RMD Open

Rheumatic & Musculoskeletal Diseases

REVIEW

Pathophysiology of osteoarthritis: canonical NF-κB/IKKβ-dependent and kinase-independent effects of IKKα in cartilage degradation and chondrocyte differentiation

Eleonora Olivotto,[1] Miguel Otero,[2] Kenneth B Marcu,[3] Mary B Goldring[2]

For numbered affiliations see end of article.

Correspondence to
Professor Mary B Goldring; goldringm@hss.edu

ABSTRACT

Osteoarthritis (OA), a whole-joint disease driven by abnormal biomechanics and attendant cell-derived and tissue-derived factors, is a rheumatic disease with the highest prevalence, representing a severe health burden with a tremendous economic impact. Members of the nuclear factor κB (NF-κB) family orchestrate mechanical, inflammatory and oxidative stress-activated processes, thus representing a potential therapeutic target in OA disease. The two pivotal kinases, IκB kinase (IKK) α and IKKβ, activate NF-κB dimers that might translocate to the nucleus and regulate the expression of specific target genes involved in extracellular matrix remodelling and terminal differentiation of chondrocytes. IKKα, required for the activation of the so-called non-canonical pathway, has a number of NF-κB-independent and kinase-independent functions in vivo and in vitro, including controlling chondrocyte hypertrophic differentiation and collagenase activity. In this short review, we will discuss the role of NF-κB signalling in OA pathology, with emphasis on the functional effects of IKKα that are independent of its kinase activity and NF-κB activation.

INTRODUCTION

Osteoarthritis (OA) is the most common joint disease and the major cause of disability in the adult population; with annual costs of knee OA being immense, this continues to be a severe health burden with respect to morbidity and expense. Age is the primary OA risk factor, and ageing-related changes also contribute to pathophysiological changes triggering OA disease. In addition, individuals with other specific OA risk factors, including obesity, altered joint mechanical loading, joint injury and inflammation, as well as genetic components,[1] may undergo an accelerated rate of changes that are similar to those associated with ageing.[2]

Although cartilage destruction is the hallmark of OA, and collagen erosion is the pivotal event that determines the irreversible progression of OA disease, it is now well established that OA is not only a disorder of cartilage homeostasis but is a whole-joint disorder involving all joint tissues, including the subchondral bone, menisci and synovial membrane.[3–5] In spite of recent advances, the mechanisms leading to cartilage destruction in patients with OA are still not clearly identified and no successful therapeutic intervention exists. This brief review elaborates on how nuclear factor (NF-κB) signalling contributes to OA pathophysiology, not only by modulating stress-related processes, but also by the hypertrophic-like conversion of OA chondrocytes.

CARTILAGE DEGRADATION IN OA DISEASE: PHENOTYPIC SHIFT OF ARTICULAR CHONDROCYTES

The principal function of articular cartilage is to adjust to biomechanical forces during joint movement; a function mediated by the extracellular matrix (ECM). Cartilage homeostasis is defined by normal cartilage ECM compensating for mechanical stress without structural or cellular damage. The ECM is produced and maintained by articular chondrocytes, the unique cell type residing in articular cartilage, which are essential for maintaining structural and functional integrity of the cartilage.[6] Mechanical, oxidative and inflammatory stresses activate signal transduction pathways in cartilage, which disseminate a phenotypic shift characterised by the release of the chondrocyte from growth arrest, imbalanced homeostasis,

hypertrophic-like conversion and aberrant expression of proinflammatory and catabolic genes.[6] As a result, OA chondrocytes are unable to maintain tissue homeostasis and fail to replace ECM, especially collagen, once it is degraded by metalloproteinases (MMPs), in particular by MMP-13, the major MMP responsible for remodelling type II collagen in cartilage tissue.[7–9] Characteristic features of OA chondrocytes in ageing and inflammatory models include prominent epigenomic alterations[10 11] leading to deregulated gene expression, exacerbated and sustained NF-κB activation, and MMP production, all of which can be modelled in vitro by interleukin 1 (IL-1β) stimulation.[12]

The NF-κB pathway is most prominent among the different gene signatures in human disease and different experimental OA models, suggesting a key regulatory role for stress and inflammatory signalling via the canonical NF-κB pathway in OA pathology.[6 12–14] Abnormal NF-κB activation provokes the loss of the growth-arrested state of articular chondrocytes, accompanied by the production of procatabolic mediators, including aggrecanases and MMPs that induce cartilage degradation, and the proinflammatory cytokines (IL-1β and tumor necrosis factor α,TNF-α) that induce them. Further, continued NF-κB activation results in the overexpression and activation of other regulatory transcription factors, including E74-like factor 3 (ELF3) and endothelial PAS domain protein 1 (hypoxia inducible factor 2 alpha) (HIF-2α), which, in turn, further perpetuate OA disease via modulation of inflammatory and catabolic mediators,[15–17] and by linking hypertrophic-like conversion with inflammation.[18 19] Together, these multilayered signalling networks collaborate to activate MMP13 expression and activity, and facilitate the progression of normal articular chondrocytes to a hypertrophic-like OA phenotype in vivo, thereby also contributing to OA onset and/or progression.[6]

