Some basic science of RA and OA

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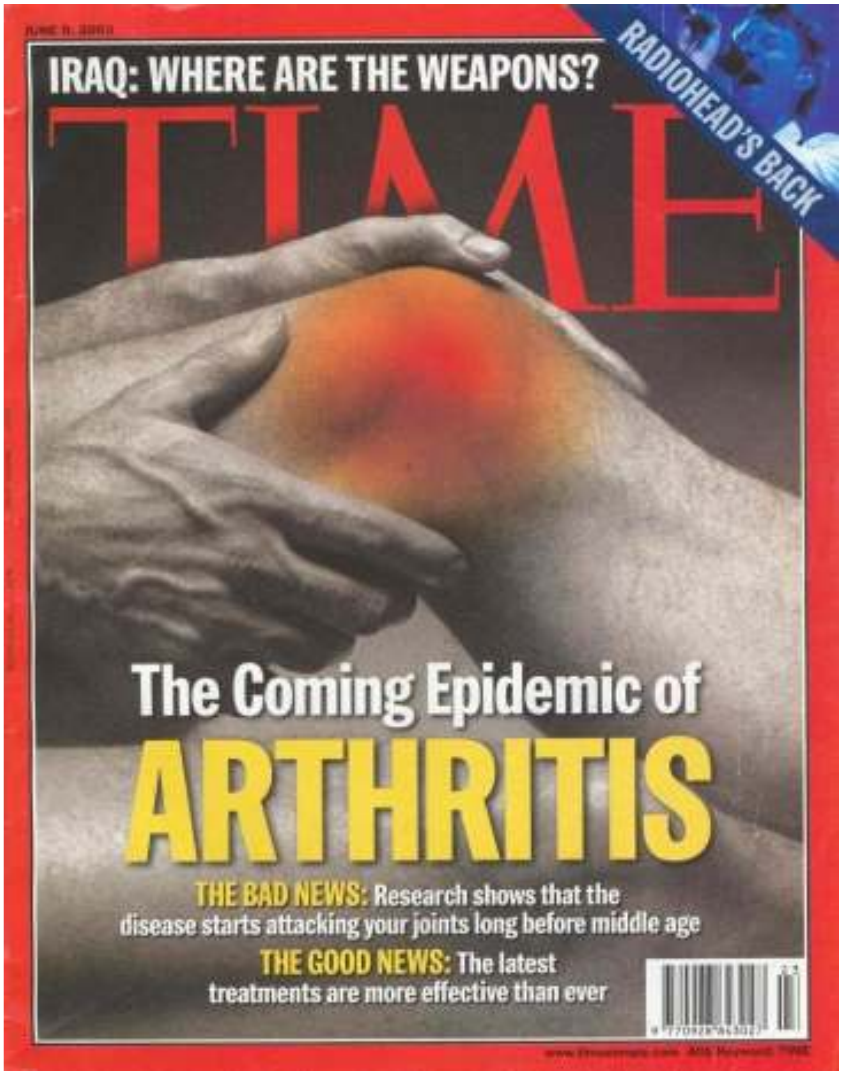
Today's talk

Cartilage

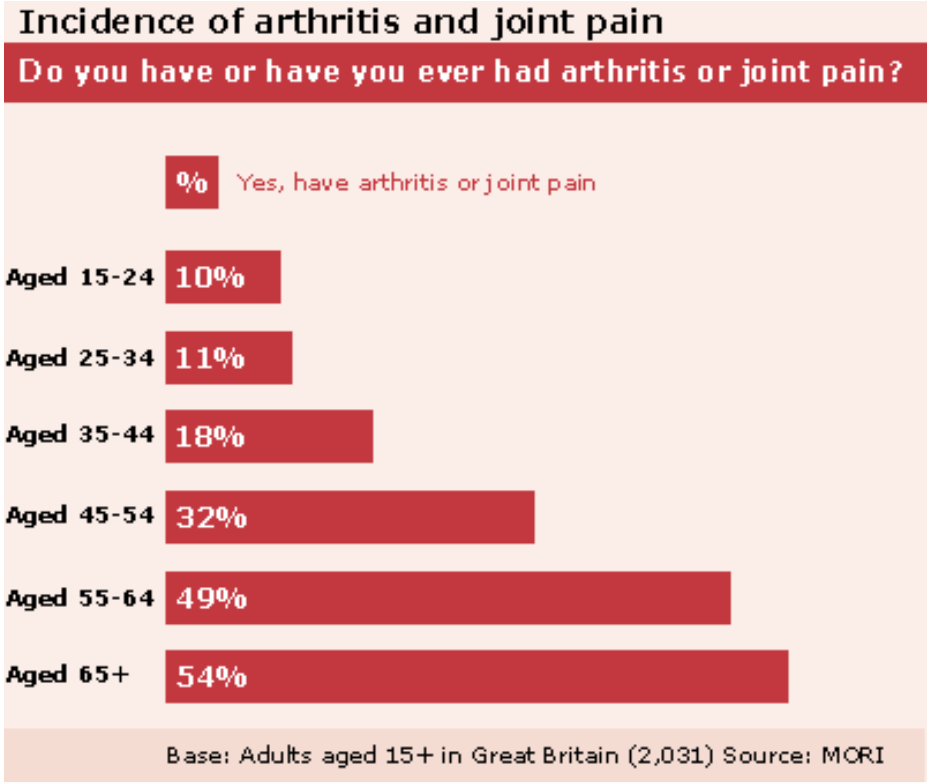
- **What it is and what it does**
- **What goes wrong in disease (RA and OA)**
- **What we know about the process(es)**
- **What the treatment options are**
- **What current research offers**
- **Why tissue is important**

Concepts rather than specifics

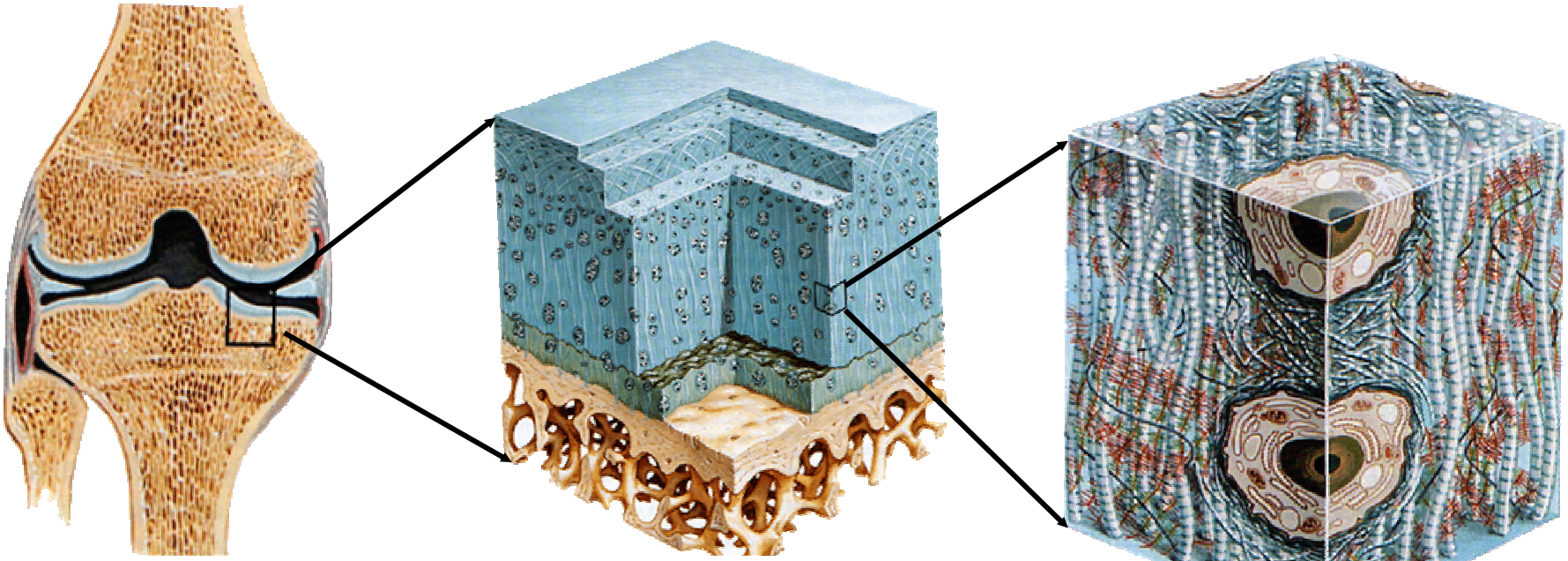
Rheumatoid (RA) and Osteoarthritis (OA)

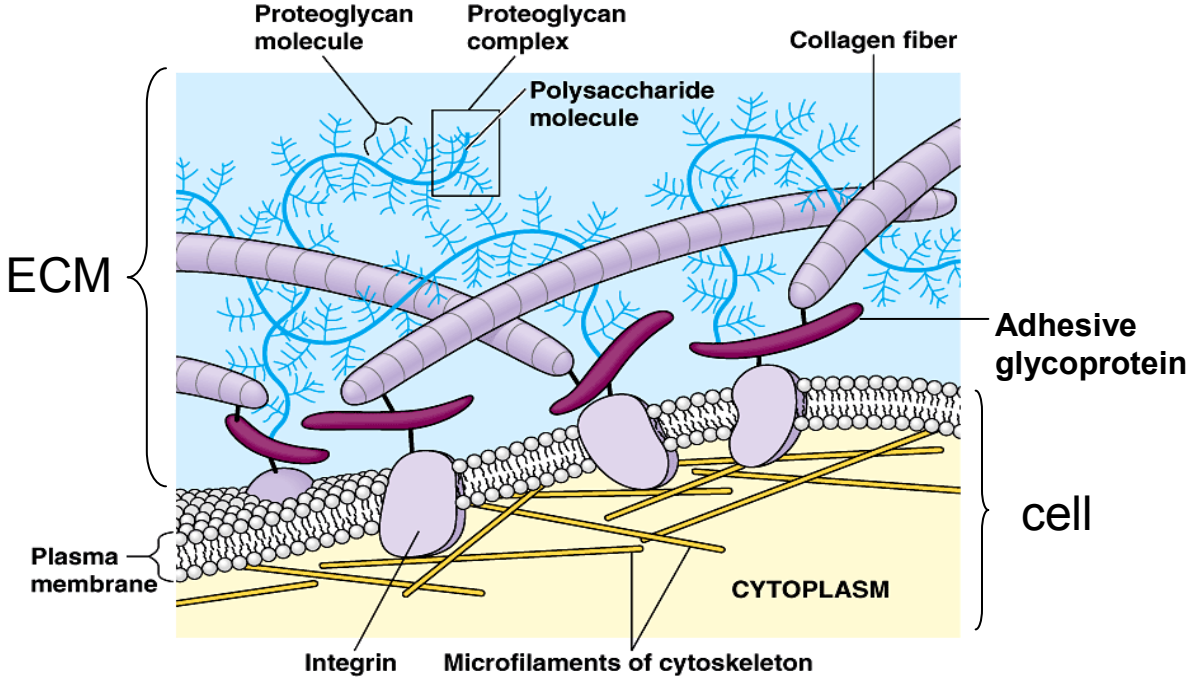


- RA; autoimmune inflammatory disease
- OA; mechanical, 'wear & tear' disease
  - high prevalence (~1% RA, ~12% OA)
  - several risk factors identified
    - gender, age, genetics, obesity
    - smoking, alcohol, coffee!

