Brief report

Retrovirally mediated correction of bone marrow–derived mesenchymal stem cells from patients with mucopolysaccharidosis type I


We have investigated the utility of bone marrow–derived mesenchymal stem cells (MSCs) as targets for gene therapy of the autosomal recessive disorder mucopolysaccharidosis type IH (MPS-IH, Hurler syndrome). Cultures of MSCs were initially exposed to a green fluorescent protein–expressing retrovirus. Green fluorescent protein–positive cells maintained their proliferative and differentiation capacity. Next we used a vector encoding α-L-iduronidase (IDUA), the enzyme that is defective in MPS-IH. Following transduction, MPS-IH MSCs expressed high levels of IDUA and secreted supernormal levels of this enzyme into the extracellular medium. Exogenous IDUA expression led to a normalization of glycosaminoglycan storage in MPS-IH cells, as evidenced by a dramatic decrease in the amount of 35SO4 sequestered within the heparan sulfate and dermatan sulfate compartments of these cells. Finally, gene-modified MSCs were able to cross-correct the enzyme defect in untransduced MPS-IH fibroblasts via protein transfer. (Blood. 2002;99:1857-1859)

Study design

Isolation and culture of MSCs

Bone marrow samples were obtained from MPS-IH patients and unaffected individuals aged from 0 to 18 years, following informed parental consent and approval from the local research ethics committee. MSCs were isolated and cultured as previously described. 

For differentiation assays, cells were plated at 5 x 10^3 per well in 6-well plates in growth medium with either 5% fetal calf serum or 10% fetal calf serum/1 mM β-mercaptoethanol. Mineralized bone was stained by the von Kossa technique, and adipocytes were stained using oil-red-O. Neurons were stained for the neuron-specific tyrosine kinase trkA using monoclonal sc-118 (Santa Cruz Biotechnology, Santa Cruz, CA). 

Transduction of MSCs

The Lid vector expressing IDUA has been previously described. This vector expresses enhanced green fluorescent protein (L-EGFP) was derived by replacing the green fluorescent protein with L-EGFP. 

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Submitted August 14, 2001; accepted October 17, 2001.

Supported by the Royal Manchester Children’s Hospital R&D fund, the Jeans for Genes appeal (Mucopolysaccharidosis Society, Amersham, United Kingdom), and the Cancer Research Campaign, London, United Kingdom.

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the IDUA complementary DNA in Lmd with a complementary DNA encoding L-EGFP. Cells (30%-40% confluent) were transduced using cell-free retroviral supernatant supplemented with 2 μg/mL polybrene. After 24 hours, medium was replaced and cells left for 5 to 7 days prior to use in assays for transgene expression, phenotypic correction, and differentiation.

Assays of IDUA activity

IDUA activity in cell homogenates and media was assayed as previously described using 4-methylumbelliferyl-alpha-D-iduronide (Glycosynth, Cheshire, United Kingdom) as substrate. Total protein was measured according to the Lowry method.

Sulfate sequestration assay

Confluent MSCs were exposed to 35S-labeled Na2SO4 (NEN Life Science Products, Boston, MA) at 20 μCi/mL (0.74 MBq/mL) in Dulbecco modified Eagle medium/fetal calf serum 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, cell pellets were solubilized in 2 mL of 6 M urea/0.15 M sodium phosphate, pH 7.0, containing 1% (vol/vol) Triton X-100. Extracts were filtered through a 0.2-μm syringe filter before application to a fast protein liquid chromatography Mono-Q HR 5/5 anion-exchange column (Pharmacia, St Albans, United Kingdom).

Nonincorporated 35SO4 was removed by washing through with 0.15 M NaCl/20 mM phosphate, pH 7.0, containing 1% (vol/vol) Triton X-100. Bound 35S-labeled material was eluted using a 60 mL 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 35S content of fractions was determined by liquid scintillation counting.

Results and discussion

Following retroviral transduction of MSCs with the L-EGFP vector, transduced MSCs maintained the same growth rate as untransduced cells (not shown) and retained the ability to differentiate into osteoblasts (Figure 1A,B), adipocytes (Figure 1C,D), and neurons (Figure 1E,F). GFP-transduced, MSC-derived osteoblasts exhibited mineral deposits that could be visualized by von Kossa staining (Figure 1A). Transduced MSC-derived adipocytes stained with oil-red-O (Figure 1C), and neurons stained positively for trkA (Figure 1E) and tau (not shown). Thus, the transduction conditions used did not compromise the proliferation and differentiation potential of the MSCs.

Following transduction of MPS-IH MSCs with the IDUA retrovirus, levels of enzyme activity were measured that equaled or exceeded those detected in normal MSCs (Table 1). In contrast, no detectable IDUA was seen in untransduced MPS-IH MSCs. When cell-free medium was assayed (Table 2), no IDUA was detectable from untransduced MPS-IH MSC cultures. IDUA could be detected in medium from normal MSCs and in higher (around 7- to 200-fold) amounts from transduced MPS-IH MSCs. This higher level of secretion of recombinant IDUA is consistent with the inclusion of a rat pre-proinsulin leader at the 5' end of the construct we have used, resulting in more efficient targeting of IDUA into the secretory pathway.

Cell-free medium from MSCs was next applied to cultures of MPS-IH fibroblasts with a view to testing the cross-correction potential of the secreted enzyme (Table 2). As expected, medium from uncorrected MPS-IH MSCs did not correct the defect in MPS-IH fibroblasts. Medium from normal MSCs did correct to a small extent but, most strikingly, medium from gene-modified MPS-IH MSCs conferred high levels of IDUA levels on MPS-IH fibroblasts.
significant amounts of $^{35}$SO$_4$ sequestration due to its accumulation in osteogenesis imperfecta. The authors are grateful to Mr Steve Bagley for assistance with imaging.

## Acknowledgment

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## References


### Table 3. $^{35}$SO$_4$ incorporation (cpm) into heparan sulfate and dermatan sulfate

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<td>Total GAGs</td>
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However, showed levels of $^{35}$SO$_4$ sequestration similar to those in MSCs from unaffected individuals, indicating a normalization of GAG levels in Lid-transduced MPS-IH MSC cultures. Thus, not only were IDUA levels corrected, but the levels of the pathological effector (namely, stored GAGs) were also corrected.

Thus, MSCs may provide a useful platform for the production of lysosomal enzymes and other bioactive molecules in patients. Clinical utility of this approach in the transplantation of gene-modified MSCs will depend upon achievement of sufficient donor chimerism in affected tissue. This has varied in studies to date, although the experience of allogeneic bone marrow transplantation in osteogenesis imperfecta has demonstrated that even very low levels of engraftment can result in clinical benefit.21

## Table 3: $^{35}$SO$_4$ incorporation (cpm) into heparan sulfate and dermatan sulfate

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