Synovitis is a feature of the OA joint, in which the activated canonical NF-κB subunits upregulate the expression of chemokines (IL-8, chemokine (C-C motif) ligand 5 (CCL5)), cytokines (receptor activator of nuclear factor kappa-B ligand (RANKL), IL-1β, IL-6, TNF-α), cyclooxygenase 2 (COX2) and angiogenic factors (vascular endothelial growth factor, fibroblast growth factor 2).[4 5] The homeostasis of the subchondral bone involves a balance between bone resorption, mediated by the receptor activator of nuclear factor kappa B (RANK)/RANKL signalling pathway that activates NF-κB transcription factors (inducing the expression of IL-1β, IL-6, prostaglandin E2 (PGE2)) and bone formation, controlled by osteoprotegerin (OPG). During OA, homeostasis is destroyed and the OPG/RANK/RANKL signalling pathway is deregulated.[14]

CONTRIBUTION OF THE NF-κB PATHWAY TO CARTILAGE DEGRADATION AND OA PATHOLOGY

NF-κB-mediated transcriptional control arises from the assembly of homodimers and heterodimers of five

different NF-κB proteins.[20] NF-κB dimers are sequestered in the cytoplasm with their transcriptional activities blocked by inhibitory IκB proteins. The two pivotal kinases IKKα and IKKβ initiate the release of active NF-κBs from IκBs, so that specific NF-κB dimers might translocate to the nucleus and regulate the expression of specific target genes. In response to a host of proinflammatory and stress-like stimuli, IKKβ is the dominant acting IκBα kinase in vivo, controlling the so-called canonical pathway.[20] However, IKKα is specifically required for the activation of the non-canonical, or alternate, NF-κB pathway, and has a number of in vivo functions as a serine–threonine kinase acting independently of NF-κB signalling (figure 1).[12 21]

In chondrocytes, IKKα and IKKβ have different functional roles in ECM remodelling and endochondral ossification, which are also developmental events recapitulated to a certain extent by hypertrophic-like chondrocytes in OA disease.[22–24] Furthermore, in activated OA chondrocytes, the canonical NF-κB pathway is an orchestrator of gene expression programmes leading to the production of catabolic enzymes, cytokines and inflammatory mediators,[12 25] and it is believed to play pivotal roles in OA disease by coordinating a complex, multilayered signalling network. Table 1 summarises some of the events controlled by IKKα and IKKβ in OA disease.

CONTROL OF INFLAMMATORY/STRESS RESPONSES BY THE CANONICAL NF-κB PATHWAY

The IKKβ-driven, canonical NF-κB pathway signalling also has effects on downstream regulators of terminal chondrocyte differentiation (including HIF-2α, β-catenin and Runx2), thus linking inflammatory and oxidative stress responses, with phenotypic and functional changes in OA chondrocytes. These events further deregulate chondrocyte homeostasis and normal function (reviewed[6 12]). In OA chondrocytes, canonical NF-κB signalling acts as a sensor of exogenous and endogenous mechanical stress and inflammatory insults, and thereby coordinates the expression and activation of an abnormal cartilage catabolic pathway leading to the onset of OA disease (see reviews[6 12]), which also includes modulation of mitogen-activated protein kinase signalling via transcriptional control of GADD45β.[44–46] In addition, NF-κB signalling plays a central role in disease progression and perpetuation, mediating a cascade of inflammatory responses triggered by advance glycation end products, Toll-like receptor ligands, or released ECM products, including fibronectin fragments, which lead to the continued expression of MMPs, aggrecanases, inflammatory cytokines and chemokines, and to an abnormal differentiation status.[26–30 33–41 47]

