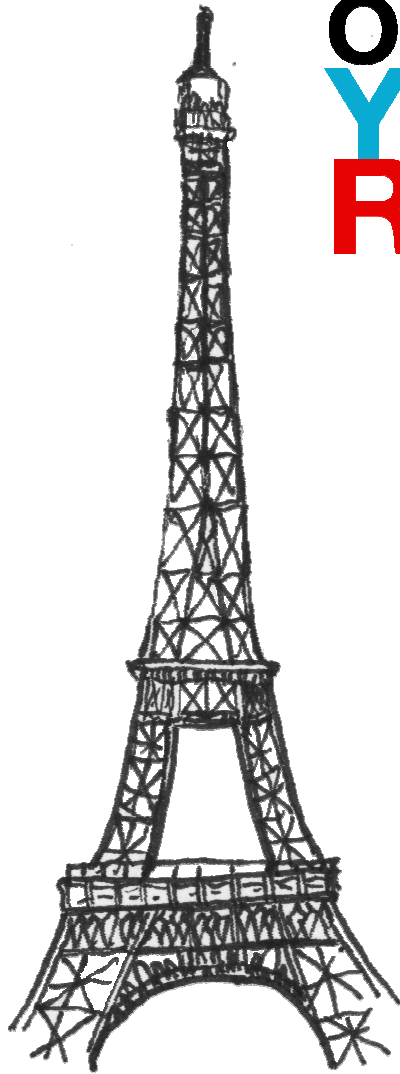
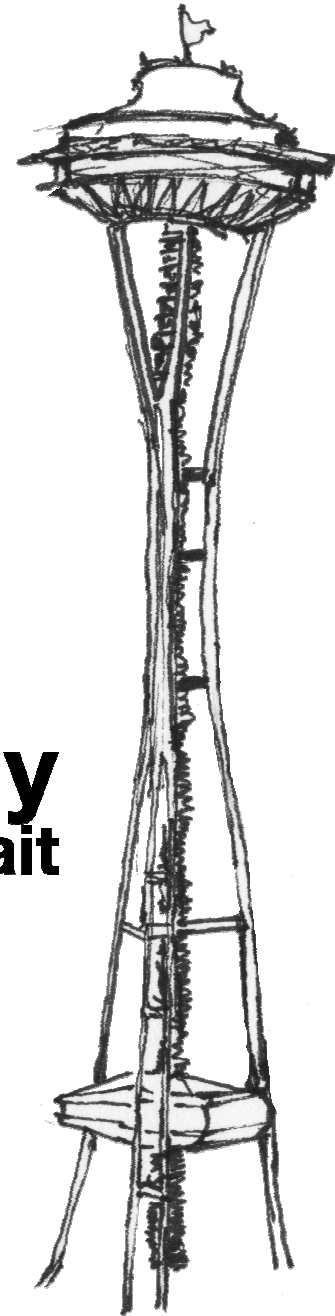


