

# We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

6,900

Open access books available

185,000

International authors and editors

200M

Downloads

Our authors are among the

154

Countries delivered to

TOP 1%

most cited scientists

12.2%

Contributors from top 500 universities



WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index  
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?  
Contact [book.department@intechopen.com](mailto:book.department@intechopen.com)

Numbers displayed above are based on latest data collected.  
For more information visit [www.intechopen.com](http://www.intechopen.com)



---

# Epigenetic Mechanisms in Osteoarthritis

---

Antonio Miranda-Duarte

Additional information is available at the end of the chapter

<http://dx.doi.org/10.5772/60533>

---

## 1. Introduction

The hallmark of osteoarthritis (OA) is the progressive degeneration of articular cartilage, although bone and synovia are also involved in the development of the disease [1]. Chondrocytes are the unique cellular component of articular cartilage and, under physiological conditions, are responsible for a subtle balance between the synthesis of extracellular matrix (ECM) components, mainly type-II collagen and aggrecan [2], and its degradation by proteolytic enzymes such as the matrix metalloproteinases (MMP) [3] and A disintegrin and metalloprotease with thrombospondin motifs (ADAMTS) [4]. In OA, there is an imbalance of this process driven by cytokines and the production of inflammatory mediators, resulting in an increase of the degradation process with respect to synthesis, and leading to articular cartilage loss [1, 5].

OA is considered a multifactorial disease in which genetics and environmental factors, such as aging, gender and obesity, among others, are strongly related with its development [6]. Primary OA possesses an important genetic component, and several genetic association studies have demonstrated that it is associated with different genes that encode molecules involved in a number of pathways, such as inflammation, Wnt signalling, bone morphogenetic proteins (BMPs), proteases and their inhibitors, and extracellular matrix proteins, among others [7, 8]. However, there has not always been consistency in the results, probably due to the low penetrance of the gene polymorphisms studied, or to different gene-gene interactions and gene-environment interactions. In this regard, epigenetics is one mechanism through which gene-environment interactions occur. Epigenetics refers to heritable changes in gene expression that occur without changes in DNA, and includes DNA methylation, histone modifications, chromatin remodelling and microRNAs (miRNAs), although debate continues concerning whether miRNA can be categorized as an epigenetic phenomenon [9]. Recent evidence has made it apparent that epigenetic changes alter the expression of genes that could participate in the pathogenesis of OA. This chapter does not intend to conduct a deep review of epigenetic modifications, but rather to review the main findings directly related with OA.

## 2. DNA methylation in osteoarthritis

DNA methylation is the most studied epigenetic mark in humans. DNA methylation involves the addition of a methyl group at CpG dinucleotides to convert cytosine into 5-methylcytosine. These CpG dinucleotides tend to cluster in regions termed 'islands', and approximately 70% of human gene promoters are associated with CpG, which are usually unmethylated. CpG-island methylation is associated with gene silencing due to the binding of methyl-CpG-binding proteins, which recruit proteins to the gene promoter, blocking its transcription. DNA methylation can also occur in CpG island shores, regions of lower CpG density that lie in close proximity to CpG islands (~2 kb); their methylation is associated with transcriptional inactivation. DNA methylation is mediated by the DNA methyltransferases (DNMTs) family of enzymes that catalyse the transfer of a methyl group to DNA. In mammals, five members of the DNMT family have been reported: DNMT1, DNMT2, DNMT3a, DNMT3b and DNMT3L, but only DNMT1, DNMT3a and DNMT3b possess methyltransferase activity. DNMTs are classified as *de novo* and maintenance enzymes. DNMT1 is the maintenance DNMT and has a preference for hemimethylated DNA; this is the most abundant DNMT in cells and is transcribed mostly during the S phase of the cell cycle. DNMT3A and DNMT3B are *de novo* DNMTs and are responsible for establishing the pattern of methylation during embryonic development [9-13].

Analysis of the overall methylation level of genomic DNA in the chondrocytes of degenerated OA cartilage shows no difference in comparison with normal chondrocytes. However, an inhibitor of cell proliferation, the cyclin-dependent kinase inhibitor 1 (*p21<sup>WAF1/CIP1</sup>*) gene, which may mediate the re-initiation of cell proliferation in OA cartilage and which has demonstrated itself to be epigenetically regulated in neoplastic cells [14], is significantly downregulated in OA chondrocytes and does not exhibit hypermethylation in its promoter [15].

In chick embryos' chondrocytes, the *Col2a1* gene shows reduced methylation in comparison with other cells, such as fibroblasts and erythrocytes [16]. In humans, it remains unclear whether the methylation of DNA alters the regulation of cartilage matrix genes in OA. Aggrecan is reduced during aging and in OA cartilage; however, the aggrecan (*ACAN*) gene expression of normal aging and osteoarthritic articular human cartilage does not correlate with increased methylation of the *ACAN*-promoter CpG island [17]. Human articular chondrocytes remain negative for type X collagen, unless they become hypertrophic. In the latter cells, the gene methylation patterns and the expression of *COL10A1* and *COL2A1* have shown that the *COL10A1* promoter is methylated, which correlates with the suppression of hypertrophy observed in articular chondrocytes, and there is no evidence of *COL2A1* regulation at the methylation level, which demonstrates a low methylation rate [18].

The nuclear transcription factor SOX9, along with other SOX family members, is required for the control of the expression of ECM components. SOX9 is an important regulator of the chondrocyte phenotype and controls the expression of the *COL2A1*, *COL9A1*, *COL11A1* and *ACAN* genes. The SOX9 protein binds to its promoter elements and forms transactivating complexes with other proteins, such as SOX5/SOX6. The SOX family maintains the chondrocytic phenotypes, and is vital for chondrogenesis in embryonic development [19, 20]. In human

synovium-derived mesenchymal stem cells (MSCs) subjected to chondrogenesis the CpG island of *SOX9* is hypomethylated; as well as in other chondrogenesis related genes such as runt-related transcription factor 2 (*RUNX2*) and fibroblast growth factor receptor 3 (*FGFR3*) [21]. While in OA chondrocytes, *SOX9*-promoter is hypermethylated, which reduces the binding affinity of the transcription factors CCAAT-binding factor/nuclear factor-Y (CBF/NF-Y) and the cAMP response element binding (CREB) [22]. This means that the methylation of the *SOX9* promoter remains low during chondrogenesis, and in OA there is change in the epigenetic status of *SOX9*, including increased DNA methylation.

