**Osteoarthritis and Cartilage**

**Review**

Epigenetic mechanisms in cartilage and osteoarthritis: DNA methylation, histone modifications and microRNAs

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**S U M M A R Y**

Osteoarthritis (OA) is a complex multifactorial disease with a strong genetic component. Several studies have suggested or identified epigenetic events that may play a role in OA progression and the gene expression changes observed in diseased cartilage. The aim of this review is to inform about current research in epigenetics and epigenetics in OA. Epigenetic mechanisms include DNA methylation, histone modifications, and microRNAs. Collectively, these enable the cell to respond quickly to environmental changes and can be inherited during cell division. However, aberrant epigenetic modifications are associated with a number of pathological conditions, including OA. Advancements in epigenetic research suggests that global analysis of such modifications in OA are now possible, however, with the exception of microRNAs, it will be a significant challenge to demonstrate how such events impact on the disease.

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**Introduction**

Osteoarthritis (OA) is the most common degenerative joint disease and although often accompanied with new bone formation, changes in the subchondral bone and low-grade synovitis, it is epitomised by focal degradation of articular cartilage. The main risk factor for OA is age and chondrocytes, the sole cell-type in cartilage, are unusual in that they are effectively post-mitotic after puberty and must survive and function throughout life. In health, chondrocytes respond to their environment to regulate the articular cartilage by balancing the synthesis of extracellular matrix (ECM) components, mainly type-II collagen and the proteoglycan aggrecan, with its turnover and degradation by proteolytic enzymes, including matrix metalloproteinases (MMP13 being the major collagenase in OA) and the ‘a disintegrin and metalloproteinase domain with thrombospondin-like motifs’ family (ADAMTS), including the aggrecanases, ADAMTS4 and ADAMTS5. Prevention of either aggrecan or collagen degradation reduces cartilage loss in murine arthritis models. Although broad-spectrum metalloproteinase inhibitors have not been beneficial in arthritis partly due to non-selectivity, the suggestion of targeting the expression/activity of specific proteases via unique mechanisms is considered an important therapeutic option to slow or halt OA progression.

OA has a strong genetic component, however numerous studies have failed to identify genes that provide the full genetic susceptibility to the disease. This could be due to low penetrance polymorphisms in the population but, it is proposed this could, in part, be accounted for by inheritance of epigenetic modifications; though in mammals such transgenerational inheritance appears limited.

Initially defined as non-genetic transgenerational inheritance, today the definition of epigenetics is a stable change in gene expression between cell divisions, and sometimes generations, that involves no change in the underlying DNA sequence. Epigenetic mechanisms include DNA methylation, histone modifications, and microRNAs (miRNAs). Collectively, these enable the cell to respond quickly to environmental changes. Aberrant epigenetic modifications are associated with a number of pathological conditions, probably occurring as a result of environmental factors and ageing, which are major risk factors for OA, and may impact on the disease via such epigenetic mechanisms.

**DNA methylation**

DNA methylation involves the addition of a methyl group to the DNA, at CpG dinucleotides, to convert cytosine to 5-methylcytosine (mC). Cpg sequences are often clustered in ‘islands’ near the promoters of around 30% of genes. Methylation of these islands correlates with suppression of gene expression and an important function of DNA methylation is the silencing of transposons.
In normal cells, CpG islands are generally unmethylated, although certain promoters become methylated during development and tissue differentiation. Regions of lower CpG density that reside close to CpG islands are termed ‘shores’. Most tissue-specific methylation appears to occur at these shores and correlates with transcriptional repression of the corresponding gene. Further complexity is added by the finding that methylation within a gene body is common in ubiquitously expressed genes probably to prevent incorrect transcriptional initiation (Fig. 1). A number of proteins (e.g., MECP2) bind specifically to methylated DNA and lead to transcriptional silencing via the recruitment of transcriptional co-repressors and histone deacetylases (HDACs), thus remodelling the local chromatin.

