

TGF- β signaling in chondrocytes

SHIFT FROM ALK5 TO ALK1 RECEPTOR USAGE

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Ageing and OA

AGEING

↓

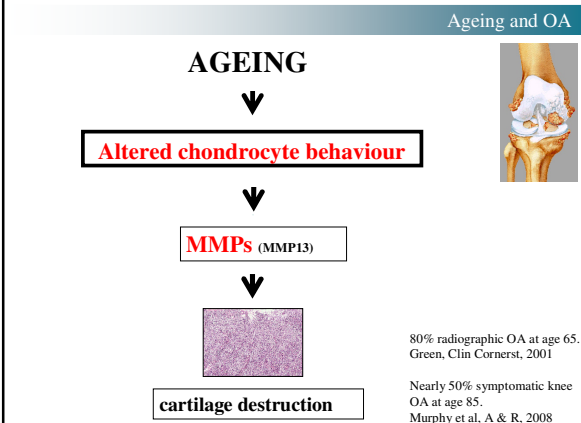
Altered chondrocyte behaviour

↓

MMPs (MMP13)

↓

cartilage destruction



80% radiographic OA at age 65.
Green, Clin Cornerst, 2001

Nearly 50% symptomatic knee
OA at age 85.
Murphy et al. A & R, 2008

Chondrocytes and TGF beta (1992)

Relation altered chondrocyte behaviour and TGF beta?

Early studies:

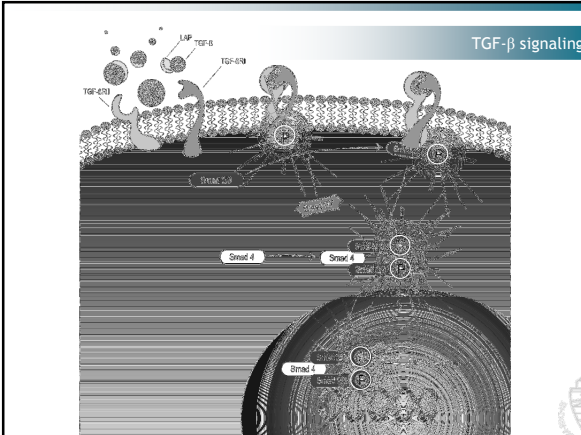
Freshly isolated chondrocytes > TGF beta **inhibited** DNA and proteoglycan synthesis

Cultured chondrocytes > TGF beta **stimulated** DNA and proteoglycan synthesis

Difference in TGF beta receptor expression?

van der Kraan P, Vitters E, van den Berg W.J *Rheumatol.* 1992 :140-5.
Differential effect of transforming growth factor beta on freshly isolated and cultured articular chondrocytes.


TGF- β signaling



Chondrocytes and TGF beta

TGF beta type II receptor isoform

25 bp difference



-TGF-bRII2
-TGF-bRII1

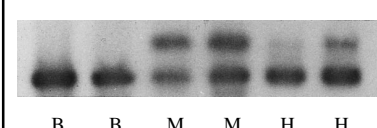
Difference in size of the TGF-beta type II receptor between freshly isolated and cultured chondrocytes.

Explains difference in size of the type II receptor the differential effect of TGF-beta on chondrocytes?

Glansbeek et al. *Ann Rheum Dis.* 1993:812-6.
Correlation of the size of type II transforming growth factor beta (TGF-beta) receptor with TGF-beta responses of isolated bovine articular chondrocytes.

Chondrocytes and TGF beta

Glansbeek et al. *Cytokine.* 1997, 9:347-51.
Species-specific expression of type II TGF-beta receptor isoforms by articular chondrocytes: effect of proteoglycan depletion and ageing.



-TGF-bRII2
-TGF-bRII1

B B M M H H

Expression of TGF-bRII1 and TGF-bRII2 mRNA in normal bovine (B), murine (M) and human (H) articular cartilage.

No changes in expression found during ageing or cartilage disease!