Metalloproteinase expression in normal cartilage is relatively low but is elevated in OA, resulting in ECM degradation [3, 23]. The altered synthesis of the cartilage-degrading enzymes in OA is the result of changes in the methylation status, as demonstrated by the analysis of the methylation of the promoter region of *MMP3*, *MMP9*, *MMP13* and *ADAMTS4* in the cartilage of patients with OA, in which the overall percentage of non-methylated sites increased in comparison with normal controls. However, not all CpG sites were equally susceptible to loss of methylation, and for each gene there was a specific site where OA demethylation was higher, namely: -635 for *MMP3*, -36 for *MMP9*, -110 for *MMP13*, and -753 for *ADAMTS4* [24]. This is interesting because it was generally thought that the methylation of many CpG sites was required to repress gene expression, and these findings suggest that methylation of a single site may be sufficient to affect gene expression. In agreement with this, demethylation of -110 in *MMP13* promoters and -299 in *IL1B* promoters is correlated with an increased gene expression. In addition, methylation of the -110 CpG site in *MMP13* decreases the hypoxia inducible factor 2 $\alpha$  (HIF-2 $\alpha$ ), binding to the *MMP13* promoter. HIF-2 $\alpha$  is a transcription factor that regulates *MMP13* expression [25]. On the other hand, demethylation of the -104 CpG region of the *MMP13* promoter correlates with increased gene expression and avoids the binding of the transcription factor CREB to its promoter [26]. *ADAMTS5* is considered to be the major aggrecanase; however, *ADAMTS4* also contributes to aggrecan degradation in OA. *ADAMTS4* is epigenetically regulated and, although methylation is lost in several promoter sites, the -753 site is that most consistently demethylated [27].

Nitric oxide (NO) is a multifunctional molecule that suppresses energy production by mitochondrial respiration. In OA, high amounts of NO are produced, a consequence of upregulation in the chondrocyte of the inducible NO synthase (iNOS) induced by inflammatory cytokines, such as interleukin 1-beta (IL-1 $\beta$ ) and tumour necrosis factor alpha (TNF $\alpha$ ), among others [28]. NO suppresses the synthesis of the cartilaginous matrix [29]. In culture, under-stimulated chondrocytes produce iNOS, and its promoter contains nuclear factor-kappa beta (NF- $\kappa$ B) binding sites; this regulates iNOS at the transcriptional level. NF- $\kappa$ B is a signalling factor activated by tissue damage and inflammation; its demethylation in specific enhancer elements favours the activation of iNOS in chondrocytes [30]. Interestingly, a study showed that glucosamine and an NF- $\kappa$ B inhibitor inhibit cytokine-induced demethylation at a specific site in the *IL1B* promoter, resulting in decreased gene and protein expression [31].

Leptin (*LEP*) is a cytokine-like peptide hormone secreted by white adipose tissue, which plays a key role in OA [32] because it has been shown that *LEP* exerts a detrimental effect on articular cartilage by promoting NO synthesis in chondrocytes [33]. In normal chondrocytes, *LEP* is

highly methylated and, in OA, it is demethylated and highly expressed. Additionally, *LEP* downregulation with small interference RNA (siRNA) decreases *MMP13* expression [34].

To date, it is well recognized that OA has an important inflammatory component in its development mediated by proinflammatory cytokines, such as IL-1 $\beta$  and TNF $\alpha$  [2, 32]. Healthy chondrocytes do not express *IL1B*, however, promoter demethylation increases the expression of the gene [35]. Suppressor of cytokine signalling (SOCS) proteins are inhibitors of cytokine signalling. There are eight SOCS proteins, including SOCS1-SOCS7 and cytokine-inducible SH2-domain-1 (CIS-1), with SOCS1, -2 and -3, and CIS-1 the best characterized. *SOCS2* and *CIS-1* expression is reduced in OA chondrocytes compared with normal chondrocytes, while *SOCS1* and *SOCS3* expression remains unchanged. In addition, the *SOCS2* promoter does not exhibit a change in its methylation status [36].

Bone morphogenetic protein-7 (BMP-7) – or osteogenic protein-1(OP-1) – is one of the most potent growth factors for cartilage maintenance and repair. It possesses a critical role in human cartilage homeostasis, regulating numerous metabolic pathways that are not only limited to its well-documented anabolic function but also to its anti-catabolic activity [37]. There is a positive correlation between age and the methylation of the *BMP7* promoter's status in aged chondrocytes; this age-related promoter methylation may contribute to a decrease in BMP7 production in cartilage, with the decreased expression of the insulin-like growth factor-1 (*IGF-1*) and the IGF-1 receptor (*IGF-1R*) genes, as well as the ECM component gene *ACAN* [38].

The growth differentiation factor 5 (*GDF5*) gene is a member of the transforming growth factor  $\beta$  (TGF $\beta$ ) superfamily that is involved in chondrogenesis and chondrocyte proliferation [39]. The rs143383 C/T single nucleotide polymorphism (SNP) in the 5' untranslated region (5'UTR) of the gene is associated with an increased risk of OA [40, 41]. This SNP is itself functional and exerts a joint-wide effect on *GDF5* expression, causing a significant reduction in the expression of the disease-associated T allele relative to the C allele in the cartilage and other joint tissues [42], a phenomenon known as 'differential allelic expression' (DAE). The transcriptional effect of rs143383 SNP is dependent on a second C-to-T SNP in the 5'UTR of *GDF5*, rs143384 C/T, with decreased expression of the T allele of rs143383 only observed in individuals which are compound-heterozygous for both SNPs. When the OA-protective C alleles are present at the rs143383 and rs143384 SNPs, they form CpG dinucleotides, which are potentially amenable to regulation by DNA methylation. In cell lines, *GDF5* is upregulated after demethylation, and methylation decreases transcriptional activity. Interestingly, CpG sites formed by the C alleles of both SNPs are methylated; however, their demethylation is associated with increased expression of the C allele of rs143383 relative to the T allele, which indicates that the OA-susceptibility conferred by rs143383 of the *GDF5* gene is regulated by methylation [43].

Methylation analysis of 23,367 sites (corresponding to 13,463 genes) through a genome-wide methylation profile of bone from patients with OA and osteoporosis (OP) revealed an inverse relationship between methylation and gene expression in both groups, with 271 CpG sites being less methylated in OP than in OA. *In silico* pathway analysis revealed genes associated in glycoprotein metabolism or cell differentiation, particularly the homeobox superfamily of transcription factors such as homeobox A9 (*HOXA9*), Iroquois homeobox 2 (*IRX2*) and msh homeobox 2 (*MSX2*), which are involved in embryonic development [44].