**OARSI**  
**YEAR IN**  
**REVIEW**



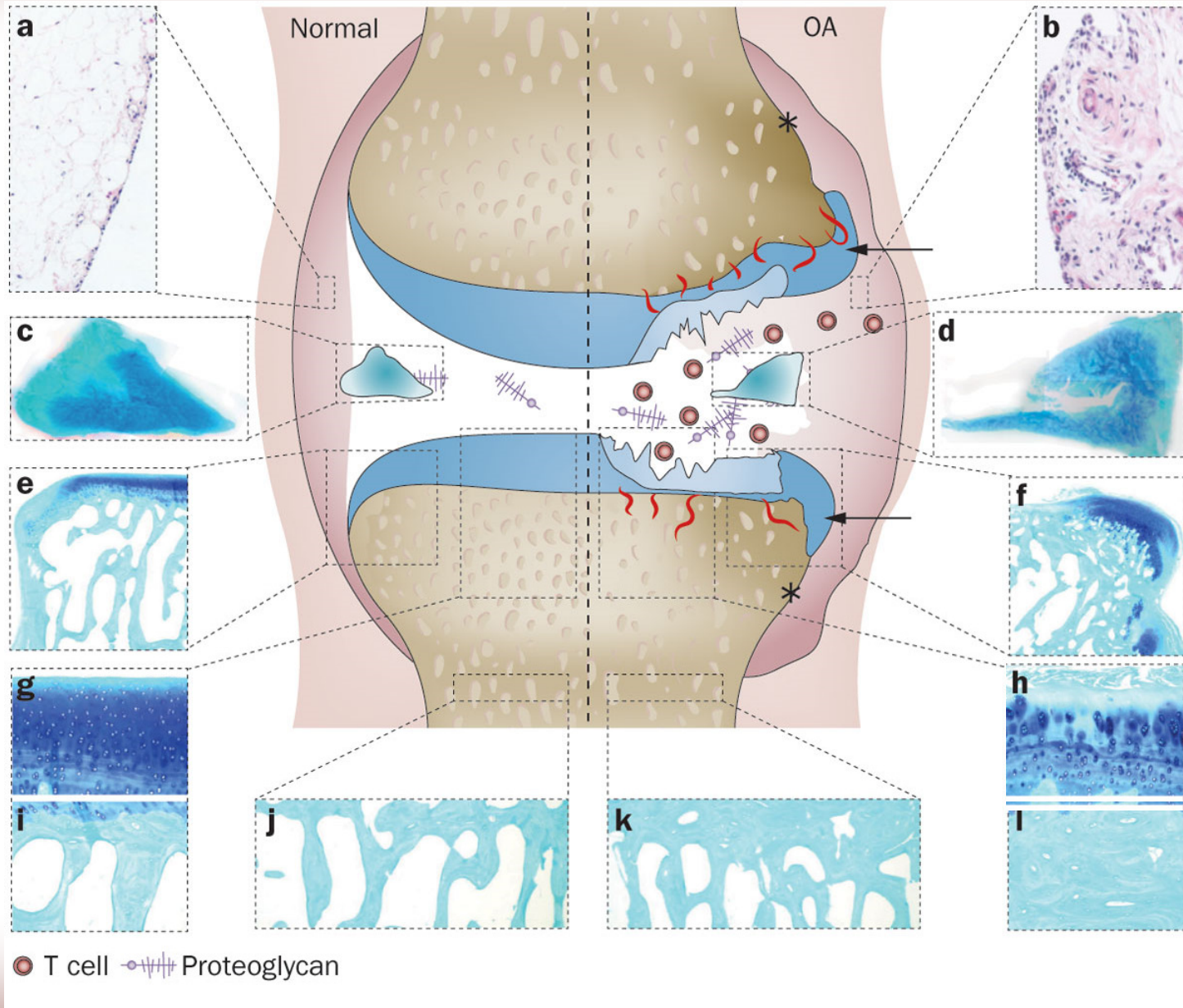
**April 2014**

**OA Biology**  
**Anne-Marie Malfait**



**April 2015**

**Osteoarthritis is a global disease with pathological changes occurring in all the joint tissues  
 What is the relative contribution at different stages? How do the tissues interact?**



## Chondrocytes in Osteoarthritis Undergo Complex Phenotypic Changes (Reprogramming)

Transcriptome-wide analysis of messenger RNA decay in normal and osteoarthritic human articular chondrocytes.

Tew SR, McDermott BT, Fentem RB, Peffers MJ, Clegg PD.  
Arthritis Rheumatol. 2014 Nov;66(11):3052-61.

### Findings:

- Majority of chondrocyte-expressed transcripts were stable, but a subset exhibited a rapid decay
- Strong bias toward shortening of mRNA half-life in OA chondrocytes.
- Among those short-lived transcripts: Genes involved in transcriptional regulation & the regulation of programmed cell death.
- First transcriptome-wide study on mRNA decay in cartilage – adds to our understanding of gene expression in OA

## Chondrocytes in Osteoarthritis Undergo Complex Phenotypic Changes (Reprogramming)

A homeostatic function of CXCR2 signalling in articular cartilage.

Sherwood J, Bertrand J, Nalesso G, Poulet B, Pitsillides A, Brandolini L, Karystinou A, De Bari C, Luyten FP, Pitzalis C, Pap T, Dell'Accio F.

Ann Rheum Dis. 2014 Aug 18. pii: annrheumdis-2014-205546.

This study uncovered an unexpected homeostatic role for CXCR2 signaling in articular cartilage:

- CXCL6 was present in territorial matrix of healthy human cartilage, but not in early OA
- Disruption in CXCR2 signaling resulted in:
  - A) *In vitro* : decreased ECM production
  - B) *In vivo*: increased susceptibility to OA after DMM

### Significance:

- Suggests that the loss of CXCL6 during cartilage breakdown contributes to the loss of chondrocyte phenotypic stability.

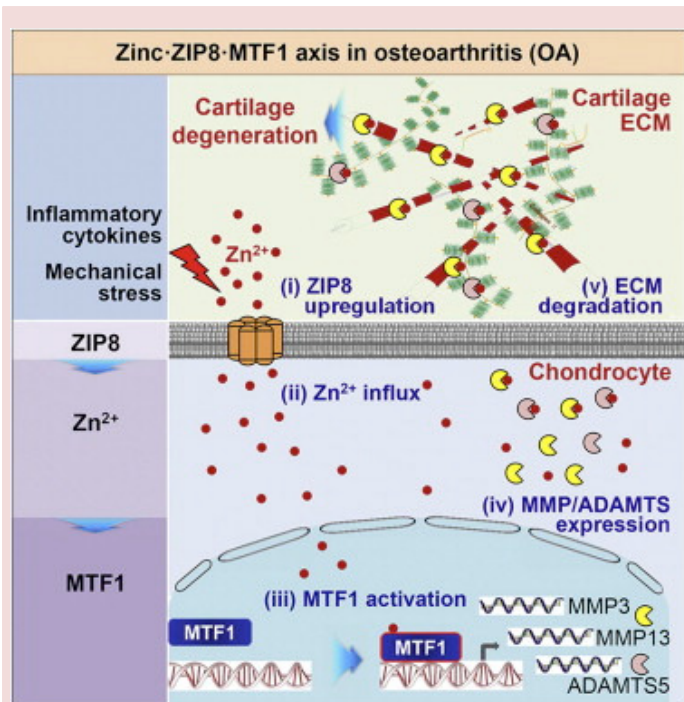
# Novel pathway that promotes the pro-catabolic phenotype

Regulation of the catabolic cascade in osteoarthritis by the zinc-ZIP8-MTF1 axis.

