Molecular Genetics and Metabolism xxx (2015) xxx–xxx

Contents lists available at ScienceDirect

Molecular Genetics and Metabolism

journal homepage: www.elsevier.com/locate/ymgme

Mucopolysaccharidosis enzyme production by bone marrow and dental pulp derived human mesenchymal stem cells

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ARTICLE INFO

Article history:
Received 26 November 2014
Received in revised form 6 February 2015
Accepted 6 February 2015
Available online xxxx

Keywords:
Mucopolysaccharidosis
Mesenchymal stem cell
Hematopoietic stem cells
Glycosaminoglycan
Differentiation

ABSTRACT

Mucopolysaccharidoses (MPS) are inherited metabolic disorders that arise from a complete loss or a reduction in one of eleven specific lysosomal enzymes. MPS children display pathology in multiple cell types leading to tissue and organ failure and early death. Mesenchymal stem cells (MSCs) give rise to many of the cell types affected in MPS, including those that are refractory to current treatment protocols such as hematopoietic stem cell (HSC) based therapy. In this study we compared multiple MPS enzyme production by bone marrow derived (hBM) and dental pulp derived (hDP) MSCs to enzyme production by HSCs. hBM MSCs produce significantly higher levels of MPS I, II, IIIA, IVA, VI and VII enzyme than HSCs, while hDP MSCs produce significantly higher levels of MPS I, IIIA, IVA, VI and VII enzymes. Higher transfection efficiency was observed in MSCs (89%) compared to HSCs (23%) using a lentiviral vector. Over-expression of four different lysosomal enzymes resulted in up to 9303-fold and up to 5559-fold greater levels in MSC cell layer and media respectively. Stable, persistent transduction of MSCs and sustained over-expression of MPS VII enzyme was observed *in vitro*. Transduction of MSCs did not affect the ability of the cells to differentiate down osteogenic, adipogenic or chondrogenic lineages, but did partially delay differentiation down the non-mesodermal neurogenic lineage.

1. Introduction

Mucopolysaccharidoses (MPS) are inherited metabolic disorders that arise from a reduction or absence of a lysosomal enzyme activity that is required for the intracellular degradation of glycosaminoglycan (GAG) chains. This results in the accumulation of undegraded or partially degraded GAG in the lysosomes of a range of cell types, interfering with normal cellular function and leading to multiple tissue and organ failure. Children with MPS display clinical symptoms that include; skeletal abnormalities, CNS deterioration, cardiac dysfunction, corneal clouding, loss of hearing, enlarged abdominal organs and obstructive airway disease [1–4].

Select MPS children are currently treated with either hematopoietic stem cell (HSC) transplant and/or enzyme replacement therapy (ERT). While treatment improves many of the soft tissue aspects of MPS disease it fails to adequately address the skeletal and neurological systems [5,6]. Systemically administered ERT does not affect neurological disease as the recombinant enzymes do not cross the blood–brain barrier (BBB) [7,8]. HSC transplant is indicated only for MPS I patients with neurological involvement [9] with optimal efficacy seen in younger patients [10]. Human trials of HSC transplant in other MPS types, such as MPS IIIA, have shown no improvement in neurological function [11–15]. Bone also responds poorly to HSC transplant [16,17] and may be due to the lack of donor chimerism for bone forming cells even under conditions of total hematopoietic engraftment [18]. Animal studies indicate that enzyme production by lymphoid and myeloid lineages is not sufficient to combat the multi-tissue symptoms associated with MPS, but that improved efficacy can be achieved by transducing HSCs with viral vectors to over-produce MPS enzymes [14,19].

In addition to HSCs, bone marrow contains an additional population of stem cells; mesenchymal stem cells (MSCs). MSCs are multi-potent stromal cells that are characterized by their intrinsic self-renewal capacity and ability to differentiate into cells of the mesenchymal lineage including; osteoblasts, chondrocytes, adipocytes, smooth muscle cells and myelo-supportive fibroblasts [20–24]. These mesenchymal-derived cells display significant GAG storage in MPS patients. MSCs can also differentiate into non-mesenchymal cells, such as epithelial cells, hepatocytes and neurons *in vitro*, however, this may be dependent upon the tissue source of MSCs and/or the associated growth factors available

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http://dx.doi.org/10.1016/j.ymgme.2015.02.001
1096-7192/

during differentiation [23,25–27]. Again many of these cells display significant GAG storage in MPS patients. MSCs therefore have the ability to differentiate into many of the cell types which are affected by MPS, and could potentially be used to replace the non-functional cell populations in MPS patients.

