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Defining the roles of inflammatory and anabolic cytokines in cartilage metabolism

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Abstract

In osteoarthritis (OA), adult articular chondrocytes undergo phenotypic modulation in response to alterations in the environment owing to mechanical injury and inflammation. These processes not only stimulate the production of enzymes that degrade the cartilage matrix but also inhibit repair. With the use of in vitro and in vivo models, new genes, not known previously to act in cartilage, have been identified and their roles in chondrocyte differentiation during development and in dysregulated chondrocyte function in OA have been examined. These new genes include growth arrest and DNA damage (GADD)45 β and the epithelial-specific ETS (ESE)-1 transcription factor, induced by bone morpho-genetic protein (BMP)-2 and inflammatory cytokines, respectively. Both genes are induced by NF- κ B, suppress COL2A1 and upregulate matrix metalloproteinase-13 (MMP-13) expression. These genes have also been examined in mouse models of OA, in which discoidin domain receptor 2 is associated with MMP-13-mediated remodelling, in order to understand their roles in physiological cartilage homeostasis and joint disease.

EXTRACELLULAR MATRIX OF ADULT ARTICULAR CARTILAGE AND ALTERATIONS DUE TO OSTEOARTHRITIS (OA)

Osteoarthritis (OA) is a chronic disease characterised by slowly progressing destruction of the articular cartilage, accompanied by changes in synovium and subchondral bone.¹² The cellular component of the cartilage is the chondrocyte, a specialised mesenchymal cell that synthesises matrix proteins such as collagens and proteoglycans, which are responsible for the tensile strength and compressive resistance, respectively, to mechanical loading.³ In adult articular cartilage, the chondrocytes maintain a low turnover rate of replacement of cartilage matrix proteins. The turnover of collagen has been estimated to occur with a half-life of >100 years,⁴ whereas the glycosaminoglycan constituents on the aggrecan core protein are more readily replaced and the half-life of aggrecan subfractions has been estimated to be in the range of 3–24 years.⁵

The adult articular chondrocyte, although quiescent in normal cartilage, can respond to mechanical injury, joint instability due to genetic factors and biological stimuli such as cytokines and growth and differentiation factors.¹⁶ In young subjects without genetic

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abnormalities, biomechanical factors due to trauma are strongly implicated in initiating the OA lesion.^{7–9} Mechanical disruption of cell–matrix interactions may lead to aberrant chondrocyte behaviour that is reflected in the appearance of fibrillations, cell clusters and changes in quantity, distribution or composition of matrix proteins.¹⁰ In the early stages of OA, a transient increase in chondrocyte proliferation and increased metabolic activity are associated with a local loss of proteoglycans occurring initially at the cartilage surface and followed by cleavage of type II collagen.^{11,12} This results in increased water content and decreased tensile strength of the matrix as the lesion progresses.

A number of studies have demonstrated enhanced biosynthesis and increased global gene expression of aggrecan and type II collagen in human OA compared with normal cartilage.^{13,14} The increased levels of factors such as bone morphogenetic protein-2 (BMP-2) and inhibin bA/activin suggest a possible mechanism for the anabolic response.^{12,14–16} However, controversy exists, based on studies in animal models^{17–19} and analysis of cartilage samples or body fluids from patients with OA,^{20–22} about whether type II collagen gene (*COL2A1*) expression is increased or suppressed, appearing to depend upon the zone of cartilage analysed and the stage of OA. Aigner and coworkers have shown that expression of the *COL2A1* is suppressed in the upper zones of early OA cartilage associated with the catabolic phenotype, but increased in late-stage OA cartilage compared with normal and early degenerative cartilage.^{23,24} Recent large-scale expression profiling, using full-thickness cartilage, showed that many collagen genes, including *COL2A1*, are upregulated in late-stage OA.^{25,26} This applies predominantly to those chondrocytes in the middle and deep zones, as found by laser capture micro-dissection, whereas the anabolic phenotype was less obvious in the degenerated areas of the upper regions.²⁷