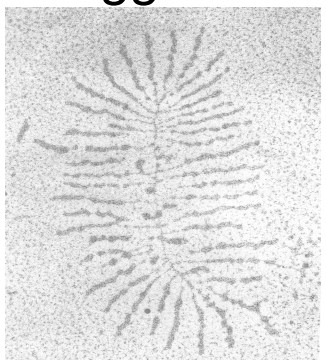


Cartilage = extracellular matrix

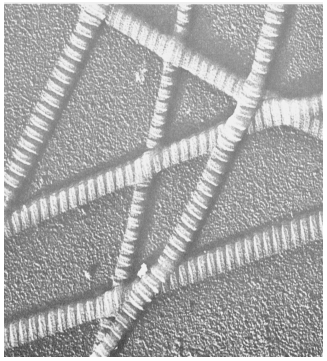




Aggrecan



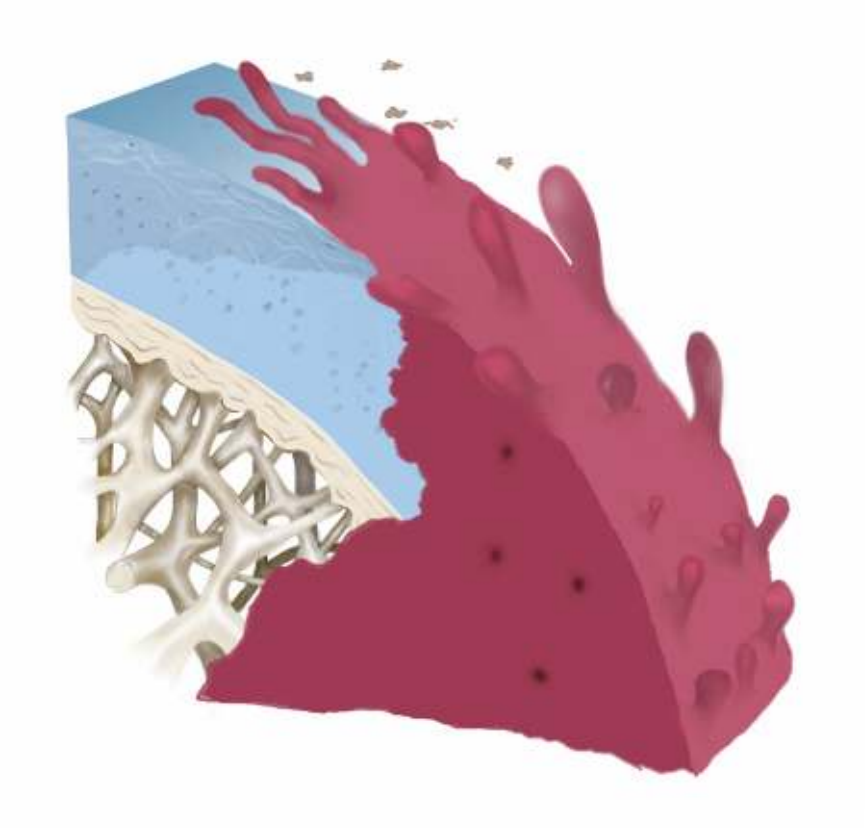
Collagen

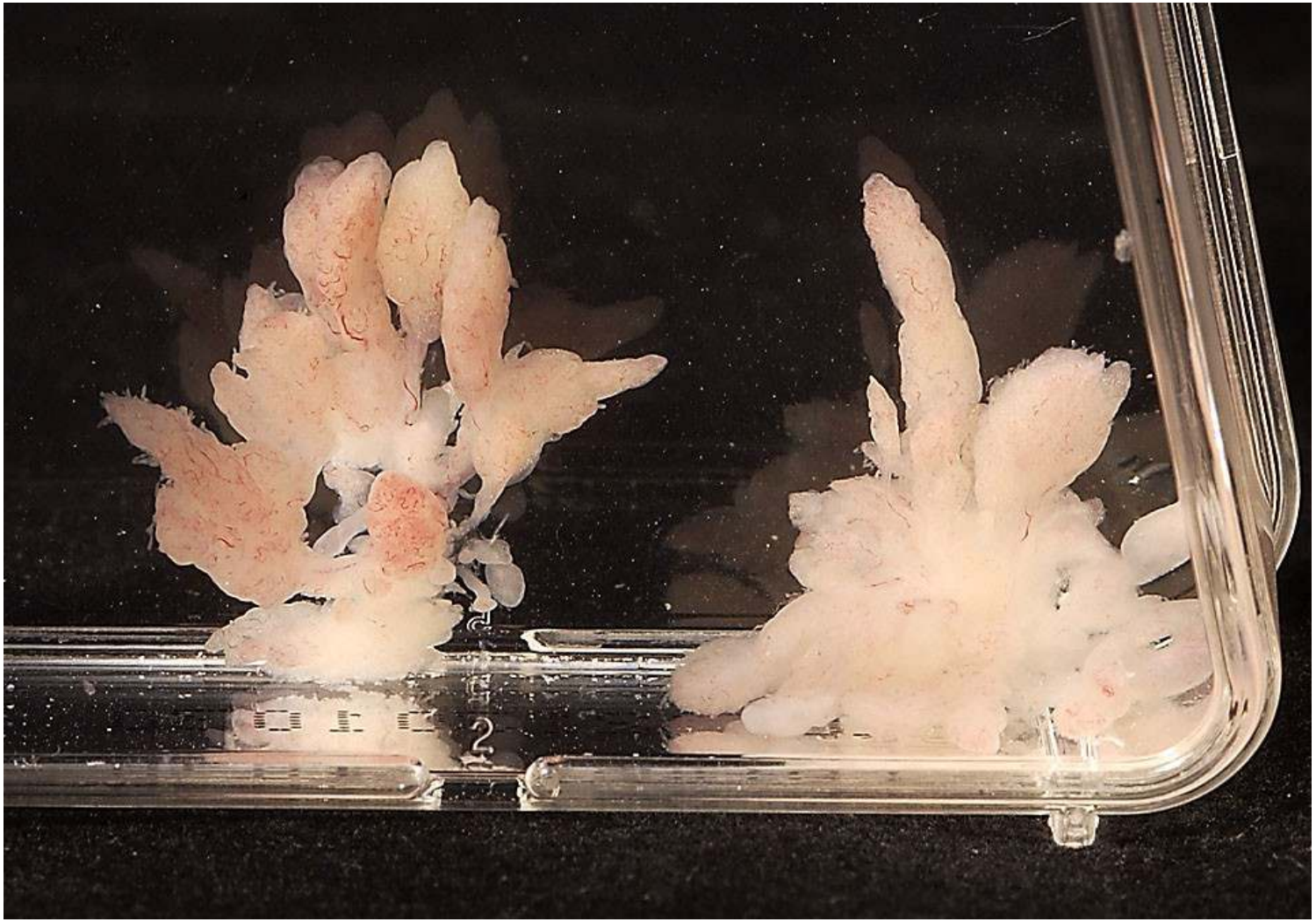


Why Cartilage?

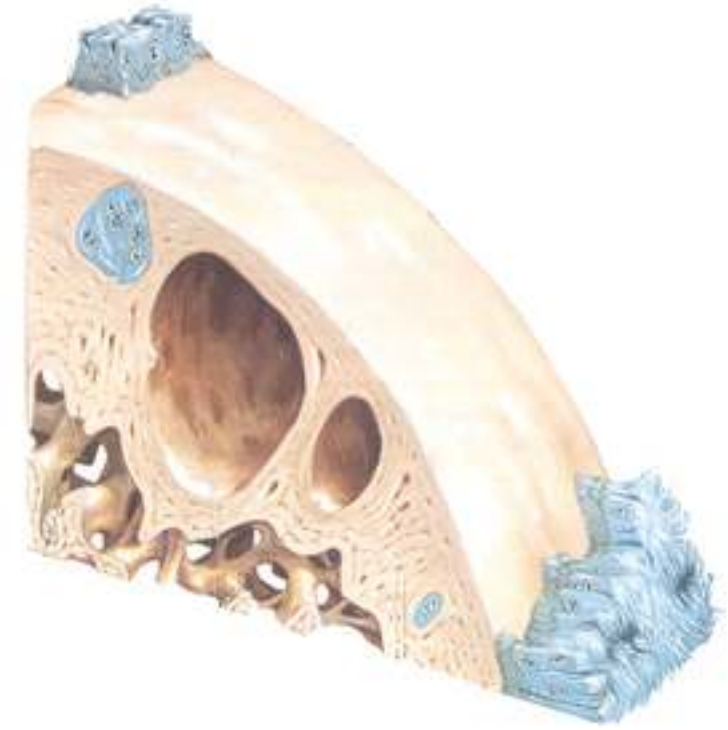
The breakdown of cartilage is a unifying feature of RA and OA irrespective of disease aetiology

RA Sequence





OA Sequence



Disease Treatments

- most modulate pain and inflammation,
NOT destruction

Biologics as therapeutics (RA)