Among the downstream effectors of IKKβ-driven NF-κB signalling that play a central role in OA disease by coordinating a complex, multilayered signalling network, is HIF-2α (for review, see ref 12). HIF-2α has been shown to have central roles in OA disease by
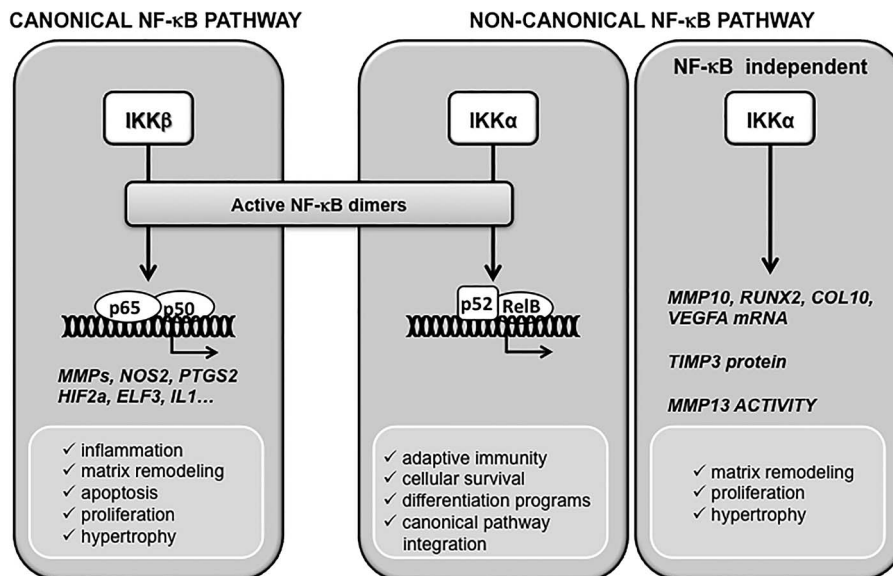
Figure 1 Contribution of IKKα and IKKβ to chondrocyte pathophysiology. IKKβ and IKKα, the activating kinases of the canonical and non-canonical NF-κB pathways, respectively, contribute to maintain normal chondrocyte homeostasis and are major players in cartilage pathology. The IKKβ-controled canonical NF-κB signaling orchestrates most stress/inflammatory responses and modulates, among others, hypertrophy or matrix remodeling, either directly or via downstream mediators including HIF2α or ELF3. The non-canonical NFκB signaling, controlled by IKKα, mediates adaptive immunity and participates in cell survival and differentiation processess in different cell types. In chondrocytes, IKKα mediates chondrocyte hypertrophic differentiation and MMP13-driven collagenase activity in a kinase-independent manner in vitro. These processes have implications in osteoarthritis disease, where abnormal chondrocyte phenotype, enhanced collagenase activity, and the imbalance between anabolism and catabolism, lead to irreversible extracellular matrix degradation and cartilage destruction.

interconnecting inflammatory ECM degradative processes with chondrocyte hypertrophic conversion due to its control of genes involved in endochondral ossification[18] and inflammatory factors,[19] and its ability to trigger MMP13 via concerted actions between CCAAT-enhancer-binding protein beta (C/EBPβ) and RUNX2.[32] ELF3 is another direct NF-κB target with central roles in cartilage catabolism and OA disease. ELF3 is an epithelium-specific member of the E26

transformation-specific sequence family of transcription factors[49] with functional roles in epithelial cell differentiation, apoptosis and gut development.[49–51] More recently, ELF3 has been shown to participate in a feedback loop with NF-κB signalling, by constitutively activating and stabilising canonical NF-κB in prostate cancer.[52] In response to stress/inflammatory stimuli and depending, at least in part, on canonical NF-κB (p65/p50) signalling, ELF3 is induced in different tissues and cell

Table 1 Summary of the contribution of IKKα and IKKβ to chondrocyte catabolism and cartilage degradative processes

Contribution of IKKα and IKKβ to chondrocyte catabolism and cartilage degradation	References
Controls MMP, PGE2, COL10 and NO expression induced by RAGE	26–28
Participates in the TLR-driven MMP expression and NO production	29, 30
Regulates the expression of mediators of inflammation: ELF3, NOS2, IL-1, COX2	5, 15, 16, 25, 31
Links inflammation and hypertrophic-like conversion via HIF-2α, RUNX2, C/EBPβ	18, 19, 32
Perpetuates cartilage damage modulating signals driven by ECM products	33–38
Triggers inappropriate differentiation via IL-8 and GROα modulation	39–41
Decreases SOX9 expression and activity leading to abnormal matrix production and phenotype	42 43
Controls MAPK signalling, ECM remodelling, apoptosis and hypertrophy via GADD45β	44–46
Controls aggrecanase activity (ADAMTS5 expression) during OA development	47
Modulates collagenase activity via IKKα-mediated control of MMP10 and TIMP3*	42, 48
Hypertrophic-like conversion via IKKα control of RUNX2, COL10 and VEGF-A expression*	42, 48

