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Identification of serum biomarkers for early-stage knee osteoarthritis using proteomics in a murine model of osteoarthritis

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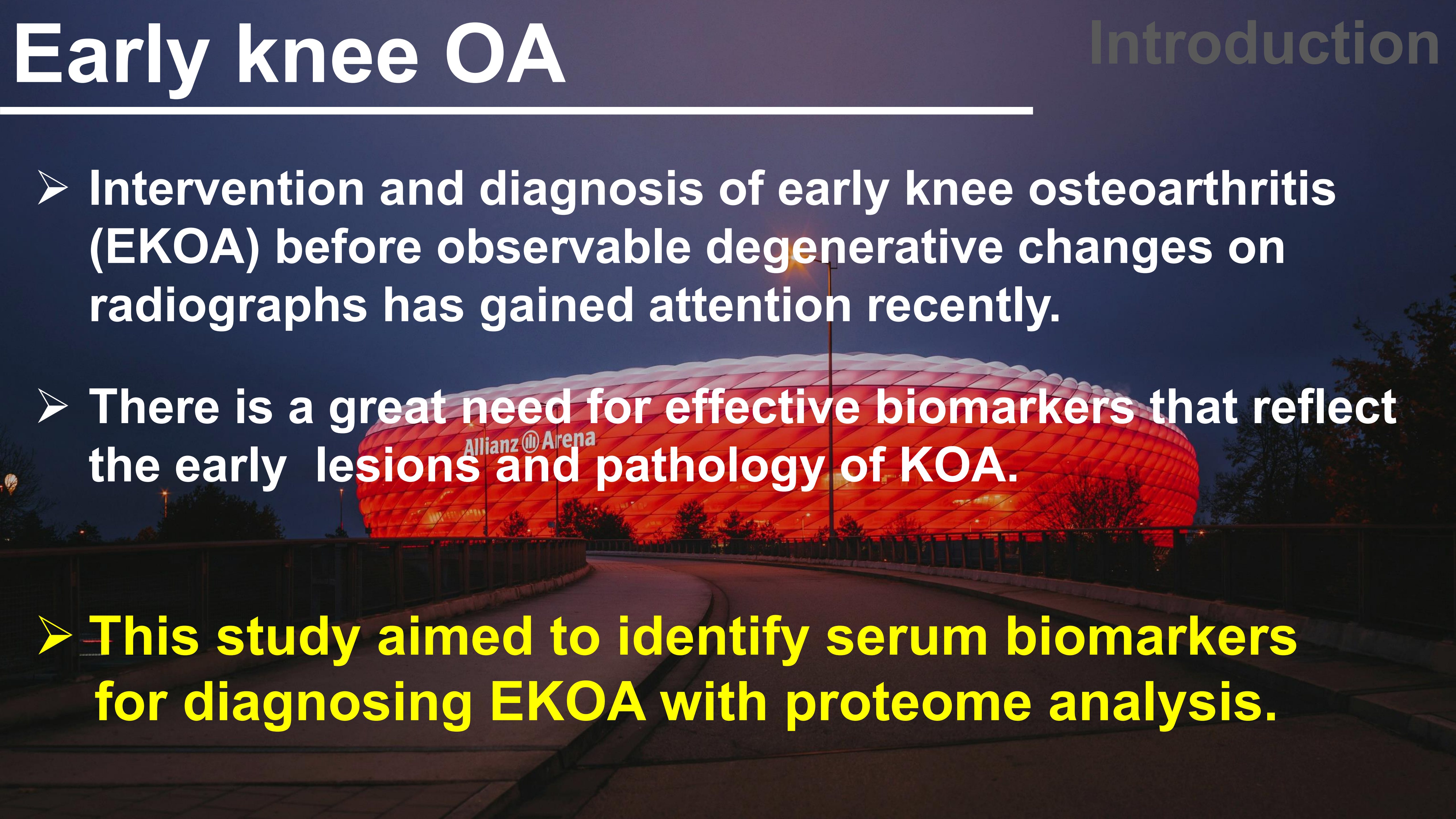
COI Disclosure

Shohei Yamauchi, MD, PhD

I have no financial conflicts to disclose.

