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AGEING AND OSTEOARTHRITIS MARKERS IDENTIFIED BY MALDI IMAGING MASS SPECTROMETRY

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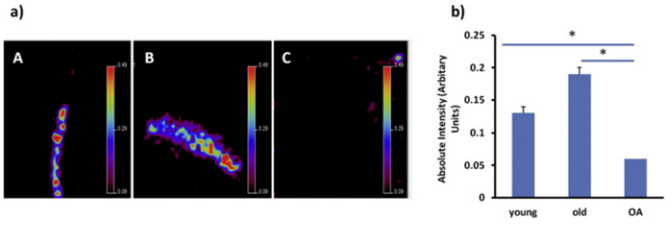
Purpose: Cartilage protein distribution and the changes that occur in cartilage ageing and disease are essential in understanding the process of cartilage ageing and age-related diseases such as osteoarthritis (OA). Matrix assisted laser desorption ionization (MALDI) imaging mass spectrometry (IMS) enables examination of proteins *in-situ* at a high spatial resolution. This study utilised this methodology to investigate the location and abundance of different cartilage proteins in ageing and OA equine cartilage in order to determine changing molecular events distinct between aging and disease.
Methods: For age-related studies samples were taken from skeletally mature young and old horses. For OA studies skeletally mature donors were chosen with mild macroscopic OA changes. 12 μm thick sections were cut in duplicate. Following washing trypsin for digestion was applied and Alpha-Cyano-4-hydroxycinnamic acid matrix deposited. Synapt HDMS MALDI-Q-TOF was used to perform the IMS experiments. Peptides were identified using MASCOT following MS/MS experiments. Biomap software was used to generate ion images and quantify peptide intensity. The data analysis workflow used Principal Component Analysis (PCA) and Discriminant Analysis (DA) for data interpretation. Following IMS samples were stained with haematoxylin and eosin and histologically assessed.
Results: Histological assessment revealed mild changes in the OA samples alone. Protein profiling experiments directly from the tissue sections identified extracellular matrix proteins including cartilage oligomeric matrix protein, fibromodulin, biglycan and type II collagen. After combining all the spectra from the different conditions and following DA, the resulting discriminant functions classified the data in three groups according to their peptide profile: young, old and OA. Interestingly there was a large contribution of the old samples to the negative part of DF1 indicating that peptides within old samples were also present in OA samples. The spectra of young, old and OA samples after MALDI-IMS experiments were analyzed independently by PCA and DA to classify peptides specific to each group (young versus old and old versus OA), thus producing a catalogue of peptides distinct in ageing and disease. Then protein distribution differences were visualised and semi-quantified, through the examination of peptide intensities in young, old and OA cartilage Biomap. A number of OA and ageing markers were identified. Significant differences were evident for the peak intensity distribution of OA specific peptides including fibronectin peptides *m/z* 1349.6 and *m/z* 1401.7 ($p=0.018$, $p=0.02$) and the hypothetical marker with *m/z* 1366.5 ($p=0.001$) between ageing and OA samples. Age-related markers were also identified for COMP *m/z* 2256.1, the hypothetical marker *m/z* 2415.9 and fibromodulin peptide ELHLDHNQISR; *m/z* 1361.7 Furthermore there was a significant reduction in the intensity of this latter peptide (Figure 1) and biglycan peptide NHLVEIPPNLPSSLVELR *m/z* 2027.2 ($p=0.001$, $p=0.02$) in OA (C) compared to young (A) and old (B) cartilage indicating peptides potentially targeted for degradation in OA.

Conclusions: MALDI-IMS based molecular imaging provided a novel platform to study cartilage ageing and disease enabling age and disease specific markers in cartilage to be elucidated and spatially resolved as well as identifying peptides targeted in early OA degradation.



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GLUCOCORTICOIDS INDUCE SENESCENCE IN PRIMARY HUMAN TENOCYTES: IN VITRO AND IN VIVO EVIDENCE

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Synthetic glucocorticoids (GCs) are frequently used to treat painful and inflamed tissues and joints. However GCs also cause unwanted side effects, inhibiting cell proliferation as well as normal cell activity. Whether these effects are transient or long-term is unknown. Inhibition of cell proliferation can lead to either quiescence or senescence. Whereas quiescence is reversible, senescence is irreversible in vivo and hence leads to a permanent loss of normal cell functionality.
Purpose: The aim of this study was to determine whether GCs induce senescence in human tenocytes in vitro and in vivo.
Methods: Characteristic features of cell senescence (β-galactsidase activity at pH6 (SA-β-gal), active mTOR in cells in cycle arrest) were examined in GC-treated primary human tenocytes. The effect of GCs on activity of the two main pathways leading to cell senescence (p38/p16INK4a and p53/p21cip) was determined by Western blotting. Gene expression changes were assessed by RT-qPCR. Tendon biopsies were taken from tendinopathy patients immediately prior to and seven-weeks following GC injection. Markers of senescence were analysed in biopsy specimens by immunohistochemistry.
Results: GC treatment of human tenocytes in vitro resulted in a higher percentage of SA-β-gal positive cells. mTOR remained active despite cell cycle arrest in GC-treated tenocytes. Increased p53 acetylation and reduced levels of the p53 deacetylase sirtuin 1 were observed in GC-treated tenocytes. Levels of p21cip, an anti-apoptosis/pro-senescence modulator of p53 activity were also increased post-GC treatment. Knockdown of p53 using RNAi or chemical inhibition of p53 activity prevented GC-induced senescence. Preclusion of the GC-induced reduction in sirtuin 1 levels either by adenoviral-mediated overexpression of exogenous sirtuin 1 or by upregulation of endogenous sirtuin 1 expression by glucose restriction (to emulate caloric restriction) or treatment with resveratrol also prevented GC-induced