A mechanism involving phenotypic modulation has been proposed with expression of collagens normally absent in adult articular cartilage or at atypical sites in OA cartilage.²⁸ Type X collagen, a marker of the hypertrophic chondrocyte that is normally absent in adult articular cartilage, as well as type III collagen and type VI collagen, which are present at low levels in normal cartilage, and the chondroprogenitor splice variant of the type II collagen gene, type IIA, have been detected during certain stages of OA or in atypical sites within OA cartilage. In addition to *COL10A1* and matrix metalloproteinase-13 (MMP-13), other chondrocyte terminal differentiation-related genes, such as MMP-9 and Indian hedgehog (*Ihh*) are detected in the vicinity of early OA lesions.²⁹ Surprisingly, decreased levels of *Sox9* mRNA are detected near the lesions,²⁹ and the expression of this factor, which is required for activation of *COL2A1* transcription and its interacting partners, α -*Sox5* and *Sox6*, does not always localise with *COL2A1* mRNA in adult articular cartilage.^{27,30} Once the cartilage is severely degraded, the chondrocyte is unable to replicate the complex arrangement of collagen laid down during development. Furthermore, the chondrocyte stress response may result in the loss of viable cells due to apoptosis or senescence.³¹ Our recent study indicates that intracellular stress response genes are upregulated in early OA, whereas a number of genes encoding cartilage-specific and non-specific collagens and other matrix proteins are upregulated in late-stage OA cartilage.²⁶ Asporin, a transforming growth factor β (TGF β) binding protein, is increased in late OA cartilage^{26,32} and a polymorphism of this gene is associated with disease severity.³³ *Runx2*, a hypertrophic chondrocyte marker, is also increased in OA cartilage and may contribute to the loss of appropriate anabolic responses in adult articular chondrocytes.³⁴

Transcriptional regulation of cartilage anabolism

Our current knowledge of transcriptional regulation of cartilage-specific genes stems largely from studies of developmental events during chondrogenesis, the process by which mesenchymal condensations form the cartilage anlagen, resulting in hypertrophy and

endochondral ossification to form bone and eventually in the formation of the growth plate and the cartilage of the articular joint. The events that control cartilage formation are mediated by cascades of both activators and repressors that interact with the promoter or enhancer regions of chondrocyte-specific genes, including those encoding type II, type IX and type XI collagen, aggrecan and the cartilage-derived retinoic acid sensitive protein (CD-RAP). The high-mobility group (HMG) protein Sox9 plays a key role in the tissue-specific transcription of these cartilage genes.^{35–39} As a requirement for chondrogenesis *in vivo*, Sox9 activates COL2A1 transcription by binding to the first intron enhancer through its HMG DNA-binding domain and acts cooperatively with α -Sox5 and Sox6.^{40,41} In articular cartilage, the anabolic effects of insulin-like growth factor-I, BMP-2 and fibroblast growth factor-2 appear to be mediated, at least in part, by Sox9.^{40,42–44} The SOX genes are regulated in a dynamic fashion during chondrogenesis by members of the BMP/TGF β family.⁴⁵ Other pathways that control chondrocyte differentiation during development include the *Ihh*/parathyroid hormone-related protein axis via Gli transcription factors, Wnt/ β -catenin via T-cell factor/lymphocyte enhancing factor and fibroblast growth factors via Stat1/p21.⁴⁶ Understanding the regulatory mechanisms of chondrogenesis in the embryo and in the postnatal growth plate may help us to identify the critical signals that disrupt articular cartilage homeostasis during OA and ageing.⁴⁷

An additional control mechanism involves the coactivator, CREB-binding protein (CBP) or its paralogue, p300, which does not interact directly with promoter DNA sequences, but serves as a bridge between DNA-binding proteins and the transcriptional machinery. Through intrinsic histone acetylase activity, CBP/p300 can directly acetylate the lysine residues of certain transcription factors that are generally activators of gene transcription, including cAMP-responsive binding protein (CREB), NF- κ B, AP-1, C/EBP, SMAD and ETS family members and thereby serves to integrate their activities resulting in transcriptional synergy.⁴⁸ CBP/p300 potentiates transcription by acetylation-dependent loosening of the chromatin structure, and its interaction with Sox9 is required for COL2A1 promoter activity.⁴⁹ CBP/p300 elicits strong positive transcription of the CD-RAP and COL2A1 genes when expressed in chondrocytes to an extent greater than that induced by Sox proteins alone.^{50,51} After binding to CBP/p300, Sox9 binds to DNA with higher affinity, thereby increasing gene transcription.⁴⁹ CBP/p300 may also bind to negative regulators such as C/EBP, thereby preventing them from binding DNA and rendering them inactive.⁵¹