### 3. Histone modification, chromatin remodelling and osteoarthritis

DNA is compacted by the tight weaving of approximately 147 base pairs around the proteins' denominated histones, forming a DNA-protein complex termed a 'nucleosome', the basic unit of chromatin. Each nucleosome consists of an octamer of two copies of the following four core histones: H2A, H2B, H3 and H4. The nucleosomes comprise a barrier to transcription that blocks the access of activators and transcription factors to their sites in DNA. The histones are subject to post-transcriptional modification; the most common are acetylation and methylation, although other modifications have been identified, including phosphorylation, ubiquitination, SUMOylation, citrullination, and adenosine diphosphate ribosylation. Histone acetylation occurs at lysine residues and is associated with DNA accessibility and transcriptional activity, whereas deacetylation is associated with transcriptional repression. Histone acetyltransferases (HATs) are enzymes that transfer the acetyl group onto the  $\epsilon$ -amino group of the lysine residues within a histone tail; this is a reversible process, and the enzymes that remove the acetyl groups are known as 'histone deacetylases' (HDACs). Classical isoforms of HDACs comprise a total of 11, and are broadly divided into two classes: HDACs 1, -2, -3 and -8 are Class I HDACs, while Class II encompasses HDAC isoforms 4, -5, -6, -7, -9, -10 and -11. The newly characterized SIR2 family of HDACs (sirtuins), termed 'Class III', operate through a nicotinamide adenine dinucleotide (NAD<sup>+</sup>)-dependent mechanism. Histone methylation is another major modification that takes place in the  $\epsilon$ -amino group of lysine residues; it is mediated by histone H3 N-lysine lysine methyltransferases (HKMTs). The effect of histone lysine methylation on gene regulation is highly complex, mediating either transcription repression or activation. Likewise, the methylation of arginine residues is catalysed by the protein arginine methyltransferase (PRMT) family. Regulation of gene transcription can also occur by chromatin remodelling. The SWItch/sucrose non-fermentable protein complex binds to the nucleosome and disconnects the DNA from the histones, creating a transient DNA loop and resulting in nucleosome repositioning, such that the transcription of targeted genes can be increased or decreased depending upon whether the gene is located in the open-chromatin or compacted chromatin region. Polycomb-group proteins are also involved in gene silencing through chromatin remodelling, repressing transcription by maintaining a heterochromatin state through particular histone modifications and DNA methylation [9-13].

Chondrocyte differentiation is controlled by transcription factors such as SOX9, among others. SOX9 requires other cofactors, such as the CREB binding protein, which activates SOX9-dependent transcription due to its intrinsic histone acetyl-transferase activity [45]. In human chondrocytes induced by IL-1 $\beta$  and the fibroblast growth factor 2 (FGF2), there is increased expression of MMPs and ADAMTS, responsible for collagen and aggrecan loss, respectively. However, HDAC inhibitors (HDACi) block the induction of these enzymes at the mRNA and protein levels [46, 47]. Thus, HDACi also suppress IL-1 $\beta$ -induced NO and prostaglandin E<sub>2</sub> (PGE<sub>2</sub>) synthesis, which plays an important role in OA as well as in proteoglycan degradation [48].

Specific HDACs appear to be involved in different processes as well as targeting different chondrocyte-specific genes. In a murine model, *HDAC4* demonstrates the regulation of

chondrocyte hypertrophy and endochondral bone formation by interacting with Runx2 and inhibiting its activity [49]. In the chondrocytes of patients with OA, HDAC1 and HDAC2 proteins are elevated with the specific downregulation of *COL2A1* and *ACAN*, though not with other cartilage marker genes. This is because the snail transcription factor acts as a mediator of the HDAC1 and HDAC2 repression of *COL2A1* via its interaction with HDACs' carboxy-terminal domains [50]. *HDAC7* shows a significant increase in OA cartilage, while its knock-down by siRNA in a chondrosarcoma cell line suppress *MMP-13* expression [51].

The role of HDACi has been explored in OA. HDAC activity decreases during chondrocyte dedifferentiation, and the inhibition of HDAC with HDACi trichostatin suppresses type II collagen expression. This is because HDACi promotes the acetylation of Wnt-5a, increasing its expression, which is known to inhibit type II collagen [52]. Human chondrocytes under mechanical stress exhibit the downregulation of *COL2A1* and upregulation of *RUNX2*, *ADAMTS4* and *MMP3*; however, after treatment with HDACi there is an increase of *COL2A1* expression, the downregulation of *RUNX2*, *ADAMTS4* and *MMP3*, and an inhibition of the mechanical stress-induced phosphorylation of mitogen-activated protein kinase (MAPK) molecules after treatment with HDACi [53]. RUNX family members regulate the gene expression involved in cellular differentiation and cell cycle progression. *RUNX2* plays a key role in bone mineralization by stimulating osteoblast differentiation [54] and it contributes to OA pathogenesis through chondrocyte hypertrophy and matrix breakdown after the initiation of joint instability [55]. MAPK pathways play essential regulatory roles in early osteoblast differentiation in response to mechanical stress via the activation of *RUNX2* [56]. *In vivo*, the effects of HDACi trichostatin on cartilage degradation in a rabbit experimental model showed that HDACi decreases cartilage degradation as well as the expression of IL1 and MMPs, such as *MMP1*, *MMP3* and *MMP13* [57].

Histone H3 lysine-4 (H3K4) methylation is associated with transcriptional activation, whereas H3K9 methylation correlates with transcriptional repression. In human osteoarthritic chondrocytes, the induction of iNOS and cyclooxygenase 2 (COX2) expressions by IL-1 $\beta$  are associated with H3K4 di and trimethylation at the *iNOS* and *COX2* promoters; these changes correlate with the recruitment of SET-1A, a HKMT. Furthermore, HKMT inhibition prevents the IL-1 $\beta$  induction of iNOS and COX2 [58].

*Nfat1* is a nuclear factor of the activated T cells' transcription factor family and is a regulator of cytokine gene-expression. It has been reported that adult *Nfat1*-deficient mice display abnormal chondrocyte differentiation in their articular cartilage and develop several articular cartilage characteristics that resemble these changes in OA in humans. These OA-like changes appear at the adult stage and an increase in *Nfat1* expression in the chondrocytes is associated with increased H3K4 methylation, whereas a decrease of *Nfat1* is associated with an increase in H3K9 methylation, which demonstrates that *Nfat1* specifically regulates the function of adult articular chondrocytes through its age-dependent expression, mediated by dynamic histone methylation [59].

NAD-dependent class III HDACs consist of SIRT1-7. SIRT1 plays a key role in the regulation of metabolism, as well in regulating cell differentiation, proliferation, survival and longevity [60, 61]. SIRT1 increases cartilage-specific gene expression, such as *ACAN*, *COL2A1*, *COL9A1*

and *COMP*. SIRT1 deacetylate SOX9 enhances the transcription of *COL2A1* and, at least for the *COL2A1* gene promoter, also enhances the acetylation of critical histone core residues in the promoter through the recruitment of activator/co-activator proteins [62]. In human chondrocytes, SIRT1 inhibits NO-induced apoptosis caused, at least partially, by caspases 3 and 9 [63]. Another mechanism by means of which SIRT1 inhibits apoptosis in human chondrocytes is that of repressing protein tyrosine phosphatase 1B (PTP1B), a potent proapoptotic protein, and there is an inverse relationship in the expression patterns of SIRT1 and PTP1B in normal and OA cartilage. In contrast, SIRT1 levels are high and PTP1B levels are low in normal cartilage, while in OA SIRT1 levels are low and PTP1B levels are high [64]. In OA, the inhibition of SIRT1 induces OA-like gene expression changes with a downregulation of *ACAN* and upregulation of *COL10A1* and *ADAMTS5*, which suggests that SIRT1 expression decreases with the development of OA, favouring chondrocyte hypertrophy and cartilage matrix loss [65].