DNA methylation patterns are established and modified in response to environmental factors by three DNA methyltransferases (DNMTs), DNMT1, DNMT3A and DNMT3B, the deletion of any of which is essentially lethal in mice. DNMT1 has a preference for hemi-methylated DNA (one strand) and is therefore the maintenance DNMT, important for transferring patterns of methylation to a newly synthesised strand after DNA replication. DNMT3A and DNMT3B have been assigned as de novo DNA methyltransferases. In cartilage while DNMT3B expression is low, DNMT1 and DNMT3A are robustly expressed suggesting that these enzymes contribute to the DNA methylation pattern of chondrocytes. DNMT activity requires the presence of the methyl donor S-adenosylmethionine (SAMe), a food supplement taken by some OA patients which, in contrast to animal studies, has shown no efficacy in clinical trials.

The mechanism of demethylation in mammals is unclear, although data suggests DNMT3A and DNMT3B may also act as demethylases. Two waves of demethylation, or reprogramming, occur in mammals; in primordial germ cells (PGCs) and the early embryo. In PGCs, although this demethylation is almost global, around 7% of CpGs, which cluster in regions of recently acquired retrotransposons, remain methylated. Thus, in mammals there is little evidence for epigenetic transgenerational inheritance, in contrast to plants where this is common. The demethylation that does occur in PGCs is partly an active process mediated by the cytosine deaminase AID (or AlcDA, activation-induced cytidine deaminase) and the base excision repair pathway.

A recent epigenetic mark, hydroxy-methylcytosine (hmC), has been identified whose function remains to be elucidated but is potentially important in gene regulation and demethylation. Also, new data suggest that unmethylated CpG islands are themselves platforms for gene regulation, being bound exclusively by the CXXC finger protein 1 (Cfp1) to regulate the local chromatin state. Together these data add further complexity to epigenetically regulated loci.

**DNA methylation in cartilage and OA**

No detailed global methylation analysis in OA has been performed but analysis of the total DNA methylation content in control relative to OA cartilage by chromatography showed no differences. However, the examination of individual gene promoters in cartilage and/or in chondrocytes suggests that a fine regulation of DNA methylation, implicating a few but strategically important CpG loci, can have a large impact on gene expression and hence disease.

An early study using chick embryos reported the possible role of DNA methylation in the regulation of type-I and II collagen genes during chondrocyte differentiation and dedifferentiation. More recently, the promoters of COL2A1 and ACAN were found to be hypomethylated in both normal and OA chondrocytes, thus not correlating with the dramatic alteration in their expression during the disease. Similarly, the promoters of the transcription factors (TFs) SOX9 and RUNX2 show a hypomethylated profile independent of their expression status during *in vitro* chondrogenesis. However, two CpG sites within the COL10A1 promoter appear to be demethylated during chondrocyte differentiation from mesenchymal stem cells (MSCs) correlating with COL10A1 expression.

**GDF5** is a factor involved in synovial joint development, maintenance and repair and is also genetically associated with OA susceptibility via a C/T single nucleotide polymorphism (SNP) located within its 5′untranslated region (UTR). There is a significant reduction in the expression of the disease-associated T allele relative to the C allele, an effect termed differential allelic expression (DAE) imbalance. Recent work suggests the C allele, which forms a CpG dinucleotide, can be methylated in joint tissues, affecting GDF5 expression and DAE. Leading to the suggestion that epigenetics could further influence the penetrance of this allele in the susceptibility to common musculoskeletal diseases.

**Metalloproteinase expression in normal cartilage** is relatively low but elevated in OA, resulting in ECM degradation. A number of metalloproteinase promoters show decreased methylation at single CpG loci in OA cartilage compared to normal, which could underlie the disease-associated change in expression; these include MMP3 (−635, relative to the transcription start site (TSS)), MMP9 (−36),

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**Fig. 1.** DNA methylation and the regulation of gene expression. **A**. Under normal circumstances, CpG islands located at the vicinity of gene promoter are hypomethylated (open circles) above the DNA representation permitting the binding of a TF and transcription by the RNA polymerase (RNAP). An aberrant hypermethylation of the CpG island (closed circles) favours the recruitment of a methyl-CpG-binding domain containing protein (MBD) and subsequently, a DNMT close to the TSS (full arrow), causing the transcriptional repression of the gene by precluding the possible binding of a TF and/or the RNAP. **B**. Less CpG-dense ‘shores’ reside about 2 kb upstream from the transcription initiation site of the gene and regulate transcription similarly to CpG islands by CpG site methylation. Conversely, gene body methylation is found in normally expressed genes and is able to prevent spurious initiation of transcription from the gene body. These tend to be demethylated in disease allowing aberrant transcription initiation (solid arrow) to occur.
MMP13 (−110) and ADAMTS4 (−753)33, the importance of these sequences remains to be elucidated but the DNA binding abilities of several TFs (e.g., AP-234) are methylation-sensitive leading to the epigenetic regulation of the corresponding gene (e.g., Ref.35).

