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Retrovirally-mediated genetic correction of mesenchymal stem cells from patients affected by mucopolysaccharidosis type II (Hunter Syndrome)

Research Article

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Abbreviations: Bone marrow transplantation, (BMT); central nervous system, (CNS); colony-forming-unit fibroblast, (CFU-F); Dulbecco's modified Eagle's medium, (DMEM); enhanced green fluorescent protein, (eGFP); enzyme replacement therapy, (ERT); fetal calf serum, (FCS); glycosaminoglycans, (GAGs); graft versus host disease, (GvHD); Human MSC, (hMSC); iduronate-2-sulphatase, (IDS); internal ribosome entry site, (IRES); mannose-6-phosphate receptor, (M-6-P); Mesenchymal stem cells, (MSC); Mucopolysaccharidosis type II, (MPSII); population doublings, (PDs)

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Summary

Mucopolysaccharidosis type II (MPSII) is an X-linked metabolic storage disorder due to the deficiency of iduronate-2-sulphatase (IDS) and accumulation of glycosaminoglycans (GAGs). Clinically it presents as a multi-system disorder with developmental delay, bone and joint disease and in the severe forms progressive mental retardation. At present little therapeutic options are available. Bone marrow transplantation is no longer recommended due to the severe side effects and lack of proven efficacy in correcting central nervous system and bone disease. Enzyme replacement therapy is under assessment and it requires weekly, expensive administration for the lifespan of the individual. Mesenchymal stem cells (MSC) are bone marrow-derived cells capable of differentiation into tissue such as bone and have been shown to contribute to bone repair. They are amenable to gene manipulation and therefore provide an excellent target for the correction of MPSII disease, especially with regard to bone disease. In this study we tested whether MSC from MPSII patients ($hMSC_{MPSII}$) could be corrected with a retroviral vector expressing the IDS gene. Following transduction $hMSC_{MPSII}$ maintained the capacity to differentiate into osteoblasts and adipocytes and showed levels of IDS enzyme over 10 fold higher than those detected in MSC from healthy donors. This led to normalization of GAGs storage in $hMSC_{MPSII}$. Such transduced cells were able to cross-correct MPSII fibroblasts by uptake of the IDS enzyme via the mannose-6-phosphate receptor. This study suggests that correction of autologous $hMSC_{MPSII}$ by retroviral gene transfer is effective and may be amenable for the improvement of the skeletal features of the disease.

I. Introduction

Mucopolysaccharidosis type II (MPSII), or Hunter syndrome, is an X-linked systemic metabolic disorder due to the deficiency of iduronate-2-sulphatase (IDS; EC 3.1.6.13) activity. This results in incomplete degradation of the glycosaminoglycans (GAGs) dermatan and heparan sulphate, which accumulate in the cells and interfere with

their functions (Lim et al, 1974; Wraith et al, 1991; Hopwood et al, 1993). Clinically, the disorder is a spectrum and at the severe end is characterized by mental retardation, skeletal deformities, joint stiffness, organomegaly, airway obstruction and premature death (usually in their teens). The attenuated end of the clinical spectrum has no central nervous system (CNS) involvement and death may occur in early to mid-adulthood (Young et al, 1982).

Bone marrow transplantation (BMT) and enzyme replacement therapy (ERT) are two treatments available for patients with MPS disease. However, despite some success with BMT in MPS types I and VI, this is not recommended for MPSII due to difficulties in engraftment, the severe side effects, including graft versus host disease (GvHD) (Peters and Krivit, 2000), and because it had limited efficacy in delaying progressive CNS deterioration (Peters and Steward, 2003). ERT is under evaluation but it is a costly treatment and has to be administered throughout the lifespan of the patient (Muenzer et al, 2006).

Genetic manipulation of stem cells is an attractive therapeutic option. Stem cells are defined by their ability to self renew and differentiate into multiple tissues for the lifespan of the individual. Therefore genetic manipulation of stem cells would guarantee lifelong enzyme production capability. Mesenchymal stem cells (MSC) can be easily isolated from bone marrow, expanded several fold and efficiently gene modified with minimal manipulation (Prockop, 1997). They are capable of differentiation into osteoblasts and chondrocytes (Pittenger et al, 1999), they have been shown to integrate and participate to bone repair in fractures (Quarto et al, 2001) and to partially correct inherited disorders of the skeleton, such as those described in osteogenesis imperfecta (Horwitz et al, 1999). Thus, they may act as a suitable enzyme delivery system to difficult tissues such as bone. Moreover as cells from the patients' bone marrow could be isolated, corrected by gene modification in vitro and reinfused into the patient they would not suffer from the same limitations as allogeneic bone marrow transplant including donor identification and the severe morbidity associated with this procedure.

Previously we have shown that hMSC could be isolated and genetically modified to correct deficiency of α-L-Iduronidase in patients affected by MPSI (Baxter et al, 2002). In this study we have shown that autologous hMSC can be isolated from MPSII patients, efficiently gene modified by retroviral vectors to express supernormal levels of IDS enzyme and correct GAGs accumulation. Moreover IDS enzyme is secreted in sufficient amount to provide uptake by the surrounding cells and correction of GAGs accumulation via the mannose-6-phosphate receptor (M-6-P).