TGF beta signals through ALK5

Franzén P et al. Cell. 1993;75:681-92. Cloning of a TGF beta type I receptor that forms a heteromeric complex with the TGF beta type II receptor.

Wrana JL et al Cell. 1992 1;71:1003-14. TGF beta signals through a heteromeric protein kinase receptor complex.

The type II receptor requires both its kinase activity and association with another TGF beta-binding protein, the type I receptor to signal.

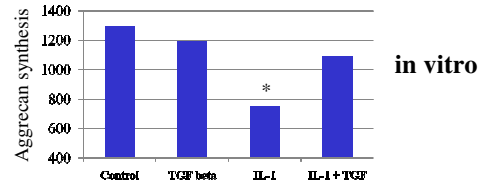
Receptor II requires receptor I (ALK5) to signal.

No alternative TGF beta receptors
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One TGF beta signaling pathway (ALK5)

Protective role of TGF beta in joint disease ?
Role in Osteoarthritis?

TGF beta protective

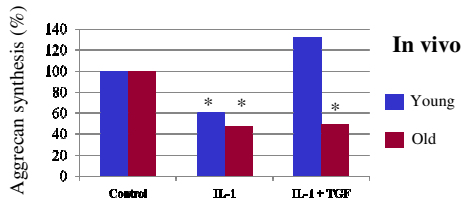
TGF beta protects cartilage against IL-1-induced cartilage damage



van Beuningen et al. Ann Rheum Dis. 1993 Mar;52(3):185-91. Protection from interleukin 1 induced destruction of articular cartilage by transforming growth factor beta: studies in anatomically intact cartilage in vitro and in vivo.

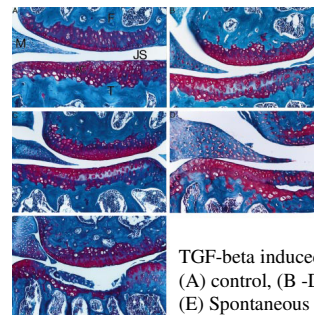
TGF beta protective

TGF beta protects cartilage against IL-1-induced cartilage damage only in young mice



van Beuningen et al Ann Rheum Dis. 1994;53:593-600. In vivo protection against interleukin-1-induced articular cartilage damage by transforming growth factor-beta 1: age-related differences.

TGF beta induces OA



van Beuningen et al Osteoarthritis Cartilage. 2000; 8:25-33. Osteoarthritis-like changes in the murine knee joint resulting from intra-articular transforming growth factor-beta injections.

TGF-beta induced changes in knee joint (A) control, (B-D) Three TGF-beta-injections, (E) Spontaneous OA in 18-month old mouse.

Conclusions (1)

In vitro:

- Isolated chondrocytes: Differential effects on freshly isolated and cultured chondrocytes
- Intact cartilage: TGF beta protects against IL-1-induced damage

In vivo:

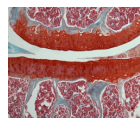
- TGF beta protects against IL-1 effects in cartilage of young animals
- TGF beta induces OA-like lesions after repeated injections, starting in deep cartilage layer

Differential, context depending effects of TGF beta

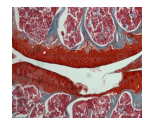
Role of TGF beta in ageing cartilage and OA?

Study role of TGF beta in Spontaneous OA in mice (age-related)