*Indicates contribution of IKKα (via kinase-independent actions).
C/EBPβ, CCAAT-enhancer-binding protein beta; ECM, extracellular matrix; ELF3, E74-like factor 3; GROα, chemokine (C-X-C motif) ligand 1; HIF-2α, endothelial PAS domain protein 1 (hypoxia inducible factor 2 alpha); IKKα, IκB kinase alpha; IL-1, interleukin 1; MAPK, mitogen-activated protein kinase; MMP, metalloproteinases; NOS2, nitric oxide synthase 2, inducible; OA, Osteoarthritis; RAGE, advanced glycosylation end product-specific receptor; SOX9, SRY (sex determining region Y)-box 9; TIMP3, tissue inhibitor of MMP 3; TLR, toll-like receptor; VEGF-A, vascular endothelial growth factor A.

types, where it mediates inflammation by controlling the expression of nitric oxide synthase 2 (NOS2) or COX2, among other targets.[31 53] In chondrocytes, ELF3 expression is increased in OA cartilage and induced in vitro by IL-1β, thereby contributing to the IL-1β-mediated repression of the collagen, type II, alpha 1 (COL2A1) promoter[16] and the activation of MMP13 transcription.[15]

NF-κB-INDEPENDENT ACTIONS OF IKKα IN ARTICULAR CHONDROCYTES

During OA, chondrocytes undergo phenotypic modulation, and display increased expression and activities of matrix-degrading enzymes, and abnormal production of matrix structural proteins, including type X collagen. Importantly, factors that drive chondrocyte hypertrophy, including Notch signalling, indian hedgehog (IHH), RUNX2 and HIF-2α, have all been implicated in OA disease.[12 18 19 32 54–58] The knockdown (KD) of either IKKα or IKKβ in three-dimensional cultures of human articular OA chondrocytes revealed essential and differential roles for IKKα and IKKβ on ECM remodelling and terminal differentiation.[42] While the contribution of IKKβ predominantly involved downregulation of SRY (sex determining region Y)-box 9 (SOX9) expression[42] and activity,[43] surprisingly, IKKα KD led to a more pronounced alteration of the hypertrophic-like conversion of articular chondrocytes. The KD of IKKα (IKKα KD) dramatically stabilised the ECM, enhanced cell viability and strongly suppressed chondrocyte differentiation towards a hypertrophic-like state. Importantly, while IKKα loss did not modify MMP-13 protein levels, it did inhibit collagenase activity, as revealed by the markedly suppressed accumulation of type II collagen fragments containing the 3/4C neo-epitope (COL2–3/4C).[42] Importantly, the functional effects of IKKα on ECM remodelling, and subsequent aspects of chondrocyte differentiation towards a hypertrophic-like state, are evolutionarily conserved between human OA chondrocytes and immature murine articular chondrocytes, indicating that these properties of IKKα are intrinsic to chondrocytes and independent of other acquired phenotypes specifically linked to OA-affected cartilage.[48] IKKα does not regulate MMP-13 gene expression, but positively modulates ECM remodelling by directly upregulating the messenger RNA (mRNA) encoding the procollagenase activator MMP-10 and by post-transcriptionally suppressing TIMP (tissue inhibitor of MMP) 3 protein levels. Together, these processes maintain maximal MMP-13 activity, which is required for ECM remodelling leading to chondrocyte differentiation. Thus, IKKα directly modulates total collagenase and MMP-13 activities both in human OA and in differentiating primary murine chondrocytes.

Previous evidence revealed that IKKα is essential for keratinocyte differentiation in murine embryonic development,[59 60] but independent of NF-κB activation and its kinase activity.[61] The abnormal skeletal development of IKKα knockout (KO) mice was due to failed epidermal differentiation, which disrupted normal epidermal–mesodermal interactions.[62] Subsequent molecular analysis uncovered the role of IKKα as a critical regulator of Smad4-independent TGFβ-Smad2/3 signalling that directly induces c-Myc antagonists, which are required for terminal epidermal cell differentiation.[63] Interestingly, we also found that kinase-independent functions of IKKα are required for primary chondrocyte differentiation towards hypertrophy.[48] Thus, rescuing IKKα expression in both IKKα KD human OA chondrocytes and IKKα KO murine chondrocytes using viral transduction of murine wild type IKKα or a recombinant IKKα kinase-dead mutant, IKKα(K44M), fully restored collagenase activity and the ability of articular chondrocytes to differentiate towards hypertrophy.[48] Detailed analyses revealed that the enforced expression of a kinase-dead IKKα mutant (even at physiological protein levels) completely rescued the expression of markers of hypertrophy, including Runx2 and Col10a1, to wild-type levels. Importantly, the kinase-dead mutant also reversed the accumulation of TIMP-3 in IKKα KD human OA chondrocytes and the suppression of MMP10 mRNA and protein levels in IKKα KO murine chondrocytes, both of which were associated with the recovery of type II collagen remodelling in these primary articular chondrocytes. Taken together, these observations represent strong evidence that the functional effects of IKKα in chondrocytes are independent of its serine–threonine kinase activity and thus also independent of the IKKα-dependent non-canonical NF-κB signalling pathway.[48] Interestingly, TGFβ-Smad2/3 signalling has also been reported to positively regulate MMP-10 transcription in breast cancer epithelial cells, but a role for IKKα in this context was not determined.[64] Future work will, in part, determine if IKKα, in a manner independent of its kinase activity, operates via TGFβ-dependent Smad2/3 signalling to activate MMP-10 transcription and simultaneously suppresses TIMP-3 levels in differentiating chondrocytes.