II. Materials and methods

A. Isolation and culture of human MSCs

Human MSC were isolated from bone marrow obtained from the posterior iliac crest of two MPSII patients ($hMSC_{MPSII}$) and two healthy donors ($hMSC_N$) aged 46 and 181 months after parental consent (in accordance with the ethical committee and with the Helsinski Declaration of 1995). Human MSC (hMSC) were isolated and cultured as previously described (Bruder et al, 1997). Briefly, mononuclear cells (MNCs) were seeded at a concentration of 8 x 10^5 MNC/cm^2 in Dulbecco's modified Eagle's medium (DMEM; Invitrogen, Paisley, UK) supplemented with 10% fetal calf serum (FCS; StemCell Technologies, London, UK). The medium was changed 24-48 h later, and cells were fed twice a week. When cultures reached confluence, hMSC were detached using 0.05% trypsin 5-mM EDTA (Invitrogen), counted using a hemocytometer; then one third of the cells were replated, and the remainder were used for differentiation assays. The number of hMSC at the start of the culture was determined by the colony-forming-unit fibroblast (CFU-F) assay, as previously described (Friedenstein et al, 1976) and was used to determine the number of population doublings that cells have undergone to reach primary confluence. Thereafter, the number of population doublings was calculated by dividing the log N by log 2, where N equals the total number of cells divided by the initial seeding number of cells. All hMSC cultures were stained with anti-CD45, anti-CD34 antigen and showed no expression when compared to cells stained with an isotype matched control. They were found positive for CD105 (Barry et al, 1999).

B. Differentiation

Human MSCs were plated at 1 x 10^4 and 3 x 10^4 per well in 6-well plates in growth medium with osteogenic or adipogenic supplements, respectively and as previously described (Pittenger et al, 1999). To detect differentiation to the osteogenic lineage alkaline phosphatase expression was assessed using an alkaline phosphatase cytochemical staining kit (Sigma Chemical Corp., Poole, Dorset, UK) according to manufacturer's instruction. Oil Red O staining was used to identify differentiation to the adipogenic lineage (Baxter et al, 2004).

C. Recombinant retroviral vector expressing IDS and transduction of hMSC

The retroviral vector used in this study (**Figure 1**) is based upon the pSFβ91 backbone (Hildinger et al, 1998) that is optimised for initiation of transcription in haemopoietic cells, (kind gift of Christopher Baum, Hannover, Germany) modified to include an internal ribosome entry site (IRES) (Jang et al, 1988), and enhanced green fluorescent protein (eGFP) sequences (Clontech, Palo Alto, USA). The IDS cDNA was cloned into the unique NotI and BamHI sites in the retroviral vector to form pSFβ91 IDS IRES eGFP that thus contains a cassette co-expressing IDS and eGFP whose translation is driven by the encephalomyocarditis virus IRES. GP+envAM12 packaging cells (Markowitz et al, 1988) were transfected with plasmids encoding the retroviral vectors described above using Transfast (Promega, Southampton, UK) as per manufacturers instructions. One week after transfection, cells harbouring a stable expressing integrant were isolated using a FACSVantage based upon GFP fluorescence. GP+envAM12 were cultured in DMEM containing 10% newborn calf serum and non essential amino acids (Invitrogen). $hMSC_{MPSII}$ (30% - 40% confluent) were transduced by incubating for 8 hours on 2 consecutive days with 2ml cell-free retroviral supernatant supplemented with 4μg/mL polybrene (Sigma Chemical Corp., Poole, UK), followed by replacement of supernatant by fresh culture medium. Seventy-two hours post-transduction, the cells were analysed for eGFP expression by flow cytometry analysis using Becton Dickinson FACScalibur equipment and the acquired data were analysed by CellQuest software.

Figure 1 SFβ91-IDS vector. Schematic representation of the vector used in this study based on the SFβ91 backbone. ψ, packaging signal; IDS, Iduronate-2-Suplhatase; IRES, internal ribosome entry site; eGFP, enhanced green fluorescent protein.

D. Determination of IDS activity

$hMSC_N$, $hMSC_{MPSII}$ and retrovirally transduced $hMSC_{MPSII}$ were tested for IDS activity using the tritium-labelled disulphated disaccharide derived from heparin sulphate basically as described by (Lim et al, 1974). Cell pellets were suspended in 120 µl distilled water, disrupted by sonication, briefly centrifuged and the supernatants dialysed against 0.15M-NaCl overnight at 4ºC. Aliquots (30 µl) were incubated with acetate buffered substrate for 2h at 37ºC. Following termination with 0.1M-Na_2HPO_4 the monosulphated product was separated on Ecteola cellulose (Sigma, Cambridge, UK) by elution in 0.6M- sodium formate and the radioactivity counted. For reference, the lysosomal enzyme β-galactosidase, was also assay by a standard fluorimetric method (Galjaard, 1980).