Early knee OA



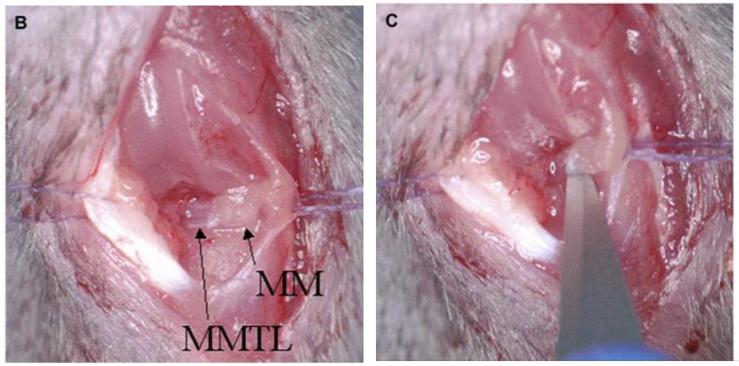
- Intervention and diagnosis of early knee osteoarthritis (EKOA) before observable degenerative changes on radiographs has gained attention recently.
- There is a great need for effective biomarkers that reflect the early lesions and pathology of KOA.
- **This study aimed to identify serum biomarkers for diagnosing EKOA with proteome analysis.**

Animal models

20 male C57BL/6J mice

Control group
- No surgery
- Sacrifice at 8 weeks old

Knee OA group
- Destabilization of medial meniscus (DMM) at 8 weeks old



Glasson SS et al. Osteoarthritis Cartilage. 2007

Divided into four groups

(1) Control (n=5)
(2) 4 weeks post-DMM (n=5, DMM4W)
(3) 8 weeks post-DMM (n=5, DMM8W)
(4) 12 weeks post-DMM (n=5, DMM12W)

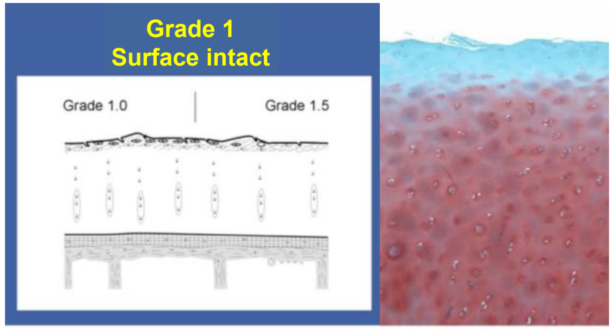
▶
- Obtained serum
- Collected right knee joint

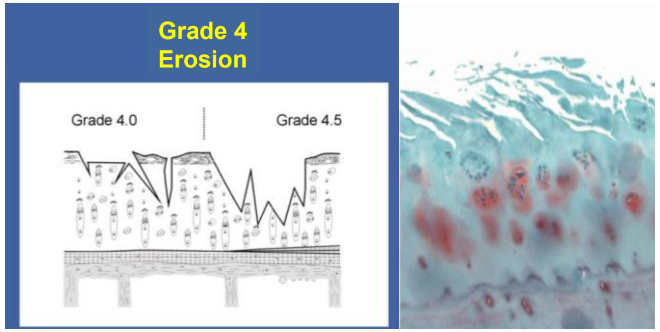
Histological analysis

OARSI Score ＝ Grade x Stage (0～24)