A region of the IL1B promoter is demethylated in human articular chondrocytes correlating with an increase in IL1B expression36. LEP (leptin) expression is regulated via DNA methylation by OA chondrocytes and interestingly, leptin down-regulation by RNA interference (RNAi), decreases MMP13 expression37. Table 1 provides a summary of the individual genes analysed in chondrocytes.

Together, the studies of DNA methylation in OA provide evidence that such mechanisms regulate metalloproteinase expression either directly or indirectly. As DNA methylation is a reversible process, a better understanding of the mechanisms involved represents an alternative approach to develop new OA therapeutic strategies.

Chromatin modifications

Closely linked with DNA methylation, gene expression is also regulated by the packaging of eukaryotic DNA into nucleosomes. Nucleosomes are considered repressive to transcription, but through the enzymatic post-translational modification of histones by such modifications as acetylation, phosphorylation, methylation and ubiquitination, this inhibition can be regulated19. The particular pattern of modifications present may ‘code’ for the factors recruited and the transcriptional state of the underlying gene19.

Acetylation is mediated by histone acetyltransferases (HATs) and occurs on specific lysine residues on the N-terminal tails of histones, loosening the histone:DNA structure and allowing access of the transcriptional machinery40. Deacetylases, which fall into two types, classical HDACs that use a zinc-catalysed mechanism of deacetylation41, and the sirtuin deacetylases that require NAD+42, remove these acetyl groups resulting in hypo-acetylation.

Numerous transcriptional activators or repressors have (or recruit) HAT or HDAC activity, respectively. Many small molecule histone deacetylase inhibitors (HDACi) have been developed, with some undergoing clinical trials for various cancers43. HDACi also show promise in rheumatoid arthritis (RA) models (see below). Non-histone acetylated proteins have been identified, including numerous TFs and signalling pathway proteins44, which has led to the postulation that acetylation is as important a post-translational regulatory mechanism as phosphorylation.

Histone methylation is important for the formation of active and inactive genomic regions and is associated with both transcriptional activation and silencing45. Methylation of histone tails on lysine or arginine residues is catalysed by histone methyl transferases (HMTs) and protein arginine methyltransferases (PRMTs) which can add one or more methyl groups to regulate transcription. These modifications are reversible via the action of histone demethylases46.

Although overall histone modifications are more dynamic than DNA methylation, some specific histone methylation modifications are thought to be actively maintained during DNA replication, therefore truly epigenetic47.

Table 1: Summary of current cartilage genes screened for regulation by DNA methylation