E. $^{35}SO_4$-GAG sequestration assay

Confluent $hMSC_N$, $hMSC_{MPSII}$ and retrovirally transduced $hMSC_{MPSII}$ were exposed to ^{35}S-labeled Na_2SO_4 (NEN Life Science Products, Boston, MA) at 20 µCi/mL (0.74 MBq/mL) in Dulbecco modified Eagle medium plus 10% fetal calf serum for 24 hours and subsequently cultured for 1 week. Cells were then trypsinized and washed in phosphate-buffered saline to remove external GAGs. Following centrifugation at 800*g* for 10 minutes, cells pellets were solubilized in 2 mL of 6 M urea/0.15 M sodium phosphate, pH 7.0, containing 1% Triton X-100 at 4ºC for 1 hour. Extracts were filtered before application to a fast protein liquid chromatography Mono-Q HR 5/5 anion exchange column (Pharmacia, St Albans, UK). Nonincorporated $^{35}SO_4$ was removed by washing through with 0.15 M NaCl/20 mM phosphate, pH 7.0, containing 1% Triton X-100. Bound ^{35}S-labeled material was eluted using a 60mL linear gradient of 0.15 to 1.5 M NaCl in 20 mM phosphate, pH 7.0, containing 1% Triton X-100 at a flow rate of 1 mL/min and collecting 1 mL fractions. The ^{35}S content of fractions was determined by liquid scintillation counting.

F. Cross-correction

To assess whether transduced IDS was secreted, fibroblasts from patients affected by MPSII were fed with IDS-conditioned medium (0.133mls/cm^2) from transduced $hMSC_{MPSII}$ at 20-24 PD. This medium was collected and filtered with a 0.45µM filter after an overnight incubation on an 80-90% confluent MSC monolayer. IDS activity was measured as described above. Medium collected from normal fibroblasts (80-90% confluent) was used as control. To assess whether recombinant IDS was endocytosed via the mannose-6-phosphate (M-6-P) receptor, fibroblasts were incubated with or without 5mM M-6-P or with the structural analog 5mM glucose-6-phosphate. Cell lysates were then analyzed for total protein content and IDS activity as described above.

III. Results

A. Isolation and culture of hMSC

In order to assess whether $hMSC_{MPSII}$ had similar characteristics to $hMSC_N$, the bone marrow frequency and expansion capacity of hMSC from two MPSII patients and age matched controls were analysed. Although the number of MSC progenitors (assessed by CFU-F assay) was in the lower range when compared to the age matched controls (**Figure 2A**) the kinetic of expansion was similar in the two groups (**Figure 2B**). Both $hMSC_N$ and $hMSC_{MPSII}$ primary cultures reached primary confluence in 9-14 days. On replating, MSC from both groups decreased their proliferation at similar rates. Cultures were examined up to 27-30 population doublings (PDs) with no noticeable differences.

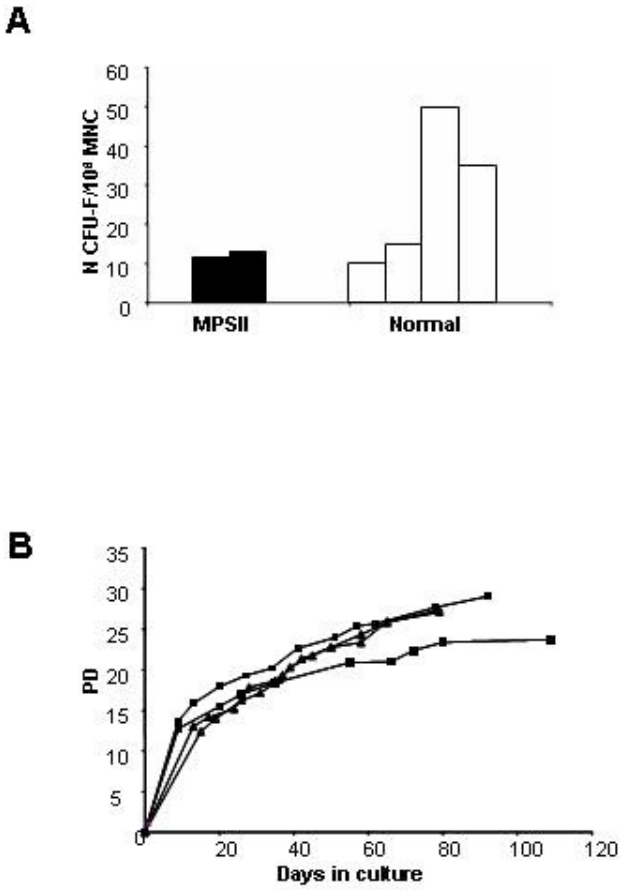

Figure 2 Bone marrow of patients affected by MPSII contains MSC with similar expansion capacity to age matched healthy individual. (**A**) The number of colony-forming-unit fibroblast (CFU-F) in patients affected by MPSII (black bars) and age matched healthy controls (white bars). Results are expressed per 10^6 mononuclear cells. (**B**) Growth kinetics of hMSC culture from patients affected by MPSII (black triangle) at the age of 46 months and 180 months; and 2 healthy donors (black square) at the age of 46 months and 181 months. The curves represent the cumulative number of population doubling (PD) versus time in culture.

hMSC from both groups were tested for their ability to undergo osteogenic and adipogenic differentiation after appropriate induction at two different times in culture (18-20 and 23-25 PDs). After 2 weeks in osteogenic medium, both $hMSC_{MPSII}$ and $hMSC_N$ cultures exhibited upregulation of alkaline phosphatase expression (**Figure 3A and C**, respectively) and mineralized deposits visualized by Von Kossa staining (data not shown). In contrast alkaline phophasphatase was very low (**Figure 3D**) and presence of Von Kossa precipitates (data not shown) were not observed in cultures not exposed to the osteogenic supplements. Induction of adipogenic differentiation was apparent by the accumulation of lipid-rich vacuoles which were stained with Oil-Red-O. No differences in the pattern of differentiation was observed between $hMSC_{MPSII}$ and $hMSC_N$ (**Figure 3E and G**, respectively). As expected no formation of adipocytes was observed in the cultures, which were not exposed to adipogenic supplements (**Figure 3H**).