Grade: 1～6

Depth progression into cartilage





Stage: 0～4

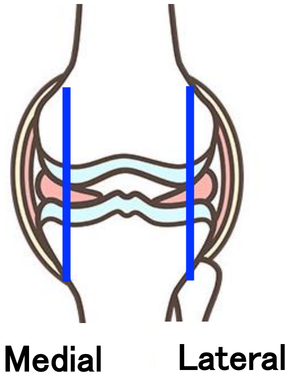
extent of joint involvement

Stage	% Involvement (surface, area, volume)
Stage 0	No OA activity seen
Stage 1	＜10%
Stage 2	10–25%
Stage 3	25–50%
Stage 4	＞50%

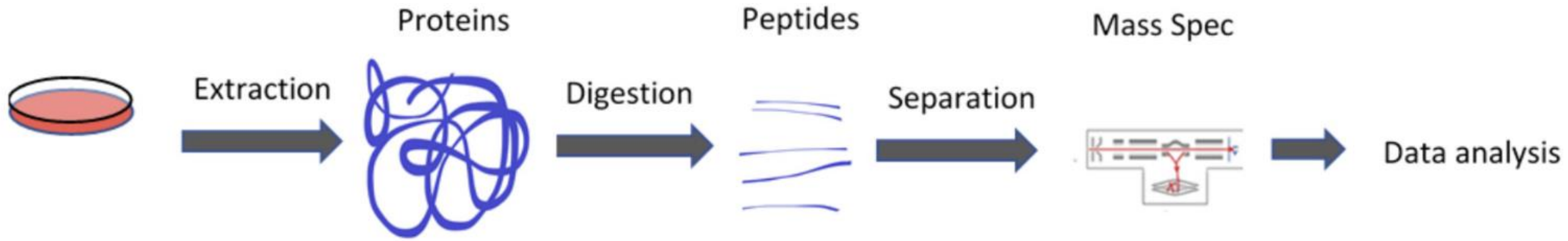
K. P. Pritzker, Osteoarthritis and Cartilage 2006

- **Knee specimens were cut sagittally**
- **OA severity was evaluated at 4 points**

  Medial tibial plateau (MTP), medial femoral condyle (MFC), lateral tibial plateau, lateral femoral condyle
- **Average OARSI scores were analyzed by the Steel-Dwass test**
- **$P < 0.05$ was considered significant**



Proteomic analysis



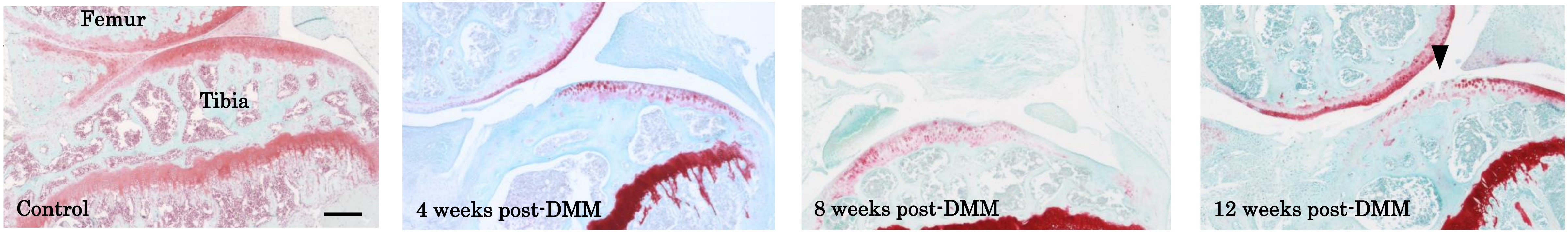
- **Minor proteins** were **enriched** by ProteoMiner kit (Bio-Rad)
- **Label-free proteomics (LC-MS/MS)** were performed with NanoLC Eksigent 400 system and Triple TOF 6600 mass spectrometer

- Protein expression levels were compared with the control group by t-test
- Protein expression level >2.0 fold-change: **Up-regulation**
  <0.5 fold-change: **Down-regulation**

