

Ex vivo Expanded Murine Mesenchymal Stem Cells as Targets for the Generation of a Cell Replacement Therapy for Type 1 Diabetes

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Gene therapy as a means of generating “artificial” insulin-producing cells is being considered as a potential cure for type 1 diabetes (T1D). The aim of this study was to evaluate the utility of *ex vivo* expanded murine mesenchymal stem cells (MSCs) as targets for gene therapy and the development of a cell replacement therapy. CD45⁻/Ly6⁺ murine MSCs were isolated from the bone marrow of non-obese diabetic (NOD) mice and nucleofected to express the bioluminescent protein *Firefly luciferase* (*Luc2*). The persistence of a subcutaneous (s.c) transplant of *Luc2*-expressing MSCs was assessed in immune-competent (NOD) (n=4) and immune-deficient (NOD/*Scid*) (n=4) animal models of diabetes. *Ex vivo* culture-expanded MSCs were subsequently transduced with the HMD lentiviral vector (MOI=10) to express furin-cleavable human insulin (*INS-FUR*), murine *NeuroD1* and *Pdx1*; followed by characterisation of pancreatic transdifferentiation via reverse transcriptase polymerase chain reaction (RT-PCR), and acute and chronic insulin secretion assays. A s.c transplant of 1x10⁷ and 5x10⁷ *INS-FUR*-expressing MSCs in NOD/*Scid* mice (n=5) was assessed for their ability to reverse diabetes. *Luc2*-expressing MSCs persisted for 2 weeks and 12weeks respectively in NOD and NOD/*Scid* mice. *INS-FUR*-expressing MSCs lacked glucose-responsiveness and secreted human insulin chronically, whereas *NeuroD1* and *Pdx1*-expressing MSCs lacked glucose-responsiveness and insulin secretion capabilities. Transduced MSCs did not undergo pancreatic transdifferentiation as determined by RT-PCR analysis, and upon transplantation did not reverse diabetes. The data suggests that *ex vivo* expanded MSCs lose their multipotent differentiation potential and may be more useful as gene therapy targets prior to expansion. This correlates with other studies where *ex vivo* expansion of MSCs is associated with a loss of MSC function and negative T1D clinical outcomes.