Kim JH, Jeon J, Shin M, Won Y, Lee M, Kwak JS, Lee G, Rhee J, Ryu JH, Chun CH, Chun JS.

Cell. 2014 Feb 13;156(4):730-43.

(Commentary by V. Byers Kraus, in News and Views, Nature March 2014)



Local interference with the zinc cascade may provide a therapeutic approach for OA

Chondrocyte-specific *Zip8* overexpression in mice (Col2a1 promoter).

By 12 months: accelerated cartilage damage + SCB sclerosis in the absence of overt synovitis.

Substantiates the idea that cartilage damage can drive changes in other joint tissues (SCB)

## Damaged cartilage as a source of DAMPs

Bioactivity in an aggrecan 32mer fragment is mediated via Toll-like receptor 2.

Fosang AJ, Lees S, Golub SB, Last K, Zeng W, Jackson DC, Sutton P.

Arthritis Rheumatol. 2015 Feb 23. doi: 10.1002/art.39063. [Epub ahead of print]

**Aim:** To determine whether an aggrecan 32-mer fragment derived from dual ADAMTS and MMP cleavage in the aggrecan interglobular domain is bioactive

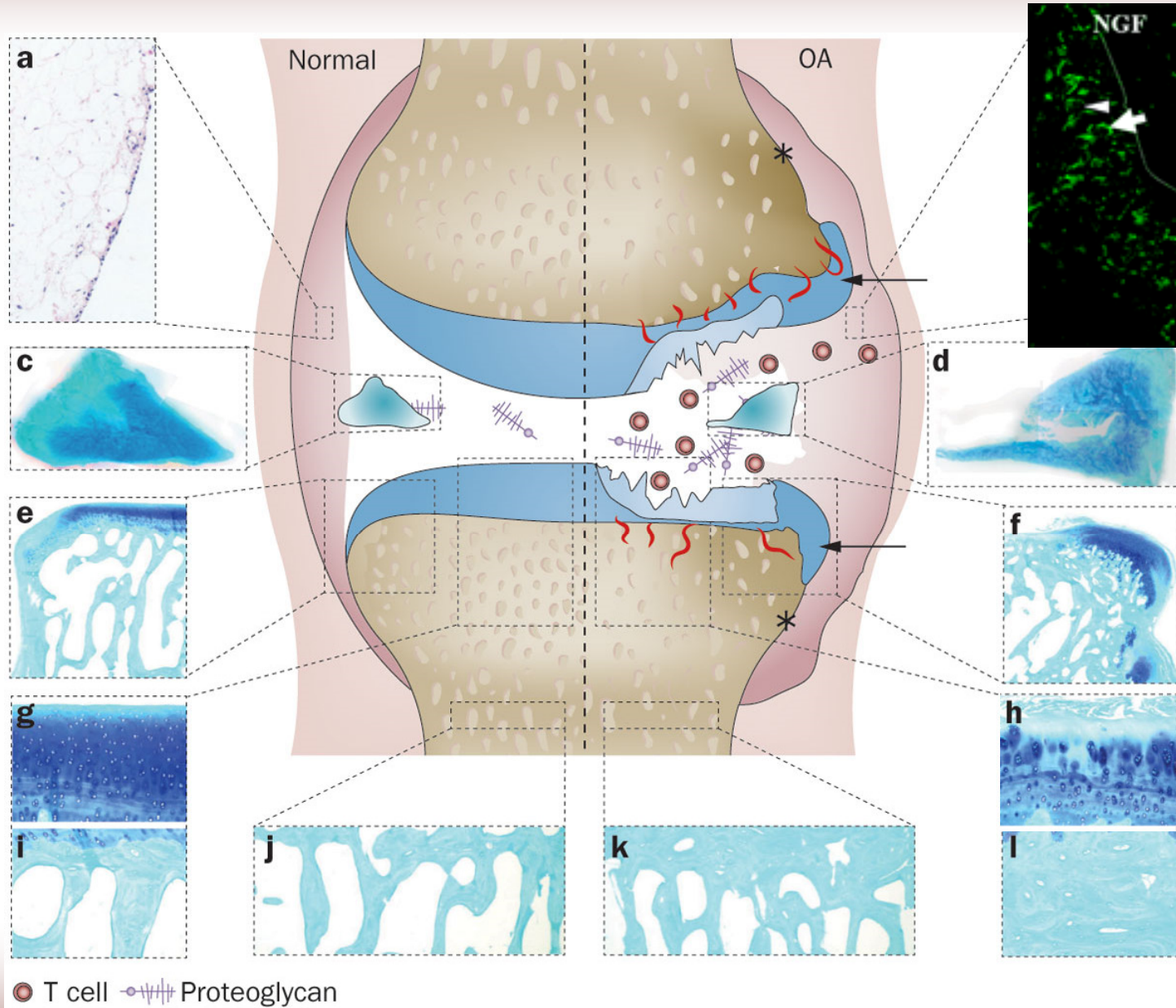
### Results:

- 32-mer had anti-anabolic, pro-catabolic and pro-inflammatory bioactivity *in vitro* on chondrocytes, synovial fibroblasts, and macrophages
- The effect is mediated through TLR2

### Significance:

- First demonstration of a TLR ligand derived from one of the major cartilage macromolecules.
- In vivo?