To date, OA is well-recognized as an inflammatory disease in which inflammatory cytokines play a central role. In human OA chondrocytes treated with TNF $\alpha$  there is an impaired SIRT1 activity due to cleavage mediated by cathepsin B, resulting in the upregulation of *MMP13* and *ADAMTS4* and reduced cartilage-specific gene expression, such as *COL2A1*, *COL11A1* and *ACAN* [66]. Null mice for SirT1 (SirT1  $-/-$ ) do not survive, and heterozygous mice for SirT1 ( $+/-$ ) are smaller and exhibit a greater increase in OA changes than normal mice. In addition, in heterozygous mice, inflammatory cytokines are upregulated and demonstrate a marked increase in apoptosis, which suggests that SirT1 may prolong the viability of articular chondrocytes in adult mice [67]. In human chondrocytes, the overexpression of SIRT1 significantly inhibits the upregulation of genes caused by the pro-inflammatory cytokines IL-1 $\beta$  and TNF $\alpha$  (*MMP1*, -2, -9, and -13, and *ADAMTS5*), while in the OA cartilage SIRT1 expression decreased while that of *MMP13* and *ADAMTS5* increased [68, 69]. Therefore, SIRT1 exerts an anti-inflammatory effect and prevents chondrocyte apoptosis.

#### 4. microRNAs in chondrogenesis and osteoarthritis

MicroRNA (miRNA) are small noncoding RNAs of ~20–25 nucleotides (nt) in length that are transcribed in the nucleus by RNA polymerase II or III into a long precursor denominated primary-miRNA (pri-miRNA). This pri-miRNA is processed by the microprocessor Drosha-DGCR8 complex, an RNase III-type enzyme, to generate a precursor of ~70–100 nt, known as 'pre-miRNA', which is translocated into the cytoplasm via exportin 5. Pre-miRNA is processed by the ribonuclease dicer, generating a miRNA duplex of ~22 nt. Finally, one of the strands is incorporated into the RNA-induced silencing complex (RISC), where it is guided to its target mRNA. miRNA is involved in post-transcriptional gene-expression regulation, targeting 30% of the encoding genes through complementary base-pairing between the miRNA and the 3'-UTR of the messenger RNA (mRNA) target, resulting in the translational suppression or direct degradation of the mRNA [9-13].



#### 4.1. Cartilage and miRNA

The influence of miRNAs in cartilage homeostasis and skeletal development has been demonstrated in recent years. Dicer is an essential enzyme for the generation of mature miRNA and for proper skeletal morphogenesis. In mouse models, the loss of *dicer1* leads to embryonic lethality, with animals surviving until embryonic day 7.5 [70] with limbs that are small in size due to the loss of processed miRNAs [71]. Dicer possesses an important function in cartilage as demonstrated in mice in which the gene was specifically deleted in cartilage. These mice exhibited a progressive reduction in the proliferating pool of chondrocytes in growth plates, leading to severe growth defects because of a decrease in proliferating chondrocytes and an accelerated differentiation into hypertrophic chondrocytes. The latter results may be explained by dicer loss having distinct functional effects at different stages of chondrocyte development [72].

miR-140 is the most studied miRNA in both cartilage and OA. This miRNA was originally identified as a cartilage-restricted miRNA in developing zebra fish, with its expression in the jaw, head and fins during embryonic development [73]. In mice, miR-140 is also expressed in cartilage during embryonic long- and flat-bone development [74]. In a murine model with a targeted deletion of miR-140, mice are borne with grossly normal skeletal development; however, postnatally they manifest skeletal deformities with short stature and craniofacial deformities, probably as a result of abnormal chondrocyte proliferation [75, 76]. miR-140 is encoded in an intronic region of the ubiquitin E3 ligase gene, *WWP2*, which plays an important role in cartilage biology. miR-140 is highly conserved among vertebrates and it is not present in invertebrates, which suggests that it plays an important role in skeletal development [77]. In mice, miR-140 is exclusively expressed in chondrocyte, is co-expressed with *Wwp2*, and is directly induced by the transcription factor SOX9. Sp1, the activator of the cell cycle regulator p15<sup>INK4b</sup>, is a target of miR-140, suggesting that it regulates chondrogenic proliferation in part via the inhibition of Sp1 [78].

In the gene expression pattern in human articular chondrocytes and human MSC, miR-140 saw the largest differences in expression. During chondrogenesis, miR-140 increases in parallel with the expression of *SOX9* and *COL2A1*, and treating chondrocytes with IL-1 $\beta$ , suppresses miR-140 expression. On the other hand, transfection of chondrocytes with miR-140 down-regulate IL-1 $\beta$ -induced *ADAMTS5* expression and rescue the IL-1 $\beta$ -dependent repression of *ACAN* expression [79]. miR-140 has offered several targets: in mice it potentially suppresses *Hdac4*, a co-repressor of Runx2, the transcription factor essential for chondrocyte hypertrophy and osteoblast differentiation [74]. Other targets include the CXC group of chemokine ligand 12 (*Cxcl12*, also known as 'stromal-derived factor 1' (*SDF-1*)) [80] and *SMAD3* [81], both of which are implicated in chondrocyte differentiation. Interestingly, miR-140 was reported as suppressing *Dnpep*, an aspartyl aminopeptidase that catalyses the sequential removal of amino acids from the unblocked N termini of peptides and proteins, which antagonize BMP signalling downstream of *SMAD* activation [76].

In addition to miR-140, miR-455-3p expression is also restricted to the cartilage and perichondrium of the developing long bones in chicks and to the long bones and joints in mouse embryos, and it contributes to chondrogenesis in humans. miR-455-3p resides in an intron of

*COL27A1*, a collagen expressed in cartilage, and its expression is regulated by TGF $\beta$  ligands and miRNA-regulated TGF $\beta$  signalling. Activin receptor type IIB (*ACVR2B*), *SMAD2*, and chordin-like protein (*CHRD1*) are targets of miR-455-3p, and may mediate its functional impact on TGF $\beta$  signalling, suppressing the *SMAD2/3* pathway; therefore, its uninhibited expression could exacerbate the OA process [82].

To study the miRNA-mediated regulation of chondrogenesis, the expression of 35 miRNAs in chondroblasts derived from MSC was analysed and it was found that miR-199a and miR-124a were strongly upregulated, while miR-96 was substantially suppressed. The potential targets of the miRNAs are the following transcriptional factors: HIF-1 $\alpha$  for miR-199a, regulatory factor X1 (RFX1) for miR-124a, and SOX5 for miR-96, which demonstrate that miRNAs and transcription factors could fine-tune cellular differentiation [83].