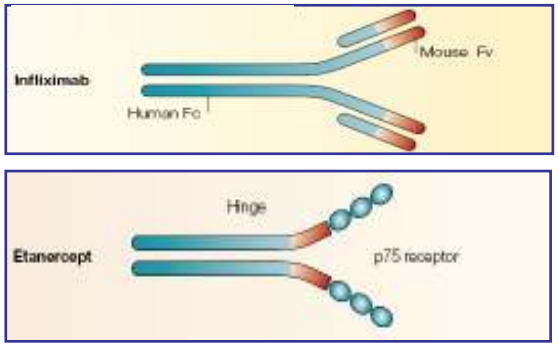
Anakinra = Anti-IL-1

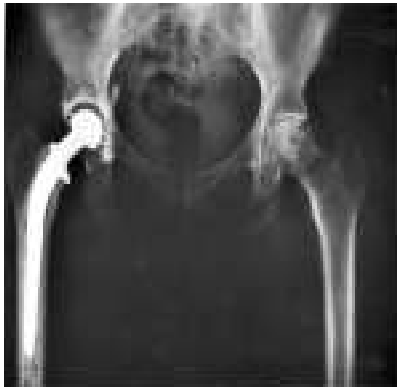
Atlizumab = Anti-IL-6

Etanercept = Anti-TNF

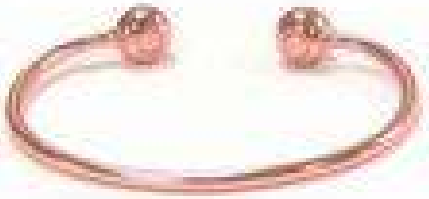
Infliximab = Anti-TNF

Retuximab = anti-CD20

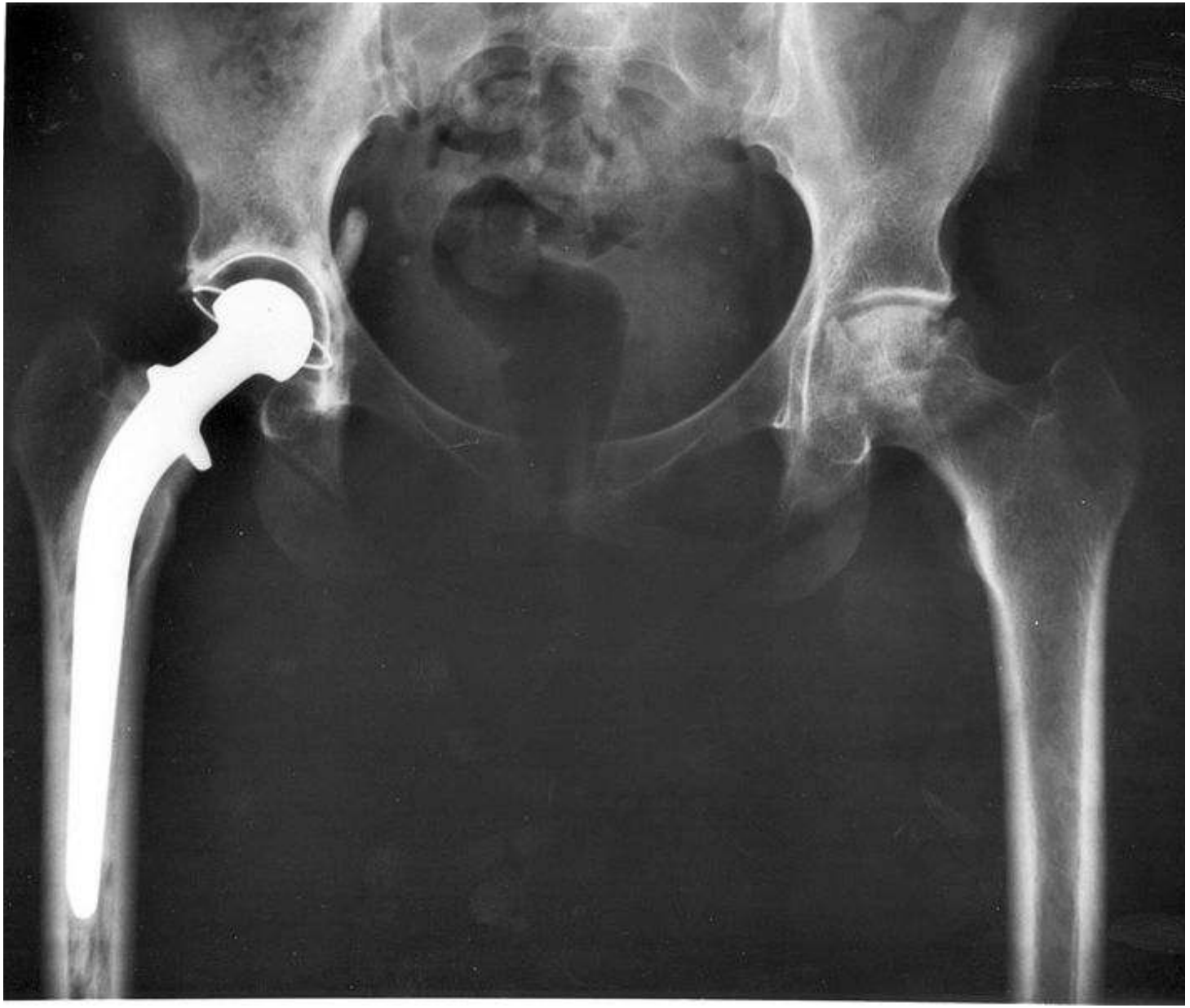




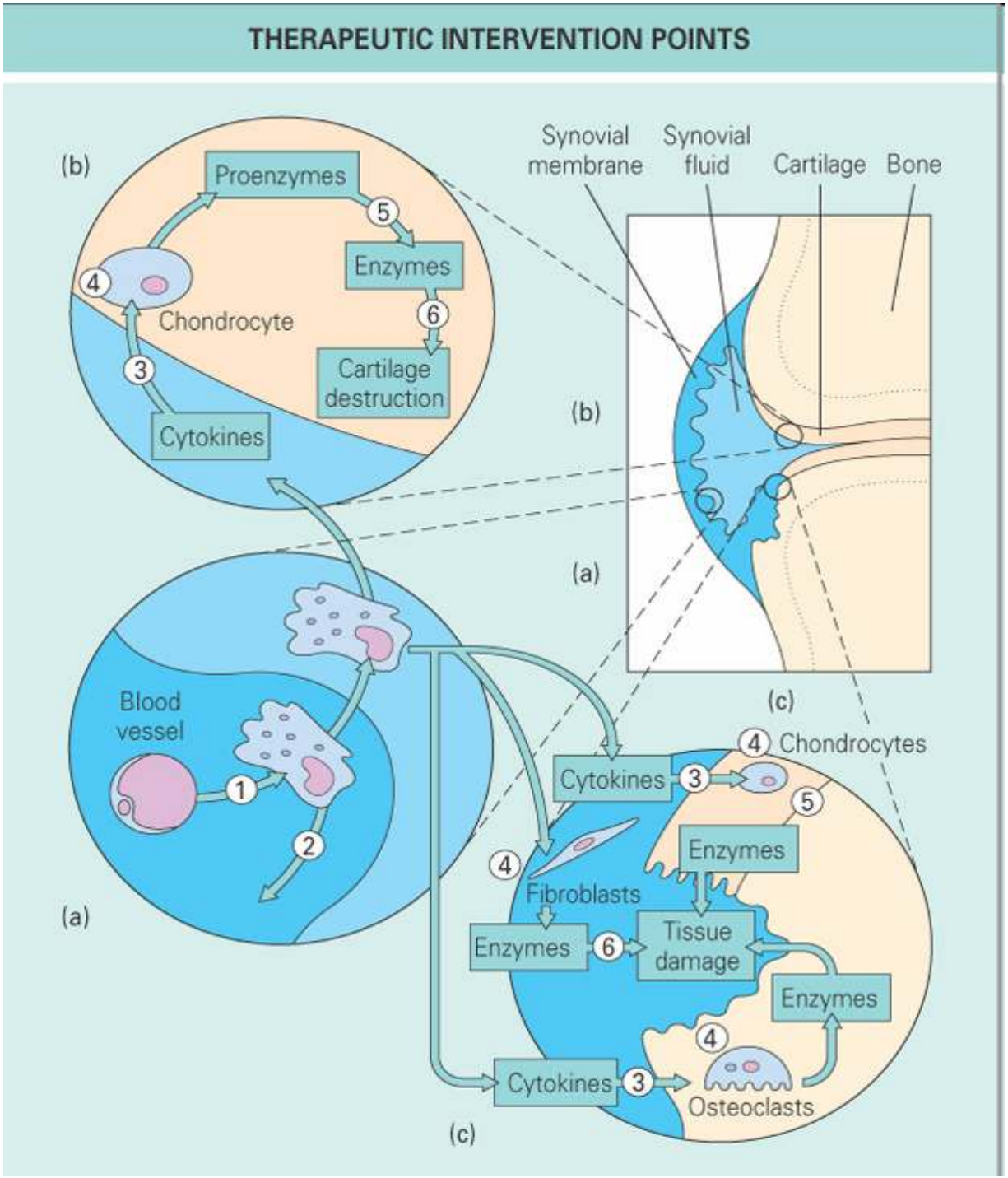
Lotions and potions as therapeutics (Arthritis)



The only cure?



Why target cartilage?



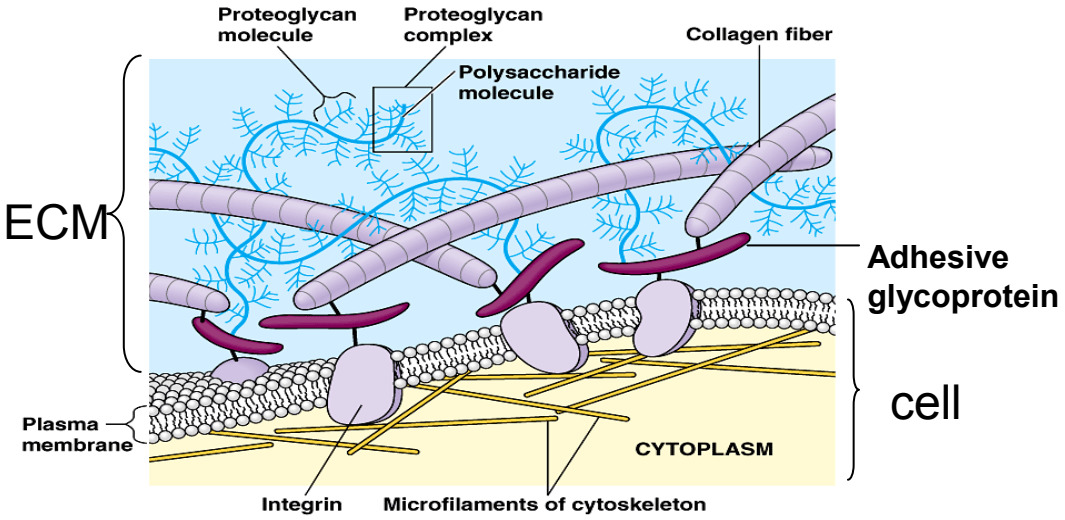
Why Cartilage?

The breakdown of cartilage is a unifying feature of RA and OA irrespective of disease aetiology

Jubb & Fell (1980) J Pathol 130: 159-167

“The preservation of the collagen network is crucial for the survival of cartilage; there is evidence from organ culture experiments that when the collagen is lost, matrix is not regenerated whereas if the proteoglycan alone is removed, it is rapidly replaced.”

Phenotypic cartilage changes (OA)



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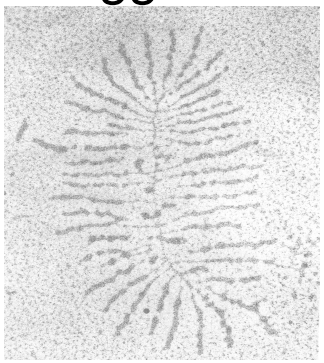
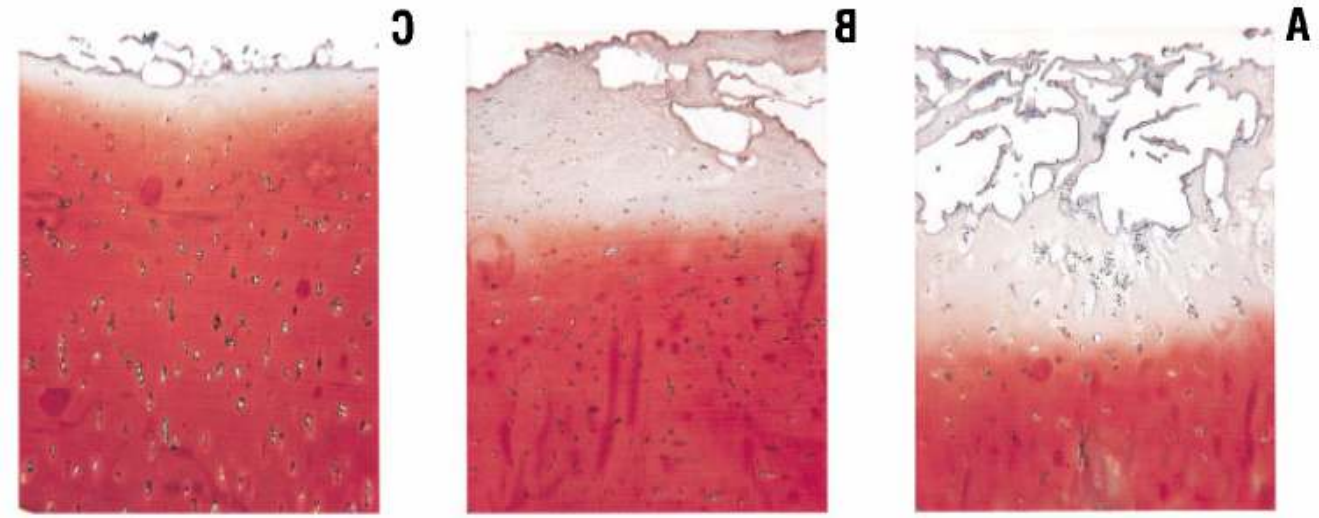
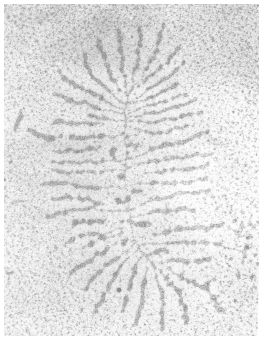