Regulation of chondrocyte phenotype by proinflammatory cytokines

In addition to the biomechanical and age-related alterations in chondrocyte function, inflammation and accompanying dysregulated cytokine activities probably contribute to the disruption of the balance between anabolism and catabolism.^{52,53} The role of the proinflammatory cytokines, particularly interleukin (IL)1 and tumour necrosis factor (TNF) α , in cartilage pathology in rheumatoid arthritis and OA is well established, based on *in vitro* and *in vivo* studies.^{54,55} The chondrocyte is the cellular target of cytokine action in cartilage, and IL1 β and TNF α and their receptors colocalise in superficial regions of OA cartilage.⁵⁶ In addition to increasing destructive proteinases, IL1 β suppresses the expression of a number of genes associated with the differentiated chondrocyte phenotype, including COL2A1 and CD-RAP.^{57–59} Early studies *in vitro* showed that IL1 and TNF α can inhibit the synthesis of type II collagen by chondrocytes by suppressing gene transcription.^{57,58,60,61} IL1 and TNF α also stimulate the synthesis of prostaglandin E₂, which feedback-regulates COL2A1 transcription in a positive manner,^{57,58,62,63} depending upon receptor utilisation.⁶⁴ The increase in anabolic activity in OA cartilage may also be associated with increased expression of BMP-2 induced by IL1 and TNF α .^{12,15} BMP-2 would, in turn, activate COL2A1 transcription and permit interaction of promoter elements with cytokine-induced factors.^{50,59} IL1 differentially regulates inhibitory Smads, transcriptional mediators of TGF β

and BMP signalling, upregulating Smad7 and downregulating Smad6 in chondrocytes.⁶⁵ However, the cytoplasmic localisation of these inhibitors in normal and OA cartilage does not correlate with downregulation of anabolic genes⁶⁶ or Sox9.³⁰

SIGNALLING AND TRANSCRIPTIONAL REGULATION BY PROINFLAMMATORY MEDIATORS

IL1 and TGF α share the ability to activate a diverse array of intracellular signalling pathways, although the cell surface receptors and associated adaptor molecules are distinct. In chondrocytes, the JNK, p38 MAPK and NF- κ B signalling pathways predominate in the regulation of IL1- and TGF α -induced genes. These pathways are also involved in the inhibition of COL2A1 expression by these cytokines,^{67,68} and non-canonical NF- κ B signalling via IKK α may also contribute to the abnormal phenotype of OA chondrocytes.⁶⁹ Along with ERK1/2, the key protein kinases in these signalling cascades are activated, particularly in the upper zones of OA cartilage.⁷⁰ Injurious mechanical stress and cartilage matrix degradation products can stimulate the same signalling pathways as those induced by IL1 and TGF α .^{71–75} Since these pathways also induce or amplify the expression of cytokine genes, it remains controversial whether inflammatory cytokines are primary or secondary regulators of cartilage damage and defective repair mechanisms in OA. Nevertheless, physiological loading on cartilage may protect against cartilage loss by inhibiting IKK β activity in the canonical NF- κ B cascade and attenuating NF- κ B transcriptional activity,⁷⁶ as well as by inhibiting phosphorylation of TAK1.⁷⁷