MSCs have been implemented in preclinical animal studies for their use in heart disease, neurodegenerative disease, diabetes, kidney and liver disease, Crohn's disease, osteogenesis imperfecta, graft-versus-host disease (GvHD) and osteoarthritis (reviewed in [28]), and to a lesser extent MPS [29–31]. MSCs were first trialed in cancer remission patients in 1995, to determine the infusion safety of *ex vivo* expanded BM MSCs [32]. Since then, nearly 400 clinical trials have been registered using MSCs (www.clinicaltrials.gov) [33], largely due to findings from animal models of disease. Clinical trials encompass 12 pathological conditions, with major areas of interest including: bone and cartilage disease, including osteoarthritis and osteogenesis imperfecta, heart disease, liver disease and GvHD (reviewed in [33]). Numerous phase I/II trials have also been conducted in diabetes, brain disease, multiple sclerosis, cancer, spinal cord damage, Crohn's disease and lung disease (reviewed in [33]). A single study of MSC infusion in MPS I patients ($n = 5$) following HSC transplantation [18], demonstrated safety and immunomodulation of GvHD due to their intrinsic immunomodulatory properties that allow the normalization of the immune system through the inhibition of T-cell proliferation [27,34].

The capacity of MSCs to produce MPS enzymes underpins their effectiveness as an MPS therapy. In this study we have assessed the ability of both bone marrow and dental pulp derived MSCs to produce the enzymes deficient in MPS I, II, IIIA, IVA, VI and VII, and to over-express the enzymes deficient in MPS I, IIIA, IV and VII. Endogenous enzyme production and over-expression were higher in both MSC types than in HSCs. Stable transduction of MSCs with the MPS VII enzyme β-D-glucuronidase and over-expression of enzyme did not inhibit the ability of these cells to differentiate down osteogenic, chondrogenic, adiopogenic or neurogenic pathways.

2. Materials

Fluorogenic substrates 4-methylumbelliferyl-β-D-glucuronide hydrate and 4-methylumbelliferyl sulfate were from Sigma-Aldrich, while 4-methylumbelliferyl-α-D-N-sulfoglucosaminide and 4-methylumbelliferyl-α-L-iduronide were from Moscerdam Substrates, Netherlands. Radiolabeled substrates for iduronate-2-sulfatase, N-acetylgalactosamine 4-sulfatase and galactose 6-sulfatase were made in house as per [35–37] respectively. Tissue culture media and FCS were from Life Technologies, Australia. HSCs and StemSpan were from Stem Cell Technologies, Australia. HIV-1 p24 ELISA kit was from XpressBio, Australia. ITS+ premix stock was from BD Biosciences, TGFβ3 was from Prospec-TanyTechnoGene Ltd. All other reagents were of analytical grade.

3. Methods

3.1. Cell culture

Human derived MSCs, immunophenotype STRO-1^{bright}/STRO-3$^+$/STRO-4$^+$/CD106$^+$/CD146$^+$/CD14$^-$/CD34$^-$/CD45$^-$/Glycophorin-A$^-$, were sourced from either bone marrow (hBM MSCs) or dental pulp (hDP MSCs) of healthy donor patients as described [22,38]. MSCs were cultured in basal growth media consisting of α-MEM supplemented with; 10% FCS, 2 mM L-glutamine, 100 μM L-ascorbate-2-phosphate, 1 mM sodium pyruvate, 50 U/mL penicillin and 50 μg/mL streptomycin. Human bone marrow CD34$^+$ HSCs were cultured in StemSpan supplemented with 10% FCS, 2 mM L-glutamine, 50 U/mL penicillin, and 50 μg/mL streptomycin. Human MPS I, human MPS IIIA and murine MPS VII skin fibroblasts were cultured in DMEM, 10% FCS, 2 mM L-glutamine, 50 U/mL penicillin, and 50 μg/mL streptomycin. All cells were maintained at 37 °C in 5% CO_2 and 90% humidity, with media changed twice weekly. On reaching 80% confluency, MSCs or fibroblasts were harvested by incubation with 0.12% trypsin, 0.02% Na_2EDTA.

