Brief report

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

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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 $^{35}SO_4$ 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)

Introduction

Mucopolysaccharidosis type IH (MPS-IH, Hurler syndrome) is an autosomal recessive disorder resulting from defects in the gene encoding the lysosomal enzyme α-L-iduronidase (IDUA). This leads to ineffective degradation of the glycosaminoglycans (GAGs) heparan sulfate and dermatan sulfate. Individuals with very low levels of IDUA present in infancy and early childhood as a consequence of the deleterious accumulation of these GAGs in different organ systems, including the central nervous system, reticuloendothelial system, and the skeleton. Such severely affected patients usually die within the first decade.[1,2]

Current therapy for MPS-IH focuses on allogeneic bone marrow transplantation from an unaffected, HLA-compatible donor. This provides normal, enzyme-competent leukocytes that secrete IDUA that can be taken up by enzyme-deficient cells via mannose-6-phosphate receptors.[3] The utility of this approach is significantly limited by the availability of donors and significant toxicity of the intense immunosuppressive conditioning therapy that the recipient requires for donor hemopoiesis to become established without rejection. Even where donor hemopoiesis is fully established (ie, all hemopoietic cells have normal enzyme levels), symptoms (particularly defects in the skeleton and central nervous system) are incompletely and variably corrected.[4,5]

Mesenchymal stem cells (MSCs) are multipotent progenitors that can be isolated from bone marrow and are capable of contributing to multiple mesenchymal tissues in vivo.[6-10] In this paper we demonstrate, for the first time, retroviral gene transfer leading to correction of these MSCs in an inherited disorder. Furthermore, there is maintenance of the proliferative and multilineage differentiation potential of these modified cells, and they are able to cross-correct non–gene-modified cells.

Numerous studies have demonstrated the presence of donor mesenchymal cells in multiple tissues following transplantation, and MSCs injected into brain are able to differentiate into nerve cells. Taken with these, our data indicate that MSCs may prove a better target than hematopoietic stem cells in the context of gene therapy of multisystem, lysosomal storage disorders.

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.[11] For differentiation assays, cells were plated at 5×10^3 per well in 6-well plates in growth medium with either osteogenic[12] or adipogenic[13] supplements. For differentiation along the neuronal lineage, cells were preincubated for 24 hours in Dulbecco modified Eagle medium/20% fetal calf serum/1 mM β-mercapotethanol and then switched into Dulbecco modified Eagle medium/5 mM β-mercapotethanol.[14] Mineralized bone was stained by the von Kossa technique,[15] and adipocytes were stained using oil-red-O.[16] Neurons were stained for the neuron-specific tyrosine kinase trkA using monoclonal sc-118 (Santa Cruz Biotechnology, Santa Cruz, CA).[14]

Transduction of MSCs

The Lid vector expressing IDUA has been previously described.[17] L–enhanced green fluorescent protein (L-EGFP) was derived by replacing

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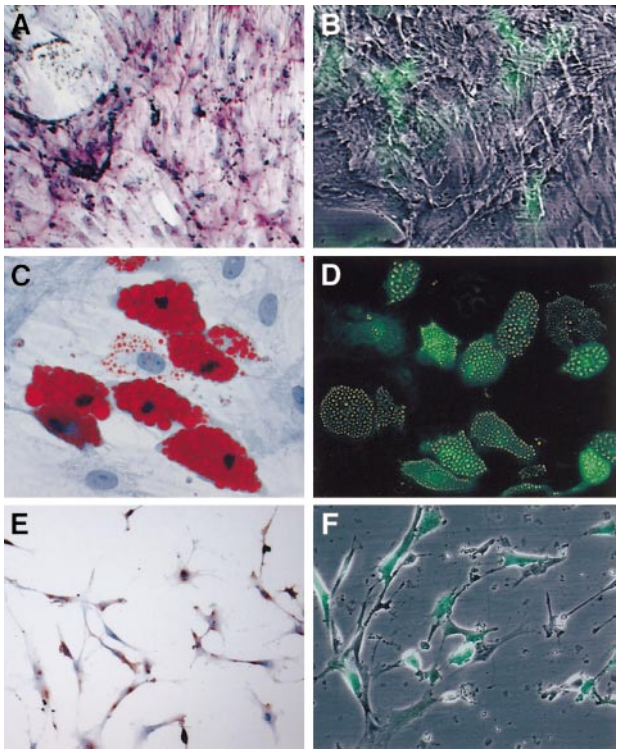