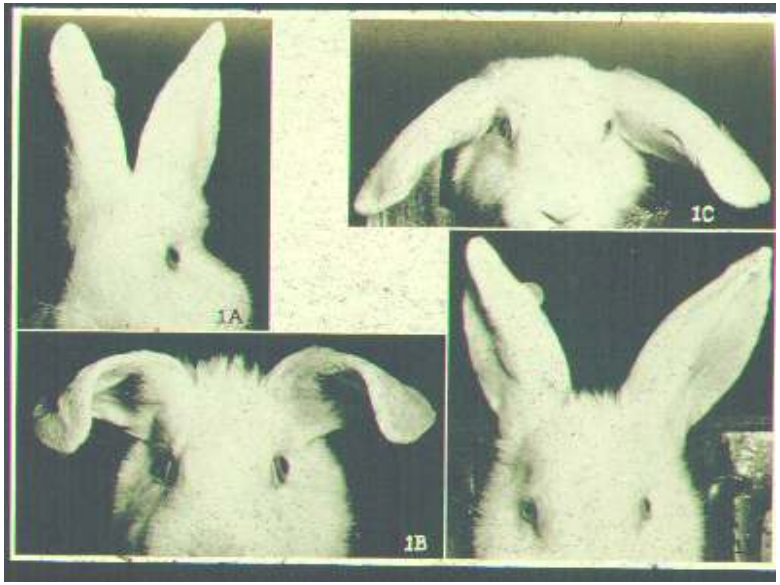
Proteolysis →



- Proteoglycans attract water, forming an hydrated gel
- results in a swelling pressure (turgor)

resistance to compression

strength and support



We need to better understand pathological proteolysis

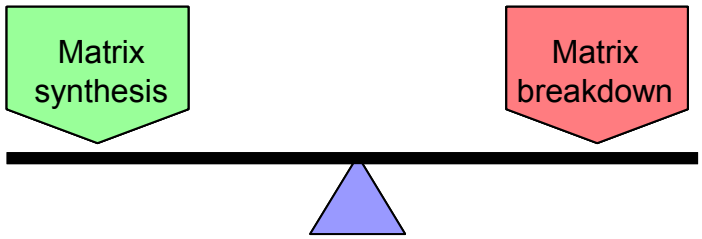
The ‘end stage’ of both RA and OA is the essentially irreversible destruction of the cartilage extracellular matrix (ECM)

Often, even limited proteolysis of this ECM is sufficient for loss of joint function

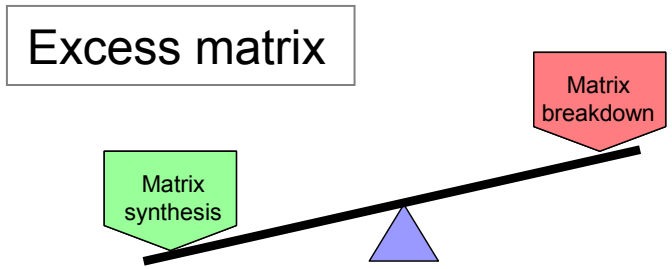
Therefore, a detailed understanding of the **molecular mechanisms** involved should reveal therapeutic targets to prevent further ECM damage

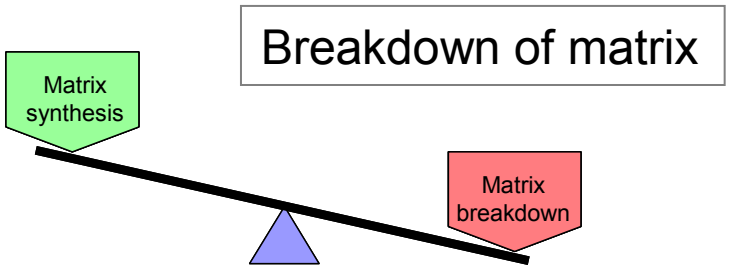
- enzymes (proteinases)
- their substrates
- inflammatory mediators

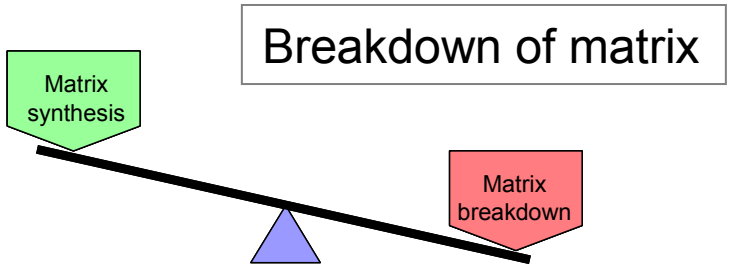
- A delicate balance between synthesis and breakdown of ECM molecules is needed to maintain a healthy matrix



- Imbalance in turnover of matrix leads to disease







Proteinases

Intracellular

- **Aspartic proteinases**
  - Cathepsin D
- **Cysteine proteinases**
  - Cathepsin B
  - Cathepsin K
  - Cathepsin L
  - Cathepsin S
- **Threonine proteinases**
  - Proteosome

Extracellular

- **Serine proteinases**
  - Elastase
  - Cathepsin G
  - Plasmin
  - PAs
  - Furin
- **Metallo-proteinases**
  - MMP-1, -8, -13
  - MMP-3, -10, -11
  - MMP-2, -9
  - MT1-6 MMP
  - ADAMs
  - ADAMTSs

Low pH → **Neutral pH**

Proteases in human diseases

Table 2 | **Human hereditary diseases of proteolysis**

Protease	Gene	Locus	Disease	OMIM	Dominant/ recessive	Function	Animal model
Loss-of-function group							
Cathepsin K	*CTSK*	1q21	Pycnodysostosis	265800	R	Loss	KO resembles disease
Cathepsin C	*CTSC*	11q14	Papillon-Lefevre and Haim-Munk syndromes	245000	R	Loss	KO does not resemble disease
Calpain 3	*CAPN3*	15q15	Limb-girdle muscular dystrophy type 2A	253600	R	Loss	KO resembles disease
Cylindromatosis protein	*CYLD1*	16q12	Cylindromatosis	132700	D	Loss	–
Ubiquitin C-terminal hydrolase 1	*UCHL1*	4p14	Parkinson disease type V	191342	D	Loss	*Gad* mouse resembles disease
Caspase-8	*CASP8*	2q33	Autoimmune lympho-proliferative syndrome (I)	601859	R	Loss	KO embryonic lethality
Caspase-10	*CASP10*	2q33	Autoimmune lympho-proliferative syndrome (II)	603909	D,R	Loss	No mouse orthologue
USP9Y	*USP9Y*	Yq11	Azoospermia and hypospermatogenesis	415000	D	Loss	–
Gelatinase A	*MMP2*	16q13	Multicentric osteolysis with arthritis	605156	R	Loss	KO does not resemble disease

Enzyme inhibition as a therapeutic: easier said than done!

Table 1 | **Matrix metalloproteinase inhibitor drugs in clinical development**

Inhibitor	Company	Structure	Specificity
Batimastat (BB-94)*	British Biotech	Peptidomimetic	Broad spectrum
Ilomastat (GM-6001)*	Glycomed	Peptidomimetic	Broad spectrum
Marimastat (BB-2516)	British Biotech; Schering-Plough	Peptidomimetic	Broad spectrum
Tanomastat (BAY-12-9566)	Bayer	Small molecule	Higher specificity towards MMP2, MMP3 and MMP9; does not target MMP1
Prinomastat (AG-3340)	Agouron; Pfizer	Small molecule	Broad spectrum
Metastat (COL-3)	Collagenex	Low-dose tetracycline derivative	Broad spectrum; higher specificity towards MMP2 and MMP9
Neovastat (AE-941)	AEterna	Shark cartilage extract	Broad spectrum
BMS-275291	Bristol-Myers Squibb; Celltech	Small molecule	Broad spectrum
MMI-270B (CGS-27023A)	Novartis	Small molecule	Broad spectrum
Trocade (Ro-32-3555)	Roche	Peptidomimetic	MMP1, MMP8 and MMP13
MMI-166	Shionoqi	Small molecule	Broad spectrum

Proteinases are biological scissors



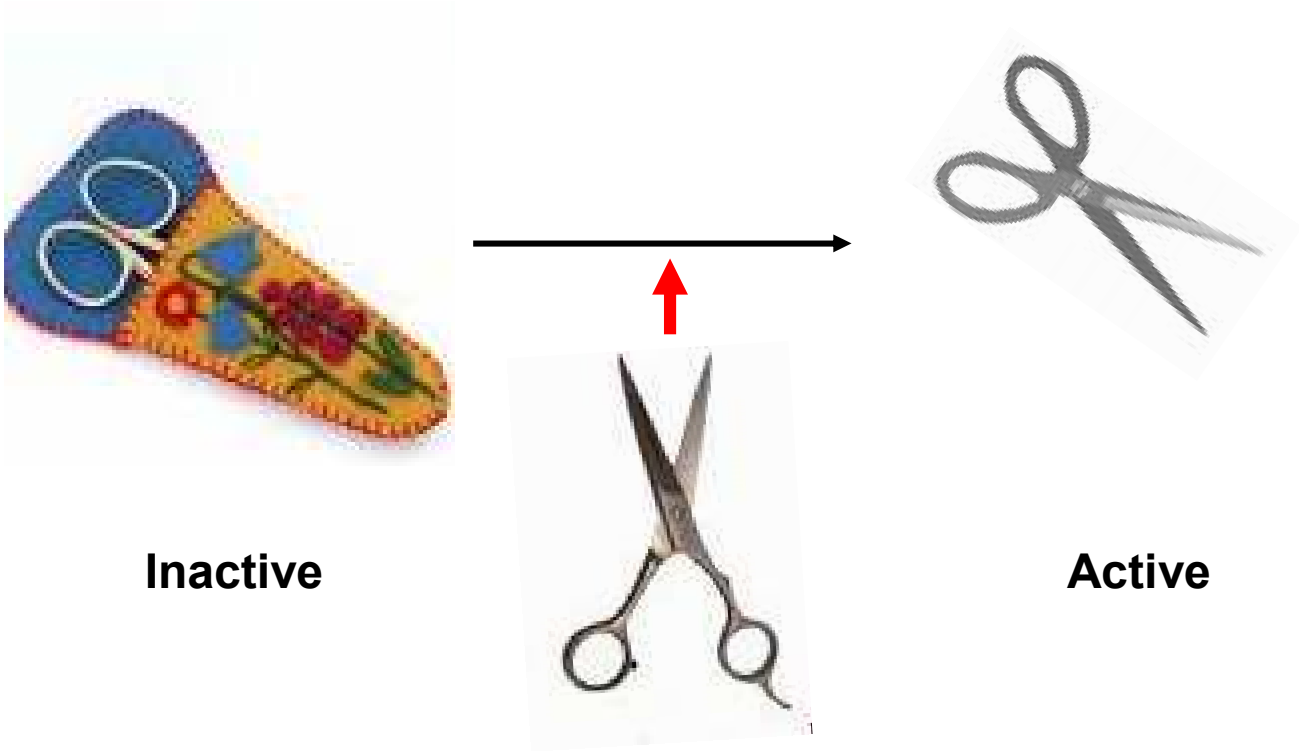









Most proteinases are made as inactive precursors



Inactive

Active

The Human Degradome

There are 570 proteinases in the human genome (this represents approx 2% of all genes) – this is called the "degradome"