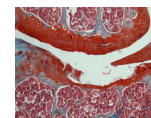
C57Bl Mice



3 months

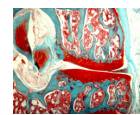


1.5 year



2 years

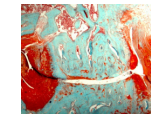
STR/ORT Mice



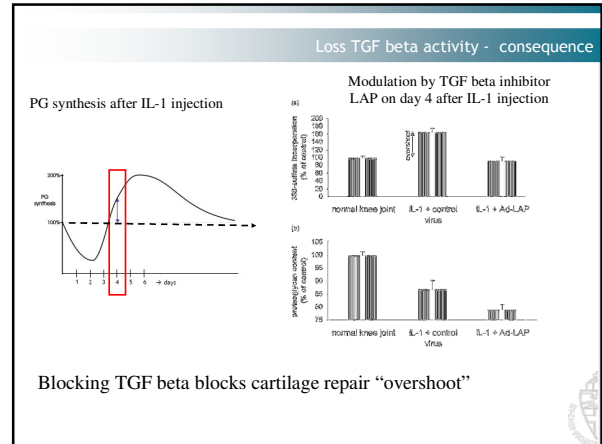
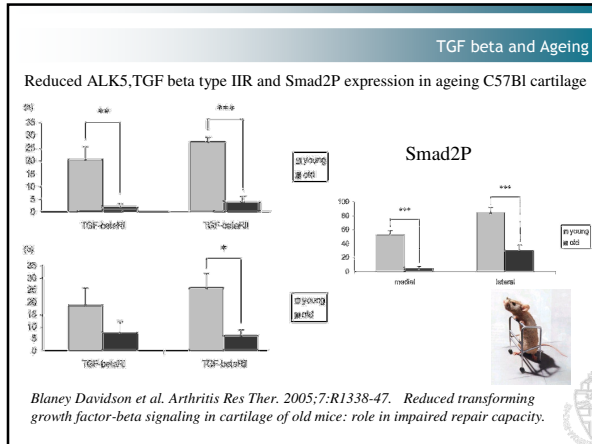
2 Months



4 months



6-12 months



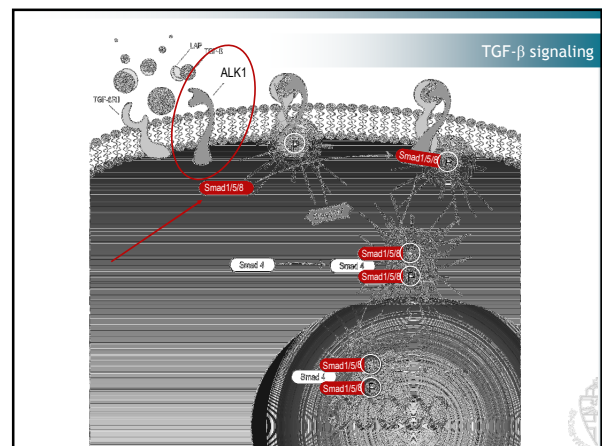
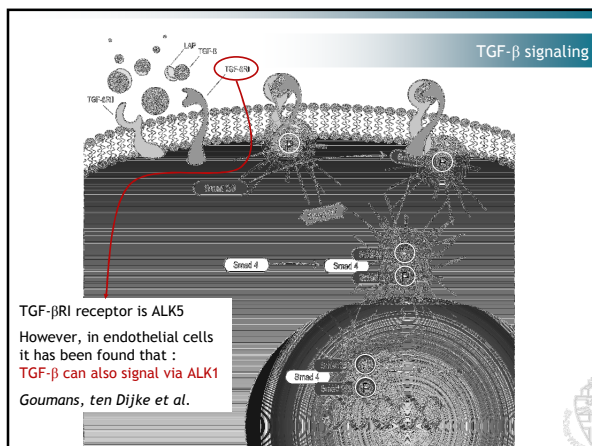
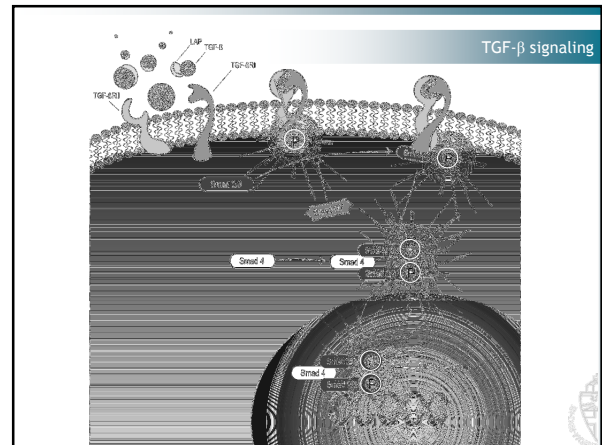
Conclusions (2)

TGF beta counteracts IL-1 effects in cartilage of young mice.