B. Transduction and enzyme reconstitution of $hMSC_{MPSII}$

Both $hMSC_{MPSII}$ cultures ($hMSC_{MPSII}$a and $hMSC_{MPSII}$b) were transduced at 17.9 and 20.5 population doublings respectively. The transduction efficiency was 52.8% and 70.5% in $hMSC_{MPSII}$a and b, respectively. Cultures maintained a growth rate similar to the untransduced $hMSC_{MPSII}$ cultures (**Figure 4**) and ability to differentiate into osteoblasts and adipocytes (**Figure 3B and F**) suggesting that the transduction procedure did not have detrimental effects on the proliferation and differentiation capacity of hMSC.

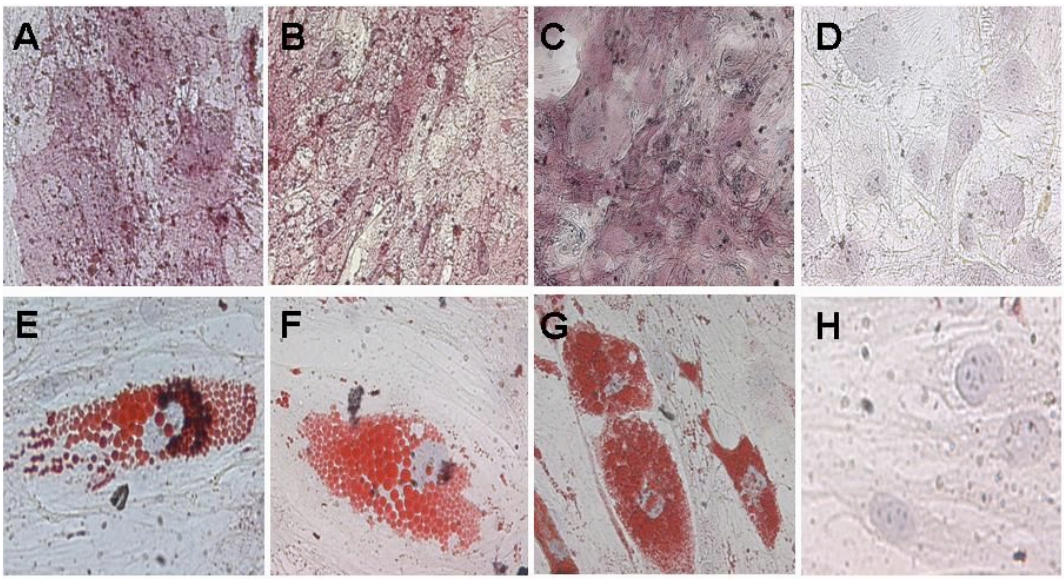

Figure 3. $hMSC_{MPSII}$ showed multipotent differentiation potential similar to $hMSC_N$. A representative example of $hMSC_{MPSII}$ before (**A**) and after (**B**) transduction, and $hMSC_N$ (**C**) exposed to osteogenic supplements and stained for the expression of alkaline phosphatase. $hMSC_{MPSII}$ before (**E**) and after (F) transduction, and $hMSC_N$ (**G**) exposed to adipogenic supplements for two weeks and staining with Oil-Red-O. All hMSC were also cultured in medium without osteogenic or adipogenic supplements and stained for the expression of alkaline phosphatase (**D**) and for Red Oil O (**H**). Panels D and H show one representative example of a culture not exposed to differentiation supplements.

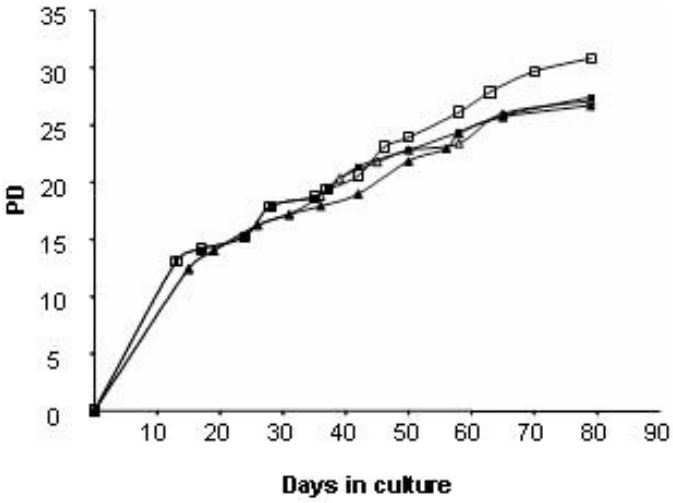

Figure 4. $hMSC_{MPSII}$ cultures retain their expansion capacity after retroviral transduction. Growth kinetics of MSC culture from patients affected by MPSII at the age of 46 months (triangle) and 180 months (square) before (close shapes) and after (open shapes) transduction with the retroviral vector containing the IDS gene.