CONCLUSION

A better understanding of the mechanisms involved in the initiation and progression of OA disease stands essential for the development of effective, specific and successful treatment options for the disease, as well for the identification of predictive biomarkers that allow us to identify early stages of disease or individuals at high risk of developing OA. The IKKβ-driven canonical NF-κB signalling pathway coordinates mechanical, inflammatory and oxidative stress-activated events leading normal chondrocytes to become activated and hypertrophic-like. In vitro experiments with primary chondrocytes indicate that IKKα also functions as a positive mediator of this process independent of its kinase activity. Thus, controlling the mechanisms of action of IKKα or IKKβ holds potential for the development of

therapeutic strategies to attenuate the onset and/or progression of OA disease pathology.

Novel strategies are being developed by selective inhibition of key molecules in the NF-κB signalling, in particular the canonical activation of RelA/p65 (by using small interfering RNA targeted to the RelA/p65 mRNA) or p50 (via annexin A4 modulatory effects).[14] Indeed, non-specific therapeutic approaches that affect the NF-κB pathway are available, such as non-steroidal anti-inflammatory drugs, glucocorticoids, glucosamine, thalidomide and nutriceuticals, such as curcumin and sirtuin,[65] as well as, potentially, anticytokine therapies such as dual acting anti-IL-1β/α, IL-1 receptor antagonists and anti-TNF-α. Novel strategies are being developed by selective inhibition of key molecules in the canonical NF-κB pathway. One in vitro study using human primary chondrocytes showed that NAPA (2-(N-acetyl-L-phenylalanylamido-2-deoxy-β-D-glucose), a derivative of glucosamine, inhibited IKKα kinase activity and its nuclear translocation.[66] However, it remains to be demonstrated that NAPA does not also inhibit the nuclear translocation of specific transcription factors required for chondrocyte differentiation or other factors involved in collagen synthesis or remodelling. Importantly, there is no in vivo study linking NF-κB and IKKs to OA. Therefore, we are currently working to validate our in vitro observations in inductive OA disease models in mice to elucidate the mechanisms of action by which IKKβ and IKKα differentially drive pathways that impact on chondrocyte homeostasis to contribute to OA disease onset and/or progression.

Author affiliations
[1]Laboratory RAMSES—Research, Innovation & Technology Department, Rizzoli Orthopedic Research Institute, Bologna, Italy
[2]Research Division, Hospital for Special Surgery and Weill Cornell Medical College, New York, USA
[3]Biochemistry and Cell Biology Department, Stony Brook University, Stony Brook, USA

Competing interests None declared.

Provenance and peer review Commissioned; externally peer reviewed.

Data sharing statement No additional data are available.