- $P < 0.05$ was considered significant

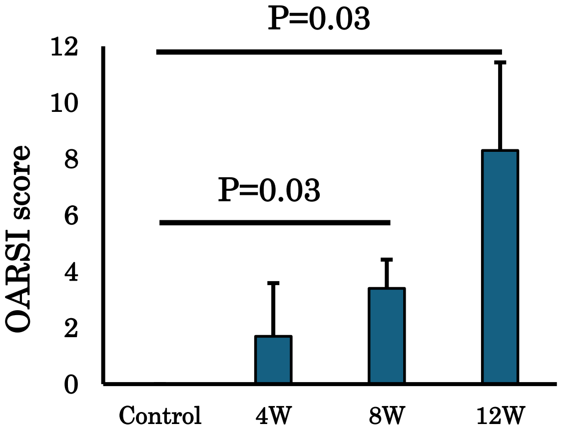
- PCA plots and heatmap analysis (MetaboAnalyst 5.0)
- Statistical extraction of canonical pathways (KeyMolnet, KM Data, Japan)

Histological OA / OARSI score

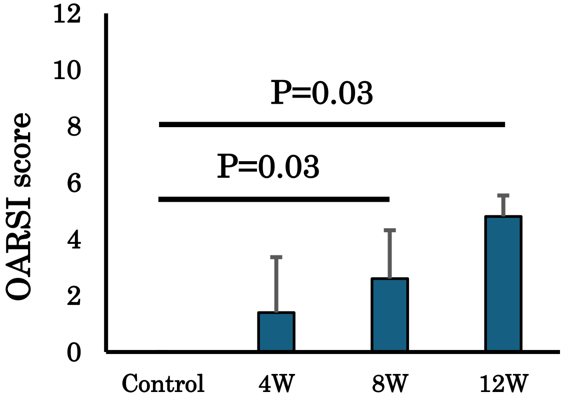


Time-dependent OA changes on MTP (Scale bar: 200 μm)

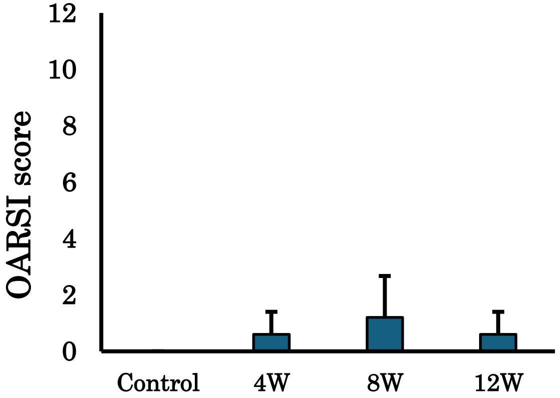
Vertical fissure (▼) on cartilage surface at **12 weeks** post-DMM



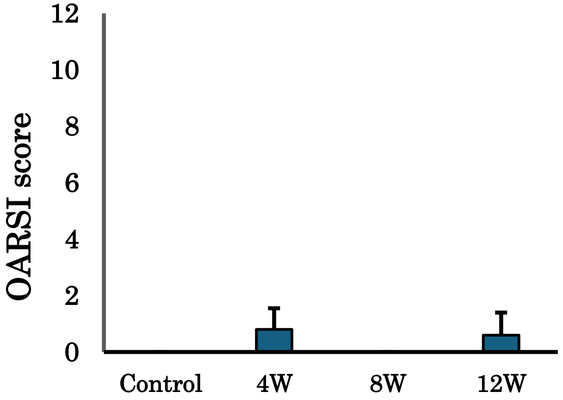
Medial Tibial Plateau



Medial Femoral Condyle



Lateral Tibial Plateau



Lateral Femoral Condyle

Score increased in MTP and MFC **after 8 weeks** post-DMM

Different expressed proteins

15 proteins were expressed differently 4 weeks post-DMM

DMM4W

Protein	Fold Change	p-value
Beta5-tublin	5.833	0.023
RELMbeta	4.755	0.02
Fiblin 1	3.037	0.029
Adk 1p	2.682	0.001
AK	2.682	0.001
b-tub	2.207	0.023
5'Ntdase	2.107	0.031
NT5C	2.107	0.031
AADISADH	0.41	0.027
IF-Ⅳ	0.28	0.001
Nestin	0.28	0.001
ALDO	0.173	0.032
AldlaseB	0.173	0.032
IGF-BP	0.171	0.006
IGF-BP5	0.171	0.006