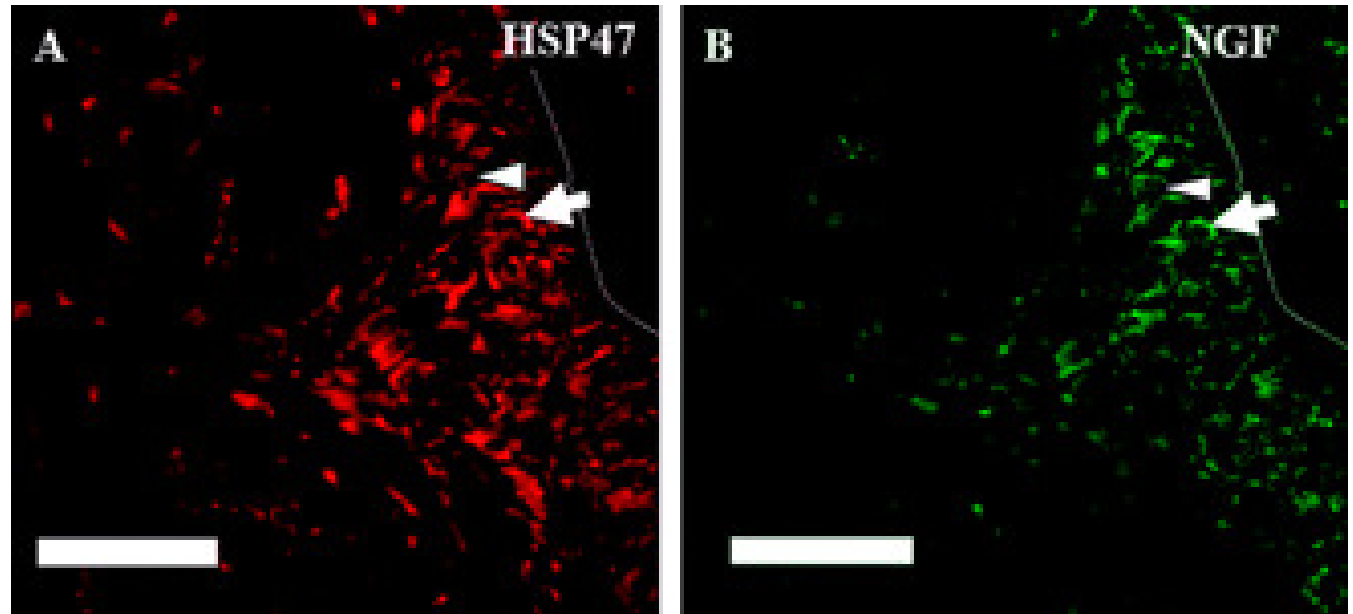
**Osteoarthritis is a global disease with pathological changes occurring in all the joint tissues  
 What is the relative contribution at different stages? How do the tissues interact?**



Structural Associations of Symptomatic Knee Osteoarthritis  
Stoppiello LA, Mapp PI, Wilson D, Hill R, Scammell BE, Walsh, DA  
Arthritis & Rheumatology 66 (11), p. 3018

HUMAN OA SYNOVIUM

NGF mostly  
co-localized with synovial  
fibroblasts



Histopathological features associated with symptomatic knee OA:

1. Synovitis
2. Synovial area positive for NGF staining



## Chondrocytes/cartilage as a source of Nerve Growth Factor (NGF). Which stimuli induce it?

1. Induction of nerve growth factor expression and release by mechanical and inflammatory stimuli in chondrocytes: possible involvement in osteoarthritis pain.

Pecchi E, Priam S, Gosset M, Pigenet A, Sudre L, Laiguillon MC, Berenbaum F, Houard X  
Arthritis Res Ther. 2014; 16 R16

murine and human chondrocytes  
stimuli: IL-1 $\beta$  and visfatin

2. TGF- $\beta$  is a potent inducer of Nerve Growth Factor in articular cartilage via the ALK5-Smad2/3 pathway. Potential role in OA related pain?

Blaney Davidson EN, van Caam AP, Vitters EL, Bennink MB, Thijssen E, van den Berg WB, Koenders MI, van Lent PL, van de Loo FA, van der Kraan PM.  
Osteoarthritis Cartilage. 2015 Mar;23(3):478-86.

bovine and human chondrocytes/explants  
TGF $\beta$ 1 is a potent inducer of NGF mRNA, in a ALK5/Smad2/3-dependent manner

Long-term analgesic effect of a single dose of anti-NGF antibody on pain during motion without notable suppression of joint edema and lesion in a rat model of osteoarthritis.

