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Proteomic Screening Identifies Novel Biomarkers of Synovial Joint Disease in Mucopolysaccharidosis I Dogs

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Introduction

The mucopolysaccharidoses (MPS) are a family of inherited lysosomal storage diseases characterized by deficient activity of enzymes that degrade glycosaminoglycans (GAGs) due to mutations in associated genes[1]. MPS I is characterized by deficient α-L-iduronidase (IDUA) activity, leading to progressive accumulation of poorly degraded heparan and dermatan sulfate GAGs in cells and tissues[2]. Synovial joint (e.g. hip, knee, hands and shoulder) abnormalities in MPS are prevalent, and patients experience significantly decreased quality of life due to pain and mobility impairment[2]. Studies suggest that progressive joint disease can be traced to a combination of developmental abnormalities and chronic inflammation, which accelerates soft tissue degeneration[3,4].There is a clinical need for specific biomarkers for assessment of joint disease progression and response to therapy. The naturally-occurring canine model of MPS I exhibits progressive synovial joint abnormalities similar to human patients, making it a clinically-relevant platform for biomarker discovery. The objectives of this study were to undertake an unbiased proteomic screen to identify molecular biomarkers upregulated in the synovial fluid (SF) of MPS I dogs and identify circulating (serum) biomarker candidates that may serve as strong predictors of synovial joint disease.

Methods

Animals and Sample Collection

With IACUC approval, serum and SF was collected from ± x MPS I affected and 5 x heterozygous control dogs at 12 months-of-age. Blood was collected from the cephalic vein, allowed to clot, then centrifuged and serum collected. Animals were then euthanized, stifle (knee) joints opened, and SF collected (~200µl) using an 18-gauge needle.

Mass Spectrometry

Total protein concentration was assessed using the bicinchoninic acid assay. SF was pretreated with hyaluronidase, and proteins in both SF and serum were denatured, reduced, alkylated, and digested into peptides. Peptide separation and mass spectrometric analyses were carried out using a Thermo Scientific UltiMate 3000 UPLC coupled to a Q Exactive HF Orbitrap LC-MS/MS System.

Bioinformatics and Statistical Analysis

Peptides were identified using Spectronaut software and Perseus was used to establish statistically-significant fold changes in protein abundance in MPS I vs control samples. Spearman's rank-order tests were used to examine correlations between serum and synovial fluid ($p<0.05$).

Results

In SF and serum samples, mass spectrometry identified 812 and 415 unique proteins, respectively. Principal component analyses (Figures 1A and B) demonstrated clustering of control and MPS I samples for both SF and serum, confirming significant effects of disease state on relative protein abundance. Examining SF, there were 151 proteins that exhibited significantly different abundance in MPS I vs control (Figure 1C), and of these, 104 exhibited a log2 fold change > 1. For serum, there were 154 proteins that exhibited significantly different abundance in MPS I vs control (Figure 1D), and of these, 64 exhibited a log2 fold change > 1. There were 50 proteins for which abundance was significantly different for both SF and serum, and of those, 32 exhibited a significant correlation in abundance between SF and serum across all 11 samples. To identify a shortlist of 10 biomarker candidates predictive of joint disease, these proteins were ranked according to their relative elevation in SF (MPS I vs control, Figure 2). Spearman correlation coefficients (SF vs serum) ranged from 0.64 to 0.90 for these 10 proteins (Figure 3).

Discussion

In this study, we identified novel candidate biomarkers of synovial joint disease in MPS I using the clinically-relevant canine model. Importantly, there were strong correlations between the abundance of these biomarkers in SF and serum, suggesting that they may serve as circulating biomarkers that specifically reflect