Proteinases are simply enzymes that degrade or cleave other proteins

These can be subdivided into -

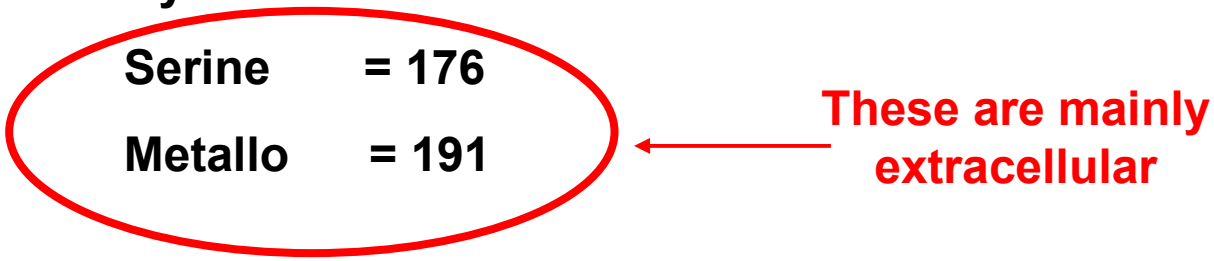
Aspartic = 21

Threonine = 28

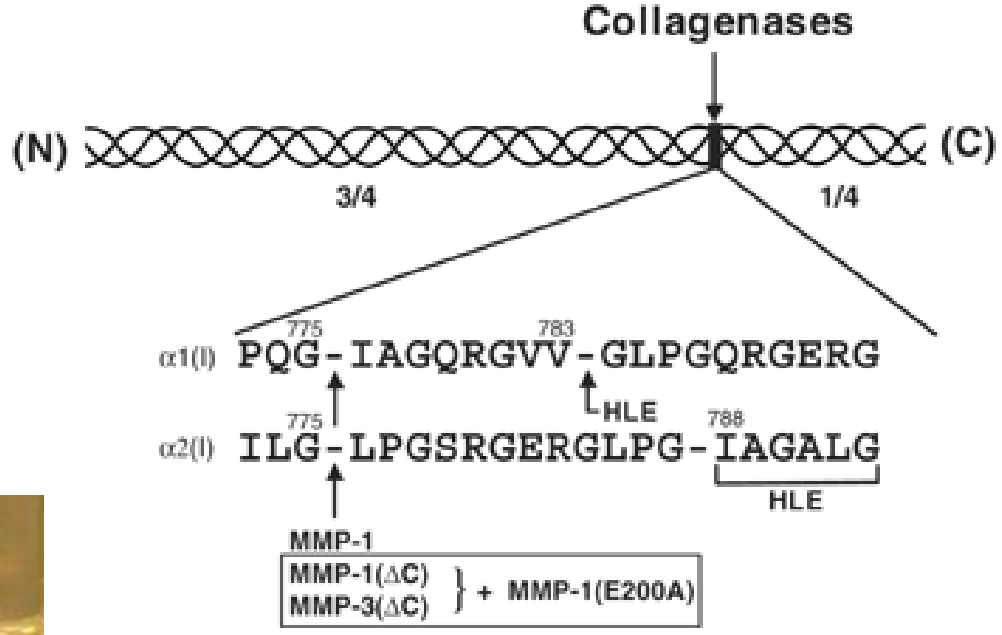
Cysteine = 154

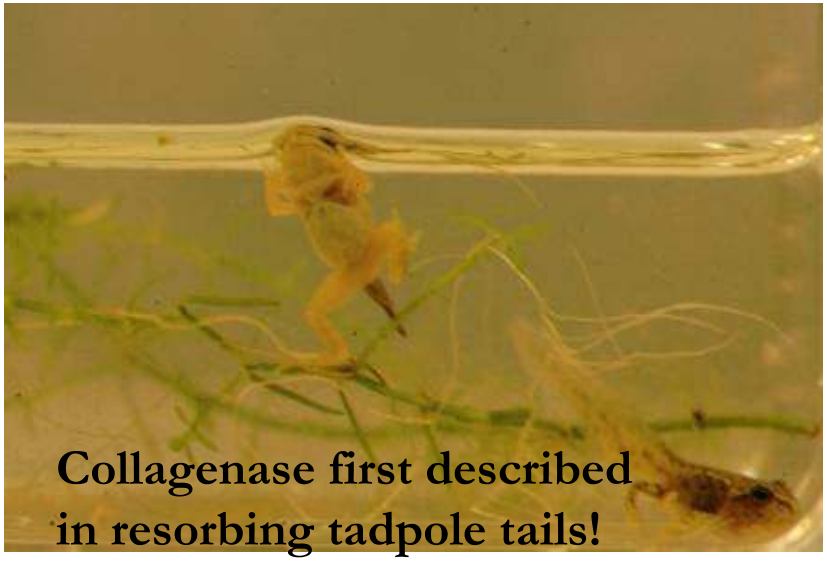
Serine = 176

Metallo = 191



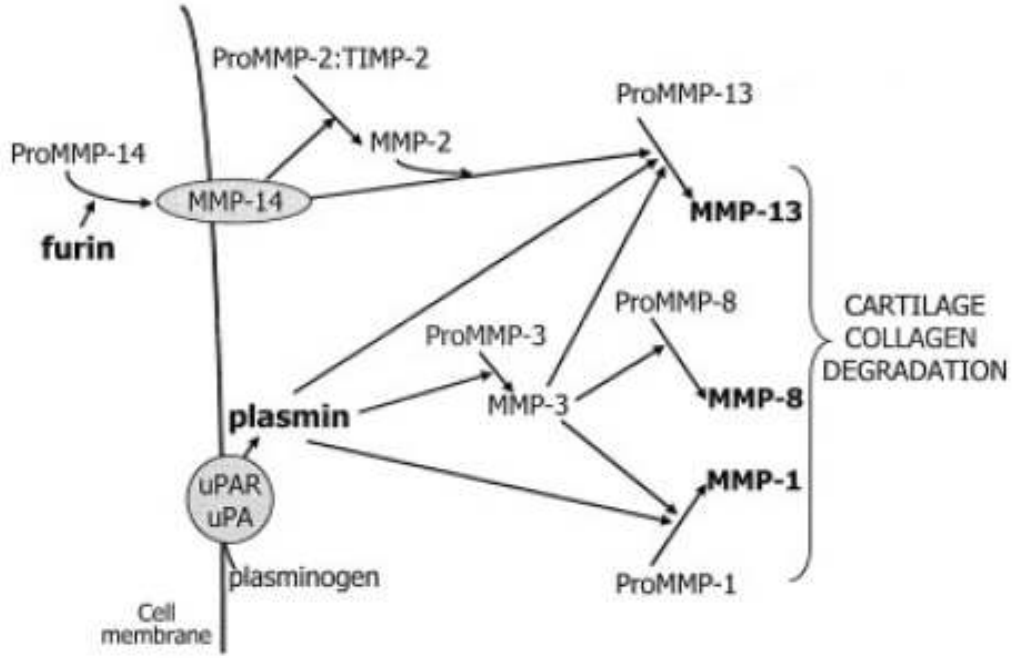
Collagen cleavage





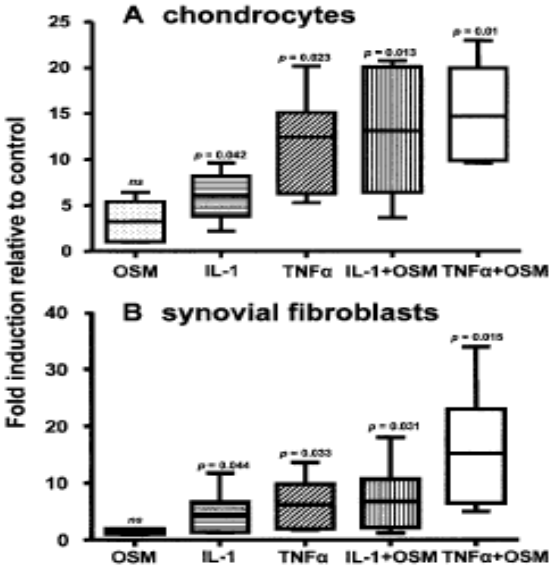
Arthritis was the first disease to be associated with a MMP (collagenase).
Harris et al. Arthritis Rheum 1969;12:92-102.

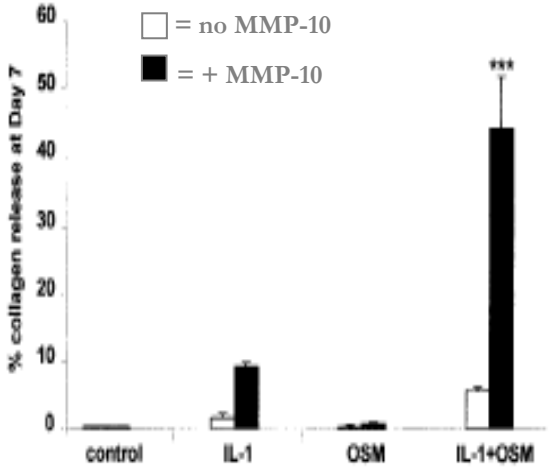
Proteinase cascades in disease are interlinked and inter-dependent

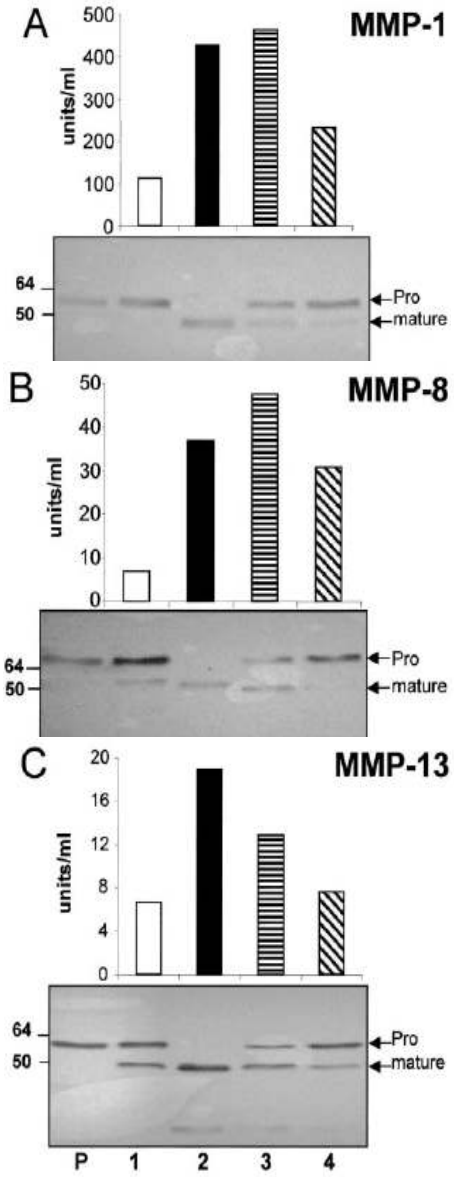


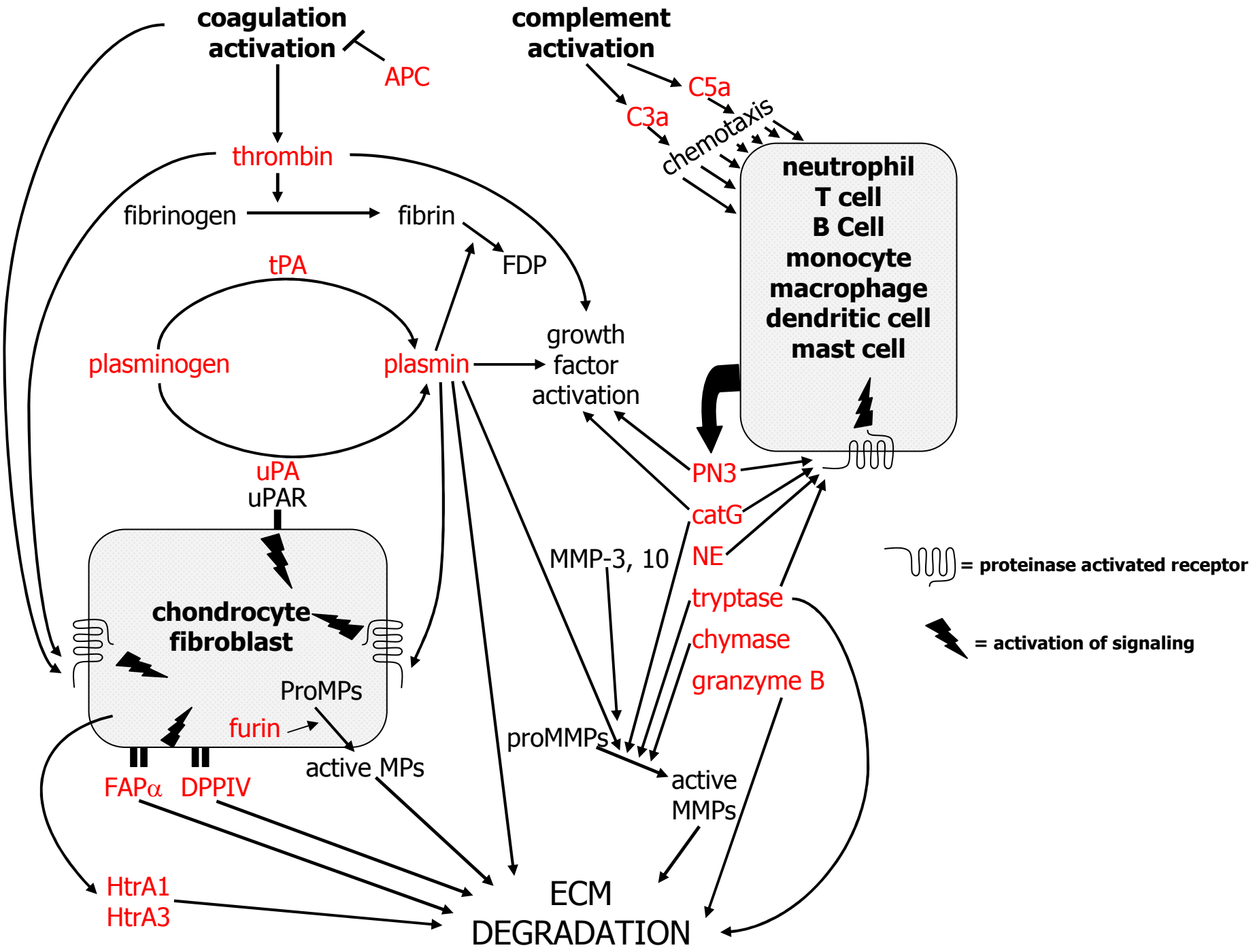
We have data that strongly indicate that serine and metallo-proteinase cascades are inter-dependent for collagen breakdown to occur