Ishikawa G, Koya Y, Tanaka H, Nagakura Y.

Osteoarthritis Cartilage. 2015 Feb 9. pii: S1063-4584(15)00043-6.

A single dose of anti-NGF antibody exerts a long-lasting analgesic effect on pain during motion (gait) in a rat MIA model (day 3-Day 35); no effect on edema or macroscopic lesions

## Synovium and Disease progression

Treatment efficacy of adipose-derived stem cells in experimental osteoarthritis is driven by high synovial activation and reflected by S100A8/A9 serum levels.

Schelbergen RF, van Dalen S, ter Huurne M, Roth J, Vogl T, Noël D, Jorgensen C, van den Berg WB, van de Loo FA, Blom AB, van Lent PL.

Osteoarthritis Cartilage. 2014 Aug;22(8):1158-66. doi

### Findings:

- IA adipose-derived stem cells were efficacious in a model with high synovial activation (collagenase-induced) but not in a model with low levels of synovitis (DMM).
- Efficacy was associated with suppression of synovial activation and suppression of S100A8 & S100A9 in joint and in serum.

Dispensable role of myeloid differentiation primary response gene 88 (MyD88) and MyD88-dependent toll-like receptors (TLRs) in a murine model of osteoarthritis.

Nasi S, Ea HK, Chobaz V, van Lent P, Lioté F, So A, Busso N.

Joint Bone Spine. 2014 Jul;81(4):320-4.

**Findings:** TLR-1, TLR-2 , TLR-4, TLR6 , or MyD88 ko mice were not protected in a surgically induced OA model (MNX -females).

**Significance:** Alarmins/TLRs may contribute to driving OA progression in subsets with high degree of inflammation/synovitis. This may have therapeutic implications.

## Synovium and Disease progression

Depletion of protease-activated receptor 2 but not protease-activated receptor 1 may confer protection against osteoarthritis in mice through extracartilaginous mechanisms. Jackson MT, Moradi B, Zaki S, Smith MM, McCracken S, Smith SM, Jackson CJ, Little CB. Arthritis Rheumatol. 2014 Dec;66(12):3337-48

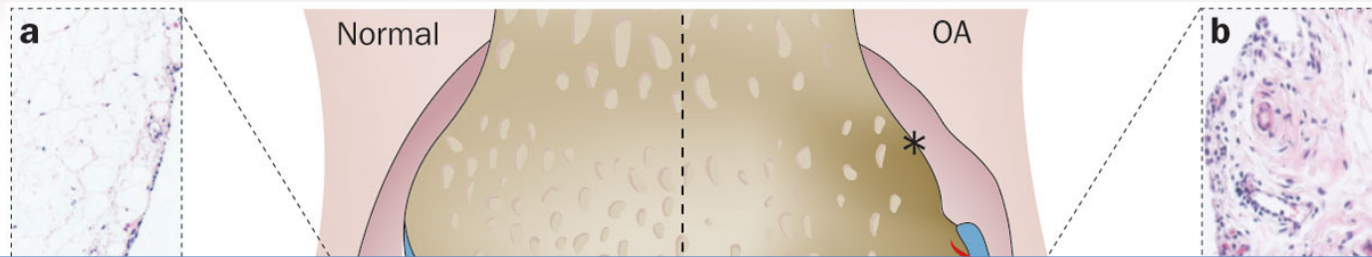
### Findings:

- PAR-2 ablation had a chondroprotective effect in DMM model (8 weeks)
- The primary protective effects of PAR2 ablation occur via modulation of subchondral bone remodeling and synovial macrophage activation

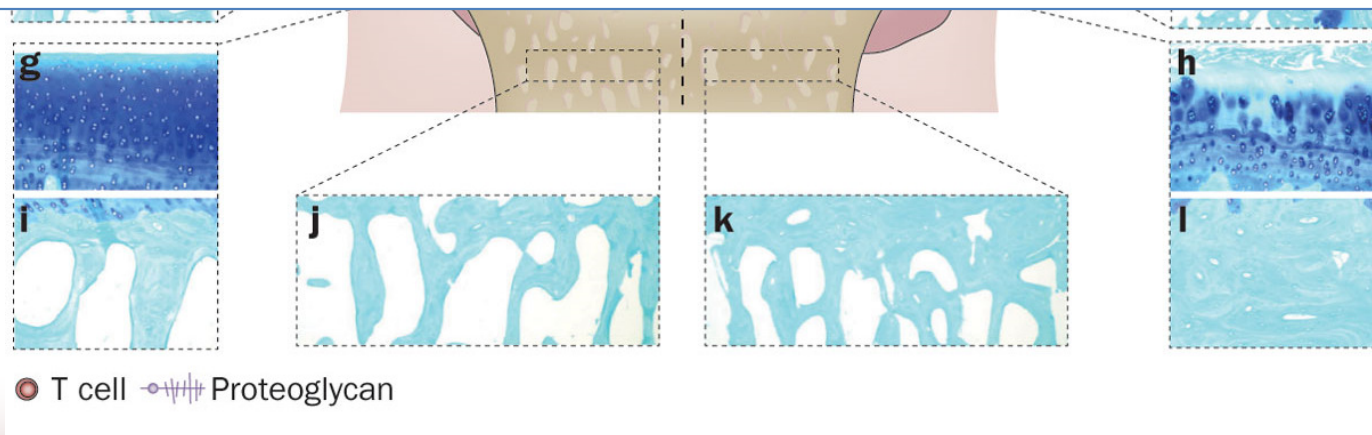
### Significance:

- There is synovitis in the DMM model, and it may contribute to driving disease.