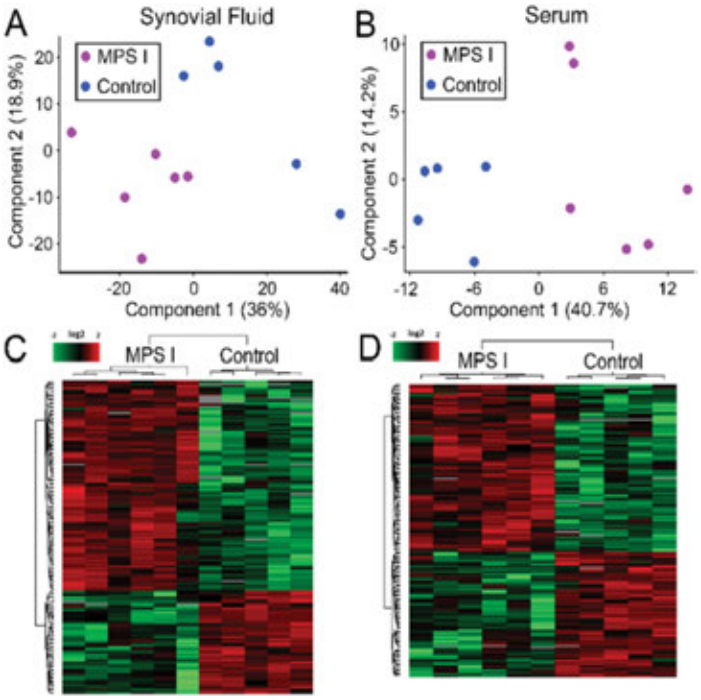

Figure 1. Principal component analysis plots showing clustering of samples as a function of disease slate for both **(A)** Synovial fluid and **(B)** Serum. Heat maps showing differences in protein abundance between MPS I and heterozygous control samplcs for **(C)** Synovial fluid and **(D)** Serun.

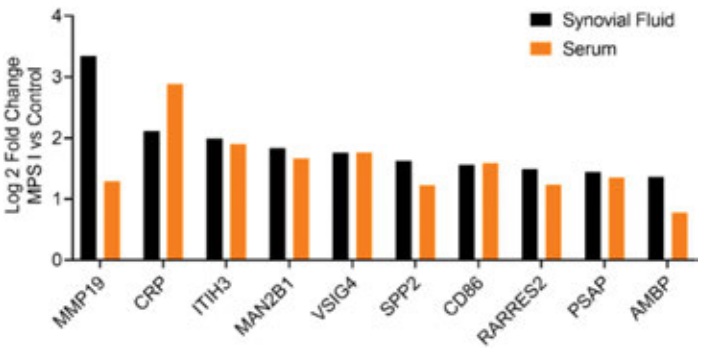

Figure 2. Candidate protein biomarkers that exhibit significantly elevated abundance in both synovial fluid and serum from MPS I dogs comparcd to heterozygous controls (all $p<0.05$).

joint disease severity and enhancing their clinical utility. These markers also provide novel insights into mechanisms of joint disease. For example, MMP19 (matrix metalloproteinase-19) cleaves aggrecan, a major component of healthy articular cartilage5. ITIH3 (inter-α-trypsin inhibitor heavy chain 3), while known to play a role in several neurological diseases, is also important for matrix stabilization[6], suggesting it may also play a role in joint health. RARRES2 (retinoic acid receptor responder 2 or chemerin) is an adipokine and inflammatory mediator that is elevated in both osteoarthritis and rheumatoid arthritis[7,8]. Finally, MAN2B1 (alpha-mannosidase) is a lysosomal hydrolyze, and its elevated abundance likely reflects broader lysosomal dysfunction secondary to IDUA deficiency. Ongoing work will further validate these biomarkers by undertaking correlations with structural and biomechanical changes in MPS I joints from both dogs and human patients.

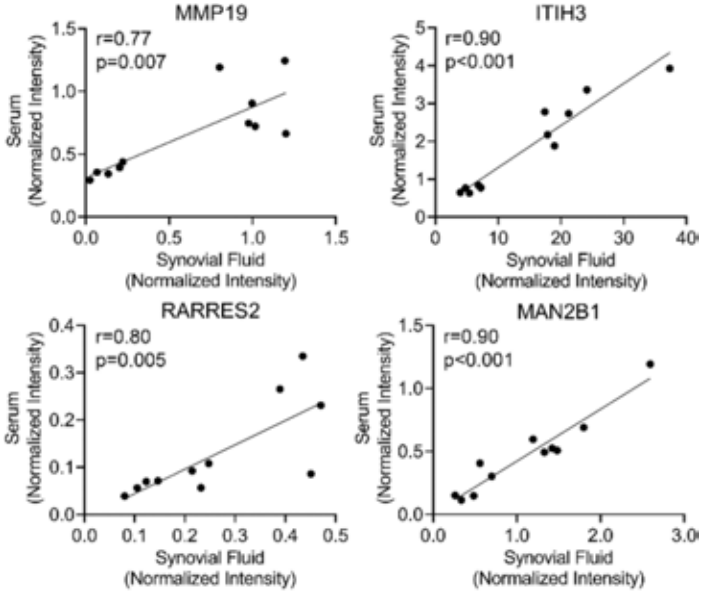

Figure 3. Plots for candidate biomarkers showing strong correlations between protein abundance in synovial fluid and serum. r = Spearman correlation coefficient.

Significance

Patients with MPS I exhibit debilitating synovial joint disease that negatively impacts quality of life. In this study we identify novel biomarker candidates that may permit minimally invasive monitoring of joint disease progression and response to therapy.

Acknowledgments

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