3.2. Determination of enzyme activity

Enzyme assays were performed on the cell layer and media of both hBM and hDP MSCs and HSCs in triplicate. Cells were seeded at 10,500 cells per cm^2 in basal growth media. Cells were fed twice weekly; with HSCs being spun at 300 ×*g* for 5 min to pellet cells prior to removal of old media. After one week, cells were fed and media collected 24 h post-feeding and stored at −20 °C, pending analysis. The cell layer was lysed for 10 min at room temperature by the addition of 100 μL of 0.1% (v/v) Triton X-100 in PBS, before being stored at −20 °C, pending analysis.

Endogenous enzyme activity for six different MPS lysosomal enzymes was determined using either fluorogenic or radio-labeled substrates. β-D-Glucuronidase, α-L-iduronidase and sulfamidase enzyme activities were determined as previously described by [39–41] respectively, using fluorogenic substrates. The samples were read on a LS 50B luminescence spectrometer using an AS 91 auto-sampler (Perkin Elmer) at excitation of 366 nm and emission of 446 nm, except for sulfamidase samples which were read on a Wallac Plate reader at excitation of 355 nm and emission of 460 nm. Iduronate-2-sulfatase, 4-sulfatase, and 6-sulfatase were measured using radiolabeled substrates as previously described [35–37]. The conversion of substrate to product was determined by HPLC using an Alltima C18-LL 5 μm column. The samples were run on a 1200 series HPLC machine (Agilent Technologies) using a HPLC–LS-pump (Raytest) and ChemStation for LC system software (B.04.02 [96]; Agilent Technologies). Activity in the cell layer was expressed as nmol/h/mg protein; with protein determined on lysed cell layers using the Bio-Rad protein assay [42]. A medium only control was included and the values if present were subtracted from those in the cell medium and activity expressed as nmol/h/mL.

3.3. Virus preparation

Lentiviral vectors were constructed encoding mouse α-L-iduronidase (pHIV-EF1αmmIDUA), β-D-glucuronidase (pHIV-EF1αmmGUS) or sulfamidase (pHIV-EF1αmmCOS), rat 4-sulfatase (pHIV-EF1αrn4S) or eYFP (pHIV-EF1αeYFP), under the transcriptional control of the EF-1α promoter. Virus was produced in HEK broad 293 T cells by transient transfection using calcium phosphate precipitation for 8 h. Transfected cells were grown in optiproSFM medium supplemented with 4 mM glutamine, 50 U/mL penicillin, and 50 μg/mL streptomycin for a further 40 h for virus production. The medium containing virus was clarified over a 0.45 μm filter and virus was then bound to Mustang Q Acrodisc anion exchange discs (2 discs in tandem, Pall Corporation). Virus was eluted from the discs by an addition of 1.5 M NaCl and the eluate centrifuged at 42,000 ×*g* at 4 °C for 90 min. The pellet was resuspended in 0.9% (w/v) saline and stored at −80 °C. Virus concentration was determined as p24 protein using the HIV-1 p24 ELISA kit.

3.4. Viral transfection

MSCs were seeded in α-MEM + 10% FCS and HSCs were seeded in StemSpan + 10% FCS at 10,500 cells per cm^2 and transfected 3 h later with the addition of 4 μg/mL polybrene and 50 μg/mL gentamycin along with varying concentrations of pHIV-EF1αeYFP (transduction efficiency); 0.0007–0.07 μg/μL p24 protein, or 0.056 μg/μL p24 protein of pHIV-EF1αmmIDUA, pHIV-EF1αmmGUS, pHIV-EF1αmmCOS and pHIV-EF1αrn4S (cross correction and over-expression study). Cells were incubated with a virus for 24 h before the media was removed and replaced with growth media, as above. Non-transduced replicates

Please cite this article as: M. Jackson, et al., Mucopolysaccharidosis enzyme production by bone marrow and dental pulp derived human mesenchymal stem cells, Mol. Genet. Metab. (2015), http://dx.doi.org/10.1016/j.ymgme.2015.02.001