In another miRNA microarray in MSC at four different stages of TGF- $\beta$ 3-induced chondrogenesis differentiation, eight significantly upregulated and five downregulated miRNA were observed. Two miRNA clusters, miR-143/145 and miR-132/212, were maintained on downregulation, while miR-140-3p was the most upregulated. By means of bioinformatics approaches, the following target genes were predicted: *ADAMTS5* for miR-140-3p; activin receptor 1B (*ACVR1B*) for miR143/145; *SOX6* for miR-132/212; and *RUNX2* for miR-30a [84]. Consistent with that finding, miR-145 decreased during TGF- $\beta$ 3-induced chondrogenic differentiation of murine MSC, and targeted the SRY-related high-mobility group-box gene 9 (SOX9), the key transcription factor for chondrogenesis. In cells overexpressing miR-145, the expression of chondrogenic markers was significantly decreased at the mRNA level, including *COL2A1*, *ACAN*, the cartilage oligomeric matrix protein (*COMP*), *COL9A2* and *COL11A1* [85], indicating that miR-145 comprises a key mediator of early chondrogenic differentiation attenuating SOX9 at the post-transcriptional level. In this way, it was reported that miR-145 is a direct regulator of SOX9 in normal human articular chondrocytes through binding to a non-conserved specific site in its human 3'-UTR. In addition, the increased expression of miR-145 in articular chondrocytes greatly reduced the expression *COL2A1* and *ACAN*, and critical cartilage ECM genes, and significantly increased the hypertrophic markers *RUNX2* and *MMP13*, responsible for the changes during the OA process [86].

miR-675 could also regulate *COL2A1* expression. miR-675 is processed from H19, a noncoding RNA, in healthy human chondrocytes. This miRNA is highly expressed and is regulated by SOX9 during chondrocyte differentiation, and upregulates the expression of *COL2A1*. The overexpression of miR-675 rescued *COL2A1* levels in H19- or SOX9-depleted chondrocytes, which suggests that the regulation of *COL2A1* by SOX9/H19 is mediated specifically by miR-675. These data suggest that miR-675 may modulate cartilage homeostasis by suppressing a *COL2A1* transcriptional repressor [87].

A comparative miRNA array of approximately 380 miRNAs in C2C12 cells induced by BMP2 found that several miRNAs, including let-7e, miR-221, miR-199a-3p, miR-374 and miR-298 were positively regulated, while miR-125a, miR-210, miR-125b, miR-21, miR-145 and miR-143 were repressed. Among these, miR-199a-3p was the most significantly upregulated at 24 h following BMP2 induction in the C3H10T1/2 cells and in an *in vitro* cell model of chondrogenesis. When these cells were transfected with miRNA-199a-3p, they exhibited a significant

decrease in mRNA expression levels of the chondrogenic markers COL2A1 and COMP, suggesting that miR-199a-3p is an inhibitor of the early stages of chondrogenic differentiation. miRNA target-prediction demonstrated that the putative target genes are *SMAD1* and *SMAD5*, which are known downstream mediators of BMP signalling in osteochondroprogenitor cells, which suggests that miR-199a-3p is a BMP2 responsive microRNA that adversely regulates early chondrocyte differentiation via the direct targeting of the *SMAD1* transcription factor [88].

To investigate the miRNA expression pattern involved in the chondrocyte dedifferentiation process, a microarray analysis was performed. Several miRNA were deregulated, including 13 upregulated and 12 downregulated miRNA in differentiated as compared with dedifferentiated chondrocytes. The most notable changes were for miR-491-3p, miR-140-3p, miR-140-5p and let-7d, which were upregulated, and for miR-548e, miR-342-5p, miR-1248 and miR-146a, which were downregulated. Bioinformatics analysis revealed 21 microRNA–gene target pairs potentially involved in chondrocyte dedifferentiation. Among these, miR-548e-*SOX9*, miR-1248-*ACAN*, miR-18a-*IGF1*, miR-193b-*SOX5*, miR-631-*RUNX1*, miR-335-*CRTAP*, miR-153-*MATN2* and miR-26a-*COL1A2* are involved in ECM proteins and homeostasis in chondrocytes, miR-365-*BCL2* in the apoptosis mechanism, and let-7a, d, f- *ITGB3*, miR-320d-*DBN1*, miR-1260-*LAMC2* and miR-222-*ITGB3* are involved in cytoskeleton organization [89]. Human primary articular chondrocytes allow the initiation of proliferation to produce ECM molecules similar to embryological chondroblasts, and are called ‘chondroblast-like’ cells. The miRNA expression profile in these cells showed five differentially expressed miRNA clusters. Among these, one cluster consisted of miR-451, four miRNA included miR-140-3p, another cluster had five miRNA including miR-221 and miR-222, and the last cluster consisted of 11 miRNA, including miR-143 and miR-145, all of these being upregulated at different differentiation stages that might inhibit the molecules from participating in the dedifferentiation process [90].

miR-194 decreased during chondrogenic differentiation in adipose-derived stem cells (ASCs) (which are capable of differentiating into cartilage lineages *in vitro*). This downregulation increases its direct target gene, *SOX5*, resulting in the chondrogenic differentiation of ASC; thus, miR-194 may mediate chondrogenic differentiation via the suppression of the transcription factor *SOX5* [91]. In a rat model, miR-337 is associated with chondrogenesis by repressing transforming growth factor-beta type II receptor (TGFBR2) expression and modulating the expression of cartilage-specific genes, such as *ACAN*, in chondrocytes [92].

During the chondrogenesis differentiation of chick-limb mesenchymal cells, miR-221 may be involved in chondrocyte apoptosis; its inhibition reverses the chondro-inhibitory actions of a Jun N-terminal protein kinase (JNK) inhibitor in the proliferation and migration of chondrogenic progenitors. A target for miR-221 is Mmd2, an oncoprotein that has been shown to inhibit the activity of the p53 tumour suppressor protein with E3 ubiquitin ligase activity, and downregulation of Mmd2 prevents the degradation of the slug protein, which negatively regulates the proliferation of chondroprogenitors. The slug protein is a snail family member – it controls the developmental process by regulating the genes involved in cell adhesion and migration [93]. In the same model, miR-34a is a negative modulator of chondrogenesis and

affects MSC migration but not proliferation. EphA5, a receptor in Eph/ephrin signalling that mediates cell-to-cell interaction, is a miR-34a target [94]. Moreover, miR-34a regulates RhoA/Rac1 cross-talk and negatively modulates the reorganization of the actin cytoskeleton, which is one of the essential processes in establishing chondrocyte morphology [95]. Table 1 depicts the miRNAs implicated in chondrogenesis.