Linking elevated expression with function

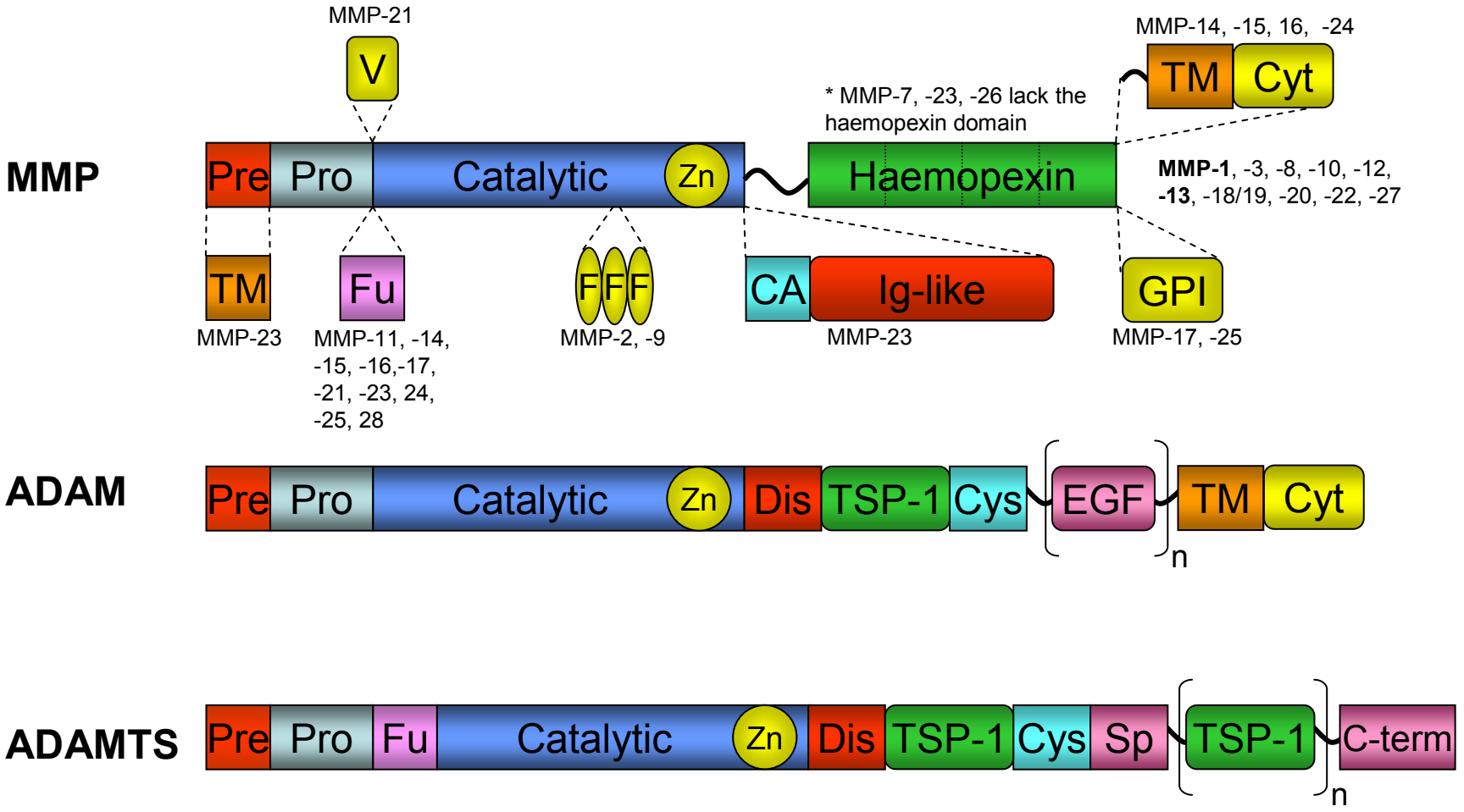







coagulation activation
APC
complement activation
C5a
C3a
chemotaxis
thrombin
fibrinogen
fibrin
FDP
tPA
plasminogen
plasmin
growth factor activation
neutrophil
T cell
B Cell
monocyte
macrophage
dendritic cell
mast cell
PN3
catG
NE
uPA
uPAR
MMP-3, 10
tryptase
chymase
granzyme B
= proteinase activated receptor
= activation of signaling
chondrocyte fibroblast
ProMPs
furin
active MPs
proMMPs
active MMPs
FAPα
DPPIV
HtrA1
HtrA3
ECM DEGRADATION

Proteinases are often multi-domain proteins

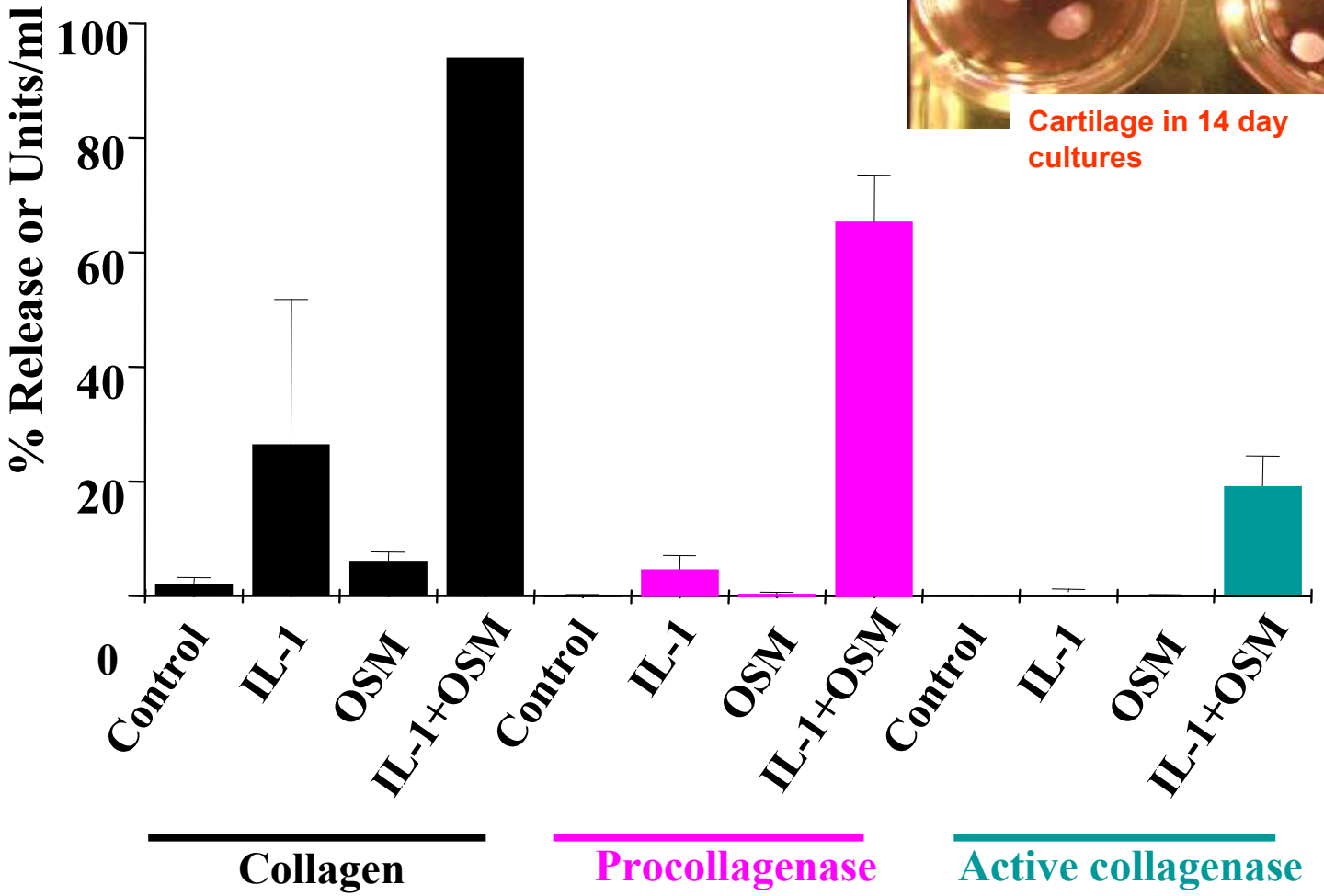


To date, we have probably over-looked the role of the non-enzymatic domains!

Inflammation and RA – how does cartilage respond to inflammatory stimuli?

IL-1+OSM-induced cartilage collagenolysis

Cartilage in 14 day cultures



Cytokine synergy is not unique to IL-1+OSM

TNFα + OSM (Hui et al., 2003a,b)

IL-17 + OSM (Koshy et al., 2002)

IL-17 + IL-1 (Koshy et al., 2002)

IL-17 + TNFα (Koshy et al., 2002)

RetA + OSM (Shingleton et al., 2006)

RetA + IL-1 (Shingleton et al., 2000)

IL-6 + IL-1 (Rowan et al., 2001)

Synergistic collagenolysis can be blocked

TGFβ (Hui et al., 2000, 2001, 2003, 2005)

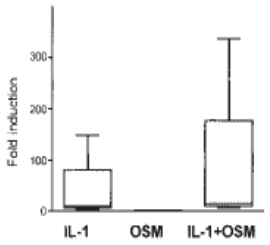
IGF-1 (Hui et al., 2001, 2005)

IL-4, IL-13 (Cleaver et al., 2001)

Microarray analyses can reveal unexpected (protective) responses

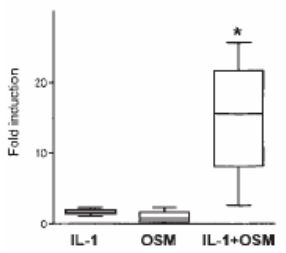
IL-8 (KC)