As expected very low levels of IDS enzyme activity were found in the untransduced $hMSC_{MPSII}$ cultures whilst IDS enzymes levels were more than 10 fold higher than those detected in $hMSC_N$ following transduction (**Figure 5A**). In order to determine whether expression of supranormal levels of IDS enzyme affected the expression of other lysosomal enzymes β-galactosidase activity was assessed in both transduced and untrasduced $hMSC_{MPSII}$ cultures and compared to the levels in $hMSC_N$ cultures. All cultures showed levels of β-galactosidase in the normal range (**Figure 5B**) suggesting that overexpression of IDS enzyme did not alter the expression of other lysosomal enzymes. In addition, in order to evaluate the capability of the recombinant enzyme to correct the metabolic defect, GAGs levels were assessed by measurement of $^{35}SO_4$ incorporation. As expected untransduced $hMSC_{MPSII}$ showed significant amounts of heparan and dermatan sulphate. In contrast transduced $hMSC_{MPSII}$ cultures, showed levels of GAGs similar to those found in $hMSC_N$ from unaffected individuals, indicating that the recombinant IDS enzyme was produced in adequate amount and was functional (**Figure 5C**).

C. Cross correction of MPSII skin fibroblasts

In order to investigate the cross-correction potential of the IDS recombinant enzyme cell-free conditioned medium was obtained from transduced $hMSC_{MPSII}$ cultures following an overnight incubation and used to feed two MPSII fibroblast cultures (MPSII fibroblast a and b) (**Figure 6A**). As expected, no enzyme activity was found when MPSII fibroblasts were incubated with medium from untransduced $hMSC_{MPSII}$ (IDS enzyme activities of 0.1 and 0.01 nmol/h/mg protein, for a and b cultures, respectively, equivalent to 2.1 and 2.9% of IDS activity in normal fibroblasts). Conditioned medium from $hMSC_N$ only modestly increased IDS enzyme levels (0.35 and 0.04 nmol/h/mg, for a and b cultures, respectively, equivalent to 7.5 and 11.7% of IDS activity in normal fibroblasts). In contrast the medium from transduced $hMSC_{MPSII}$ cultures conferred high IDS levels (38.9 and 4.42 nmol/h/mg, for a and b cultures, respectively, equivalent to 837 and 1300% of IDS activity in normal fibroblasts). This was well above levels observed in normal skin fibroblasts. Cross-correction was inhibited by mannose-6-phosphate, but not by the structural analogue glucose-6-phosphate, confirming that uptake was dependent on the mannose-6-phosphate receptor.To test whether the endocytosed IDS enzyme was capable of degrading GAGs storage in MPSII fibroblasts, cultures were exposed to $^{35}SO_4$ to label glycosamonoglycans (**Figure 6B**). Fibroblasts from MPSII patients showed significant amounts of $^{35}SO_4$ incorporation due to its accumulation in the GAGs. MPSII fibroblasts exposed to transduced $hMSC_{MPSII}$ conditioned medium, however, showed levels of $^{35}SO_4$ incorporation similar to those in fibroblasts from unaffected individuals, showing that transduced $hMSC_{MPSII}$ can secrete the IDS enzyme in a form capable of cross-correction. Surprisingly despite the modest enzyme levels obtained following cross-correction of MPSII fibroblasts with cell free conditioned medium from MSC_N these were sufficient to degrade intracellular GAGs to normal levels.