<table>
<thead>
<tr>
<th>Function</th>
<th>Gene symbol</th>
<th>Context</th>
<th>Expression level</th>
<th>Methylation status</th>
<th>Differential ΔCpGs</th>
<th>Correlation</th>
<th>Refs</th>
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<tbody>
<tr>
<td>ECM components</td>
<td>COL1A4</td>
<td>Differentiation</td>
<td>⌧</td>
<td>Hypo, UN</td>
<td>None</td>
<td>–</td>
<td>34</td>
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<tr>
<td></td>
<td>COL2A1</td>
<td>Differentiation</td>
<td>⌧</td>
<td>Hypo, UN</td>
<td>None</td>
<td>–</td>
<td>25</td>
</tr>
<tr>
<td></td>
<td>COL2A1</td>
<td>OA, chondrogenesis</td>
<td>–</td>
<td>Hypo, UN</td>
<td>None</td>
<td>–</td>
<td>25</td>
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<tr>
<td></td>
<td>COL10A1</td>
<td>Chondrogenesis</td>
<td>⌧</td>
<td>Hyper, DIFF</td>
<td>(−1680, −1674) in MSCs</td>
<td>+</td>
<td>28</td>
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<tr>
<td></td>
<td>ACAN</td>
<td>OA</td>
<td>–</td>
<td>Hypo, UN</td>
<td>None</td>
<td>–</td>
<td>27</td>
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<tr>
<td></td>
<td>MATN4</td>
<td>Chondrogenesis</td>
<td>⌧</td>
<td>Hypo, UN</td>
<td>None</td>
<td>–</td>
<td>29</td>
</tr>
<tr>
<td>Matrix-degrading enzymes</td>
<td>MMP3</td>
<td>OA</td>
<td>⌧</td>
<td>Hypo, DIFF</td>
<td>−635</td>
<td>+</td>
<td>33</td>
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<tr>
<td></td>
<td>MMP9</td>
<td>OA</td>
<td>⌧</td>
<td>Hypo, DIFF</td>
<td>−36</td>
<td>+</td>
<td>33</td>
</tr>
<tr>
<td></td>
<td>MMP13</td>
<td>OA</td>
<td>⌧</td>
<td>Hypo, DIFF</td>
<td>−110</td>
<td>+</td>
<td>33, 107</td>
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<tr>
<td>Signalling</td>
<td>IL1B</td>
<td>OA</td>
<td>⌧</td>
<td>Hyper, DIFF</td>
<td>(−256, −299)</td>
<td>–</td>
<td>36</td>
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<tr>
<td></td>
<td>SOCS2</td>
<td>OA</td>
<td>–</td>
<td>Hyper and hypo, UN</td>
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<td>–</td>
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<td>GREM1</td>
<td>Chondrogenesis</td>
<td>–</td>
<td>Hypo, UN</td>
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<td>–</td>
<td>29</td>
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<td>SDF1</td>
<td>Chondrogenesis</td>
<td>–</td>
<td>Hyper, DEC</td>
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<tr>
<td></td>
<td>BMP7</td>
<td>OA, ageing</td>
<td>–</td>
<td>NS, INC</td>
<td>Not mentioned</td>
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<tr>
<td>TFs</td>
<td>SOX9</td>
<td>Chondrogenesis</td>
<td>⌧</td>
<td>Hypo, UN</td>
<td>None</td>
<td>–</td>
<td>29</td>
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<tr>
<td></td>
<td>RUNX2</td>
<td>Chondrogenesis</td>
<td>⌧</td>
<td>Hypo, UN</td>
<td>None</td>
<td>–</td>
<td>29</td>
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<td></td>
<td>SOX4</td>
<td>Chondrogenesis</td>
<td>–</td>
<td>Hypo, UN</td>
<td>None</td>
<td>–</td>
<td>29</td>
</tr>
<tr>
<td>Others</td>
<td>SOD2</td>
<td>OA</td>
<td>–</td>
<td>Hypo, DIFF</td>
<td>(−222, −183, −154)</td>
<td>+</td>
<td>37</td>
</tr>
<tr>
<td></td>
<td>LEP</td>
<td>OA</td>
<td>⌧</td>
<td>Hyper, DEC</td>
<td>Not mentioned</td>
<td>–</td>
<td>17</td>
</tr>
<tr>
<td></td>
<td>p21WAF1/CIP1</td>
<td>OA, ageing</td>
<td>–</td>
<td>Hypo, UN</td>
<td>None</td>
<td>–</td>
<td>29</td>
</tr>
<tr>
<td></td>
<td>CHM1</td>
<td>Chondrogenesis</td>
<td>⌧</td>
<td>Hypo, UN</td>
<td>None</td>
<td>–</td>
<td>29</td>
</tr>
<tr>
<td></td>
<td>FGFR3</td>
<td>Chondrogenesis</td>
<td>⌧</td>
<td>Hypo, UN</td>
<td>None</td>
<td>–</td>
<td>29</td>
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<tr>
<td></td>
<td>GPR39</td>
<td>Chondrogenesis</td>
<td>–</td>
<td>Hypo, UN</td>
<td>None</td>
<td>–</td>
<td>29</td>
</tr>
</tbody>
</table>

Genes are classified according to their function/role during physiological and pathophysiological conditions. The methylation status is listed as: hyper, hypermethylation; hypo, hypomethylation; UN, unchanged; DIFF, differential; DEC, decreased; NS, not specified; INC, increased.

* Refers to chick.
HDACi increase histone H4 acetylation in the promoters of MMP1 and MMP13 following interleukin (IL)-1 stimulation, suggesting repression by histone-independent mechanisms. Certainly, many signalling pathway proteins are subject to reversible acetylation, however in chondrocytes HDACi do not appear to modulate NF-κB signalling.

