PROTEOMIC IDENTIFICATION OF SECRETED BIOMARKERS IN AN EXPLANT MODEL OF EARLY OSTEOARTHRITIS

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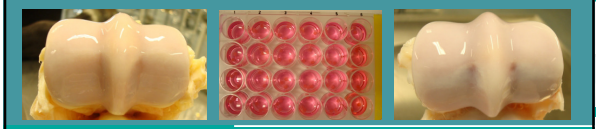


Introduction

- Molecular changes in early OA are poorly understood
- Comparative models are beneficial for researching OA - equine
- Proteomics;
  - increasingly being applied in OA research and in basic cartilage biology to understand the composition of the chondrocyte proteome *
  - not extensively applied to serum free explant cultures

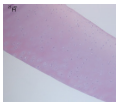
* Ruiz-Ramero, C., López-Armada, M. & Blanco, F.J. (2005) *Proteomics*, 5: 3048-3059.

Aims and Objectives

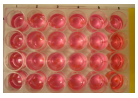
- Can proteomics and cross-species peptide matching be applied to a serum free equine cartilage explant model to identify secreted proteins in early OA?
- The cross-species approach may help validate biomarkers identified in studies using experimental animals and human cartilage samples

Method- Explant Model

- Distal forelimbs of 3 horses euthanized for reasons other than research

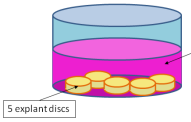


- Articular cartilage obtained from MCP joints and washed
- Full depth cartilage discs excised and placed into the wells of a 24 well plate containing serum free DMEM + 2% antibiotic



Method- Study Design

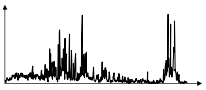
- After overnight incubation at 37°C, the media was removed and replaced
- Explants were incubated at 37°C for 5 days with;



Sample Preparation

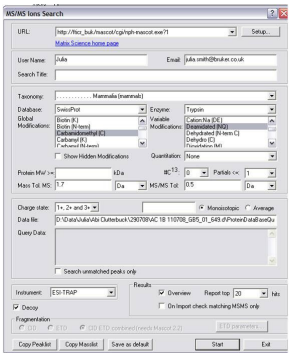
- Reduction of soluble proteins – DTT
- Blocking the thiol groups - iodoacetamide
- Protein precipitation - ice-cold acetone
- Protein quantification - Lowry Assay
- Protein resuspension - ammonium bicarbonate (at 2µg/µl)
- Peptide fragmentation - trypsin
- Termination of trypsin digestion - formic acid

Separation & Detection

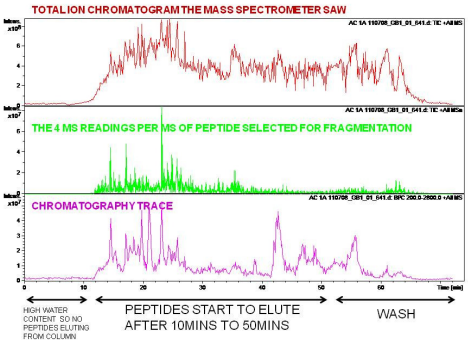


- Separation
  - on a C18 pepmap column using a Dionex U3000 chromatography platform
- Detection
  - Bruker HCT PTM discovery **ion trap** instrument.

MS/MS Ion Search

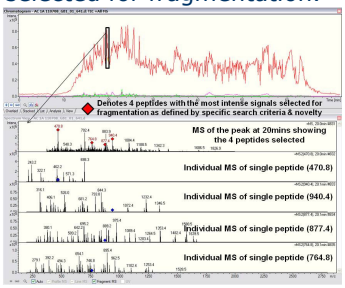


Chromatographic Profiles



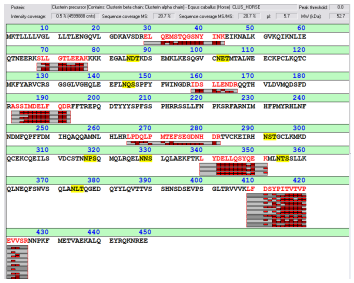
MS Scan

- The 4 most abundant ions in each MS scan were selected for fragmentation.



Sequence Matching

- Fragment patterns for each peptide were compared to the mammalian entries in the SwissProt database using MASCOT search engine



Mascot Search http://www.matrixscience.com/

Results- Cross Species Matching

Protein	Bovine	Dog	Chimp	Horse	Human	Mouse	Pig	Rabbit	Rat	Sheep
FMOD	*					*			*	
CILP-1					*	*	*			
CHAD	*				*	*			*	
MMP-1				*						
PRELP					*	*				
PGS-1	*			*						
PGS-2	*	*		*	*			*	*	
TR11B						*				

Results- Consistently Identified Proteins

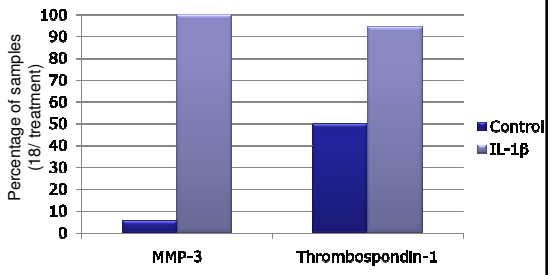
- The most abundant proteins secreted into the media were identified
  - Proteins constitutively expressed by cartilage
  - Found in other proteomic studies *

Protein	Control (18)	IL-1β (18)
Aggrecan Core Protein	18	18
Fibronectin	17	18
Cartilage Oligomeric Matrix Protein	18	17
Clusterin	18	16

- Validate the model

* Stevens, A.L., Wishnok, J.S., Chai, D.H., Grodzinsky, A.J. & Tannenbaum, S.R. (2008) *Arthritis & Rheumatism*, 58 (2): 489-500

Differentially secreted proteins- IL-1β specific



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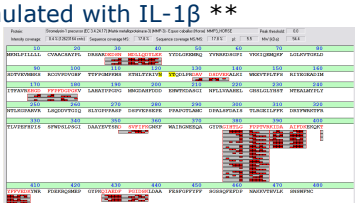
MMP-3 -*horse*

- Increased activity in SF of equine OA joints*
- Increased in equine chondrocyte cultures stimulated with IL-1β **

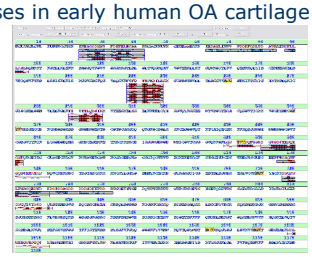


* Brama, P.A. *et al.* (2000) *Annals of the Rheumatic Diseases*, 59(2): 155-157.

** Tung, J.T. *et al.* (2002) *Canadian Journal of Veterinary Research*, 66(1):19-25.

TSP-1 -*bovine, human, mouse*

- Adhesive glycoprotein that mediates cell-to-cell and cell-to-matrix interactions.
- Increases in early human OA cartilage lesions*



* Pfander, D., *et al.* (2000) *Annals of the Rheumatic Diseases*, 59: 448-454.

Conclusions

- First proteomic study of a serum free equine cartilage explant model
- Identified several proteins with well-established ECM functions
- Other proteomic studies have identified some of the proteins being up-regulated in early OA thus validating the model
- Identified proteins that could be potential biomarkers in the supernatants of IL-1β-stimulated equine cartilage explants
- Some of the proteins identified may participate in early repair responses and may be useful for the detection of early changes in OA

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