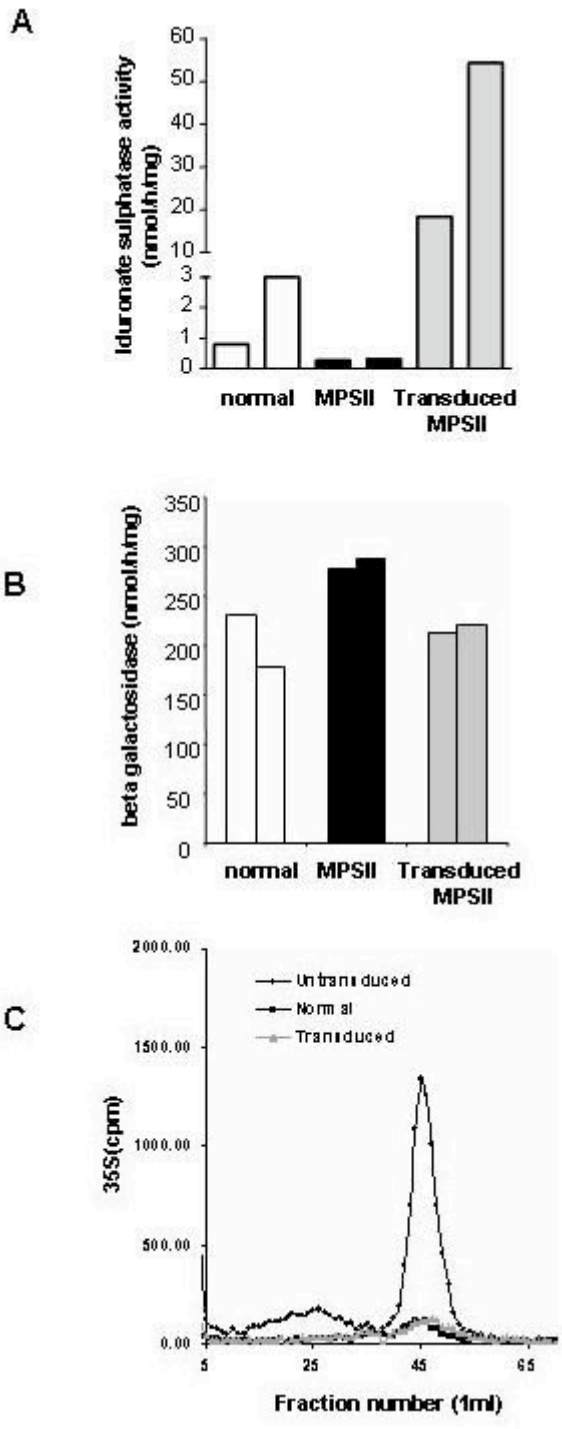

Figure 5. Following retroviral transduction $hMSC_{MPSII}$ express supernormal levels of IDS enzyme capable of degrading GAGs to normal levels. (**A**) IDS activity in $hMSC_N$ (white bars, n=2), $hMSC_{MPSII}$ (n=2) before (black bars) and after (grey bars) transduction. (**B**) Beta galactosidase activity in $hMSC_N$ (white bars, n=2), $hMSC_{MPSII}$ (n=2) before (black bars) and after (grey bars) transduction. Columns represent levels of enzymes expressed as nmol/h/mg. (**C**) Levels of GAGs accumulation in hMSC cells measured by sulphate sequestration assay (a representative example). Black diamond represents levels of GAGs in untransduced $hMSC_{MPSII}$, gray triangle in transduced $hMSC_{MPSII}$ and black square in $hMSC_N$

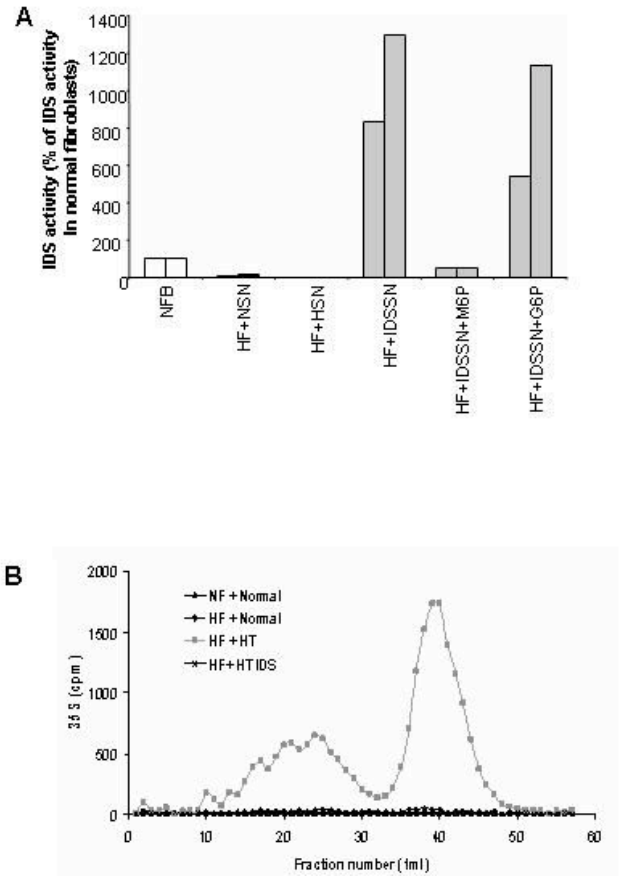

Figure 6. $hMSC_{MPSII}$ transduced to express supranormal levels of IDS enzyme secrete the IDS enzyme and is capable to cross-correct GAGs storage in fibroblasts from MPSII patients. (**A**) IDS activity expressed as percentage of IDS activity in normal fibroblasts. White bars represent IDS activity in fibroblast obtained from healthy donors (NFB); grey bars represent fibroblasts from patients affected by MPSII (HF) exposed to cell free conditioned medium from hMSC culture from a healthy donor (NSN), from a donor affected by MPSII (HSN), from a donor affected by MPSII and transduced with the IDS expressing retroviral vector (IDSSN) in presence of mannose-6-phosphate (M6P) or glucose-6-phosphate (G-6-P). (**B**) A representative experiment of $^{35}SO_4$-GAG sequestration assay. Levels of ^{35}S-GAG accumulation in fibroblasts from healthy donors (black triangle), from MPSII patients exposed to cell-free conditioned medium from $hMSC_N$ (black diamond), IDS transduced $hMSC_{MPSII}$ (black star) and untransduced $hMSC_{MPSII}$ (grey square) were measured.

IV. Discussion

There is a strong rational in using gene modification of mesenchymal stem cells for the therapy of MPSII. In contrast to MPSI, the two main treatments currently available, BMT and ERT, are unlikely to result in long term benefit in patients with severe MPSII. Moreover, BMT requires a suitable donor and has often side effects such as GvHD; ERT is a lifelong and expensive treatment. Therefore the possibility of correcting the patients' autologous cells overcome some of the problem of BMT such as finding a suitable donor and the severe side effects associated to GvHD. MSC have also been shown to have immunosuppressive properties, reducing the risk of immune response to the IDS enzyme, which is seen in patients with a total absence of protein expression.