HDACi also modulate the expression of ECM components, although the duration of administration appears important, for example, short-term treatment of chondrocytes (<24 h) induces anabolic cartilage gene expression such as COL2A1, COL9A1, COMP, and ACAN, while extended treatment represses many of the same transcripts. The early positive effects may be a direct result of HDAC inhibition since HDAC1 and HDAC2 overexpression represses ACAN and COL2A1, whereas the long-term inhibition may be due to the up-regulation of repressive factors such as Wnt-5A or NAB1.

In vivo, the only assessment of the role of HDACs in OA was in a rabbit anterior cruciate ligament transaction (ACLT) model, where animals given a weekly injection of HDACi developed significantly less cartilage erosion, concomitant with reduced expression of collagenases and IL1. Several studies have shown that HDACi can exhibit beneficial effects on animal models of RA, mainly by suppressing synovitis and reducing the levels of inflammatory cytokines (e.g., Ref. 57).

In human disease, HDAC7 was reported to be differentially expressed between OA and normal cartilage, where its depletion controlled MMP13 expression, HDAC1 and HDAC2 are reportedly up-regulated in OA chondrocytes, although overall HDAC activity is similar between OA and normal synovial tissue. HDAC activity decreases during human articular chondrocyte dedifferentiation, correlating with a reduction in COL2A1 expression, and HDACi prevent the redifferentiation of dedifferentiated chondrocytes, suggesting that HDAC activity is crucial for the maintenance of the chondrocyte phenotype.

In terms of histone methylation in chondrocytes, IL1 induces histone H3K4 di- and tri-methylation around the COX2 and iNOS promoters, but not MMP13, which correlated with the recruitment of SET-1A, a methyltransferase with increased expression in OA compared with normal tissue. Histone methyltransferase inhibition or depletion of SET-1A prevented IL1 induction of COX2 and iNOS (Fig. 2).
**Sirtuins**

In cartilage, the sirtuin SirT1 is essential for chondrocyte survival by enhancing insulin-like growth factor signalling to inactivate p53 and prevent apoptosis. SirT1 levels decrease in dedifferentiating chondrocytes, in OA cartilage and in cartilage exhibiting OA-like damage, which could account for the loss of chondrocytes in OA. The inhibition of SirT1 leads to an increase in chondrocyte apoptosis while addition of resveratrol, a sirtuin activator, protects chondrocytes from cell death. Exposure of chondrocytes to ionising radiation significantly increases the number of senescent cells by reducing SirT1 levels, since this can be blocked by SirT1 overexpression or resveratrol. Interestingly, the function of SirT1 is closely linked to hypoxia where, potentially, the sirtuin can directly deacetylate and thus activate HIF-2α, resulting in the up-regulation of metalloproteinases and cartilage destruction.

Aside from its role in survival, SirT1 also promotes cartilage matrix gene expression such as ACAN, COL2A1, COL9A1 and COMP, potentially via the deacetylation of SOX9, while also inhibiting ADAMT55 expression. SirT1 also plays a role in skeletal homeostasis and osteoporosis since haplo-insufficient female mice exhibit a significant reduction in bone mass characterised by decreased bone formation and increased marrow adipogenesis. The role for SirT1 in muscle physiology and disease has also been extensively studied.

**miRNAs**

Small non-coding RNAs known as miRNAs are important regulators of gene expression in metazoan genomes. They are short, 20- to 23-nucleotide-long single-stranded non-coding RNAs, transcribed from either intergenic regions with their own promoter or as part of a gene where they are usually intronic. Following processing to short stem-loop structures, pre-miRNA, they are exported from the nucleus. The cytoplasmic RNase III enzyme Dicer removes the stem-loop forming a miRNA duplex consisting of two arms of the loop (often named -5p and -3p respectively). One miRNA duplex is then integrated into the RNA-induced silencing complex (RISC), where it base pairs, via nucleotides 2–8 of the miRNA ‘seed-sequence’, with generally the 3’UTR of target mRNAs. Depending on the level of complementarity between miRNA and target mRNA, RISC either cleaves or suppresses translation of the mRNA, the latter being predominant, but still somewhat controversial, in mammals.

miRNAs were originally mooted as ‘fine tuning’ gene expression. Recently, particularly through the generation of knockout and transgenic mice for specific miRNAs, it has become clear that they can be major regulators in development and play a significant role in various pathologies.