IL-1+OSM



Activin A

IL-1+OSM



PTX-3

IL-1+OSM

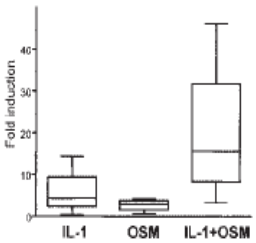
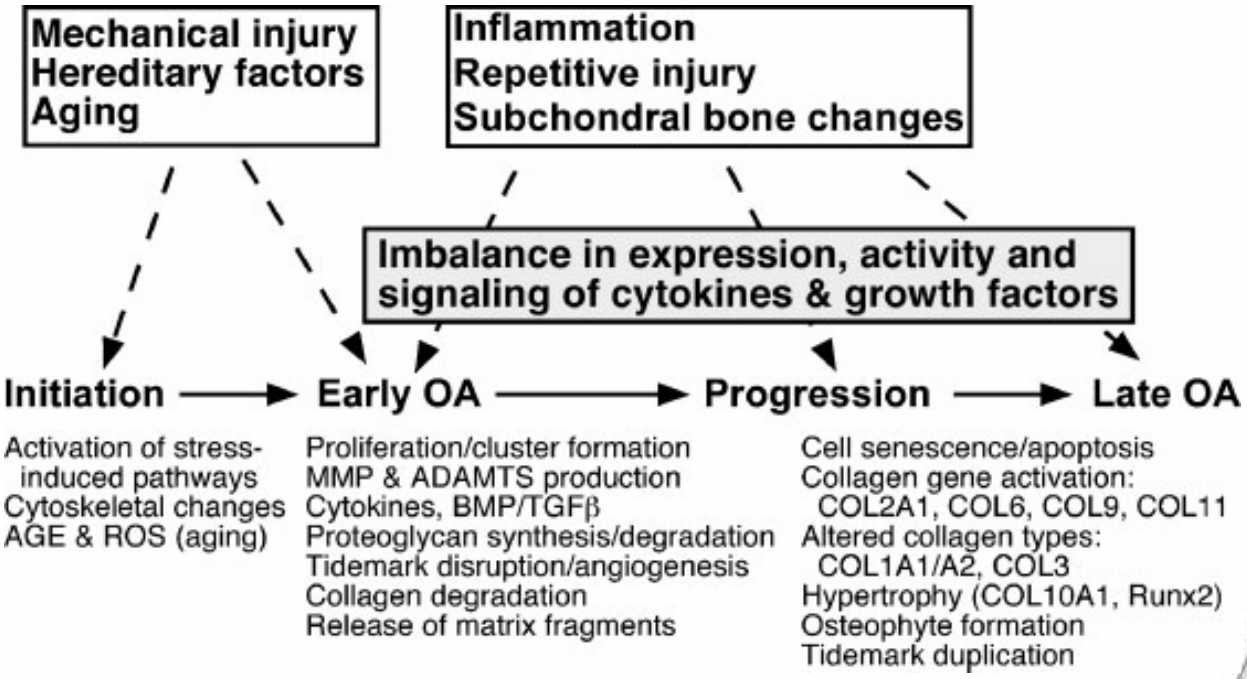


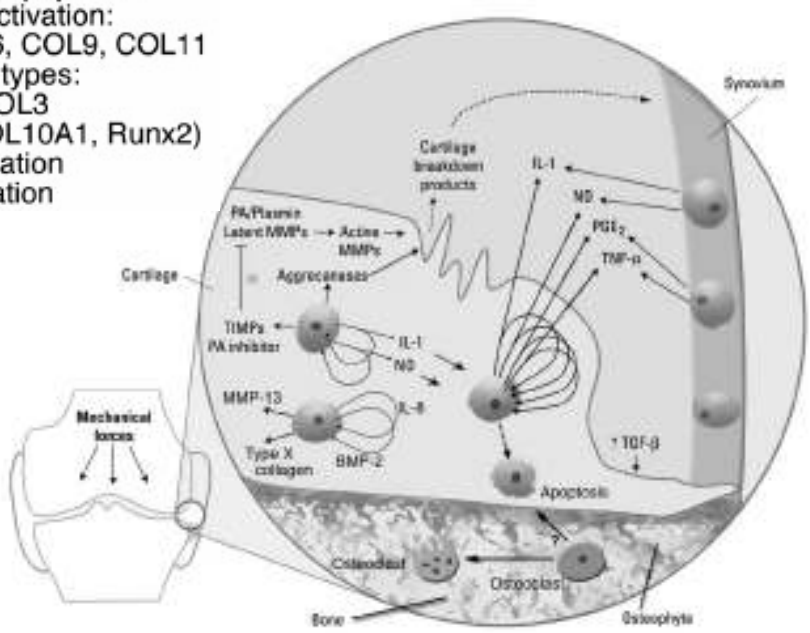
Table 1. Genes synergistically induced in chondrocytes following stimulation with IL-1 plus OSM*

Gene	Signal intensity				Signal log ratio vs. control			Fold change vs. control		
	Control	IL-1	OSM	IL-1 + OSM	IL-1	OSM	IL-1 + OSM	IL-1	OSM	IL-1 + OSM
Proteases and inhibitors										
MMP-1	22A	1,875P	28P	4,217P	6.1	0.3	7.3	67.6	1.3	157.6†
MMP-3	376P	5,862P	318P	8,910P	3.4	0.2	4.4	10.2	1.1	21.1†
MMP-10	30A	99P	14A	177A	1.2	−0.6	2.3	2.3	−0.7	4.8†
MMP-12	57A	1,015P	37A	2,447P	3.6	−0.3	4.9	12.5	−0.8	30.7†
MMP-13	3A	280P	18P	1,548P	5.9	2.0	8.4	58.9	4.1	342.5†
MMP-14	22A	28A	97A	248P	0.0	1.8	3.2	1.0	3.5	8.9
Antileukopeptidase	112P	175P	68A	400P	0.4	−0.7	2.4	1.1	−0.6	6.9
SCCA-2	4A	20A	525P	1,125P	2.2	5.8	7.2	4.4	55.7	147.0
C1r	274P	1,217P	509P	1,912P	2.2	1.2	2.9	4.6	2.3	7.5
Chemokines, cytokines, receptors, and signal transduction										
IL-8	2A	896P	2A	1,972P	7.7	−0.2	9.0	207.9	−0.9	512.0†
IL-1β	24A	150A	48A	560P	2.9	0.8	4.2	7.2	1.7	18.4
MCP-1	3A	175P	43A	285P	5.0	3.9	6.3	32.0	14.4	78.8†
MCP-3	43A	369P	89P	734P	3.8	1.6	7.3	13.9	3.0	28.8†
IL-6	53A	251P	43A	1,486P	1.7	−0.1	4.3	3.3	−0.9	20.4†
LIF	12A	125M	9A	226P	3.1	−0.1	4.0	4.3	−0.9	10.2†
OSMβR	25P	71P	155P	253P	1.2	2.7	3.3	2.4	6.5	9.2
ENA-78	9A	10A	6A	217P	0.6	−0.2	4.4	1.5	−0.3	20.4†
PBEF	305P	928P	604P	2,303P	1.6	1.0	3.0	3.0	2.0	8.1†
Activin A	58A	90P	17A	125A	0.7	−2.3	1.5	1.8	−0.7	2.3†
Jak 2 kinase	28A	88P	98P	371P	1.4	1.5	3.2	2.7	2.8	9.1
Extracellular proteins										
Decorin variant A	14P	64P	23P	126P	1.8	0.1	3.3	2.3	1.2	18.4†
Decorin variant C	98P	255P	99P	826P	1.7	0.0	3.5	3.3	1.0	11.3
Fibronectin	130P	140P	397P	709P	0.11	1.6	2.7	1.0	2.9	6.3
Serum amyloid A2	5A	289P	9A	2,288P	5.15	0.9	8.9	85.0	1.1	362.0
Calcium binding protein A9	7A	45A	18A	339P	1.51	1.4	338.9	2.8	2.6	45.3
Calcium binding protein A8	33A	63A	19A	604P	0.91	−0.2	4.0	1.9	1.7	16.0
PTX-3	18A	123P	24P	407P	2.59	0.7	5.1	6.0	1.6	33.6†
Chitinase-3–like 2	79A	215P	71A	1,750P	1.56	−0.3	3.9	2.9	1.0	14.9
Chitinase-3–like 1	243P	579P	1,666P	2,320P	1.21	2.7	3.2	2.4	6.4	9.2
SOD-3	24P	232P	25A	404P	3.45	−0.2	5.7	24.8	1.5	41.6

- **IL-8** is chemotactic
- **Activin A** promotes TIMP and collagen synthesis
- **Pentraxin-3** is anti-angiogenic

Molecular changes in OA ↔ dysregulation





Diseases caused by mutations in collagen genes or collagen processing enzymes

Gene	Disease
COL1A1;COL1A2	OI, EDS (EHLERS-DANLOS SYNDROME) types I, II, VIIA and VIIB, osteoporosis
COL2A1	Several chondrodysplasias §
COL3A1	EDS type IV, arterial aneurysms
COL4A3;COL4A4;COL 4A5	Alport syndrome
COL4A5; COL4A6	Alport syndrome with diffuse oesophageal leiomyomatosis
COL5A1; COL5A2	EDS types I and II
COL6A1; COL6A2; COL6A3	Bethlem myopathy
COL7A1	EB, dystrophic forms
COL8A2	Some forms of corneal endothelial dystrophy
COL9A1; COL9A2; COL9A3	Multiple epiphyseal dysplasia, intervertebral disc disease, osteoarthritis
COL10A1	Schmid metaphyseal dysplasia
COL11A1; COL11A2	Several mild chondrodysplasias §, non-syndromic hearing loss, osteoarthritis
COL17A1	Generalized atrophic benign EB
COL18A1	Knobloch syndrome
Lysyl hydroxylase 1	EDS type VI
Procollagen N-proteinase	EDS type VIIC

Molecular changes ↔ altered "phenotype"

Toll-like receptor (TLR) changes in OA

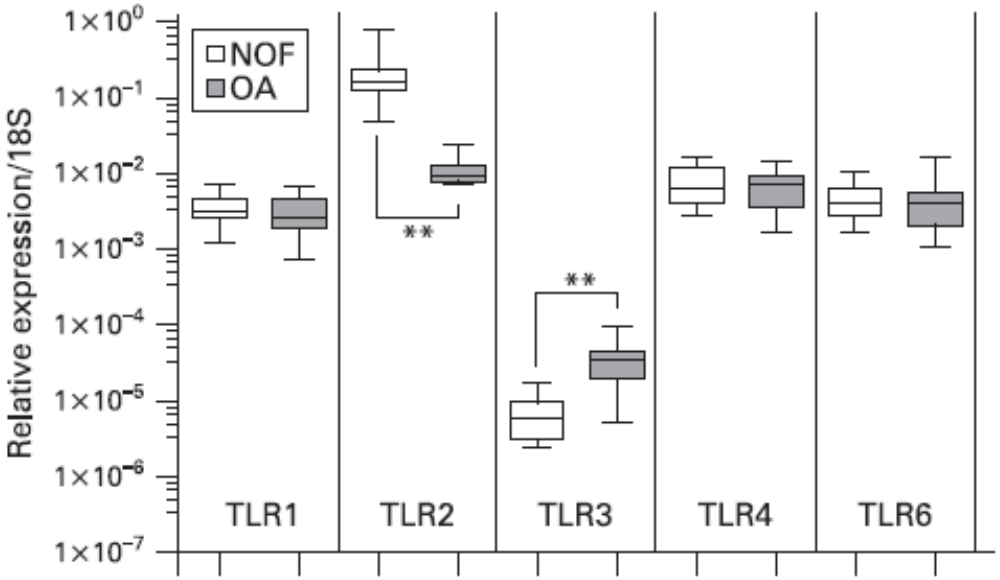
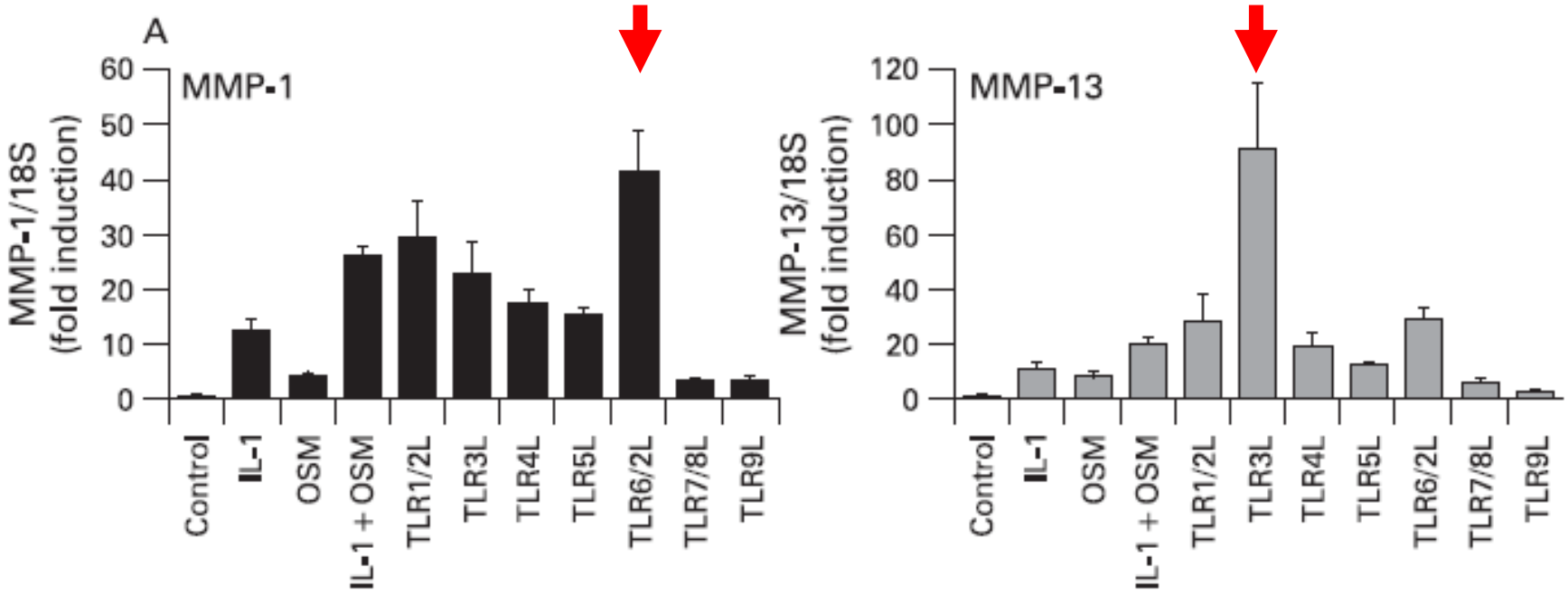