DMM8W

Protein	Fold Change	p-value
beta5-tublin	4.293	0.024
SAA	3.661	0.007
SAA4	3.661	0.007
ECM1	2.989	0.006
Lumican	2.721	0.029
PSMA3	2.64	0.047
TIMP	2.488	0.021
TIMP-3	2.488	0.021
PDXK	2.423	0.036
Arginase1	0.464	0.008
adipokine	0.448	0.021
AADISADH	0.414	0.038
Cyclophilin	0.339	0.008
PPlase	0.339	0.008
PPIC	0.339	0.008
IF-Ⅳ	0.23	0.001
Nestin	0.23	0.001
SLPI	0.205	0.032
WAP	0.205	0.032
Vitronectin	0.116	0.024
Gelsolin	0.101	0.038
Angiostatin	0.042	0.022
Plasmin	0.042	0.022
Plasminogen	0.042	0.022
Acylation-SP	0.01	0.021
C3c	0.01	0.021

DMM12W

Protein	Fold Change	p-value
GRP94	5.029	0.045
C5 convertase	4.579	0.001
Factor B	4.579	0.001
Factor B-Ba	4.579	0.001
Factor B-Bb	4.579	0.001
PDXK	4.266	0.033
Adk 1p	3.413	0.012
AK	3.413	0.012
HSP90	2.87	0.045
ApoC-Ⅳ	2.524	0.048
PLA1	2.439	0.024
ADF	2.383	0.047
HSP	2.336	0.045
PLA	2.309	0.024
C3bBb	2.294	0.001
C3bBb3b	2.294	0.001
iC3Bb	2.294	0.001
Copine	2.001	0.041
Copine1	2.001	0.041

Protein	Fold Change	p-value
HDL	0.498	0.033
HDL2	0.498	0.033
HDL3	0.498	0.033
AADISADH	0.453	0.036
PGADH	0.431	0.005
PSP	0.429	0.011
Adipokine	0.406	0.021
LPL	0.395	0.024
C4b2a3b	0.335	0.021
PP2AA	0.323	0.043
PP2AAa	0.323	0.043
IGF-BP	0.306	0.013
IGF-BP5	0.306	0.013
SLP1	0.247	0.038
WAP	0.247	0.038
C1complex	0.199	0.035
compl-c1q	0.199	0.035
C1CC	0.125	0.035
ApoD	0.115	0.033
Angiostatin	0.107	0.026
Plasmin	0.107	0.026
Plasminogen	0.107	0.026
DENND2	0.095	0.038
DENND2A	0.095	0.038
LCAT	0.093	0.039
Vitronectin	0.044	0.021
Gensolin	0.04	0.032
Acylation-SP	0.01	0.021
C3c	0.01	0.021

Activated pathways

DMM4W

Rank	Name	Score
1	Intermediate filament signaling pathway	53.244
2	p160 SRC signaling pathway	52.418
3	Microtubule-associated protein signaling pathway	41.202
4	GSK3 signaling pathway	41.158
5	Mst(Hippo) signaling pathway	39.963
6	Wnt signaling pathway	39.54
7	Autophagy-related protein signaling pathway	39.329
8	MAPK signaling pathway	38.142
9	Transcriptional regulation by SMAD	32.659
10	Arrestin signaling pathway	31.252

DMM8W

Rank	Name	Score
1	Complement pathway	108.621
2	MMP signaling pathway	74.647
3	Alternative complement pathway	63.445
4	Fibrinolysis system	59.351
5	Calpain signaling pathway	53.875
6	SIBLING signaling pathway	52.855
7	S100 family signaling pathway	45.455
8	Transcriptional regulation by STAT	43.866
9	ADAM signaling pathway	40.541
10	PIN1 signaling pathway	39.831

