Overcoming arthritis:

Science offers hope for removing achy joints from the aging equation

Martin Lotz, MD
More than 60 million Americans are affected by arthritis.

More than half of individuals with symptomatic knee osteoarthritis are younger than 65.

More than 1 million total joint replacements per year. Expected to increase to 4 million by 2030.
Osteoarthritis

- Disease impact and mechanisms of disease
- Drug target discovery
- FoxO transcription factors for Osteoarthritis therapy
- Mohawk transcription factor for meniscus healing and Osteoarthritis prevention
Osteoarthritis is a Serious Disease*

- Leading cause of pain
- Affects sleep quality, mood, and participation in every-day life
- 3rd most rapidly rising condition associated with disability, just being diabetes and dementia
- Limits person’s ability to self-manage other conditions such as hypertension or diabetes
- Increases risk for developing heart disease by 50%
- Reduced levels of physical activity, comorbid conditions and adverse effects of medications lead to a 55% increase in all cause mortality

*FDA 2018
Osteoarthritis affects all joint tissues

Normal 24-year-old

OA 64-year-old

Joint cartilage and biomechanical function

Cartilage cells:
- Chondrocytes
- Progenitor cells

No nerve fibers
No blood vessels

Collagen framework with proteoglycans
- Collagen type II
- Structure
- Tensile strength

Aggrecan
- Core protein
- Glycosaminoglycan side chains
- Water binding: swelling pressure

Superficial zone
Mid
Deep
Calcified
Pathways of cartilage destruction

Clinical Risk Factors
- Biochemical Stimuli
- Mechanical Stress
- Aging

Chondrocyte
- Activation
- Differentiation
- Senescence
- Death

Mediators of extracellular matrix destruction and inflammation
Osteoarthritis treatment

**Disease-modifying OA Drugs: Clinical Trial Failures**
- MMP inhibitors
- Risedronate
- Doxycyclin
- IL-1RA
- Glucosamine/
- Chondroitin
- Vitamin D3
- iNOS inhibitor
- Strontium
- Senolytic
- ADAMTS5 inhibitor

**Treatment guidelines 1985**
- NSAIDs
- Acetaminophen
- IA steroids
- Glucosamine
- Chondroitin sulfate

**Treatment guidelines 2021**
- NSAIDs
- COX2 inhibitors
- Acetaminophen
- IA steroids
- Opioids
- Self-management programs
Potential reasons for failures of DMOAD clinical trials

Disease-modifying OA drugs (DMOAD) that slow, or halt radiographic structural disease progression are not available

- **Clinical trials design**
  - Patient heterogeneity
  - Advanced stages of disease: calcification, amyloid
  - Burden of clinical risk factors (aging, obesity, overuse, injury)

- **Drug targets**
  - Large number of effector molecules in tissue destruction
  - Spectrum of inflammatory mediators
  - Diverse disease pathways

- Need for better understanding of disease mechanisms and identification key drivers of tissue damage and pain

- Better drug targets
Osteoarthritis

- Disease impact and mechanisms of disease
- **Drug target discovery using Omics analyses**
- FoxO transcription factors for Osteoarthritis therapy
- Mohawk transcription factor for meniscus healing and Osteoarthritis prevention
Joint Omics Analysis

Objectives

Characterize the transcriptomic and epigenomic landscapes of normal and OA articular cartilage and meniscus to identify genes and pathways that are central to OA pathophysiology and prioritize potential therapeutic targets.
Focus on Transcription Factors

Rationale

Transcription factors are critical determinants of tissue identity by regulating expression of tissue specific genes.

Dysregulated transcription factors control expression of disease promoting genes and are promising therapeutic targets.
RNA-sequencing analysis of normal vs. Osteoarthritis human cartilage

- 18 normal and 20 OA samples
- 12,467 genes considered expressed
- 1332 Differentially expressed genes
- 630 upregulated, 702 downregulated in OA

Fisch et al. Osteoarthritis Cartilage. 2018
Dysregulated transcription factors in Osteoarthritis

- 12,467 cartilage expressed genes
- 1332 Differentially expressed genes
- 93 transcription factors are differentially expressed in OA
  - 73/93 suppressed

Transcription factors targeting differentially expressed genes

Fisch et al. Osteoarthritis Cart. 2018
FoxO suppression in pathogenic cell subsets

Hannah Swahn in preparation
Summary of RNA-seq study

- OA cartilage has high expression of genes involved in ECM remodeling and reduced expression of genes involved in tissue homeostasis (HIF-1, FoxO, circadian rhythm).

- Deficiency of FoxO transcription factors is a mechanism for disease-promoting gene expression patterns in OA cartilage.
Osteoarthritis

- Disease impact and mechanisms of disease
- Drug target discovery using Omics analyses
- **FoxO transcription factors for Osteoarthritis therapy**
- Mohawk transcription factor for meniscus healing and Osteoarthritis prevention
Forkhead box O (FoxO) transcription factors and autophagy

FoxO play a crucial role in regulation of oxidative stress resistance to promote longevity and reduce age-related disease.
FoxO expression in osteoarthritis mouse joints

A.

4 months

12 months

24 months

Surgical OA

B.

