Orthopaedic Surgery
School of Medicine
University of Missouri Health

Thompson Laboratory for Regenerative Orthopaedics

Relationships among Pro-Inflammatory and Degradation-Related Biomarkers released by Articular Cartilage from Osteoarthritic Knees

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Introduction

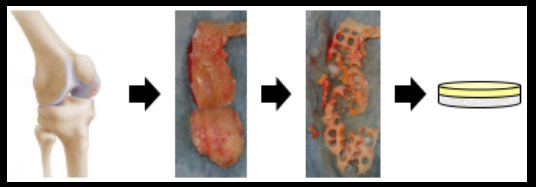
- Osteoarthritis (OA) is a multifactorial disease often progressing from an initial insult or injury to whole-joint inflammation and degeneration causing pain and dysfunction
- Previous studies have indicated moderate to weak linear correlations between inflammatory and degradation related biomarker production levels by OA cartilage during culture
- This study was designed to characterize non-linear relationships among pro-inflammatory and degradation-related biomarkers produced by articular cartilage recovered from patients with knee OA

Hypothesis

- **There will be significant ($p<0.5$) differences in the production of inflammatory and degradative biomarkers (Testing) when grouped based on the production level of another biomarker (ranking) produced by OA cartilage.**

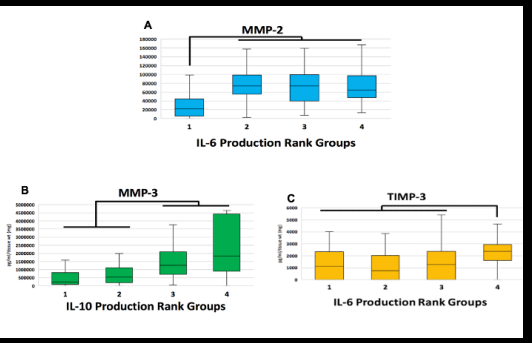
Methods

1. With IRB approval (IRB# 1208392) and informed consent, tissues normally discarded after total knee arthroplasty were recovered from OA patients (n=11), and 6mm osteochondral explants (n=359) were created
2. Cartilage was separated from bone, and cartilage explants were cultured for 3 days
3. After 3 days culture, media were collected and stored at -20˚C for subsequent biomarker analyses and the cartilage explant was weighed
4. Media were tested for MMP-1, MMP-2, MMP-3, MMP-7, MMP-8, MMP-13; TIMP-1, TIMP-2, TIMP-3, TIMP-4, PDGF-AA, IL-6, IL-8, IL-10, IL-13, IL-1RA, IL-1β, FRACTALKINE GRO-α, MCP-1, MCP-3, MIP-1α, MIP-1β, TNF-α, and VEGF using commercially available assays.
5. The media biomarker data were standardized to the weight of the explant for analysis. Each biomarker was sorted lowest to highest, and placed into 4 quartiles based on production level, approximately 25% of all samples in each quartile. A Kruskal Wallace test with post-hoc analysis and Bonferroni correction were used to determine significant differences between groups with significance ($p<0.05$)



Results-Positive Biomarker Patterns

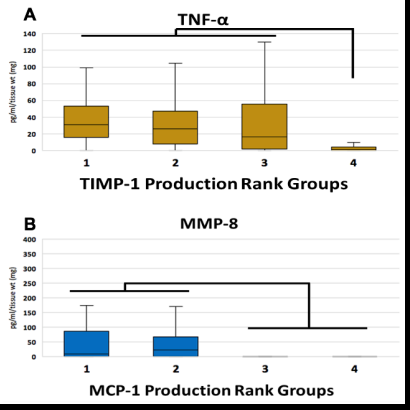
(H1) 2 3 4 > 1		(H1) 2 3 4 > 1		(H2) 3 4 > 1 2		(H3) 4 > 1 2 3	
RANKING	TESTING	RANKING	TESTING	RANKING	TESTING	RANKING	TESTING
Gro-α	MMP-7	MMP-1	IL-8	IL-10	MMP-1	Gro-α	MMP-1
IL-13	MMP-7	MMP-1	VEGF	IL-10	MMP-3	Gro-α	MMP-3
IL-6	MMP-2	MMP-2	IL-6	IL-10	MMP-7	Gro-α	MMP-13
IL-6	MMP-1	MMP-2	MCP-1	IL-6	TIMP-2	IL-10	TIMP-2
IL-6	MMP-3	MMP-3	Gro-α	MCP-1	TIMP-2	IL-10	TIMP-4
IL-6	MMP-13	MMP-3	IL-6	MCP-1	TIMP-3	IL-6	TIMP-3
IL-6	MMP-7	MMP-3	IL-8	MIP-1α	MMP-1	IL-8	TIMP-1
IL-8	MMP-1	MMP-3	MCP-1	MIP-1α	MMP-3	IL-8	TIMP-2
IL-8	MMP-3	MMP-3	VEGF	MIP-1α	MMP-13	IL-8	TIMP-3
MIP-1α	MMP-7	MMP-7	IL-10	MIP-1β	MMP-3	MCP-1	TIMP-2
VEGF	MMP-1	MMP-13	VEGF	PDGF-AA	TIMP-4	MIP-1β	MMP-13
VEGF	MMP-2	TIMP-3	PDGF-AA	VEGF	TIMP-3	PDGF-AA	MMP-7
VEGF	MMP-3	TIMP-3	Gro-α	MMP-1	MIP-1α	MMP-13	MIP-1α
VEGF	MMP-7	TIMP-3	IL-6	MMP-2	MIP-1α	MMP-13	IL-10
VEGF	MMP-13	TIMP-3	IL-8			MMP-3	MIP-1α
Gro-α	TIMP-3	TIMP-3	MCP-1				
TNF-α	TIMP-3	TIMP-3	MCP-3				
MMP-1	Gro-α	TIMP-3	MIP-1β				
MMP-1	IL-10	TIMP-3	TNF-α				
MMP-1	IL-6	TIMP-3	VEGF				



