Post-transcriptional gene regulation in chondrocytes

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Abstract

The control of gene expression in articular chondrocytes is an essential factor in maintaining the homoeostasis of extracellular matrix synthesis and turnover necessary in healthy articular cartilage. Although much is known of how steady-state levels of gene expression and rates of transcription are altered, there has been a poorer understanding of gene control at the post-transcriptional level and its relevance to cartilage health and disease. Now, an emerging picture is developing of the importance of this tier of gene regulation, driven by *in vitro* studies and mouse genetic models. This level of cellular regulation represents an as yet unexplored area of potential intervention for the treatment of degenerative cartilage disorders such as osteoarthritis.

Introduction

Articular chondrocytes are the resident cell type within articular cartilage and play the major role in maintaining this tissue within the synovial joint throughout life. The key role which they play in remodelling the cartilage extracellular matrix, through a carefully controlled balance of anabolic and catabolic processes, depends upon well controlled expression of genes encoding ECM (extracellular matrix) proteins, proteases and regulatory molecules [1,2]. Chondrocytes express high levels of collagen type II encoded by the *COL2A1* gene, the proteoglycan aggrecan and a myriad of other collagens, proteoglycans and glycoproteins [3]. Articular cartilage pathologies such as OA (osteoarthritis) result in loss of cartilage at the joint surface owing to an imbalance in the production of ECM molecules and the degradative proteases which specifically cleave them [4]. An understanding of how this imbalance occurs is an ongoing feature of research into degenerative joint diseases.

A great deal is now known about the mechanisms which regulate gene expression in articular chondrocytes, particularly at the level of mRNA transcription. Many of the changes in mRNA levels that are observed in degenerative diseases can be replicated *in vitro* by treating cultured chondrocytes with inflammatory cytokines such as IL-1β (interleukin 1β) or TNFα (tumour necrosis factor α) [5,6]. Transduction of these extracellular stimuli occurs directly and indirectly through many common signalling mechanisms including the MAPKs (mitogen-activated protein kinases), NF-κB (nuclear factor κB) and JAK (Janus kinase)/STAT (signal transducer and activator of transcription) pathways [7,8]. A large body of work has now led us to an understanding of many of the transcription factors which are affected by these signalling mechanisms and how they control molecular regulatory elements in and around ECM and protease genes [9].

The control of mRNA levels in articular chondrocytes post-transcriptionally is now emerging as a further tier of regulation with important consequences for our understanding of the progression of diseases such as OA and with potential for therapeutic intervention. This is particularly being driven by current research into the expression of miRNAs (microRNAs) in chondrocytes in health and disease. However, our understanding of the scope of post-transcriptional gene control and the molecular mechanisms underlying it in articular chondrocytes is still in its infancy.

Altered mRNA decay rates in chondrocytic cells

There have been few studies solely aimed at examining whether regulation of mRNA decay rates control gene expression in chondrocytes. This may be in part due to the additional processes and difficulties that are required to measure RNA decay rates compared with measuring steady-state levels. Regardless, there is now evidence that a number of important cartilage genes can be regulated post-transcriptionally by chondrocytic cells in cell culture experiments. The transcription factor SOX [SRY (sex determining region Y)-box] 9 is essential for chondrocyte differentiation during development and forms a part of transactivational complexes that control the expression of many important cartilage ECM genes, including *COL2A1* [10,11]. Interestingly, it is also down-regulated during OA [12,13]. Recent studies using human articular chondrocytes

Key words: cartilage, chondrocyte, microRNA (miRNA), osteoarthritis, post-transcriptional regulation.