REFERENCES

1. Sandell LJ. Etiology of osteoarthritis: genetics and synovial joint development. *Nat Rev Rheumatol* 2012;8:77–89.
2. Lotz M, Loeser RF. Effects of aging on articular cartilage homeostasis. *Bone* 2012;51:241–8.
3. Heinegard D, Saxne T. The role of the cartilage matrix in osteoarthritis. *Nat Rev Rheumatol* 2011;7:50–6.
4. Loeser RF, Goldring SR, Scanzello CR, *et al*. Osteoarthritis: a disease of the joint as an organ. *Arthritis Rheum* 2012;64:1697–707.
5. Scanzello CR, Goldring SR. The role of synovitis in osteoarthritis pathogenesis. *Bone* 2012;51:249–57.
6. Goldring MB, Otero M. Inflammation in osteoarthritis. *Curr Opin Rheumatol* 2011;23:471–8.
7. Knauper V, Lopez-Otin C, Smith B, *et al*. Biochemical characterization of human collagenase-3. *J Biol Chem* 1996;271:1544–50.
8. Mitchell PG, Magna HA, Reeves LM, *et al*. Cloning, expression, and type II collagenolytic activity of matrix metalloproteinase-13 from human osteoarthritic cartilage. *J Clin Invest* 1996;97:761–8.
9. Reboul P, Pelletier JP, Tardif G, *et al*. The new collagenase, collagenase-3, is expressed and synthesized by human chondrocytes but not by synoviocytes. A role in osteoarthritis. *J Clin Invest* 1996;97:2011–19.
10. Goldring MB, Marcu KB. Epigenomic and microRNA-mediated regulation in cartilage development, homeostasis, and osteoarthritis. *Trends Mol Med* 2012;18:109–18.
11. Loughlin J, Reynard LN. Osteoarthritis: epigenetics of articular cartilage in knee and hip OA. *Nat Rev Rheumatol* 2015;11:6–7.
12. Marcu KB, Otero M, Olivotto E, *et al*. NF-kappaB signaling: multiple angles to target OA. *Curr Drug Targets* 2010;11:599–613.
13. Poulet B, Ulici V, Stone TC, *et al*. Time-series transcriptional profiling yields new perspectives on susceptibility to murine osteoarthritis. *Arthritis Rheum* 2012;64:3256–66.
14. Rigoglou S, Papavassiliou AG. The NF-kappaB signalling pathway in osteoarthritis. *Int J Biochem Cell Biol* 2013;45:2580–4.
15. Otero M, Plumb DA, Tsuchimochi K, *et al*. E74-like factor 3 (ELF3) impacts on matrix metalloproteinase 13 (MMP13) transcriptional control in articular chondrocytes under proinflammatory stress. *J Biol Chem* 2012;287:3559–72.
16. Peng H, Tan L, Osaki M, *et al*. ESE-1 is a potent repressor of type II collagen gene (COL2A1) transcription in human chondrocytes. *J Cell Physiol* 2008;215:562–73.
17. Hashimoto K, Otero M, Imagawa K, *et al*. Regulated transcription of human matrix metalloproteinase 13 (MMP13) and interleukin-1beta (IL1B) genes in chondrocytes depends on methylation of specific proximal promoter CpG sites. *J Biol Chem* 2013;288:10061–72.
18. Saito T, Fukai A, Mabuchi A, *et al*. Transcriptional regulation of endochondral ossification by HIF-2alpha during skeletal growth and osteoarthritis development. *Nat Med* 2010;16:678–86.
19. Yang S, Kim J, Ryu JH, *et al*. Hypoxia-inducible factor-2alpha is a catabolic regulator of osteoarthritic cartilage destruction. *Nat Med* 2010;16:687–93.
20. Hoffmann A, Natoli G, Ghosh G. Transcriptional regulation via the NF-kappaB signaling module. *Oncogene* 2006;25:6706–16.
21. Perkins ND. Integrating cell-signalling pathways with NF-kappaB and IKK function. *Nat Rev Mol Cell Biol* 2007;8:49–62.
22. Goldring MB, Marcu KB. Cartilage homeostasis in health and rheumatic diseases. *Arthritis Res Ther* 2009;11:224.
23. Tchetina EV, Squires G, Poole AR. Increased type II collagen degradation and very early focal cartilage degeneration is associated with upregulation of chondrocyte differentiation related genes in early human articular cartilage lesions. *J Rheumatol* 2005;32:876–86.
24. van der Kraan PM, van den Berg WB. Chondrocyte hypertrophy and osteoarthritis: role in initiation and progression of cartilage degeneration? *Osteoarthritis Cartilage* 2012;20:223–32.
25. Roman-Blas JA, Jimenez SA. NF-kappaB as a potential therapeutic target in osteoarthritis and rheumatoid arthritis. *Osteoarthritis Cartilage* 2006;14:839–48.
26. Cecil DL, Johnson K, Rediske J, *et al*. Inflammation-induced chondrocyte hypertrophy is driven by receptor for advanced glycation end products. *J Immunol* 2005;175:8296–302.
27. DeGroot J, Verzijl N, Wenting-van Wijk MJ, *et al*. Accumulation of advanced glycation end products as a molecular mechanism for aging as a risk factor in osteoarthritis. *Arthritis Rheum* 2004;50:1207–15.
28. Yammani RR, Carlson CS, Bresnick AR, *et al*. Increase in production of matrix metalloproteinase 13 by human articular chondrocytes due to stimulation with S100A4: Role of the receptor for advanced glycation end products. *Arthritis Rheum* 2006;54:2901–11.
29. Kim HA, Cho ML, Choi HY, *et al*. The catabolic pathway mediated by Toll-like receptors in human osteoarthritic chondrocytes. *Arthritis Rheum* 2006;54:2152–63.
30. Zhang Q, Hui W, Litherland GJ, *et al*. Differential Toll-like receptor-dependent collagenase expression in chondrocytes. *Ann Rheum Dis* 2008;67:1633–41.