Limited studies are available on the feasibility of gene therapy for the correction of Hunter syndrome (Friso et al, 2005; Cardone et al, 2006). Very recently excellent results have been obtained in a murine model of MPSII. AAV2-IDS viral particles were administered intravenously to adult MPSII mice (Cardone et al, 2006). The plasma and tissue IDS activities were completely restored in all of the treated mice with normalization of the GAGs levels and correction of the skeletal malformation, suggesting that the gene therapy approach has the potential for the systemic treatment of MPSII. However clinically, there are safety concerns in directly injecting viral particles at high titre. Moreover it is known that transduction of murine cells not always reflect what is observed in human cells (Heim and Dunbar, 2000). In contrast data are limited with regard to ex vivo correction of human cells from patients affected by Hunter syndrome. Ex vivo gene modification of lymphocytes has been attempted. However efficiency was poor as lymphocytes are notoriously difficult to transduce (Stroncek et al, 1999). Moreover little is known about their lifespan and therefore it is unknown whether they would provide long term cure.

In this study we propose hMSC as a delivery system for the IDS enzyme. Stem cells have the ability to self-renew and differentiate into multiple tissues, contributing to tissue repair and maintenance. Correction of stem cells by genetic modification can potentially lead to a definitive cure for the disease.

Human MSC are easy to isolate from the patient bone marrow, can be expanded in vitro and efficiently transduced by a variety of viral vectors. Due to their ability to differentiate in several cell types including osteoblasts, they can directly participate to regeneration of tissues such as bone. Bone malformations are present in MPSII patients and are difficult to correct with other therapeutic modalities. Indeed, so far hMSC have been shown to be able to home to several organs including bone (Pereira et al, 1998; Devine et al, 2001) following intravenous infusion in both animal models and a phase I clinical trial with some benefits.

Here we show that hMSC can be efficiently trasduced with a retroviral vector using a simple two days transduction protocol. This does not alter the expansion and differentiation capacity of hMSC and result in supranormal levels of enzyme that is functionally active being capable of degrading the accumulated GAGs and cross-correct the surrounding cells. Of interest is that we observed very low enzyme uptake following exposure of fibroblasts obtained from patients affected by MPSII to conditioned medium from hMSC derived from normal donors. Even if very low levels of enzyme activity seem to be sufficient to completely correct GAGs accumulation in the cells in vitro this may not be the case in vivo where the circulating enzyme may be diluted in the circulation. These data emphasize how a gene therapy approach may be particularly beneficial in the case of MPSII, where overexpression of the IDS gene may be required to prevent poor cross-correction.

In vivo studies with long term follow up are now required to demonstrate the establishment of hMSC long-term residence within the BM, bone and possibly other tissues in sufficient number to contribute to the correction of the systemic accumulation of GAGs; to improve bone regeneration for the life-span of the individual with no adverse effects such as clonal transformation due to insertional mutagenesis. This may be difficult to achieve in murine models as murine MSC display marked differences from human MSC (Aguilar et al, 2007). Immunocompromised models and/or non human primate models may be required to assess successful engraftment of human cells and long term follow up.

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References

Aguilar S, Nye E, Chan J, Loebinger M, Spencer-Dene B, Fisk N, Stamp G, Bonnet D, Janes SM (**2007**) Murine but not human mesenchymal stem cells generate osteosarcoma-like lesions in the lung. **Stem Cells** 25, 1586-1594.

Barry FP, Boynton RE, Haynesworth S, Murphy JM, Zaia J (**1999**) The monoclonal antibody SH-2, raised against human mesenchymal stem cells, recognizes an epitope on endoglin (CD105). **Biochem Biophys Res Commun** 265, 134-139.

Baxter MA, Wynn RF, Deakin JA, Bellantuono I, Edington KG, Cooper A, Besley GT, Church HJ, Wraith JE, Carr TF, Fairbairn LJ (**2002**) Retrovirally mediated correction of bone marrow-derived mesenchymal stem cells from patients with mucopolysaccharidosis type I. **Blood** 99, 1857-9.

Baxter MA, Wynn RF, Jowitt SN, Wraith JE, Fairbairn LJ, Bellantuono I (**2004**) Study of telomere length reveals rapid aging of human marrow stromal cells following *in vitro* expansion. **Stem Cells** 22, 675-682.

Bruder Sp, Jaiswal N, Haynesworth SE (**1997**) Growth kinetics, self-renewal, and the osteogenic potential of purified human mesenchymal stem cells during extensive subcultivation and following cryopreservation. **J Cell Biochem** 64, 278-294.

Cardone M, Polito VA, Pepe S, Mann L, D'Azzo A, Auricchio A, Ballabio A, Cosma MP (**2006**) Correction of Hunter syndrome in the MPSII mouse model by AAV2/8-mediated gene delivery. **Hum Mol Genet** 15, 1225-1236.

Devine SM, Bartholomew AM, Mahmud N, Nelson M, Patil S, Hardy W, Sturgeon C, Hewett T, Chung T, Stock W, Sher D, Weissman S, Ferrer K, Mosca J, Deans R, Moseley A, Hoffman R (**2001**) Mesenchymal stem cells are capable of homing to the bone marrow of non-human primates following systemic infusion. **Exp Hematol** 29, 244-255.