Abbreviations used: ARE, AU-rich element; AUF1, AU-rich RNA-binding factor 1; BMP, bone morphogenetic protein; ECM, extracellular matrix; HDAC4, histone deacetylase 4; IL-1β, interleukin 1β; iNOS, inducible nitric oxide synthase; JAK, Janus kinase; MAPK, mitogen-activated protein kinase; miRNA, microRNA; NF-κB, nuclear factor κB; OA, osteoarthritis; siRNA, small interfering RNA; SOX, SRY (sex determining region Y)-box; STAT, signal transducer and activator of transcription; TNFα, tumour necrosis factor α; TTP, tristetraprolin; UTR, untranslated region.

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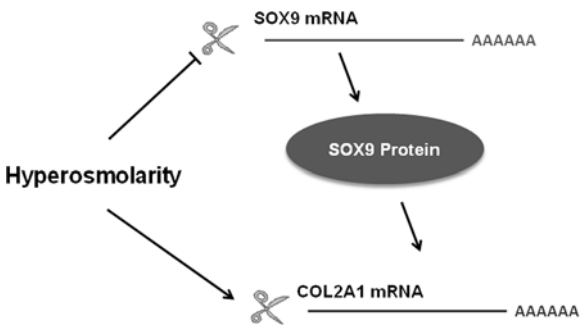
Figure 1 | The opposing effects of hyperosmolarity on post-transcriptional gene regulation in articular chondrocytes

SOX9 mRNA decay is stabilized by hyperosmolarity, leading to increased SOX9 protein and increased stimulation of COL2A1 transcriptional enhancer regions. However hyperosmotic stimulation also acts to destabilize the *COL2A1* mRNA and, on balance, leads overall to a slight reduction in *COL2A1* mRNA levels.



have demonstrated that cell stress, invoked by either cycloheximide stimulation or by exposure to hyperosmotic conditions, leads to increased levels of *SOX9* mRNA and protein and that this is associated with reduced rates of *SOX9* mRNA decay [14,15]. This process is controlled at least in part by signalling through the stress-activated p38 MAPK signalling pathway. Interestingly, in the case of hyperosmotic stimulation of the articular chondrocytes, analysis of COL2A1 expression revealed that it is down-regulated by the stress conditions and that its mRNA is destabilized [15]. Given that increased levels of SOX9 protein would be expected to lead to increased transactivation of COL2A1, this is a somewhat conflicting observation. It suggests that the hyperosmotic stimulus might be influencing COL2A1 expression in conflicting ways by, on one hand, increasing transcription through elevated SOX9 and, on the other, promoting increased turnover of the *COL2A1* transcript (Figure 1).

A pattern of involvement of stress signals such as the p38 MAPK pathway in the control of post-transcriptional gene regulation in chondrocytes has emerged over recent years. In primary human articular chondrocytes and in the murine chondrocytic ATDC5 cell line, there is post-transcriptional regulation of *BMP2* (bone morphogenetic protein 2) mRNA in response to stimulation with TNFα [16]. This process requires AREs (AU-rich elements) within the *BMP2* mRNA to proceed and is regulated, similarly to SOX9, through p38 MAPK signalling. There is also indirect evidence for regulation of the decay rates of *iNOS* (inducible nitric oxide synthase) mRNA in the human C28/I2 chondrocytic cell line. In these cells, an up-regulation in iNOS caused by exposure to a cocktail of cytokines (including IL-1β and TNFα) is inhibited in cells that overexpress the RNA-binding protein KSRP [KH (K homology)-type splicing regulatory protein], a well-characterized modulator of RNA decay rate [17]. Pharmacological inhibition demonstrated that induction of iNOS in the cells was again sensitive to blocking the p38 MAPK pathway as well as to blocking JAK/STAT and NF-κB signalling [17]. More recently, it has been shown that hyperosmotic stimulation of human articular chondrocytes stabilizes the mRNA for COX2 (cyclo-oxygenase 2) and that this also requires p38 MAPK signalling [18]. In addition, other factors have also been demonstrated to control chondrocyte RNA decay rates. In chicken chondrocyte lysates, the multi-functional protein nucleophosmin can modulate the RNA stability of the *ccn2* gene through interactions with 3'-UTR (untranslated region) elements [19]. *ccn2* is the chick homologue of the *CTGF* (connective tissue growth factor) gene in humans which can regulate cellular differentiation, motility and adhesion. Interestingly, application of growth factors such as TGFβ1 (transforming growth factor β1), BMP2 or CCN2 itself is able to regulate the rate of decay of the *ccn2* mRNA. Furthermore, although these growth factors act to stabilize the rate of *ccn2* mRNA decay in chondrocytes from the lower sternum, which chiefly contains proliferating chondrocytes, the opposite is true in chondrocytes from the upper sternum where they promoted *ccn2* mRNA destabilization [19].