**miRNAs and chondrocytes**

Loss of the miRNA processing enzyme Dicer in mice leads to embryonic day 7.5 lethality. However using conditional Dicer-null mice, an overall role for miRNAs in limb mesenchyme and skeletogenesis has been demonstrated. In terms of skeletogenesis, the severe growth defects observed in these mice are caused by a decrease in proliferating chondrocytes in the growth plate and a more rapid onset of hypertrophy. Cartilage miRNA research to date has focussed largely on miR-140 which was originally identified as a cartilage-restricted miRNA in developing zebrafish. Similarly, during murine skeletal development miR-140 is specifically expressed in cartilage tissues. In humans, the expression of miR-140 increases during chondrogenesis and is more abundant in articular cartilage, but reduced in OA. In vitro murine targets of miR-140 include Hdac4, a repressor of Runx2 and MEF2C, and Smad7, while in humans miR-140 decreases Cxcl12 (SDF-1) and SMAD2, and increases ACAN expression.

miR-140-null mice are born normally but within 4 weeks have impaired growth, due to shorter long bones, and exhibit craniofacial deformities. The articular joint cartilage initially appears normal, but the mice show an early onset OA-like disease and greater susceptibility to surgically-induced OA. Conversely, in an antigen-induced arthritis model, transgenic overexpression of miR-140 protected against cartilage damage. Although also a target in mice, the inhibition of Adamps5 by miR-140 is only modestly effective.
which suggest further targets of miR-140 are to be identified. To this end, a second miR-140-null mouse with a skeletal phenotype was recently reported and the authors show that miR-140 targets Dnpep (aspartyl aminopeptidase), which may antagonise BMP signalling.

miR-140 resides within an intron of Wwp2, an E3 ubiquitin ligase recently shown to have an important role in cartilage biology via its mono-ubiquitination of TF Goosecoid. In addition, miR-140 regulates Pdgf (platelet-derived growth factor) signalling and impinges on palatogenesis, a process for which Wwp2 is essential. The chondrocyte TF Sox9 directly promotes the expression of Wwp2 and miR-140, but can also regulate Il1 signalling, as does miR-146a, to modulate Mmp13 levels. Mmp13 is also a direct target of miR-27, and Type-II collagen is a major component of cartilage and appears to be regulated by the hypoxia induced miR-675.

Other miRNAs

The role of other miRNAs in OA is as yet unclear. Two screens of miRNA expression in normal compared with OA cartilage have been published. Firstly, Jones et al. highlighted the altered expression of miR-9, miR-98 and miR-146 in OA cartilage and showed that their overexpression reduced IL1-induced TNFα production, whilst inhibition or overexpression of miR-9 modulated Mmp13 secretion. Secondly, Lipopoulos et al. identified several miRNAs altered in OA cartilage. They focused on miRNAs whose expression correlated with body mass index and demonstrated that miR-22 targets Ppara and Bmp7 with indirect effects on Il1, Mmp13 and Acan expression. Consistent with the findings of Jones et al., Yamasaki et al. found that the expression of miR-146a decreases with increased OA severity. IL1 stimulation induces miR-146a expression in chondrocytes, which via a feedback loop, represses IL1-induced gene expression, consistent with its role in the 3’UTR-mediated repression of Ira1k and Traf6 to suppress Tlr (toll-like receptor) signalling. Recently, miR-146a has also been linked to the pain-related pathophysiology of OA.

In contrast, IL1 stimulation represses miR-27b expression in chondrocytes. miR-27b expression is reduced in OA cartilage where it inversely correlates with Mmp13, which directly targets Traf6. Dudek et al. identified Hif1a, a non-coding RNA, processed to form miR-675, as tightly regulated by Sox9 during chondrocyte differentiation. miR-675 upregulates Col2a1 expression, albeit indirectly. In rat chondrocytes Il1 induces miR-34, which appears to counteract the effect of IL1 on Col2a1 and Nos2 (NOS) expression. Stimulation of chondrocytes by cyclical loading regulates the expression of a number of miRNA including miR-365 which, like miR-140, directly targets the expression of Hdac4.