During ageing TGF type I receptor expression (ALK5) and TGF type II receptor is decreased.

Blocking TGF beta activity inhibits cartilage repair in young animals

Ageing > Reduced ALK5 expression > Reduced protective effects



Hypothesis

- During OA reduced TGF- β -signaling via ALK5
- In OA chondrocyte behavior is similar to terminal differentiated chondrocytes \rightarrow strong MMP13 up regulation
- Smad2/3 route (=ALK5) blocks terminal differentiation
- Smad1/5/8 route (=ALK1) stimulates terminal differentiation

\rightarrow During ageing and OA more TGF- β signaling via ALK1 than ALK5?

Chondrocytes can signal via ALK1

TGF beta

TGF- β 1 ng/ml	control	15 min	30 min	2 hours	5 hours
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TGF- β activates both Smad-2P and Smad1/5/8P

Constitutive active ALKs

	control	Ad-caALK1	Ad-caALK5	Ad-LACZ
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ALK1 activated Smad1/5/8, ALK5 activates Smad2(3)

Chondrocytes can signal via ALK1

Chondrocytes can signal via ALK1, thereby activating the Smad1/5/8 pathway.

Are there changes in ALK1 expression with age?
Are there changes in ALK1 expression during OA?

Methods:
Isolate knee joints of young and old C57Bl/6 mice
Stain sections immunohistochemically for ALK5 and ALK1
Score positive cells in tibial cartilage

Are there changes in ALK1 during ageing?

ALK5 Young vs Old

ALK1 Young vs Old

ALK1/ALK5 ratio Young vs Old

ALK5 is downregulated more than ALK1 with age
 \rightarrow Shift in balance towards ALK1 with age

Chondrocytes can signal via ALK1

ALK5 decreases faster than ALK1 with age.

What happens to ALK5 and ALK1 during OA?

Methods:
Isolate knee joints of STR/ort mice aged 3, 6, 9 or 12 months for histology
Stain sections immunohistochemically for ALK5 and ALK1
Score positive cells in tibial cartilage

Are there changes in ALK1 during OA?

STR/ort ALK5

STR/ort ALK1

ALK1/ALK5 ratio STR/ort

ALK5 is already strongly reduced by 3 months of age
ALK1 expression is reduced, but compared to ALK5 still abundantly present
 \rightarrow Strong shift in balance towards ALK1

Downstream alterations

- Both ALK5 and ALK1 are reduced in OA cartilage
- ALK5 decrease is faster than ALK1
- The balance is shifted towards ALK1

ALK5 signaling via Smad2/3 → PAI1 is expressed
 ALK1 signaling via Smad1/5/8 → Id1 is expressed

Is there a change in Id1/PAI1 balance as well?

Downstream alterations

Id1/PAI1 ratio
ST/Coit

medial tibia lateral tibia

Legend: 3 months (black), 6 months (grey), 9 months (white), 1 year (light grey)

Increase in Id1/PAI1 ratio with OA progression

Downstream alterations

What is the functional consequence of signaling via ALK5 or ALK1 in chondrocytes?

Methods

Overexpression of Ad-caALK5 or Ad-caALK1 in chondrocytes
 Inhibition of ALK1 and ALK5 by siRNA

Measure mRNA expression of collagen type II, aggrecan, MMP13

Functional consequence in chondrocytes

Constitutive active ALK1 and ALK5

Legend: Ad-ALK1 (black), Ad-ALK5 (grey)

ALK1 and ALK5 siRNA

Legend: siRNA ALK1 (black), siRNA ALK5 (grey)

Active ALK1 > up regulation of MMP13 !!
 Blocking ALK5 > upregulation of MMP13 !!

From mice to humans

Previous data all in mice...

Is there a correlation between ALK1 expression and MMP13 expression in *human* cartilage?