DMM12W

Rank	Name	Score
1	Complement pathway	186.506
2	Classical complement pathway	94.511
3	Lectin complement pathway	71.22
4	Alternative complement pathway	66.695
5	HSP90 signaling pathway	53.913
6	MMP signaling pathway	45.727
7	Fibrinolysis system	44.639
8	AMPK signaling pathway	41.908
9	p160 SRC signaling pathway	36.3
10	adipokine signaling pathway	36.161

(Statistically significant for scores > 20.0)

DMM 8W/12W

Alternative, classical, lectin pathways are activated

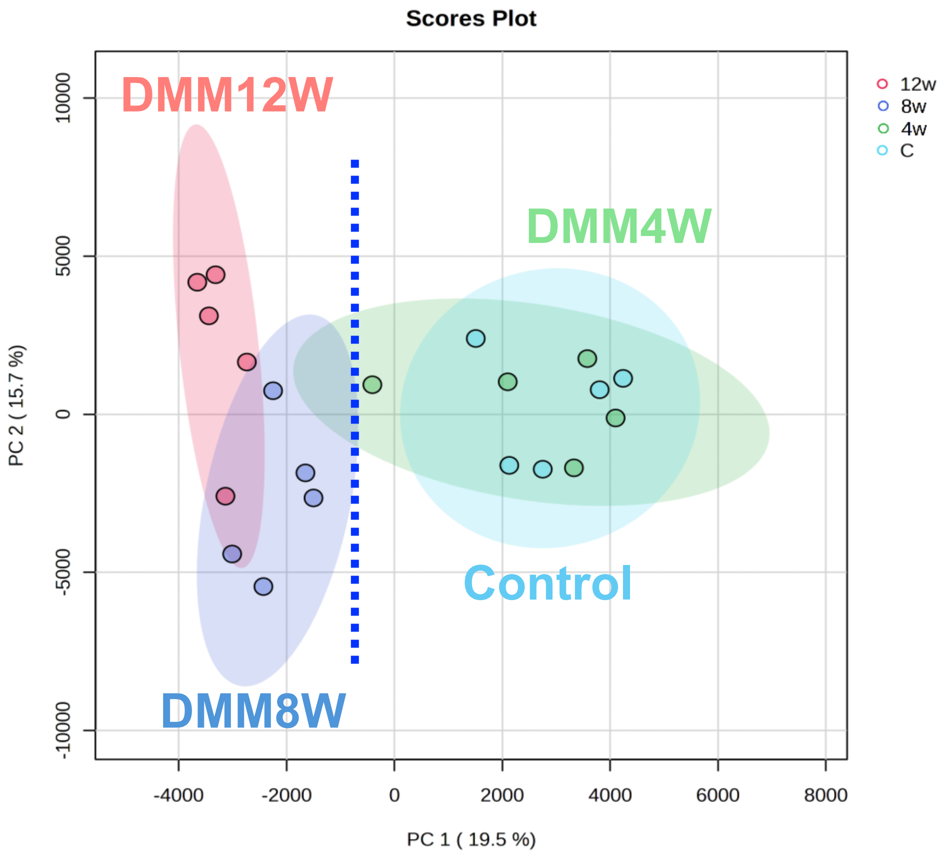
Complement system is usually activated in OA patients