microRNA	Expression	Target	Ref.
miR-140	↑	<i>HDAC4</i>	Histone Deacetylase 4
		<i>Sp1</i>	Transcription Factor Sp1
		<i>ADAMTS5</i>	A Disintegrin-Like And Metalloproteinase With
		<i>CXCL12</i>	Thrombospondin Type 1 Motif, 5
		<i>SMAD3</i>	Chemokine, Cxc Motif, Ligand 12
		<i>DNPEP</i>	Mothers against decapentaplegic homolog 3 Aspartyl aminopeptidase
miR-96	↓	<i>SOX5</i>	SRY-Related HMG-Box gene 5
miR-199a	↑	<i>HIF1α</i>	Hypoxia-inducible factor 1-alpha
miR-124a	↑	<i>RFX1</i>	Regulatory factor x 1
miR-199a-3p	↑	<i>Smad1</i>	Mothers against decapentaplegic homolog 1
		<i>Smad5</i>	Mothers against decapentaplegic homolog 5
miR-221		<i>Mdm2</i>	p53-binding protein Mdm
miR-675	↑	<i>COL2A1</i>	Type II collagen
miR-143/145	↓	<i>ACVR1B</i>	Activin a receptor, Type IB
miR-140-3p	↑	<i>ADAMTS5</i>	
miR-132/212	↓	<i>SOX6</i>	SRY-Related HMG-Box gene 6
miR-30a	↑	<i>RUNX2</i>	Runt-related transcription factor 2
miR-145	↑	<i>SOX9</i>	SRY-Related HMG-Box gene 9
miR-let-7a,d, f	↑	<i>ITGB3</i>	Integrin, beta 3
miR-631		<i>RUNX2</i>	
miR-548e	↓	<i>SOX9</i>	SRY-Related HMG-Box gene 9
miR-365	↓	<i>BCL2</i>	B-Cell CLL
miR-342-5p	↓	<i>IGFBP5</i>	Insulin-Like Growth Factor-Binding Protein 5
miR-335	↓	<i>CRTAP</i>	Cartilage-Associated Protein
miR-320d	↑	<i>DBN1</i>	Drebrin 1
miR-26a	↑	<i>COL2A1</i>	Type II collagen
miR-222	↑	<i>ITGB3</i>	Integrin, beta 3
miR-193b	↓	<i>SOX5</i>	SRY-Related HMG-Box gene 5



microRNA	Expression	Target	Ref.
miR-18a	↓	IGF1	Insulin-Like Growth Factor I
miR-153		MATN2	Matrilin-2
miR-1248	↓	ACAN	Aggrecan
miR-142-3p		ADAM9	A Disintegrin And Metalloproteinase Domain 9
miR-455-3p	↑	ACVR2B SMAD2 CHRD1	Activin a receptor, Type IIB Chordin-Like 1
miR-194	↓	SOX5	Chondrogenic differentiation
miR-337	↑	TGFBR2	Transforming Growth Factor-Beta Receptor, Type II
miR-181b	↓	MMP13	Matrix Metalloproteinase 13

Table 1. microRNAs implicated in chondrogenesis

4.2. Osteoarthritis and miRNAs

The effects of miRNA deregulation on OA are evident in studies comparing the expression of miRNAs between OA tissue specimens and their normal articular counterparts. A study tested 365 miRNA expression in articular cartilage obtained from patients with OA undergoing total joint replacement surgery as well as from normal controls. The study identified 16 miRNA differentially expressed in OA cartilage, of which nine were upregulated and seven downregulated. Through an *in silico* analysis, miRNA-gene targets were potentially involved in cartilage homeostasis and its structure (*miR-377-CART1*, *miR-140-ADAMTS5*, *miR-483-ACAN*, *miR-23b-CRTAP*, *miR-16-TPM2*, *miR-223-GDF5*, *miR-509-SOX9* and *miR-26a-ASPN*), in biomechanical pathways (*miR-25-ITGA5*), in apoptotic mechanisms (*miR-373-CASP6* and *miR-210-CASP10*), and in lipid metabolism pathways (*miR-22-PPARA*, *miR-22-BMP7*, *miR-103-ACOX1*, *miR-337-RETN* and *miR-29a-LEP*). The comparison of the molecular and clinical data revealed that miR-22 and miR-103 were highly correlated with body mass index (BMI) [97]. In another study, the expression profiling of 157 miRNA in chondrocytes obtained from OA cartilage identified 17 miRNAs that showed differential expression in comparison with normal controls. The most notable changes were observed for miR-9, miR-25 and miR-98, which were upregulated, and for miR-146 and miR-149, which were downregulated. A bioinformatics analysis performed to identify the potential gene targets suggested that a significant number of genes involved in inflammation were related with miR-9, miR-98 and miR-146. In overexpression experiments involving these miRNAs, miR-9, miR-98 and miR-146 were implicated in the control of TNF $\alpha$ , and miR-9 was implicated in MMP13 regulation [98]. To identify and characterize the expression profile of miRNA in the chondrocytes of III and IV grade OA, 723 miRNA were analysed and seven exhibited differential expression, of which one was upregulated and six were downregulated. In the bioinformatics prediction for knowing potential target genes regulated by these miRNA, it was found that the genes were involved in TGF- $\beta$ , Wnt, MAPK and the mammalian target of rapamycin (mTOR) signalling pathways, as well in

focal adhesion, cytoskeleton regulation, ubiquitin-mediated proteolysis and the cell cycle. Interestingly, TGF- $\beta$  and Wnt both played a role in OA [99].

Some miRNAs were examined as potential biomarkers of patients with OA of the knee, and miR-16, miR-132, miR-146a and miR-22 were significantly lower in the synovial fluid than in plasma. miR-132 in plasma exhibited a number of miRNAs in plasma, some of which were found at different levels between patients with rheumatoid arthritis (RA) and with OA. Concentrations of miR-16, miR-132, miR-146a and miR-223 are reduced in the synovial fluid of individuals suffering from OA compared with healthy controls [100]. More recently a profiling of 384 miRNAs was developed in the plasma of patients with radiographic OA of the knee, and 12 miRNAs were found to be differentially expressed with a clear differentiation of OA samples from those of healthy controls. *In silico* analysis revealed that potential miRNA targets belonged to OA-related pathways, such as those of chondrocyte maintenance, osteocyte modulation, inflammation, proteases, extracellular matrix (ECM) molecules and signalling pathways. Interestingly, some specific target genes are also involved in OA development, such as fibroblast growth factor receptor 1 (*FGFR1*), histone deacetylase 4 (*HDAC4*), (*FGF2*), vascular endothelial growth factor A (*VEGFA*), the insulin-like growth factor I receptor (*IGF1R*), A disintegrin-like and metalloproteinase with thrombospondin type 1 motif-5 (*ADAMTS5*), tissue inhibitor of metalloproteinase 2 (*TIMP2*), and WNT1-inducible signalling pathway protein 1 (*WISP1*) [101].

miR-140 has also been implicated in OA pathogenesis. In mice, the disruption of miR-140 *in vivo* induced the early onset of spontaneous OA-like changes in articular cartilage, in part due to elevated ADAMTS5, characterized by proteoglycan loss and articular cartilage fibrillation in the age-related model, and more severe OA-like changes in the surgical model [75]. In OA cartilage chondrocytes, *COL2A1* expression is low and *ADAMTS5* is increased. In response to IL-1 $\beta$  stimulation, miR-140 expression decreases while that of *ADAMTS5* and *MMP13* increases. These results demonstrate that miR-140 regulates cartilage-specific genes, playing an important role in regulating the balance between ECM synthesis and degradation [79]. Cartilage degradation in OA is due to factors such as MMP and to insulin-like growth factor protein 5 (IGFBP-5). In human OA chondrocytes, miR-140 significantly decreases *IGFBP5* expression, and anti-miR-140 exerts the contrary effect; therefore, these data suggest that *IGFBP5* is a direct target of miR-140 [102]. miR-140 was shown to mediate *MMP13* expression directly *in vitro*. In C28/I2 cells, a model cell of OA, stimulation by IL-1 $\beta$  increases the expression of *MMP13* [103].