Cytokine-activated transcription factors of the NF- κ B, C/EBP and ETS families, as well as EGR-1, are involved in repressing, directly or indirectly, COL2A1 promoter activity.^{50,59,78} During trauma or inflammation, the chondrogenic transcription factors may be inactivated by direct or indirect interactions with cytokine-induced transcription factors. It has been proposed that cytokine-mediated repression of COL2A1 transcription is dependent upon inhibition of Sox9 expression,^{44,79,80} although expression of Sox9 and COL2A1 does not always correlate.^{50,81–83} However, Sox9 overexpression in chondrocytes may either increase or decrease COL2A1 transcription depending upon its concentration and the differentiation state of the cells.⁸⁴ During chondrocyte hypertrophy the suppression of Sox9 expression and activity by transcription factors such as Runx2 has been proposed as a mechanism essential for endochondral ossification.^{85,86} Although Runx2 is required for IL1 induction of MMP-13 gene transcription in articular chondrocytes,⁸⁷ its role in the suppression of chondrocyte phenotype by inflammatory cytokines has not been defined. IL1 may also induce chondrocytes to synthesise other cytokines such as IL6, which downregulates COL2A1, aggrecan and link protein via the JAK/STAT pathway in association with suppression of Sox9.⁸⁸

COL2A1 is one of the cytokine-responsive genes without functional binding sites for NF- κ B or AP-1, indicating that downregulation of COL2A1 promoter activity by IL1 cannot be ascribed to direct interaction of these transcription factors with DNA elements. A recent study also showed that NF- κ B is not required for modulation of Sox9-dependent COL2A1 promoter activity by Bcl-2.⁸⁹ Despite previous findings,⁷⁹ we observe that IL1 β treatment does not decrease the constitutive levels of Sox9, ι -Sox5 and Sox6 mRNA,⁵⁰ similar to our findings regarding the inhibition of COL2A1 transcription by interferon γ .⁸¹ Sox9 and related HMG factors are architectural proteins that act to maintain the nucleosomes in an open configuration, thereby exposing the endogenous, chromatin-integrated COL2A1 promoter to regulatory binding proteins.⁹⁰ In differentiated chondrocytes at a post-developmental stage of low matrix turnover, the COL2A1 promoter cannot respond to negative regulation by cytokines unless it is activated. In support of this concept are findings

that the integration of the responses of cytokine-induced promoters requires the assembly of higher-order nucleoprotein complexes orchestrated by HMG-I(Y) factors.⁹¹⁹²

In addition to members of the AP-1 (Jun/Fos) family, EGR-1 is an immediate early growth response factor that plays a role in IL1 β -regulated transcriptional events. We reported that EGR-1 inhibits COL2A1 promoter activity by binding to the -131/+125 bp core promoter and displacing Sp1 from at least one of the GGGCG boxes that overlap with the EGR-1 binding site.⁵⁰ A similar mechanism may account for the increased ratio of Sp3 to Sp1 binding to the Sp1 sites observed in response to IL1.⁹³ Since overexpression of CBP reverses the inhibition, it is probable that activation of EGR-1 and its binding to DNA disrupts the interactions among Sp1, CBP and TATA-binding proteins.⁵⁰ This early response appears to be transient, suggesting that complete transcriptional repression of COL2A1 promoter activity may be dependent upon the binding to DNA of other IL1 β -induced factors, such as C/EBP β and C/EBP δ .⁵⁹ In contrast to EGR-1, C/EBP β and δ are induced by IL1 β in a time-dependent manner over 48 h, suggesting that they are late regulators.⁹⁴ Both IL1 β and TGF α can act together to decrease expression of the cartilage-characteristic proteins, but the response may be additive owing to the involvement of different combinations of C/EBP sites.⁵¹⁹⁵ However, C/EBP proteins act as negative regulators of chondrogenesis during skeletal development, during which IL1 β is not known to play a role, and their presence in muscle and in other non-cartilage tissues may contribute to the lack of expression of cartilage-characteristic genes.⁹⁴⁹⁵

