Cellular pathogenesis in mucopolysaccharidosis dogs at the onset of postnatal growth

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Introduction

The mucopolysaccharidoses are a family of inherited lysosomal storage disorders caused by deficiencies of enzymes that degrade glycosaminoglycans (GAGs).[1] GAGs accumulate in cells and tissues resulting in multi-organ manifestations. Progressive skeletal abnormalities, including kyphoscoliosis and joint dysplasia, are hallmarks of most subtypes including MPS I (alpha-L-iduronidase deficiency) and VII (beta-glucuronidase deficiency). In previous work using naturally-occurring canine models of these diseases, we showed that both MPS I and VII dogs exhibit failures of endochondral ossification during postnatal growth, including delayed cartilage-bone conversion in secondary ossification centers,[2-4] and low bone volume and mineral density in primary ossification centers.[4] The underlying cellular basis of these abnormalities remains poorly understood. The objective of this study was to conduct an ultrastructural examination of lysosomal storage and quantify pathological changes to other organelles across different skeletal cell types in MPS I and VII dogs at the onset of postnatal growth.

Methods

With IACUC approval, vertebral bodies were obtained postmortem from 9-day-old normal (control), MPS I and MPS VII-affected dogs (each n = 5), fixed in glutaraldehyde/paraformaldehyde overnight and decalcified. Samples were post-fixed in 2% osmium tetroxide prior to *en bloc* staining with 2% uranyl acetate. Thin (80nm) sections were stained with uranyl acetate and lead citrate and imaged using transmission electron microscopy (TEM; JEOL JEM-1010). Ultrastructural analyses were performed for resting, proliferating and hypertrophic growth plate chondrocytes (RC, PC and HC, respectively), and osteoblasts (OB) and osteocytes (OCY) in primary ossification centers. The following parameters were measured using ImageJ software: cell area occupied by vacuoles (lysosomal storage, %), rough endoplasmic reticulum (ER) lumen diameter, and number of mitochondria and Golgi. For each sample, measurements were performed for 3 cells of each type, with results averaged prior to statistics. Detection of apoptotic cells was carried out on paraffin sections from thoracic vertebrae using in situ cell death (TUNEL assay) detection kit (Sigma, USA) following manufacture's instruction. Statistical differences were established via ANOVA with pairwise post-hoc Tukey's tests ($p < 0.05$).

Results

All skeletal cell types examined from MPS VII vertebrae exhibited significantly elevated lysosomal storage (vacuoles as a percent of cell area) compared to control cells (Figs. 1 and 2). Storage was greatest and most striking for MPS VII osteocytes, occupying ~ 50% of the total cell area. Storage was also elevated in MPS I compared to control, but did not reach significance for any cell type. Rough ER lumen were significantly dilated for MPS I resting chondrocytes compared to both control and MPS VII (Figs. 3A and B). Rough ER lumen were also dilated in MPS VII resting chondrocytes, but not significantly compared to control. There were no significant differences in ER lumen diameter between groups for other cell types. There was elevated TUNEL staining in MPS VII epiphyseal cartilage compared to controls, indicating increased apoptosis of resting chondrocytes secondary to storage (Fig. 3C). There were no significant differences in the number of mitochondria or Golgi between groups for any cell type.

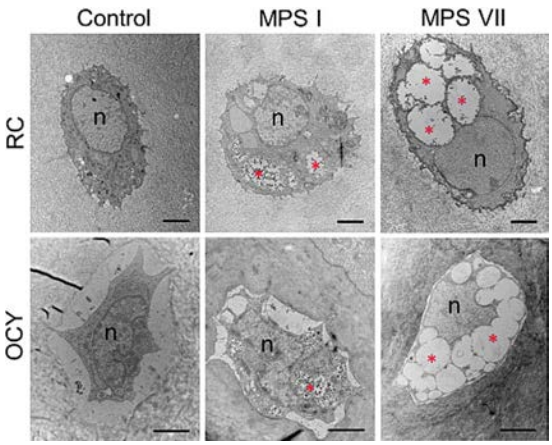

Figure 1. TEM of resting chondrocytes (RC) and osteocytes (OCY) in the vertebrae of 9-day-old control, and MPS I and VII-affected dogs. Bar =2μm. Asterisks = vacuoles (lysosomal storage); n=nucleus