Osteoarthritis is a global disease with pathological changes occurring in all the joint tissues  
What is the relative contribution at different stages? How do the tissues interact?



**Subsets of Osteoarthritis**  
**Can be driven by distinct risk factors**  
**e.g. obesity, age, PTOA**  
**Specific disease mechanisms?**



## Increased focus on mechanisms that link obesity and OA

Not just mechanical factors related to weight

But focus on inflammatory and metabolic factors associated with obesity.

Life-long caloric restriction does not alter the severity of age-related osteoarthritis.

McNeill JN, Wu CL, Rabey KN, Schmitt D, Guilak F.

Age (Dordr). 2014;36(4):9669

C57BL/NIA mice were fed either a calorie-restricted (CR) or an *ad libitum* (AL) diet (14 weeks- 24 months).

### Findings:

- Although AL mice were heavier than CR mice, there was no difference in Mankin score or synovitis

**Significance:** Dietary composition may be important

Dietary fatty acid content regulates wound repair and the pathogenesis of osteoarthritis following joint injury.

Wu CL, Jain D, McNeill JN, Little D, Anderson JA, Huebner JL, Kraus VB, Rodriguiz RM, Wetsel WC, Guilak F. Ann Rheum Dis. 2014 Jul 10. doi: 10.1136/annrheumdis-2014-205601 (ePub)

The ratio of  $\omega$ -6 to  $\omega$ -3 polyunsaturated fatty acids (PUFAs) is considered one of the most important dietary mediators of inflammation.

Mice were fed a HFD – DMM at 16 weeks:

- HFD led to obesity
- But when enriched in  $\omega$ -3 PUFAs, OA was much milder, with less synovitis
- SFA and  $\omega$ 6- PUFA independently acted as a detrimental factor in OA following DMM
- Injury-induced OA was associated with dietary content and serum levels of inflammatory adipokines but not with body weight

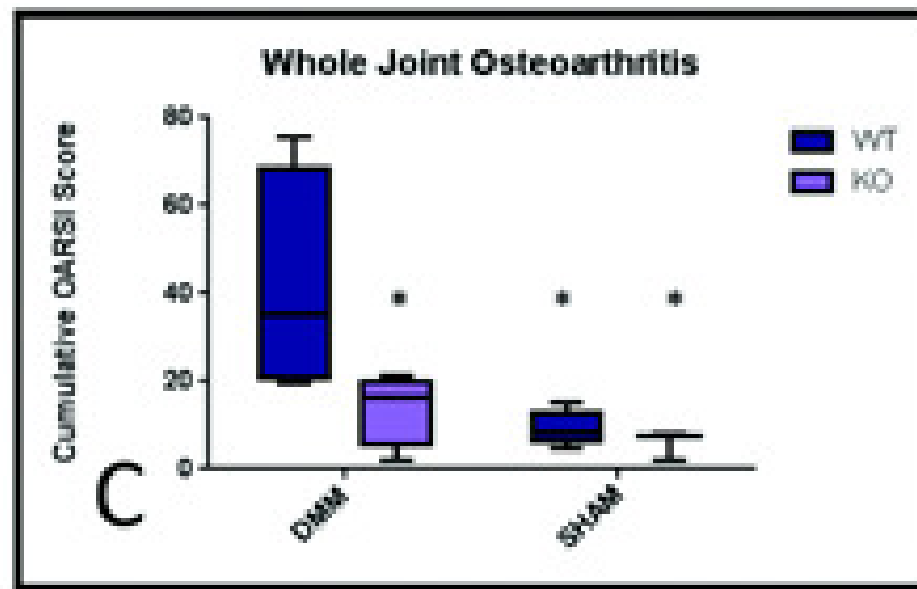
**Significance:** May provide a path toward clinical studies of dietary fatty acids supplements

## Metabolic component of OA

Peroxisome proliferator-activated receptor  $\delta$  promotes the progression of posttraumatic osteoarthritis in a mouse model.

Ratneswaran A, LeBlanc EA, Walser E, Welch I, Mort JS, Borradaile N, Beier F.  
Arthritis Rheumatol. 2015 Feb;67(2):454-64

- PPARs: a family of nuclear receptors activated by lipid ligands
- PPAR $\delta$  activation promotes catabolic processes in chondrocytes & cartilage
- Cartilage-specific (Col II-cre) *Ppard* knockout mice were protected after DMM surgery (cartilage damage)



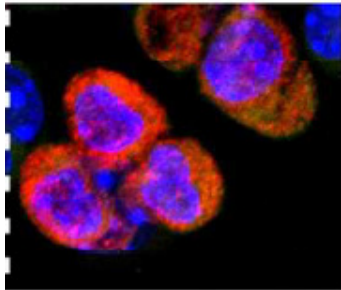
## Aging and Osteoarthritis: Autophagy

The relationship of autophagy defects and cartilage damage during joint aging in a mouse model.