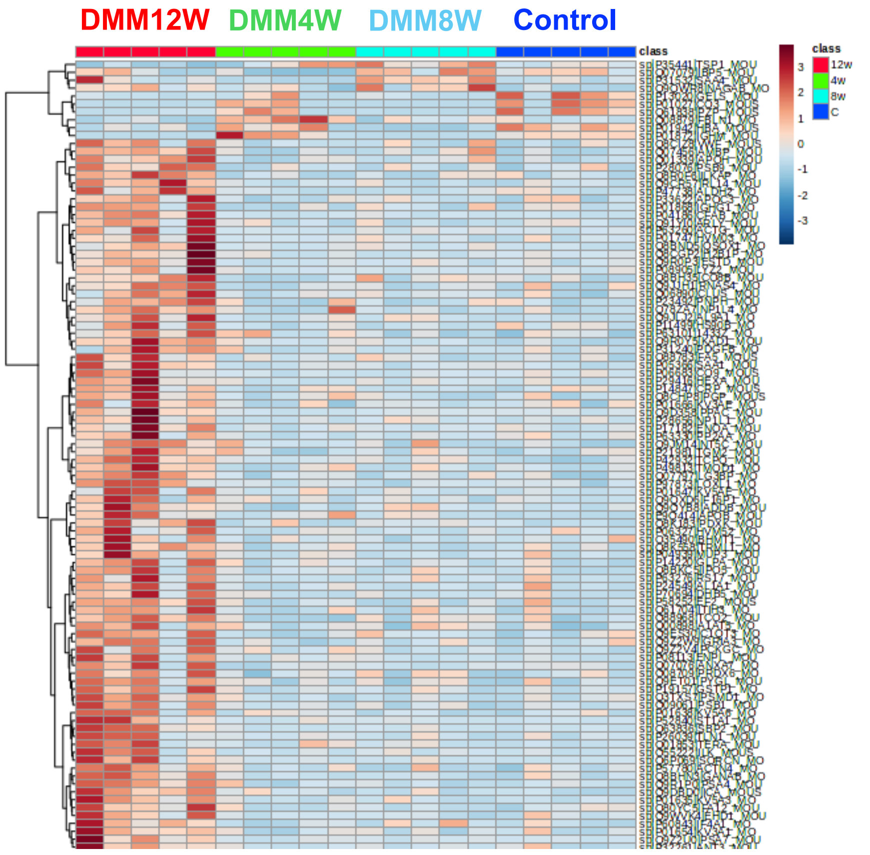
Struglics A et al, Arthritis Res Ther, 2016

DMM4W

Pathways differ from those of over 8 weeks post-DMM

PCA analysis / Heat map





PLS-DA modeling

Marked group separations

between Control / DMM4W

and DMM8W / DMM12W

Heatmap of clustered protein

- **Top 100 differentially expressed proteins**
- **Specific protein upregulation in DMM12W**

EOKA and DMM mice

Human EKOA

Defined as OARSI grade ≤ 3 and OARSI score ≤ 12

Madry H et al, KSSTA, 2012

Mouse EKOA

Beyond **5 weeks** post-DMM mice represents late stage knee OA

Fang H et al, Sci Rep, 2018

How many weeks post-DMM are equivalent to **human EKOA ?**

▼

This study

4-week post-DMM model is **Closest** to **human EKOA**

(Under 5 weeks post-DMM, OARSI grades < 3, OARSI scores < 12)