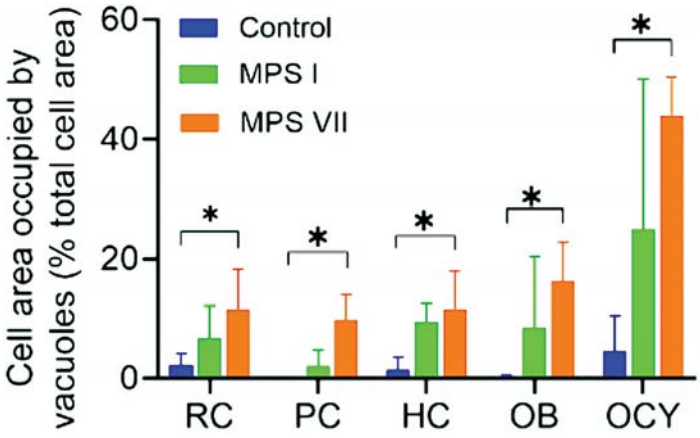

Figure 2. Relative lysosomal storage (cell area occupied by vacuoles) in resting (RC), proliferating (PC) and hypertrophic (HC) chondrocytes, osteoblasts (OB) and osteocytes (OCY) of 9-day-old control, and MPS I and VII affected dogs. *p < 0.05 vs control.

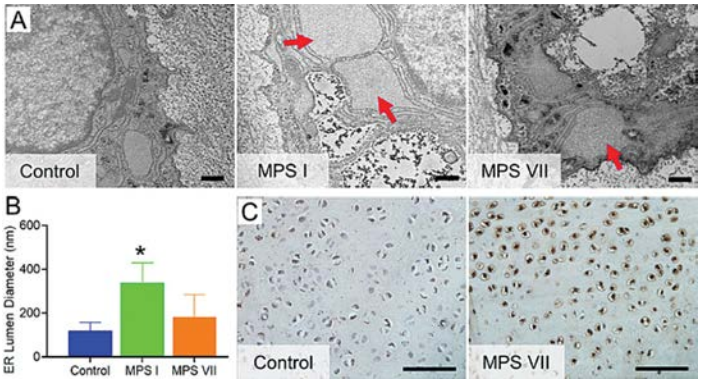

Figure 3. A. Representative images showing dilated rough ER lumen (arrows) in resting chondrocytes of 9-day-old MPS I and VII dog vertebrae. Bars = 500 nm. **B.** Quantification of rough ER limen diameter; * p < 0.05 vs control and MPS VII. **C.** TUNEL staining showing elevated numbers of apoptotic resting chondrocytes in MPX VII vertebral epiphyseal cartilage. Bars = 100μ.

Discussion

Abnormal development of the vertebrae and long bones is a hallmark of skeletal disease in MPS patients; however, the underlying cellular mechanisms remain poorly understood. In general, skeletal manifestations are more severe in MPS VII compared to MPS I. In the current study we showed that both bone and cartilage cells from MPS VII dog vertebrae exhibit significantly elevated lysosomal storage from early in postnatal life. Storage in chondrocytes may impair proliferation and differentiation ability, contributing to delayed epiphyseal cartilage-bone conversion and longitudinal bone growth. Storage in osteoblasts and osteocytes likely negatively impacts bone formation and turnover. Interestingly, storage was greatest in MPS VII osteocytes, potential reflecting the relative age of these cells. Once entombed within the bone matrix, osteocytes are relatively inaccessible to exogenous drugs, which may in part explain why bone disease is recalcitrant to treatments such as enzyme replacement therapy. Rough ER dilation (highest for MPS I resting chondrocytes) is a marker of ER stress and may negatively impact protein synthesis and cell health. In conclusion, these results highlight the importance of very early diagnosis and intervention for preventing the progression of skeletal manifestations of MPS, and the need for new therapies that effectively target skeletal cells that reside in dense, avascular microenvironments.

Significance

MPS patients exhibit severe skeletal disease for which there are currently no effective treatments. The results of this study provide insights into how storage differentially effects major skeletal cell types, and highlights the need for early and target delivery of therapeutic agents to these cells to prevent progression of crippling skeletal deformities.

References

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