31. Grall F, Gu X, Tan L, *et al*. Responses to the proinflammatory cytokines interleukin-1 and tumor necrosis factor alpha in cells derived from rheumatoid synovium and other joint tissues involve nuclear factor kappaB-mediated induction of the Ets transcription factor ESE-1. *Arthritis Rheum* 2003;48:1249–60.

32. Hirata M, Kugimiya F, Fukai A, *et al*. C/EBPbeta and RUNX2 cooperate to degrade cartilage with MMP-13 as the target and HIF-2alpha as the inducer in chondrocytes. *Hum Mol Genet* 2012;21:1111–23.

33. Ding L, Guo D, Homandberg GA. Fibronectin fragments mediate matrix metalloproteinase upregulation and cartilage damage through proline rich tyrosine kinase 2, c-src, NF-kappaB and protein kinase Cdelta. *Osteoarthritis Cartilage* 2009;17:1385–92.

34. Forsyth CB, Pulai J, Loeser RF. Fibronectin fragments and blocking antibodies to alpha2beta1 and alpha5beta1 integrins stimulate mitogen-activated protein kinase signaling and increase collagenase 3 (matrix metalloproteinase 13) production by human articular chondrocytes. *Arthritis Rheum* 2002;46:2368–76.

35. Loeser RF, Forsyth CB, Samarel AM, *et al*. Fibronectin fragment activation of proline-rich tyrosine kinase PYK2 mediates integrin signals regulating collagenase-3 expression by human chondrocytes through a protein kinase C-dependent pathway. *J Biol Chem* 2003;278:24577–85.

36. Pulai JI, Chen H, Im HJ, *et al*. NF-kappa B mediates the stimulation of cytokine and chemokine expression by human articular chondrocytes in response to fibronectin fragments. *J Immunol* 2005;174:5781–8.

37. Saito S, Yamaji N, Yasunaga K, *et al*. The fibronectin extra domain A activates matrix metalloproteinase gene expression by an interleukin-1-dependent mechanism. *J Biol Chem* 1999;274:30756–63.

38. Stanton H, Ung L, Fosang AJ. The 45 kDa collagen-binding fragment of fibronectin induces matrix metalloproteinase-13 synthesis by chondrocytes and aggrecan degradation by aggrecanases. *Biochem J* 2002;364(Pt 1):181–90.

39. Borzi RM, Mazzetti I, Macor S, *et al*. Flow cytometric analysis of intracellular chemokines in chondrocytes in vivo: constitutive expression and enhancement in osteoarthritis and rheumatoid arthritis. *FEBS Lett* 1999;455:238–42.

40. Merz D, Liu R, Johnson K, *et al*. IL-8/CXCL8 and growth-related oncogene alpha/CXCL1 induce chondrocyte hypertrophic differentiation. *J Immunol* 2003;171:4406–15.

41. Sandell LJ, Xing X, Franz C, *et al*. Exuberant expression of chemokine genes by adult human articular chondrocytes in response to IL-1beta. *Osteoarthritis Cartilage* 2008;16:1560–71.

42. Olivotto E, Borzi RM, Vitellozzi R, *et al*. Differential requirements for IKKalpha and IKKbeta in the differentiation of primary human osteoarthritic chondrocytes. *Arthritis Rheum* 2008;58:227–39.

43. Rockel JS, Kudirka JC, Guzi AJ, *et al*. Regulation of Sox9 activity by crosstalk with nuclear factor-kappaB and retinoic acid receptors. *Arthritis Res Ther* 2008;10:R3.

44. Ijiri K, Zerbini LF, Peng H, *et al*. A novel role for GADD45beta as a mediator of MMP-13 gene expression during chondrocyte terminal differentiation. *J Biol Chem* 2005;280:38544–55.

45. Ijiri K, Zerbini LF, Peng H, *et al*. Differential expression of GADD45beta in normal and osteoarthritic cartilage: potential role in homeostasis of articular chondrocytes. *Arthritis Rheum* 2008;58:2075–87.

46. Tsuchimochi K, Otero M, Dragomir CL, *et al*. GADD45beta enhances Col10a1 transcription via the MTK1/MKK3/6/p38 axis and activation of C/EBPbeta-TAD4 in terminally differentiating chondrocytes. *J Biol Chem* 2010;285:8395–407.