- Positive Biomarker Production patterns indicate that similar factors stimulate production of the ranking and testing biomarker by the OA cartilage
- The H1 pattern (A) indicates that factors that initiate production of both biomarkers may be shared, but factors that increase the production of the ranking biomarker do not increase the testing biomarker
- The H2 pattern (B) indicates a stronger positive relationship between biomarkers, with potentially similar factors regulating production of both.
- The H3 pattern (C) indicates that factors that stimulate the highest level of production by the ranking and testing biomarker may be shared.

Results-Negative Biomarker Patterns

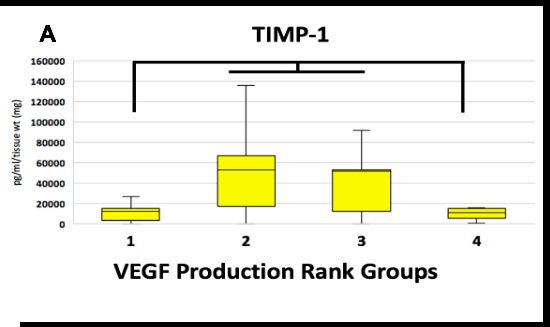
(L1) 1 2 3 > 4		(L2) 1 2 > 3 4	
RANKING	TESTING	RANKING	TESTING
MMP-1	MCP-3	MCP-1	MMP-8
MMP-1	MIP-1β	MCP-1	MMP-13
MMP-8	VEGF	MCP-1	TIMP-4
TIMP-1	MCP-3	MCP-3	TIMP-4
TIMP-1	MIP-1β	PDGF-AA	MMP-13
TIMP-1	TNF-α	PDGF-AA	TIMP-1
TIMP-4	PDGF-AA	TIMP-1	TIMP-4
TIMP-4	MIP-1α	VEGF	MMP-8
		MMP-13	MCP-3
		MMP-13	TNF-α
		MMP-8	MCP-1
		TIMP-4	MIP-1β
		TIMP-4	TNF-α



- Negative Biomarker Production Patterns indicate that factors that stimulate the ranking biomarker production may inhibit the production of the testing biomarker
- The L1 pattern (A) indicates that factors that stimulate the highest production of the ranking biomarker may inhibit the production of the testing biomarker
- The L2 pattern indicates that factors increase production of the ranking biomarker may inhibit the production of the testing biomarker

Results-Complex Biomarker Patterns

(M1) 2 3 > 1 4	
RANKING	**TESTING**
MCP-3	TIMP-1
MCP-3	TIMP-3
RANTES	MMP-3
RANTES	MMP-13
TNF-α	MMP-7
VEGF	TIMP-1
VEGF	TIMP-2
VEGF	TIMP-4
MMP-1	MCP-1
TIMP-2	PDGF-AA
TIMP-2	MCP-1
TIMP-2	MCP-3
TIMP-2	TNF-α
TIMP-4	PDGF-AA
TIMP-4	IL-6
TIMP-4	IL-8
TIMP-4	Gro-α



- **Negative Biomarker Production Patterns indicate that factors that stimulate the ranking biomarker production may inhibit the production of the testing biomarker**
- **The L1 pattern (A) indicates that factors that stimulate the highest production of the ranking biomarker may inhibit the production of the testing biomarker**
- **The L2 pattern indicates that factors increase production of the ranking biomarker may inhibit the production of the testing biomarker**

Conclusions

- The data from this study indicates that there are non-linear relationships between inflammatory and degradation-related biomarker production.
- Further analysis of these production relationships between inflammation-related biomarkers and degradation-related biomarkers produced my OA cartilage may provide insight into novel regulatory pathways that contribute to the development and progression of OA clinically.
- Identifying these mechanistic intracellular signaling pathways and relating them to severity of OA may allow for the identification of novel targets for therapeutic intervention for the treatment and prevention of OA clinically