The post-transcriptionally controlled genes described in the present paper have all been implicated in having critical roles in regulating the balance between ECM production and destruction in chondrocytes. As such, their biology has been investigated extensively in relation to OA and other degenerative joint diseases. The potential that post-transcriptional gene control of these and other genes might form part of the progression of such diseases is therefore a distinct possibility.

Insights from *in vivo* models of impaired mRNA decay

A number of mouse genetic models have now been developed which examine the roles of various molecular factors that affect mRNA decay rates. Many of these involve targeting of genes encoding proteins which bind to and regulate the stability of ARE-containing mRNAs. Given the importance of the chondrocyte phenotype in cartilage and bone development, examination of the phenotypes of these animals could reveal potentially interesting links with chondrocyte function. To date, the strongest skeletal phenotype observed in an ARE-binding protein knockout model is caused by HuR deficiency. HuR (also known as ELAVL1) is a member of a family of 3′-UTR RNA-binding factors, related to the *Drosophila* ELAVL protein [20]. Katsanou et al. [21] have demonstrated that knockout of HuR leads to impaired placental development, resulting in early embryonic death. However, in the same study, restricting the loss of HuR to cells in the epiblast, using floxed HuR alleles and a *SOX2* promoter-driven Cre recombinase, allowed the embryos to survive for longer. The phenotype of these mice was characterized by a delay in endochondral ossification in many skeletal structures. In addition, other skeletal abnormalities were observed such as incomplete fusion of the thoracic

cage, syndactyly in the fore and hind limbs and frequent fusion of the radius and ulnar bones. These observations implicate HuR as having fundamental developmental roles in the regulation of chondrocyte differentiation during endochondral ossification as well as in the control of limb patterning [21]. Interestingly, knockout of another ELAVL family member, HuD (ELAVL4), does not lead to a skeletal phenotype [22]. Other knockout models of ARE-binding proteins have failed to demonstrate a direct role on chondrocyte differentiation. TTP (tristetraprolin)/ZFP36 (zinc-finger protein 36) and AUF1 (AU-rich RNA-binding factor 1) are proteins with well-established roles in the control of many mRNAs [23–25]. Mice lacking TTP exhibit severe cartilage erosion caused by deregulation of TNFα RNA expression, resulting in severe immune arthritis [26]. Application of anti-TNFα antibodies or additional knockout of each of the two TNFα receptors is able to rescue the effects of the TTP$^{-/-}$ arthritis phenotype [26,27]. AUF1$^{-/-}$ mice are growth-retarded, but demonstrate no developmental defects [28]. Like the TTP$^{-/-}$ mice, AUF$^{-/-}$ mice exhibit deregulation of the decay of mRNA encoding TNFα and also IL-1β. However, unlike the chronic effect of TTP on TNFα mRNA decay rates, AUF1 appears to regulate RNA cytokine mRNA turnover following endotoxin challenge, resulting in severe endotoxic shock.

Another mouse genetic model which interferes with post-transcriptional gene control in a different way has also been developed. Mice whose chondrocytes are unable to express the miRNA-processing enzyme Dicer suffer from severe disruption to skeletal growth caused by a reduction in chondrocyte proliferation and an increase in their terminal differentiation [29]. Dicer, a cytoplasmic enzyme, is critical for the formation of mature miRNA molecules which perform a major role in the control of genes at the post-transcriptional and translational level [30]. The importance of Dicer to chondrocyte differentiation has made it clear that miRNA-based regulation of genes is essential for chondrocyte function during development.