47. Kobayashi H, Hirata M, Saito T, *et al*. Transcriptional induction of ADAMTS5 protein by nuclear factor-kappaB (NF-kappaB) family

48. Olivotto E, Otero M, Astolfi A, *et al*. IKKalpha/CHUK regulates extracellular matrix remodeling independent of its kinase activity to facilitate articular chondrocyte differentiation. *PLoS ONE* 2013;8:e73024.

49. Oettgen P, Alani RM, Barcinski MA, *et al*. Isolation and characterization of a novel epithelium-specific transcription factor, ESE-1, a member of the ets family. *Mol Cell Biol* 1997;17:4419–33.

50. Cabral A, Fischer DF, Vermeij WP, *et al*. Distinct functional interactions of human Skn-1 isoforms with Ese-1 during keratinocyte terminal differentiation. *J Biol Chem* 2003;278:17792–9.

51. Reddy SP, Vuong H, Adiseshaiah P. Interplay between proximal and distal promoter elements is required for squamous differentiation marker induction in the bronchial epithelium: role for ESE-1, Sp1, and AP-1 proteins. *J Biol Chem* 2003;278:21378–87.

52. Longoni N, Sarti M, Albino D, *et al*. ETS transcription factor ESE1/ELF3 orchestrates a positive feedback loop that constitutively activates NF-kappaB and drives prostate cancer progression. *Cancer Res* 2013;73:4533–47.

53. Rudders S, Gaspar J, Madore R, *et al*. ESE-1 is a novel transcriptional mediator of inflammation that interacts with NF-kappa B to regulate the inducible nitric-oxide synthase gene. *J Biol Chem* 2001;276:3302–9.

54. Hosaka Y, Saito T, Sugita S, *et al*. Notch signaling in chondrocytes modulates endochondral ossification and osteoarthritis development. *Proc Natl Acad Sci U S A* 2013;110:1875–80.

55. Kamekura S, Kawasaki Y, Hoshi K, *et al*. Contribution of runt-related transcription factor 2 to the pathogenesis of osteoarthritis in mice after induction of knee joint instability. *Arthritis Rheum* 2006;54:2462–70.

56. Wei F, Zhou J, Wei X, *et al*. Activation of Indian hedgehog promotes chondrocyte hypertrophy and upregulation of MMP-13 in human osteoarthritic cartilage. *Osteoarthritis Cartilage* 2012;20:755–63.

57. Ruan MZ, Erez A, Guse K, *et al*. Proteoglycan 4 expression protects against the development of osteoarthritis. *Sci Transl Med* 2013;5:176ra34.

58. Zhou J, Chen Q, Lanske B, *et al*. Disrupting the Indian hedgehog signaling pathway in vivo attenuates surgically induced osteoarthritis progression in Col2a1-CreERT2; Ihhfl/fl mice. *Arthritis Res Ther* 2014;16:R11.

59. Hu Y, Baud V, Delhase M, *et al*. Abnormal morphogenesis but intact IKK activation in mice lacking the IKKalpha subunit of IkappaB kinase. *Science* 1999;284:316–20.

60. Li Q, Lu Q, Hwang JY, *et al*. IKK1-deficient mice exhibit abnormal development of skin and skeleton. *Genes Dev* 1999;13:1322–8.

61. Hu Y, Baud V, Oga T, *et al*. IKKalpha controls formation of the epidermis independently of NF-kappaB. *Nature* 2001;410:710–14.

62. Sil AK, Maeda S, Sano Y, *et al*. IkappaB kinase-alpha acts in the epidermis to control skeletal and craniofacial morphogenesis. *Nature* 2004;428:660–4.

63. Descargues P, Sil AK, Sano Y, *et al*. IKKalpha is a critical coregulator of a Smad4-independent TGFbeta-Smad2/3 signaling pathway that controls keratinocyte differentiation. *Proc Natl Acad Sci USA* 2008;105:2487–92.

64. Sundqvist A, Zieba A, Vasilaki E, *et al*. Specific interactions between Smad proteins and AP-1 components determine TGFbeta-induced breast cancer cell invasion. *Oncogene* 2013;32:3606–15.

65. Mobasheri A, Henrotin Y, Biesalski HK, *et al*. Scientific evidence and rationale for the development of curcumin and resveratrol as nutraceuticals for joint health. *Int J Mol Sci* 2012;13:4202–32.

66. Scotto d'Abusco A, Politi L, Giordano C, *et al*. A peptidyl-glucosamine derivative affects IKKalpha kinase activity in human chondrocytes. *Arthritis Res Ther* 2010;12:R18.