Friedenstein AJ, Gorskaja JF, Kulagina NN (**1976**) Fibroblast precursors in normal and irradiated mouse hematopoietic organs. **Exp Hematol** 4, 267-274.

Friso A, Tomanin R, Alba S, Gasparotto N, Puicher EP, Fusco M, Hortelano G, Muenzer J, Marin O, Zacchello F, Scarpa M (**2005**) Reduction of GAG storage in MPS II mouse model following implantation of encapsulated recombinant myoblasts. **J Gene Med** 7, 1482-1491.

Galjaard H (**1980**) *Genetic Metabolic Diseases: early diagnosis and prenatal analysis. (Elsevier, Amsterdam/New York).*

Heim DA, Dunbar CE (**2000**) Hematopoietic stem cell gene therapy: towards clinically significant gene transfer efficiency. **Immunol Rev** 178, 29-38.

Hildinger M, Eckert HG, Schilz AJ, John J, Ostertag W, Baum C (**1998**) FMEV vectors: both retroviral long terminal repeat and leader are important for high expression in transduced hematopoietic cells. **Gene Ther** 5, 1575-1579.

Hopwood JJ, Bunge S, Morris CP, Wilson PJ, Steglich C, Beck M, Schwinger E, Gal A (**1993**) Molecular basis of mucopolysaccharidosis type II: mutations in the iduronate-2-sulphatase gene. **Hum Mutat** 2, 435-442.

Hopwood JJ, Bunge S, Morris CP, Wilson PJ, Steglich C, Beck M, Schwinger E, Gal A (**1999**) Transplantability and therapeutic effects of bone marrow-derived mesenchymal cells in children with osteogenesis imperfecta. **Nat Med** 5, 309-313.

Jang SK, Krausslich HG, Nicklin MJ, Duke GM, Palmenberg AC, Wimmer E (**1988**) A segment of the 5' nontranslated region of encephalomyocarditis virus RNA directs internal entry of ribosomes during in vitro translation. **J Virol** 62, 2636-2643.

Lim TW, Leder IG, Bach G, Neufeld EF (**1974**) An assay for iduronate sulfatase (Hunter corrective factor). **Carbohydr Res** 37, 103-109.

Markowitz D, Goff S, Bank A (**1988**) Construction and use of a safe and efficient amphotropic packaging cell line. **Virology** 167, 400-406.

Muenzer J, Wraith JE, Beck M, Giugliani R, Harmatz P, Eng CM, Vellodi A, Martin R, Ramaswami U, Gucsavas-Calikoglu M, Vijayaraghavan S, Wendt S, Puga AC, Ulbrich B, Shinawi M, Cleary M, Piper D, Conway AM, Kimura A (**2006**) A phase II/III clinical study of enzyme replacement therapy with idursulfase in mucopolysaccharidosis II (Hunter syndrome). **Genet Med** 8, 465-73.

Pereira RF, O'Hara Md, Laptev AV, Halford KW, Pollard MD, Class R, Simon D, Livezey K, Prockop DJ (**1998**) Marrow stromal cells as a source of progenitor cells for nonhematopoietic tissues in transgenic mice with a phenotype of osteogenesis imperfecta. **Proc Natl Acad Sci U S A** 95, 1142-1147.

Peters C, Krivit W (**2000**) Hematopoietic cell transplantation for mucopolysaccharidosis IIB (Hunter syndrome)

Peters C, Steward CG (**2003**) Hematopoietic cell transplantation for inherited metabolic diseases: an overview of outcomes and practice guidelines. **Bone Marrow Transplant** 31, 229-239.

Pittenger MF, Mackay AM, Beck SC, Jaiswal RK, Douglas R, Mosca JD, Moorman MA, Simonetti DW, Craig S, Marshak DR (**1999**) Multilineage potential of adult human mesenchymal stem cells. **Science** 284, 143-147.

Prockop DJ (**1997**) Marrow stromal cells as stem cells for nonhematopoietic tissues. **Science** 276, 71-74.

Quarto R, Mastrogiacomo M, Cancedda R, Kutepov SM, Mukhachev V, Lavroukov A, Kon E, Marcacci M (**2001**) Repair of large bone defects with the use of autologous bone marrow stromal cells. **N Engl J Med** 344, 385-386.

Stroncek DF, Hubel A, Shankar RA, Burger SR, Pan D, Mccullough J, Whitley CB (**1999**) Retroviral transduction and expansion of peripheral blood lymphocytes for the treatment of mucopolysaccharidosis type II, Hunter's syndrome. **Transfusion** 39, 343-350.

Wraith JE, Cooper A, Thornley M, Wilson PJ, Nelson PV, Morris CP, Hopwood JJ (**1991**) The clinical phenotype of two patients with a complete deletion of the iduronate-2-sulphatase gene (mucopolysaccharidosis II--Hunter syndrome). **Hum Genet** 87, 205-6.

Young ID, Harper PS, Newcombe RG, Archer IM (**1982**) A clinical and genetic study of Hunter's syndrome. 2. Differences between the mild and severe forms. **J Med Genet** 19, 408-411.

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