In human OA chondrocytes, miR-27a expression is decreased in OA and treatment with anti-miR-27a increases the expression of *IGFBP5* and *MMP-13*, which suggests that miR-27a may indirectly regulate the levels of both genes by targeting upstream positive effectors of both genes [102]. In agreement with these results, miR-27b is downregulated in IL-1 $\beta$ -stimulated OA chondrocytes stimulated with an inverse correlation of MMP-13 expression [104].

Members of the miR-34 family are induced by p53, leading to apoptosis, cell cycle arrest and senescence through targeting E2F3, cyclin E2 and CDK6, etc. [105]. miR-34a is the most significantly induced miRNA after the activation of p53. In chondrocytes of a rat model of OA,

miR-34a is upregulated after IL-1 $\beta$  stimulation and its silencing prevents the IL-1 $\beta$ -induced upregulation of iNOS and the downregulation of Col2a1 [106].

miR-146a/b has been described as a key molecule in the inflammatory response [107] and is expressed in all layers of human articular cartilage, especially in the superficial zone. In grade I OA, the expression of miR-146a and COL2A1 is significantly increased, and is decreased in grades II and III, along with MMP13. Thus, their expression gradually decreased with progressive tissue degeneration [108]. In a rat model with surgically induced OA, miR-146a is upregulated in articular chondrocytes in response to treatment with IL-1 $\beta$ . *SMAD4* is a direct target of miR-146a, and the inhibition of *SMAD4* results in the upregulation of the vascular endothelial growth factor (VEGF) and apoptosis in chondrocytes. This VEGF induction by miR-146a may affect angiogenesis and inflammation during OA pathogenesis [109]. Interestingly, miR-146a has been implicated in the control of knee joint homeostasis and OA-associated algesia by balancing the inflammatory response in cartilage and synovia with pain-related factors in glial cells [110], because miR-146a is induced by joint stability resulting from medial collateral ligament transaction and medial meniscal tearing in the knee joints of an OA mouse model, suggesting that miR-146a might be a mechano-responsive miRNA in articular cartilage [109]. Previously, miR-365 was described as a mechano-responsive miRNA in chicken primary proliferative chondrocytes under mechanical stimulation. This miRNA stimulates chondrocyte proliferation and differentiation, and increases the expression of the hypertrophic marker COL10A1. Additionally, it targets *HDAC4*, which modulates cell growth and inhibits chondrocyte hypertrophy and endochondral bone formation by inhibiting Runx2 [111]. Table 2 depicts the miRNAs implicated in OA.

microRNA	Expression	Target	Ref.
miR-140	↓	<i>ADAMTS5</i>	A Disintegrin-Like And Metalloproteinase With
		<i>MMP13</i>	Thrombospondin Type 1 Motif, 5
		<i>IGFBP5</i>	Matrix Metalloproteinase 13
			Insulin-Like Growth Factor-Binding Protein 5
miR-146	↓	<i>IRAK1</i>	Interleukin 1 Receptor-Associated Kinase 1
		<i>TRAF6</i>	TNF Receptor-Associated Factor 6
miR-146a	↑	<i>MMP13</i>	Matrix Metalloproteinase 13
		<i>SMAD4</i>	Mothers against decapentaplegic homolog 4
miR-103	↑	<i>ACOX1</i>	AcyL-CoA Oxidase 1
miR-16	↑	<i>TIPM2</i>	Tissue Inhibitor Of Metalloproteinase 2
miR-210	↓	<i>CASP10</i>	Caspase 10
miR-22	↑	<i>PPAR</i>	Peroxisome Proliferator-Activated Receptor
		<i>BMP7</i>	Bone Morphogenetic Protein 7
miR-223	↑	<i>GDF5</i>	Growth/Differentiation Factor 5
miR-23b	↑	<i>CRTAP</i>	Cartilage-Associated Protein

microRNA	Expression	Target	Ref.
miR-25	↓	<i>ITGA5</i> Integrin, Alpha 5	97
miR-26a	↓	<i>ASPN</i> Asporin	97
miR-29a	↓	<i>LEP</i> Leptin	97
miR-337	↓	<i>RETN</i> Resistin	97
miR-373	↓	<i>CASP6</i> Caspase 6	97
miR-377	↑	<i>CART1</i> TNF Receptor-Associated Factor 4	97
miR-483	↑	<i>ACAN</i> Aggrecan	97
miR-509	↑	<i>SOX9</i> SRY-Related HMG-Box gene 9	97
miR-9	↑	<i>MMP13</i> Matrix Metalloproteinase 13	98
miR-27a	↓	<i>MMP13</i> Matrix Metalloproteinase 13	102
		<i>IGFBP5</i> Insulin-Like Growth Factor-Binding Protein 5	
miR-27b	↓	<i>MMP13</i> Matrix Metalloproteinase 13	104
miR-365	↑	<i>HDAC4</i> Histone Deacetylase 4	111
miR-488	↓	<i>ZIP8</i> Solute carrier family 39 (zinc transporter), member 8	112

**Table 2.** microRNAs implicated in Osteoarthritis

## 5. Conclusions

At present, there is increasing progress in the description of epigenetic mechanisms under normal conditions, as well in disease, and much of the current knowledge has focused on cancer. Yet epigenetics research has provided new insights in relation to other entities, such as neurological and autoimmune diseases. Epigenetics research into OA continues to be developed, but could shed light on its pathological mechanisms. One promising field is related with OA treatment, such as HDACi or miRNAs. However, although HDACi and miRNAs could inhibit several genes related with its development, they also inhibit ECM genes. To date, there is no appropriate biomarker for OA. Epigenetics marks in OA have been associated with the condition, and even with its progression, and could be biomarkers of disease and progression, as miRNAs determined in plasma. However, research into epigenetics continues to be required.

## Acknowledgements

This work was supported by the Consejo Nacional de Ciencia y Tecnología (CONACYT), proyecto SALUD-2012-C01-180720.



## Author details

Antonio Miranda-Duarte

Address all correspondence to: fovi01@prodigy.net.mx

Department of Genetics, National Rehabilitation Institute (INR), Mexico City, Mexico

To Ivonne, Ivana, and Romina, with love

To my father, Antonio Miranda-Novoa, in loving memory.