Detected Proteins and Pathways in DMM4W

KOA-related Proteins

Nestin: Cytoskeletal protein classified as a type IV intermediate filament

Zhen G et al, *Nat Med, 2013*

IGFBP-5: One of the insulin-like growth factor-binding protein

Clemmons DR et al, Arthritis Rheum, 2002

KOA-related Pathways

Wnt signaling: Involved in homeostasis / degradation of cartilage matrix

GSK3 signaling : Control canonical Wnt signaling pathway

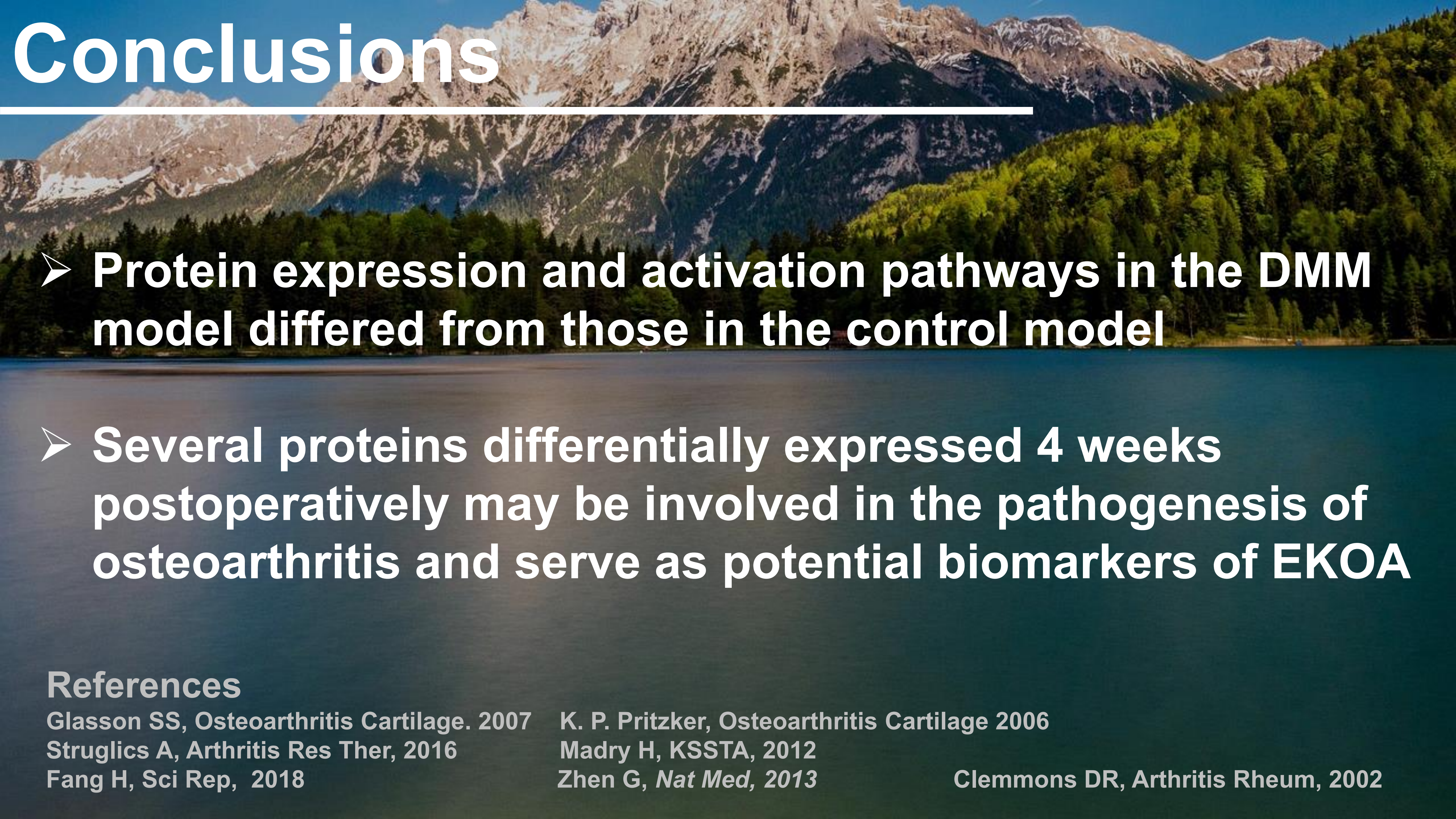
MAPK signaling: Induce of MMP-13 activity

Proteins may **serve** as potential **EKOA biomarkers**

and

Pathways may **contribute** to **EKOA pathogenesis**

Conclusions



- Protein expression and activation pathways in the DMM model differed from those in the control model
- Several proteins differentially expressed 4 weeks postoperatively may be involved in the pathogenesis of osteoarthritis and serve as potential biomarkers of EKOA

References

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