Methods

Isolate cartilage of patients undergoing joint replacement surgery
 Perform Q-PCR for ALK5, ALK1, Collagen type II, aggrecan, MMP13

ALK1 is correlated with MMP13

Human OA cartilage

	ALK5		ALK1	
	Pearson	p-value	Pearson	p-value
Aggrecan	0.768	0.000 **	0.508	0.053
Collagen II	0.547	0.028 *	0.448	0.082
MMP-13	0.326	0.201	0.548	0.005 *

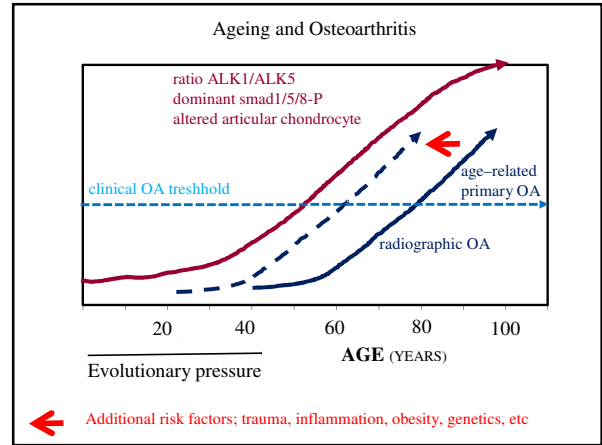
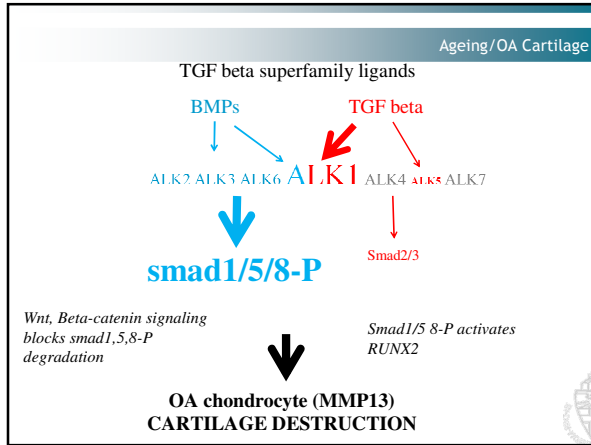
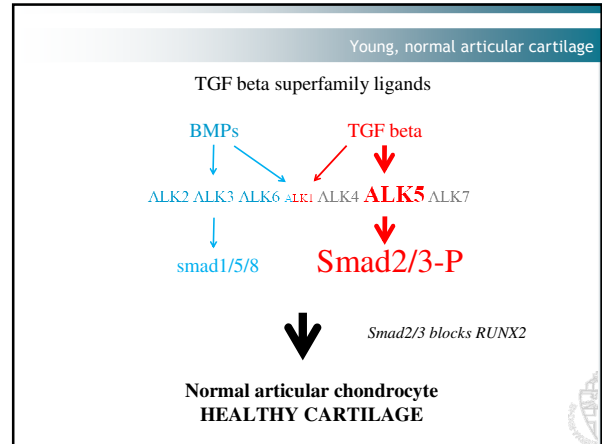
- ALK5 correlates with aggrecan and collagen type II
- ALK1 correlates with MMP13

Conclusions (3)

- TGF- β can signal via ALK1 in chondrocytes (BMP9, BMP10)
- Balance ALK5 and ALK1 expression can determine effect of TGF beta on chondrocytes (*protective, OA induction*)
- Shift in balance from ALK5 to ALK1 with age and OA
- Shift in balance from PAI1 to Id1 during OA progression
- ALK1 overexpression results in MMP13 up regulation
- ALK1 expression correlates with MMP13 expression in human cartilage

Differential ALK5 and ALK1 expression can explain enigmatic, context depending effects of TGF beta

The shift in balance from signaling via Smad2/3 (ALK5) to signaling via Smad1/5/8 (ALK1) is a potential cause for MMP13 up regulation in OA



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