% positive cells

FoxO1

FoxO3

% positive cells

4 months 12 months 24 months Surgical OA

FoxO1

p-FoxO1 cytoplasmic

FoxO3 p-FoxO3 cytoplasmic

4 months 12 months 24 months Surgical OA

Spontaneous cartilage degradation in Col2CreFoxO triple KO and Col2CreFoxO1 KO mice

Matsuzaki et al. Sci Transl Med. 2018
Postnatal FoxO deletion

Control
- FoxO1/3/4\textsuperscript{Lox/lox} ; Aggrecan-Cre\textsuperscript{ERT-}

Triple KO
- FoxO1/3/4\textsuperscript{Lox/lox} ; Aggrecan-Cre\textsuperscript{ERT+}

Intraperitoneal Tamoxifen injection
Day0 1 2 3 4

RNA collection
2W 3W

Spontaneous OA model
2M 5M

Treadmill: 6W
DMM surgery: 4,8W

4 months of age

Spontaneous OA model
Spontaneous OA in Aggrecan-Cre-ERT FoxO TKO mice

Matsuzaki et al. Sci Transl Med. 2018
Mechanisms of cartilage damage in FoxO KO mice: Autophagy and oxidant defense

Cartilage RNA expression

Autophagy

Antioxidant

Matsuzaki et al. Sci Transl Med. 2018
Mechanisms of cartilage damage in FoxO KO mice: FoxO1 regulates *Prg4* expression

FoxO-deficient mice

**1-month-old**

- Control
- Triple KO
- FoxO1 KO
- FoxO3 KO
- FoxO4 KO

**2-month-old**

- Control
- Triple KO
- FoxO1 KO

Overexpression of FoxO1

- Human Chondrocytes
- SW1353
- Mouse IMACs

**PRG4 Log2 fold change**
(normalized to Ad-GFP)

Matsuzaki et al. Sci Transl Med. 2018
Spontaneous intervertebral disc degeneration in mice with conditional deletion of FOXO

Alvarez-Garcia et al. Aging Cell 2018
Summary

- FoxO1 and FoxO3 are suppressed in OA cartilage, meniscus and degenerated intervertebral disc.
- FoxO-deficient mice develop spontaneous cartilage degradation.
- Autophagy and antioxidant genes in cartilage cells are controlled by FoxOs.
- FoxO1 enhances PRG4 gene expression.
- Ad-FoxO1 normalizes gene expression in OA chondrocytes.
- FoxO1 and FoxO3 deficient mice spontaneously develop meniscus and intervertebral disc degeneration.

FoxO are potential therapeutic targets for OA and intervertebral disc degeneration.
Drug screening for FoxO1

FoxO1 promoter constructs

ReFRAME Library of ~12,000 compounds for drug repositioning

Created by CALIBR at Scripps Research
Janes at al. PNAS 2018
Drug screening for FoxO1

ReFrame library screening
  Hits n=113

Compound selection n=18

In Vitro testing
  Chondrocytes
  Meniscus cells
  Synoviocytes

Lead compound selection:
  Panobinostat

In Vivo testing
  OA animal model
Panobinostat

- Histone deacetylase (HDAC) inhibitor
- HDACs have important functions in transcription regulation and in protein modification
- Pan HDAC inhibitor with potent activity against Class 2a HDACs
- More favorable in vitro profile than 3 other pan HDAC inhibitors
Panobinostat in human OA chondrocytes

IL6

MMP13

PTGES2

iNOS

Injection in mouse knee joints

Ohzono et al. under review
Panobinostat reduced joint damage and pain in mouse OA model

Cartilage damage

Pain behavior

Red 1g filament
Blue 5g filament
Summary

- HDAC inhibitors were among the top hits in high throughput drug screening of ReFRAME library with FoxO1 promoter reporter.

- Panobinostat was the most promising compound in testing of candidates in human chondrocytes, meniscus and synovial cells.

- In an animal model of experimental OA, Panobinostat reduced the severity of histological changes in cartilage, synovium and subchondral bone and improved pain behaviors.

  - Panobinostat has a clinically relevant activity profile and is a candidate for OA symptom and structure modification.
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Meniscus destruction and OA

- Menisci are essential to the biomechanical function of the knee joint
- Acute meniscus injury can lead to Post-traumatic osteoarthritis (PTOA)
- Aging-associated degenerative meniscus tears are highly prevalent and promote OA progression
Targeting meniscus destruction and OA

Discover key regulators of meniscus cell identity and normal function by comparing transcription factors that are enriched in meniscus compared to other human tissues.

Test candidate in cell and tissue models of meniscus injury.

Test candidate in animal model of meniscus injury and osteoarthritis.
Mohawk (MKX) is the most meniscus-enriched transcription factor.
Mohawk plus TGF-β3 induces stem cell differentiation to a meniscus cell.
Mohawk suppresses genes that promote meniscus damage
Ad-MKX promotes meniscus repair and suppresses genes associated with meniscus calcification and ECM degradation in human explants

Lee. Sci Transl Med. 2020
Ad-MKX injection into mouse knees protects against meniscus and cartilage damage induced by surgical meniscus destabilization.
Summary

- MKX is the transcription factor that is most enriched in meniscus.

- MKX induces differentiation of mesenchymal stem cells to a meniscus cell phenotype.

- In menisci from human OA joints, Ad-MKX promotes meniscus repair and suppresses genes associated with meniscus calcification and ECM degradation.

- In a mouse model of meniscus injury, intraarticular Ad-MKX injection leads to healing of experimental meniscus tear and reduces cartilage damage.

- MKX gene delivery is a promising approach to prevent post-traumatic OA and slow progression of aging-related OA.
Ongoing studies

Develop local therapies for injections into joints and intervertebral discs

Sustained release formulations for small molecules: PLA/PLGA Microspheres

Day 1  Week 26

AAV-mediated gene delivery

AAV-GFP
Rat
Intervertebral disc

AAV-GFP
Mouse knee
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Transforming the face of research:
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Julia Moore Vogel, MBA, PhD
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All of Us Research Program
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