Role of ETS transcription factors in cytokine responses

The ETS factors constitute a family of at least 30 members that play central roles in regulating genes involved in development, differentiation and cell proliferation.⁹⁶⁹⁷ The epithelial-specific ETS factor (ESE)-1, also called ELF3, ESX, ERT or JEN, epithelial tissues was identified originally.⁹⁸⁹⁹ ESE-1 is expressed in nonepithelial tissues, particularly those undergoing inflammation, including rheumatoid and, to a limited extent, OA synovium and in chondrocytes, as well as glioma cells, smooth muscle cells, synovial fibroblasts, osteoblasts and monocytemacrophages, after treatment with IL1 β , TGF α or lipopolysaccharide.¹⁰⁰ This induction relies on the translocation of the NF- κ B (p50/p65) to the nucleus and transactivation of the ESE-1 promoter via a high affinity NF- κ B binding site.¹⁰⁰¹⁰¹ After induction, ESE-1 can directly activate transcription of cyclooxygenase 2¹⁰² and nitric oxide synthase 2¹⁰¹ by binding to two or more functional ETS sites in the respective promoters. Together these studies indicate that increased expression of these IL1 β -induced genes is mediated indirectly by NF- κ B via induction of ESE-1, which then serves as a primary transcription factor that binds to and regulates promoter activity of the target gene. Furthermore, after induction, ESE-1 interacts with NF- κ B.¹⁰⁰⁻¹⁰² Since cytokines activate other ETS factors, such as ETS-1 and Elk-1, via the p38, JNK and PI3K pathways,¹⁰³¹⁰⁴ it is likely that these pathways are involved in activating ESE-1.

We also reported recently that ESE-1 is one of the factors that mediate the downregulation of the COL2A1 promoter by IL1.⁷⁸ ESE-1 acts as a potent repressor of COL2A1 promoter activity by binding to at least two tandem ETS sites upstream of -131 bp and accounts, in part, for the sustained suppression by IL1 β . Adenoviral overexpression of I κ B in chondrocytes blocks the suppression of COL2A1 mRNA, consistent with previous findings,⁷⁹¹⁰⁵ but also the induction of ESE-1 mRNA by IL1 β in chondrocytes.⁷⁸ Of the ETS factors tested, the suppression of COL2A1 transcription is specific to ESE-1, since ESE-3, which is also induced by IL1 β , suppresses COL2A1 promoter activity only weakly. In contrast, overexpression of ETS-1 increases COL2A1 promoter activity and blocks the inhibition by IL1. In transient cotransfections, the inhibitory responses to ESE-1 and IL1 β colocalise in the -577/-132 bp promoter region, ESE-1 binds specifically to tandem ETS

sites at -403/-381 bp, as observed by electrophoretic mobility shift assay and confirmed in vivo by chromatin immunoprecipitation. These results suggest for the first time a mechanism involving a balance among ETS factors in the control of *COL2A1* transcription that is disrupted during inflammation owing to the induction of ESE-1.⁷⁸

CARTILAGE DESTRUCTION IN OA

During ageing and joint disease, this equilibrium between the synthesis and the degradation of cartilage matrix is disrupted and the rate of loss of proteoglycans from the matrix may exceed the rate of deposition of newly synthesised molecules, exposing the collagen network to irreversible destruction. In contrast, in rheumatoid arthritis, cartilage is degraded primarily in areas contiguous with the synovial pannus owing to the direct action of synovial proteinases and cytokines.¹⁰⁶ However, synovitis is common in advanced OA involving infiltration of mononuclear cells, and the expression of proinflammatory mediators is seen in early and late OA.^{107,108} Recent evidence indicates that IL1 β and TGF α are produced independently in OA synovial cells associated with differential expression of matrix-degrading enzymes.¹⁰⁹ Cytokines originating from the cartilage, synovium and other surrounding tissues can increase the gene expression levels and activities of MMPs and ADAMTS (a disintegrin and MMP with thrombospondin motifs) in chondrocytes. These include MMP-1, MMP-3, MMP-8, MMP-13, MMP-14 and the aggrecanases, ADAMTS-4 and -5. MMP-13 has a prominent role in degrading the collagen network in cartilage owing to its particularly potent ability to cleave type II collagen.^{110,111} Furthermore, MMP-13-specific type II collagen cleavage products have been immunolocalised in OA cartilage,^{56,112} and constitutive expression of MMP-13 in cartilage in mice produces OA-like changes in knee joints.¹¹³