Figure 1. GFP-transduced MSCs maintain their multipotentiality. GFP-transduced MSCs following differentiation down the osteoblast (A,B), adipocyte (C,D), or neuronal lineages (E,F). Lineage-specific staining for bone (von Kossa staining [A]), fat (oil-red-O [C]), or neuronal (trkA [E]) markers is shown along with UV visualization of GFP (B,D,F). Original magnification A-B, × 100; C-F, × 200.

the IDUA complementary DNA in Lid 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[18] using 4-methylumbelliferyl-α-L-iduronidase (Glycosynth, Cheshire, United Kingdom) as substrate. Total protein was measured according to the Lowry method.[19]

Sulfate sequestration assay

Confluent MSCs 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/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 at 4°C for 1 hour. 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 $^{35}SO_4$ was removed by washing through with 0.15 M NaCl/20 mM phosphate, pH 7.0, containing 1% (vol/vol) Triton X-100. Bound ^{35}S-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 ^{35}S content of fractions was determined by liquid scintillation counting.

Table 1. Enzyme and GAG levels in transduced and cross-corrected cells

Lysosomal enzyme levels in transduced MSCs (patients A–C, passages P1–P3)				
	MPS-IH A	MPS-IH B	MPS-IH C	Normal (mean ± SEM)
Intracellular IDUA (μM/g.h)				
Untransduced	0	0	0	134.8 ± 37.8
Transduced P1	254	568	918	(n = 5)
Transduced P2	280	346	409	
Transduced P3	nd	192	605	
Extracellular IDUA (nM/mL.h)				
Untransduced	0	0	0	0.90 ± 0.37
Transduced P1	8	141	148	(n = 4)
Transduced P2	7	133	167	
Transduced P3	6	81	197	

nd indicates not done.

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.[20]

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

Table 2. IDUA levels in MPS-1H fibroblasts exposed to different conditioned media (μM/g.h)

Conditioned medium	Fresh medium	Normal MSCs	MPS-IH MSCs	Transduced MPS-IH MSCs	Transduced MPS-IH MSCs + m-6-p	Transduced MPS-IH MSCs + g-6-p
MPS-IH fibroblasts	0	1.5	0	208.9	4.9	360.7
Normal fibroblasts	71.9	nd	nd	nd	nd	nd

m-6-p indicates mannose-6-phosphate; g-6-p, glucose-6-phosphate.

Table 3. $^{35}SO_4$ incorporation (cpm) into heparan sulfate and dermatan sulfate

	Normal MSCs	MPS-IH A	MPS-IH A (transduced)	MPS-IH B	MPS-IH B (transduced)
Heparan sulfate	4 973	54 671	14 957	34 515	6 120
Dermatan sulfate	32 567	227 044	19 923	175 135	22 151
Total GAGs	37 540	281 715	34 880	209 650	28 271

fibroblasts. This cross-correction was inhibited by mannose-6-phosphate but not by the structural analog glucose-6-phosphate, confirming that uptake was dependent on the mannose-6-phosphate receptor.[3] Thus, gene-corrected MPS-IH MSCs secrete IDUA in an appropriate form that may be taken up by non–gene-corrected cells.

To test the effect of exogenous IDUA expression on the storage of GAGs in transduced MPS-IH MSCs, cultures were exposed to $^{35}SO_4$ to radiolabel proteoglycans (Table 3). MSCs from 2 separate individuals with MPS-IH were tested. MPS-IH MSCs showed significant amounts of $^{35}SO_4$ sequestration due to its accumulation in the GAGs dermatan and heparan sulfate and a lack of subsequent catabolism of these. IDUA-expressing MPS-IH MSC cultures, 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]

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