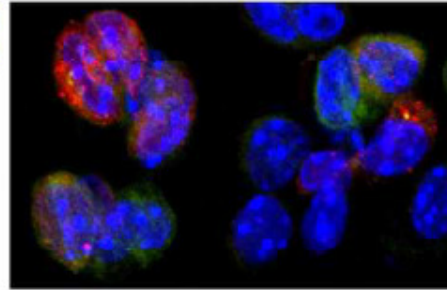
Caramés B, Olmer M, Kiosses WB, Lotz M.

Arthritis Rheumatol. 2015 Feb 23. doi: 10.1002/art.39073.

Young (6 mo)



Old (28mo) mouse



- *In vivo* analysis of basal autophagy activation in cartilage, using GFP-LC3 reporter mice
- Reduction in number of autophagic vesicles in chondrocytes with age
- This preceded cartilage damage

**Significance:** Suggests that autophagy decreases with age, which contributes to joint damage.

PPAR $\gamma$  deficiency results in severe, accelerated osteoarthritis associated with aberrant mTOR signalling in the articular cartilage.

Vasheghani F, Zhang Y, Li YH, Blati M, Fahmi H, Lussier B, Roughley P, Lagares D, Endisha H, Saffar B, Lajeunesse D, Marshall WK, Rampersaud YR, Mahomed NN, Gandhi R, Pelletier JP, Martel-Pelletier J, Kapoor M.

Ann Rheum Dis. 2015 Mar;74(3):569-78.

- Inducible cartilage-specific PPAR $\gamma$  KO mice, subjected to DMM : accelerated cartilage degradation, chondrocyte apoptosis, with increased mTOR expression and suppression of autophagy markers.
- PPAR $\gamma$ -mTOR double KO rescued phenotype

**Significance:** PPAR $\gamma$  maintains articular cartilage homeostasis, partly through the mTOR pathway

## ***In vitro* studies on aging cartilage and chondrocyte senescence**

AMPK inhibits chondrocyte pro-catabolic responses

AMPK signaling decreases with age

Peroxisome proliferator-activated receptor  $\gamma$  coactivator 1 $\alpha$  and FoxO3A mediate chondroprotection by AMP-activated protein kinase.

Zhao X, Petursson F, Viollet B, Lotz M, Terkeltaub R, Liu-Bryan R.

Arthritis Rheumatol. 2014 Nov;66(11):3073-82.

- The chondroprotective effect of AMPK is at least partly mediated by 2 major downstream targets, PGC-1 and FoxO3A.
- PGC-1 and FoxO3A expression levels decreased in murine aging and OA cartilage (MnX)



## ***In vitro* studies on aging cartilage and chondrocyte senescence**

Aging and oxidative stress reduce the response of human articular chondrocytes to insulin-like growth factor 1 and osteogenic protein 1.

Loeser RF, Gandhi U, Long DL, Yin W, Chubinskaya S.  
Arthritis Rheumatol. 2014 Aug;66(8):2201-9.

- Age-related decline in proteoglycan synthesis stimulated by IGF-1 (24-81yrs).
- Oxidative stress inhibited IGF-1-stimulated Akt phosphorylation and increased phosphorylation of ERK. These effects were greater in cells from older donors

Depletion of SIRT6 causes cellular senescence, DNA damage, and telomere dysfunction in human chondrocytes.

Nagai K, Matsushita T, Matsuzaki T, Takayama K, Matsumoto T, Kuroda R, Kurosaka M.  
Osteoarthritis Cartilage. 2015 Mar 26. pii: S1063-4584(15)00866-3

- The sirtuin, SIRT6, is preferentially expressed in superficial zone chondrocytes (nucleus)
- Depletion of SIRT6 causes cellular senescence, DNA damage, and telomere dysfunction in human chondrocytes

## Post-traumatic OA

Targeting pro-inflammatory cytokines following joint injury: acute intra-articular inhibition of interleukin-1 following knee injury prevents post-traumatic arthritis.

Furman BD, Mangiapani DS, Zeitler E, Bailey KN, Horne PH, Huebner JL, Kraus VB, Guilak F, Olson SA. Arthritis Res Ther. 2014 Jun 25;16(3):R134

Closed intra-articular fracture in the lateral tibial plateau of the mouse knee  
8 week model of PTOA that is inflammation driven.