Figure 1 Differential Toll-like receptor (TLR) gene expression in normal and osteoarthritic cartilage. The gene expression levels of TLR1, 2, 3, 4 and 6 in hip cartilage from patients with osteoarthritis (OA) (shaded bars; n = 12) or normal controls (fractured neck of femur (NOF)) (open bars; n = 12) were determined as described in Materials and methods and normalised to the level of 18S rRNA. Since all primer/probe combinations amplify with essentially equal efficiencies, TLR expression levels are directly comparable. Significant differences between the normal and OA groups were determined using a two-sided Mann–Whitney U test, where ** $p<0.01$. Lines within the boxes represent the median, the boxes represent the 25th and 75th percentiles and the lines outside the boxes correspond to the minimum and maximum values.

Cells will become more or less responsive dependent on the altered phenotype



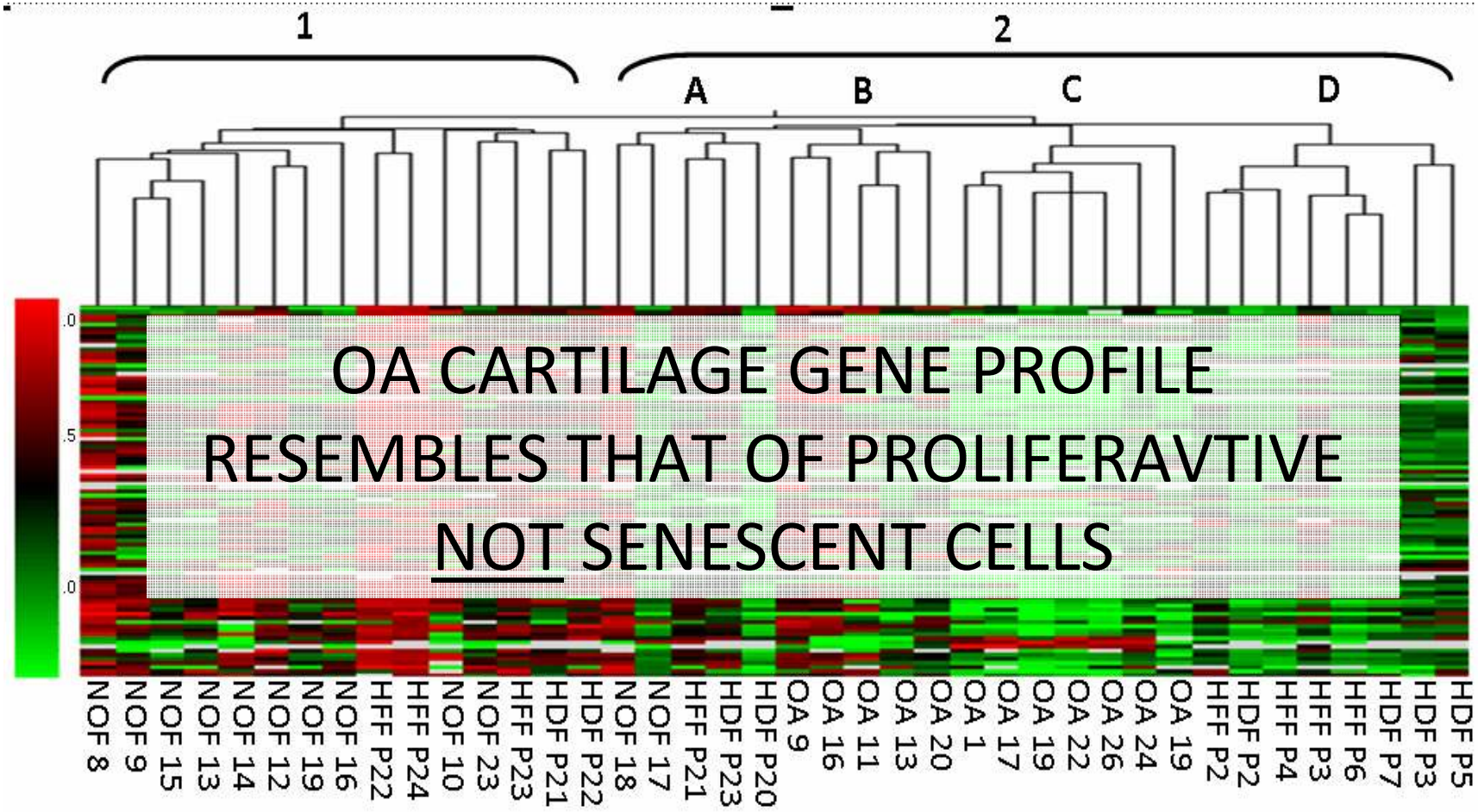
Based on our own data, OA cartilage will make less MMP-1 via TLR6/2 but more MMP-13 via TLR3

TLR3 responds to nucleic acid from necrotic cells

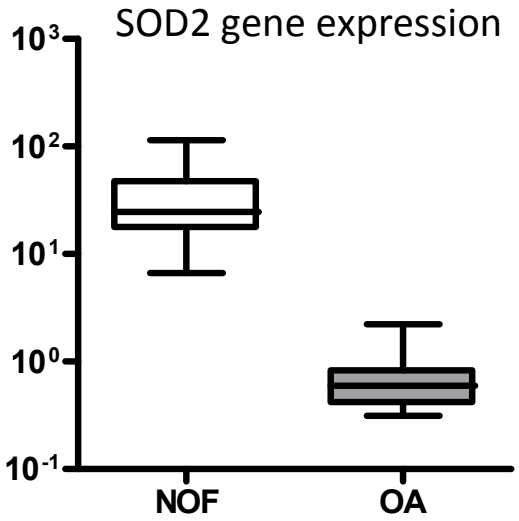
But is this altered phenotype a cause or an effect of disease?

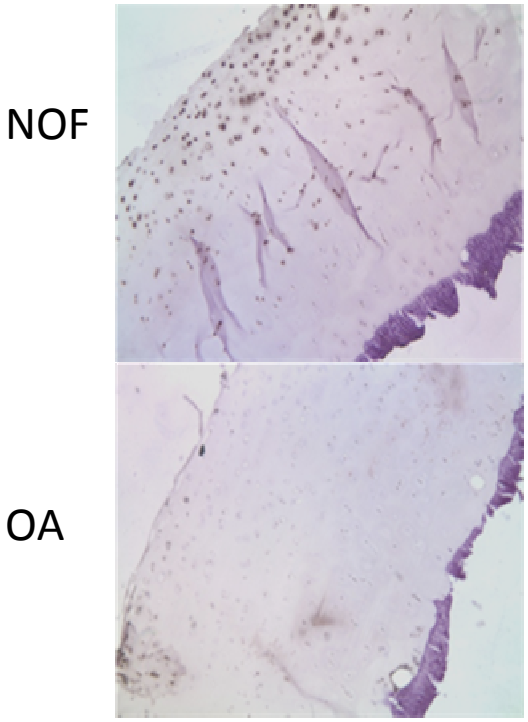
Identification of gene expression changes in cartilage with age and OA progression

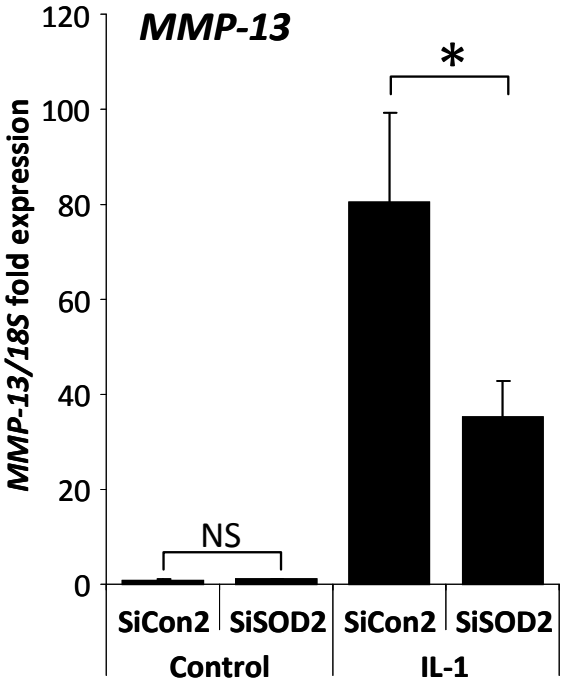
Q. Do OA chondrocytes have a senescent transcriptome?

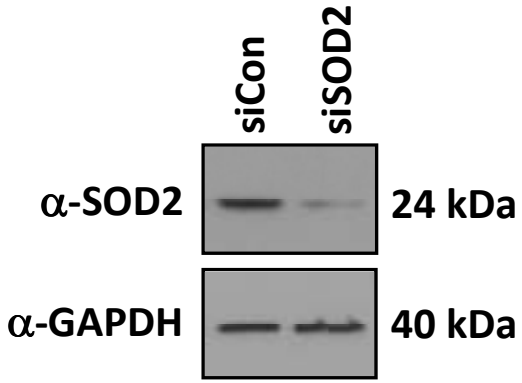


Superoxide dismutase expression is down in OA cartilage









Why do we need human tissue?

Relevant to human disease

OA tissue is diseased tissue and comparison with 'normal' tissue can tell us what differences exist and how this might make tissue more susceptible to proteolysis

Each joint is n = 1

To generate data with high confidence, we need to repeat and repeat! Biological samples vary, so this is an essential part of what we have to do

Individual samples are a limited resource

To generate data with high confidence, we need to repeat and repeat! Cartilage samples vary, so this is an essential part of what we have to do

MRG is establishing a biobank of joint tissues

Where possible, we want to begin to generate a biobank of joint tissue samples (especially RNA and DNA) for future studies that will require high numbers of age- and sex-matched samples

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