The emerging role of miRNAs in cartilage function and disease

Many studies have now been able to demonstrate in detail the miRNA molecules that are expressed in articular chondrocytes and the roles that they may play. The first miRNA to be associated with the chondrocyte phenotype was *miR-140*. It was identified in a screen of zebrafish embryos as being specific to cartilages of the head, jaw and fins [31]. Further work demonstrated that this cartilage-specific distribution was replicated in the developing mouse embryo [32]. Computer-based predictions were used to determine potential *miR-140* targets and identified the histone deacetylase HDAC4. This gene made a good candidate as HDAC4-null mice have a skeletal phenotype, again characterized by early-onset terminal differentiation of chondrocytes, which results in premature ossification of developing cartilage templates [33]. Using siRNA (small interfering RNA) which mimics the *miR-140* seed sequence, the *in silico* predictions have been confirmed. The siRNA led to reduction of HDAC4 protein levels and inhibited reporter gene expression controlled by the HDAC4 3′-UTR [32]. Further examination of *miR-140*'s role in chondrocyte biology has demonstrated that its expression not only correlates with increased levels of chondrocyte marker genes during chondrogenic differentiation, but also is reduced in OA tissue [34,35].

Other recent examinations of miRNAs in chondrocytes have sought to determine whether any further differences in expression occur during diseases such as OA. These studies have identified a raft of regulated miRNAs and identified that they could play roles in the regulation of cytokines and growth factors such as TNFα and BMP7 as well as ECM-degrading proteases such as MMP13 (matrix metalloproteinase 13) [35–37]. Interestingly, changes in miRNA levels are likely to be altered as joint disease progresses, as there is evidence that expression levels of *miR-146a* are highest during the early stages of OA, but decrease as the disease becomes more severe [36,38]. This raises the interesting prospect that deregulated miRNA expression occurs during distinct phases of joint diseases such as OA.

Articular cartilage is a tissue which is subjected to changing mechanical conditions, and chondrocytes are able to sense and respond to their mechanical environment. There is now evidence that this response involves differential miRNA expression. Comparisons of cartilage tissue from different areas of bovine stifle joints has demonstrated that miRNAs such as *miR-221* and *miR-222* are expressed at higher levels in chondrocytes which are exposed to greater loads within the joint [39]. These data provide us with evidence that miRNAs are involved in the regulation of cartilage homoeostasis necessary for joint function.

In vitro studies have demonstrated that miRNA expression levels can be regulated by inflammatory cytokines and growth factors. For instance, *miR-140* is suppressed by IL-1β, and evidence suggests that this is linked to the increased catabolic and reduced anabolic effect attributed to this cytokine [34]. Conversely, *miR-146a* expression levels can be increased in human articular chondrocytes by stimulation with IL-1β [38]. Finally, a number of miRNAs are regulated during BMP2-induced chondrogenic differentiation, one of which (*miR-199a*) modulates the chondrogenic process by targeting the BMP pathway mediator Smad1 [40].

Overview

There is a growing body of evidence which identifies an important role for post-transcriptional gene regulation in the development and disease of cartilage tissues. The importance of this process to articular chondrocytes and to their ability to maintain articular joint surfaces has yet to be fully understood, but the studies to date demonstrate that cytokine signalling, biophysical and biomechanical factors can control chondrocyte post-transcriptional control. These

are stimuli which are often implicated in the control of cartilage homoeostasis, which suggests that control of gene activity at the level of RNA decay and translation does play an important role. These processes will therefore make excellent candidates for intervention as we seek to control the progression of degenerative joint diseases, and develop techniques for cartilage regeneration.

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