Screens in dedifferentiating articular chondrocytes, bovine cartilage, chondrosarcoma and osteochondroma have established miRNA profiles for which further studies are required to identify targets in cartilage. Recent work based upon such screens has identified miR-145 and miR-455-3p as miRNAs that warrant further investigation in chondrogenesis and OA. miR-145 directly targets Sox9 to control chondrogenesis, while miR-455-3p appears to regulate Tgfb signalling by suppressing the Smad2/3 pathway.

miRNAs are also found in plasma and may even be able to enter recipient cells, opening up the possibility of exogenous regulation of gene expression. Murata et al. examined the potential of miRNA as diagnostic biomarkers from OA patients and found a number of miRNAs in plasma some of which were found at different levels between RA and OA patients.

Epigenetic analysis

Epigenetics methodologies have until recently been small-scale, low throughput focussing on the analysis of a single gene, many using polymerase chain reaction (PCR) as the final (or penultimate) analytical or quantitative based step. However, many of these techniques now employ tiling genome hybridisation technology or high-throughput DNA sequencing as their final read-outs to make whole epigenome analysis a reality.

Analysis of DNA methylation

After isolation of genomic DNA, methylation analysis can have a number of initial steps. Many original techniques used methylation-sensitive and insensitive restriction endonucleases followed by either PCR or gel-based methods to compare the DNA fragments generated after digestion. Newer techniques use an initial affinity enrichment step, either an antibody against 5-mC (or more recently 5hmC) or an antibody against, or a tagged, methyl-binding protein (e.g., Mbd2). After 5mC DNA enrichment PCR, tiling arrays or high-throughput DNA sequencing can then be employed. The most frequently used method for DNA methylation analysis is that of bisulphite conversion, which leads to the initial affinity enrichment step, either an antibody against, or a tagged, methyl-binding protein (e.g., Mbd2). After 5mC DNA enrichment PCR, tiling arrays or high-throughput DNA sequencing methods can be employed. The most frequently used method for DNA methylation analysis is that of bisulphite conversion, which leads to the deamination of unmethylated cytosine residues in preference to those methylated. The deaminated cytosines are converted to thymidines (via uracil) turning an epigenetic into a genetic difference. This DNA is then subjected to PCR using bisulphite specific PCR primers. The amount of methylation in the amplicon can then be determined by either direct pyrosequencing, mass spectrometric analysis (Sequenom Epityper) or cloning and Sanger sequencing. Bisulphite converted DNA can also be subjected to array-based
null animals clearly indicates that further important miRNAs remain to be identified. Conditional-null animals, including for miR-140, still need to be generated in order to distinguish the role of miRNAs in development and disease. Many other challenges also remain, especially in terms of miRNA target identification; such targets are likely to identify new pathways involved in OA. Therapeutically, inhibitors of specific miRNAs, modified locked-nucleic acids, are already undergoing evaluation and could be explored in OA.

To date, there have been no global chromatin or DNA methylation analyses in OA. A number of chromatin modifying enzymes certainly have important roles in skeletogenesis, such as HDAC470, and will undoubtedly in OA. Small molecule inhibition of these enzymes is an intriguing therapeutic option and at least for HDAC4 has shown efficacy in small animal arthritis models including OA56. However, current HDACi target multiple family members and although some HDAC specific inhibitors exist the appropriate HDACs to target in OA remain to be identified. Candidate gene approaches have led to some interesting observations with regards promoter methylation which suggests that global epigenetic analysis would be valuable. Many of the technical and data related issues with global epigenetic analysis will clearly be resolved, and it is likely that soon the ultimate in epigenetic analysis, whole genome single cell analysis, will be possible. However, significant hurdles remain, in particular how to distinguish between cause and effect for specific epigenetic modifications or patterns on gene expression changes, and the major question, if epigenetic changes that lead to OA are identified, how could this be therapeutically targeted to slow or prevent disease?

Contributions

All three authors, MJB, CB and DAY, were involved in the conception of the article, analysis and interpretation of reported data, drafting the article and revising it critically for important intellectual content and approved the final version for submission. David A. Young (d.a.young@ncl.ac.uk) takes responsibility for the integrity of the work as a whole, from inception to finished article.

Conflict of interest

The authors declare no competing interests.

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