Transcriptional regulation of cartilage catabolism

The MMPs have been classified according transcriptional mechanisms of regulation such that the group 1 promoters (MMP-1, 3, 7, 9, 10, 12, 13, 19, 26) contain both a TATA box and AP-1 sites, located at approximately -30 bp and -70 bp, respectively, the group 2 (MMP-8, 11, 21) contain TATA but no AP-1 site, and group 3 (MMP-2, 14, 28) contain no TATA or AP-1 site.¹¹⁴ MMP-1, the gene encoding collagenase-1, was among the first whose promoter was characterised. The early work showed that UV light and phorbol ester induced promoter activity via AP-1 (c-Jun/c-Fos), and NF- κ B was found to be a primary cytokine-responsive factor. The MMP-9 promoter has a functional NF- κ B binding site, whereas the dependence of other MMP gene responses on this factor may relate to indirect stimulation of intermediate genes, the presence of non-canonical NF- κ B sites, or interaction with other factors that bind DNA. For example, TGF β induction of MMP-13 promoter activity requires activation of Smad3 and its interaction with JunD and Runx2, which bind to DNA elements.¹¹⁵ MMP gene expression may also be modified by JAK/STAT signalling, although direct binding to STAT sites in MMP promoters has not been demonstrated. Oncostatin M augments IL1-induced expression of several MMPs and ADAMTSs by activating Stat3,^{116–118} whereas interferon γ antagonises cytokine induction of MMP-1, 9 and 13 through mechanisms that involve interaction of Stat1 α with AP-1 or CBP/p300.¹¹⁹

C/EBPs and ETS family members are also involved in the cytokine-induced expression of MMP genes, as well as cyclooxygenase 2 and nitric oxide synthase 2, in chondrocytes and other cell types.^{101,102,114,120–122} IL1 stimulates MMP-1 transcription through ERK-dependent activation of C/EBP β .^{122,123} IL1 stimulates ETS-1 expression in fibroblasts, and other ETS family members, including ETS-1 and Erg, have also been implicated in the regulation of MMP promoters in a cell type and gene-specific manner.^{87,124–126} Most of the MMP promoters, including MMP-13, contain ETS sites adjacent to the AP-1 sites. IL1, TGF α and a large number of other cytokines and growth factors transactivate MMP

promoters by convergence of AP-1 and ETS elements in a manner dependent upon p38 and JNK signalling and increased transcription of Fos and Jun.^{127–129} The JNK pathway, which mediates phosphorylation of AP-1/Jun family members, as well as Elk-1 and ATF-2, is required for cytokine induction of MMP-1 and MMP-13 in fibroblasts,¹³⁰ and MMP-13, but not MMP-1, induction in chondrocytes.¹³¹ The ETS/PEA3 sites in the MMP-13 promoter are required for MMP-13 promoter activity in fibroblasts,¹³² and the induction by cytokines involves cooperation among ETS/PEA3, AP-1 and Runx2 in chondrocytes.⁸⁷ The critical roles of these factors in MMP gene expression are supported by studies showing that retinoid receptor ligands and the nuclear orphan receptor NURR1 attenuate the binding of AP-1 and ETS factors, respectively, to MMP-1 or 13 promoter elements.¹²⁵¹³³ The AGRE site between the Runx2 and ETS/PEA3 binds a nucleocytoplasmic shuttling, zinc finger protein, Nmp4/CIZ and appears to be important for basal activity of the MMP-13 promoter.¹³⁴

