice underwent irradiation then received wild-type whole bone marrow via the retro-orbital sinus, followed by intramedullary delivery of a 106 donor BMSCs in the right femur. Histology: Mice were sacrificed 4 weeks after the transplantation. Expression of Col2.3GFP and the presence of the mineralizing front in relation to the transgene expression was evaluated in undecalcified frozen sections. To evaluate new bone formation mice were injected with xylene orange (XO). Stem cell engraftment To assess long-term engraftment of transplanted progenitors, primary BMSC cultures were established from the mice after 4 weeks after transplantation. Cells were cultured under osteogenic conditions and monitored for the presence of mineralizing colonies. The presence of Col2.3GFP expressing colonies would indicate that BMSCs have been established from transplanted cells. Secondary transplantation: Primary BMSC cultures derived from transplanted OIM mice were expanded under non-differentiating conditions (5% oxygen) and transplanted by intra-bone marrow injections into OIM mice. Four weeks after the transplantation, presence of mature osteoblasts was assessed by histological analysis.

Results: Histological evaluation. We detected differentiation of donor cells into Col2.3GFP+ osteoblasts and osteocytes in cortical and trabecular bone of the transplanted femurs (Figure 1). New bone formation was detected by XO deposition in the proximity to the Col2.3GFP+ osteoblasts. Col2.3GFP+ cells were not found in the contralateral femur indicating that transplanted osteogenic cells did not disseminate by circulation. Following systemic transplantation of BMSCs via the retro-orbital sinus we did not observe any engraftment of Col2.3GFP+ cells.

Stem cell engraftment. Primary cultures derived from transplanted femurs of mice after one month showed no presence of Col2.3GFP+ cells after 7 days in culture, but numerous Col2.3GFP+ colonies were present by day 14. In addition, these colonies showed mineralization potential as assessed by von Kossa staining. This observation indicates the presence of donor derived progenitor cells with capability to form mineralized colonies. This was not observed in cultures from the contralateral femur or from mice that underwent systemic transplantation. To evaluate the self-renewal potential of transplanted BMSCs we performed secondary transplantation of cells recovered and expanded in vitro from recipient femurs. These cells also engrafted and differentiated into Col2.3GFP+ osteoblasts and osteocytes indicating the persistence of donor stem/progenitor cells.

Discussion: Our results indicate that BMSCs delivered locally in OIM femurs are able to engraft, differentiate into osteoblasts and osteocytes and maintain their progenitor potential in vivo. This result shows that local delivery is a promising approach for delivery of autologous MSC in which mutations have been corrected. We were not able to detect any cells with osteogenic differentiation capability following systemic transplantation, or in the contralateral limbs following intra-bone marrow transplantation. These results suggest that mesenchymal stem cells cultured in this manner do not circulate. This is currently an area of research with conflicting reports. Discrepancy between in vitro and in vivo evidence for the presence of circulating MSCs, where in vitro data in number of studies would support the presence of circulating cells by assaying their in vitro potential. In contrast the in vivo evidence often does not utilize very stringent criteria for defining a cell on a bone surface as a functional and matrix producing osteoblast.
Significance: This is the first study that utilized a bone specific marker to trace a population of primary mesenchymal progenitor cells following direct intra-bone marrow transplantation in the OIM model. Our work also suggests that mesenchymal progenitor cells do not engraft by systemic circulation.

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References: None

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