Two treatment protocols:

- 1) Single IA injection of IL-1RA (anakinra) or sTNFRII (etanercept)
- 2) Systemic administration of IL-1RA or sTNFRII for 4 weeks after fracture

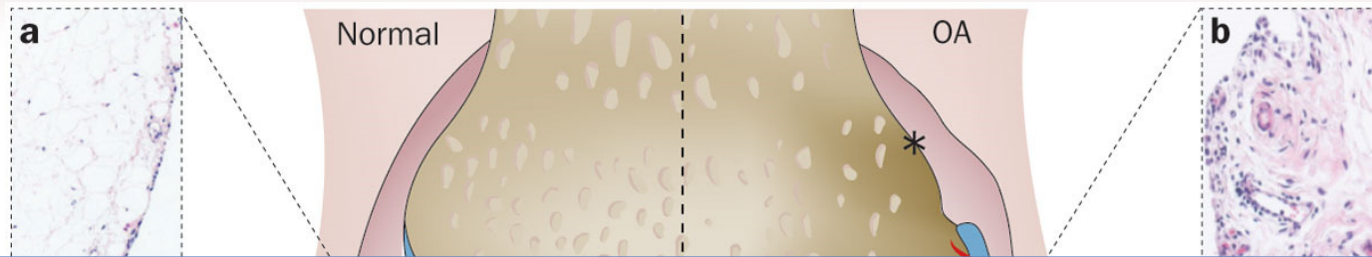
### Findings:

- Intra-articular inhibition of IL-1 significantly reduced cartilage degeneration & synovial inflammation.

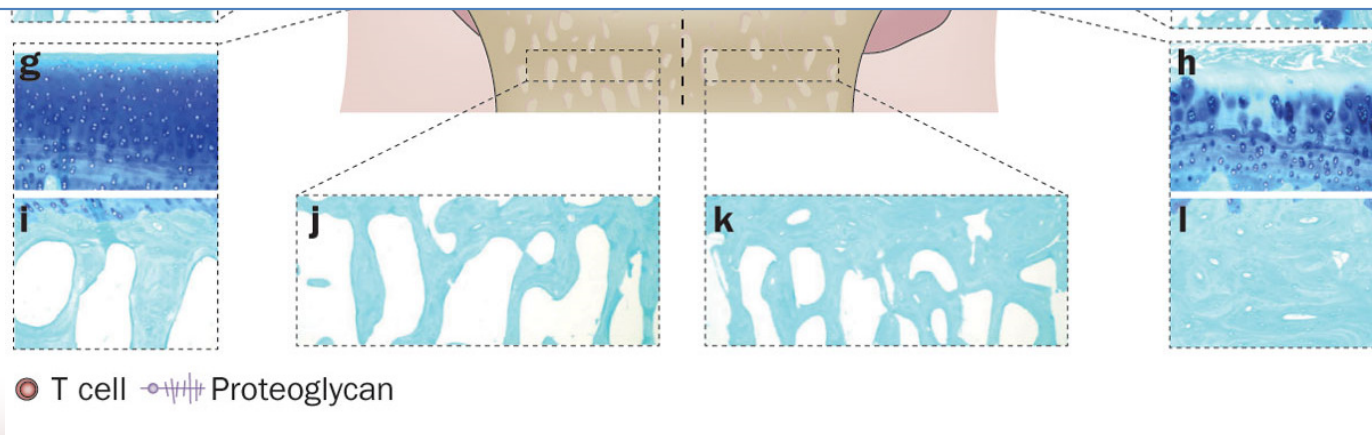
### Significance:

- Supports a role for IL-1, rather than TNF- $\alpha$ , in the acute phase following joint injury
- Acute treatment with local IL1RA can prevent cartilage degeneration and synovitis in PTOA.

Osteoarthritis is a global disease with pathological changes occurring in all the joint tissues  
What is the relative contribution at different stages? How do the tissues interact?



**Subsets of Osteoarthritis**  
Can be driven by distinct risk factors  
e.g. obesity, age, PTOA  
Specific disease mechanisms?



## Targets under exploration for OA pain (in preclinical studies using OA models)

### CGRP

Peripheral calcitonin gene-related peptide receptor activation and mechanical sensitization of the joint in rat models of osteoarthritis pain.

Bullock CM, Wookey P, Bennett A, Mobasheri A, Dickerson I, Kelly S.  
Arthritis Rheumatol. 2014 Aug;66(8):2188-200

Development of a novel antibody to calcitonin gene-related peptide for the treatment of osteoarthritis-related pain.

Benschop RJ, Collins EC, Darling RJ, Allan BW, Leung D, Conner EM, Nelson J, Gaynor B, Xu J, Wang XF, Lynch RA, Li B, McCarty D, Oskins JL, Lin C, Johnson KW, Chambers MG.  
Osteoarthritis Cartilage. 2014 Apr;22(4):578-85. doi

### Na-channels, Nav1.7 and Nav1.8

Osteoarthritis-dependent changes in antinociceptive action of Nav1.7 and Nav1.8 sodium channel blockers: An in vivo electrophysiological study in the rat.

Rahman W, Dickenson AH.  
Neuroscience. 2015 Jun 4;295:103-16. doi: 10.1016/j.neuroscience.2015

### Transient receptor potential cation channel, TRPM2 – sensor for ROS

Involvement of TRPM2 in a wide range of inflammatory and neuropathic pain mouse models.

So K, Haraguchi K, Asakura K, Isami K, Sakimoto S, Shirakawa H, Mori Y, Nakagawa T, Kaneko S.  
J Pharmacol Sci. 2015 Mar;127(3):237-43. doi:



Thank you