SIGNALLING MECHANISMS INVOLVED IN INTERACTIONS OF CHONDROCYTES WITH CARTILAGE MATRIX

Alteration of chondrocyte metabolic responses may also result from mechanical disruption of chondrocyte–matrix associations.¹³⁵ More rapid matrix turnover may occur in the immediate pericellular zones compared with the interterritorial zones of cartilage.¹¹²¹³⁶¹³⁷ This suggests roles for chondrocyte cell surface receptors such as integrins and discoidin domain receptor 2 (DDR2) in the response to mechanical stress that may result in the disruption of normal remodelling of matrix components.^{138–141} The engagement of integrin receptors by fibronectin or collagen fragments activates focal adhesion kinase signalling and transmits signals intersecting with ERK, JNK and p38 signalling pathways, which converge on AP-1 and ETS sites to transactivate the MMP-13 promoter.^{124142–144} The upregulation of MMP-13 due to upregulation and activation of DDR2 is somewhat distinct, since it requires direct interaction with native type II collagen fibrils rather than fragments and is not associated with upregulation of other MMP or ADAMTS genes.¹⁴¹ This mechanism was discovered in the type XI collagen haploinsufficient Cho/+ mouse with chondrodysplasia and the Col9a–/– mouse, both of which increase DDR2 at 6 months associated with MMP-13 gene expression and activity. In these mice, the collagen fibrils are modified and are present in increased amounts in the pericellular matrix compared with the relatively low amounts in wild-type cartilage.¹⁴¹¹⁴⁵¹⁴⁶ This mechanism is not restricted to genetic abnormalities, since DDR2 expression associated with MMP-13-dependent collagen destruction is seen in human OA, as well as in surgical mouse OA, where loss of proteoglycans would permit interaction of the chondrocyte cell surface with denuded collagen fibrils.¹⁴⁷ The activation of DDR2 by intact type II collagen fibrils requires the PTK core and tyrosine phosphorylation at the Y740 site and leads to increased *MMP-13* gene expression via Ras/Raf/MEK/ERK pathway and p38 signalling. Neither IL1-mediated nor integrin-mediated signalling is involved.¹⁴⁷

GADD45 β AND MMP-13 RECAPITULATE DEVELOPMENTAL EVENTS IN OA

During a microarray study to identify BMP-2-induced early genes involved in signalling and transcriptional regulation in chondrocytes, we identified growth arrest and DNA damage-inducible (GADD)45 β as one of the most highly expressed genes at 1 h.¹⁴⁸ GADD45 β (*MyD118*) is member of a family of stress-induced proteins inducible by NF- κ B,¹⁴⁹¹⁵⁰ but its role in cartilage had not been explored previously. We also found that Runx2 plays a synergistic role in both the induction and activity of GADD45 β by enhancing BMP-2-induced Smad1 signalling and AP-1 (JunB or D/Fra2)-dependent MMP-13 gene expression.¹⁴⁸ The GADD45 β gene product has been implicated as an anti-apoptotic factor during genotoxic stress and cell cycle arrest in other cell types. In a microarray analysis, we

observed upregulation of GADD45 β in normal cartilage and in early OA compared with late OA, whereas anabolic genes, including COL2A1, were upregulated in late OA.²⁶ Intracellular staining for GADD45 β was present in chondrocytes throughout normal adult articular cartilage and in chondrocyte clusters and deep-zone chondrocytes with hypertrophic morphology in OA cartilage. Consistent with a potential role for GADD45 β as a survival factor, knockdown of GADD45 β enhances TGF α -induced apoptosis. In contrast, IL1 β is a less effective inducer of chondrocyte apoptosis, suggesting the ESE-1 may not have a role in this process. Similar to ESE-1, however, GADD45 β is induced by NF- κ B and its overexpression downregulates COL2A1 promoter activity and mRNA levels.²⁶

CONCLUSION

Despite our increasing knowledge of the mechanisms regulating the production and activities of anabolic and catabolic factors involved in OA, the application of the principles of cell and molecular biology, such as those described in this review, to the development of disease-modifying treatments has been elusive.¹⁵¹ Diverse stimuli induce MMP-13 expression and activity in chondrocytes and both similar and distinct signalling and transcription events are involved. Some of these events also downregulate COL2A1 and other anabolic genes that might be recruited for cartilage repair. However, targeting these events in vivo may be difficult, since they occur at different times and at localised sites in cartilage, as well as in other joint tissues.¹⁵² Nevertheless, specific approaches should take into account these events, given the failure of broad-spectrum MMP inhibitors against osteoarthritic processes in clinical trials and their unexpected side effects on muscle and the skeleton.¹⁵³ We also need to consider other mediators of these events, such as Toll-like receptors,^{154–157} intermediate cytokines and chemokines^{158,159} and alternate signalling kinases, as well as the crosstalk between anabolic and catabolic signals. The role of epigenetics in the “unsilencing” of genes not expressed normally in cartilage and other joint tissues might also be a fruitful area of investigation.¹⁶⁰

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