Unique degeneration of intervertebral discs adjacent to Modic changes

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Background

Modic changes (MC) are painful vertebral bone marrow lesions and are often found in patients with chronic low back pain. The adjacent intervertebral disc (IVD) seems to play an important role: the rapidly degrading disc stands in an inflammatory cross-talk with the MC bone marrow and MC develop almost always simultaneously cranial and caudal to a degenerated IVDs. Few studies have investigated expression of inflammatory cytokines and proteases expressed by IVD cells adjacent to MC, however, how this affects the ECM degeneration has not been determined.

Objective

The aim of this study was to identify MC-specific cleavage of the extracellular matrix (ECM). We hypothesize that the 'Modic discs' have a distinct disc matrix degradome (Fig. 1).

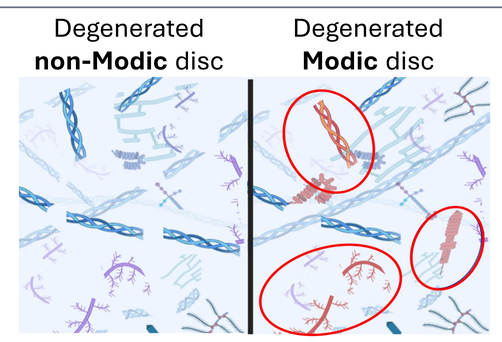

Fig. 1: *Degradome of non-Modic and Modic discs*

Methods

Degenerated lumbar IVDs from MC1 (n=29), MC2 (n=24) and non-MC (n=25) levels from gender and age matched patients undergoing spinal fusion surgery were collected. Degradome was measured with N-terminal amine isotopic labeling of substrates (TAILS) liquid chromatography tandem mass spectrometry (LC-MS/MS) (Fig. 2). TAILS allows to identify degraded proteins by detecting de novo N-terminal peptides. Sequence motifs were calculated using TwoSample Logo web application to identify significantly enriched amino acids around the cleavage site of the top 50 upregulated peptides. Since proteases have preferences for amino acid sequences, different sequence motifs can indicate activity of different proteases. Proteases were matched to cleavage sites of the top 50 enriched MC1 and MC2 peptides using TopFINDer database.

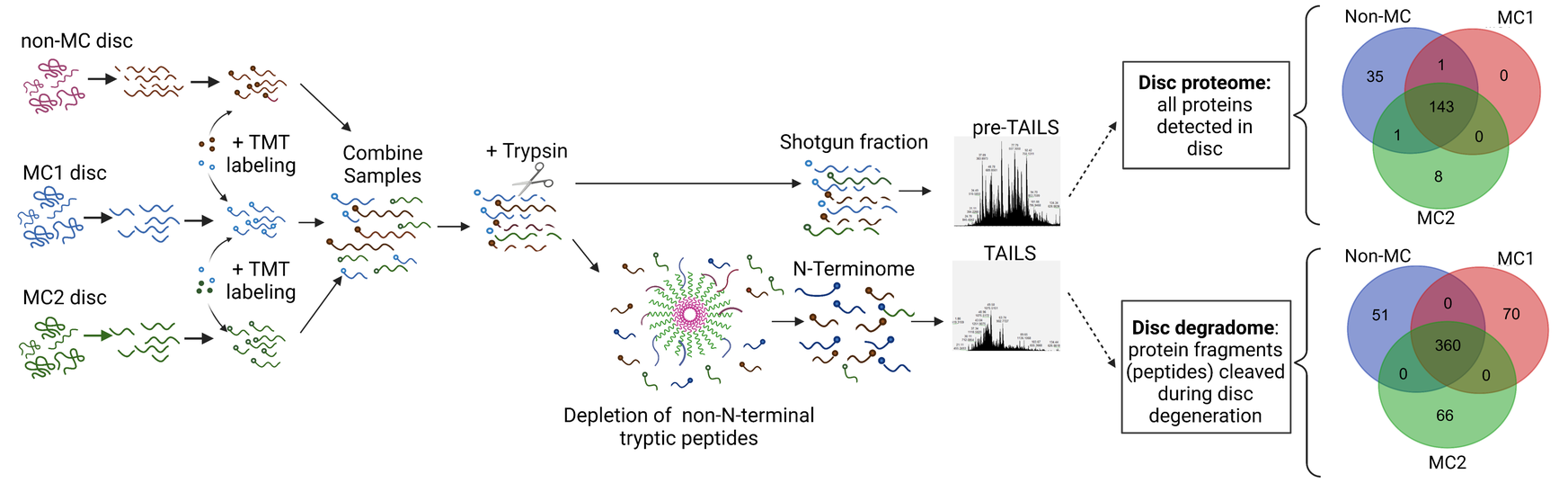

Fig. 2: *TAILS workflow for proteomic shotgun and N-terminome profiling of the MC1, MC2 and non-Modic discs. Venn diagrams show the proteins found for each group using the pre-TAILS fraction (top) and unique N-terminal cleaved peptides for each group (bottom).*

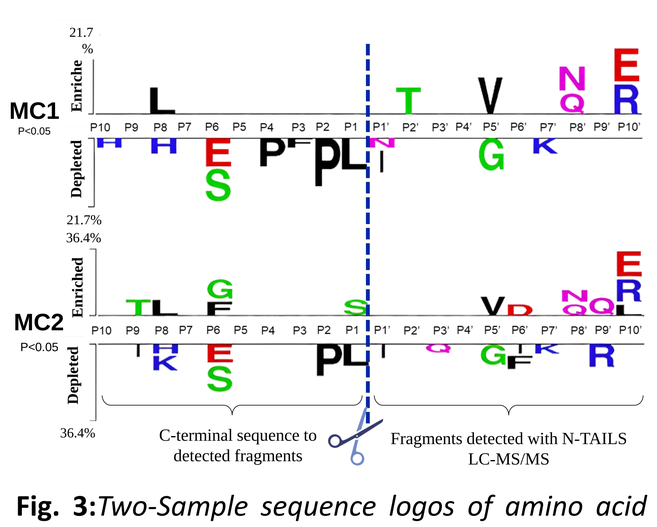

Fig. 3:*Two-Sample sequence logos of amino acid sequences around the cleavage site showing only significantly enriched amino acids.*

Results

Mean degree of disc degeneration as measured by Pfirrmann grade was not significantly different between groups (MC1: 3.8 ± 0.9, MC2: 4.2 ± 0.6, non-MC: 3.6 ± 0.8). Pre-TAILS comparable to a shotgun measurement of the disc detected a total of 188 proteins with 152 detected in the MC1 group, 152 in the MC2 group and 180 in the non-MC group. The TAILS fraction, corresponding to the degenerative products through proteolytic cleavage in-vivo, detected three unique degradomes. A total of 360 de-novo N-termini are found in all groups. The two MC groups have a greater variation of peptides than the non-MC group. With 32 of the 70 unique MC1 peptides, 17 of the 66 unique MC2 peptides and 13 of 51 unique non-MC proteins deriving from the extracellular matrix protein fibronectin, it is shown that the same protein is uniquely cleaved into different fragments.

Sequence motifs show unique cleavage preferences for each group indicating activity of different proteases in MC1 and MC2 discs potentially responsible for the unique cleavages of the same proteins (Fig. 3). TopFinder identified neutrophil elastase as an important protease in all groups targeting cleavage of different proteins. In MC1 and MC2 discs kallikrein 3 as well as MMP9 and MMP2 are associated with found cleavage products. Finally, in MC1 TopFINDer uniquely identifies multiple fragments created by MMP2, MMP7, MMP12, MMP13 or cathepsin B.

Conclusion

The findings reveal a difference in the breakdown of the 'Modic discs' both in number of distinct fragments produced and in cleavage of these fragments. Further research will allow us to determine specifically which fragments play a role in the development of MC, finally presenting new treatment targets.

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