## References

- [1] Goldring MB, Goldring SR. Articular cartilage and subchondral bone in the pathogenesis of osteoarthritis. *Ann N Y Acad Sci* 2010; 1192: 230-7.
- [2] Maldonado M, Nam J. The role of changes in extracellular matrix of cartilage in the presence of inflammation on the pathology of osteoarthritis. *Biomed Res Int* 2013; 2013: 284-873. doi: 10.1155/2013/284873.
- [3] Knapinska A, Fields GB. Chemical biology for understanding matrix metalloproteinase function. *Chembiochem* 2012; 13(14): 2002-20.
- [4] Verma P, Dalal K. ADAMTS-4 and ADAMTS-5: key enzymes in osteoarthritis. *J Cell Biochem.* 2011; 112(12): 3507-14.
- [5] Houard X, Goldring MB, Berenbaum F. Homeostatic mechanisms in articular cartilage and role of inflammation in osteoarthritis. *Curr Rheumatol Rep* 2013; 15(11): 375.
- [6] Johnson VL, Hunter DJ. The epidemiology of osteoarthritis. *Best Pract Res Clin Rheumatol.* 2014; 28(1): 5-15.
- [7] Valdes AM, Spector TD. The contribution of genes to osteoarthritis. *Rheum Dis Clin North Am* 2008; 34(3): 581-603.
- [8] Reynard LN, Loughlin J. The genetics and functional analysis of primary osteoarthritis susceptibility. *Expert Rev Mol Med.* 2013; 15: e2.
- [9] Tammen SA, Friso S, Choi SW. Epigenetics: the link between nature and nurture. *Mol Aspects Med.* 2013; 34(4): 753-64.
- [10] Kim JK, Samaranayake M, Pradhan S. Epigenetic mechanisms in mammals. *Cell Mol Life Sci* 2009; 66(4): 596-612.
- [11] Portela A, Esteller M. Epigenetic modifications and human disease. *Nat Biotechnol* 2010; 28(10): 1057-68.

- [12] Golbabapour S, Abdulla MA, Hajrezaei M. A concise review on epigenetic regulation: insight into molecular mechanisms *Int J Mol Sci.* 2011; 12(12): 8661-94.
- [13] Walters DM. Epigenetic considerations in medicine. *N C Med J* 2013; 74(6): 534-6.
- [14] Fang JY, Lu YY. Effects of histone acetylation and DNA methylation on p21(WAF1) regulation. *World J Gastroenterol* 2002; 8(3): 400-5.
- [15] Sesselmann S, Söder S, Voigt R, Haag J, Grogan SP, Aigner. DNA methylation is not responsible for p21WAF1/CIP1 down-regulation in osteoarthritic chondrocytes. *Osteoarthritis Cartilage* 2009; 17(4): 507–512.
- [16] Fernandez MP, Young MF, Sobel ME. Methylation of type II and type I collagen genes in differentiated and dedifferentiated chondrocytes. *J Biol Chem* 1985; 260(4): 2374-8.
- [17] Pöschl E, Fidler A, Schmidt B, Kallipolitou A, Schmid E, Aigner T. DNA methylation is not likely to be responsible for aggrecan down regulation in aged or osteoarthritic cartilage. *Ann Rheum Dis* 2005; 64(3): 477–480.
- [18] Zimmermann P, Boeuf S, Dickhut A, Boehmer S, Olek S, Richter W. Correlation of COL10A1 induction during chondrogenesis of mesenchymal stem cells with demethylation of two CpG sites in the COL10A1 promoter. *Arthritis Rheum* 2008; 58(9): 2743–2753.
- [19] Goldring MB, Tsuchimochi K, Ijiri K. The Control of Chondrogenesis. *J Cell Biochem* 2006; 97(1): 33-44.
- [20] Hardingham TE, Oldershaw RA, Tew SR. Cartilage, SOX9 and Notch signals in chondrogenesis *J Anat* 2006; 209(4): 469-80..
- [21] Ezura Y, Sekiya I, Koga H, Muneta T, Noda M. Methylation status of CpG islands in the promoter regions of signature genes during chondrogenesis of human synovium-derived mesenchymal stem cells. *Arthritis Rheum* 2009; 60(5): 1416-26.
- [22] Kim KI, Park YS, Im GI. Changes in the epigenetic status of the SOX-9 promoter in human osteoarthritic cartilage. *J Bone Miner Res* 2013; 28(5): 1050-60.
- [23] Kevorkian L, Young DA, Darrah C, Donell ST, Shepstone L, Porter S, Brockbank SM, Edwards DR, Parker AE, Clark IM. Expression profiling of metalloproteinases and their inhibitors in cartilage. *Arthritis Rheum* 2004; 50(1): 131-41.
- [24] Roach HI, Yamada N, Cheung KS, Tilley S, Clarke NM, Oreffo RO, Kokubun S, Bronner F. Association between the abnormal expression of matrix-degrading enzymes by human osteoarthritic chondrocytes and demethylation of specific CpG sites in the promoter regions. *Arthritis Rheum* 2005; 52(10): 3110–3124
- [25] Hashimoto K, Otero M, Imagawa K, de Andrés MC, Coico JM, Roach HI, Oreffo RO, Marcu KB, Goldring MB. Regulated transcription of human matrix metalloproteinase

- 13 (MMP13) and interleukin-1 beta (IL1B) genes in chondrocytes depends on methylation of specific proximal promoter CpG sites. *J Biol Chem* 2013; 288(14): 10061-72.
- [26] Bui C, Barter MJ, Scott JL, Xu Y, Galler M, Reynard LN, Rowan AD, Young DA. cAMP response element binding (CREB) recruitment following a specific CpG demethylation leads to the elevated expression of the matrix metalloproteinase 13 in human articular chondrocytes and osteoarthritis. *FASEB J*. 2012; 26(7): 3000-11.
- [27] Cheung KS, Hashimoto K, Yamada N, Roach HI. Expression of ADAMTS-4 by chondrocytes in the surface zone of human osteoarthritic cartilage is regulated by epigenetic DNA de-methylation. *Rheumatol Int* 2009; 29(5): 525-34.
- [28] de Andrés MC, Maneiro E, Martín MA, Arenas J, Blanco FJ. Nitric oxide compounds have different effects profiles on human articular chondrocyte metabolism. *Arthritis Res Ther* 2013; 15(5): R115.
- [29] Taskiran D, Stefanovic-Racic M, Georgescu H, Evans C. Nitric oxide mediates suppression of cartilage proteoglycan synthesis by interleukin-1. *Biochem Biophys Res Commun* 1994; 200(1): 142-8.
- [30] de Andres, MC, K Imagawa, K Hashimoto, et al. Loss of methylation in CpG sites in the NF-kappaB enhancer elements of iNOS is responsible for gene induction in human articular chondrocytes. *Arthritis Rheum* 2013; 65(3): 732-42.
- [31] Imagawa K, de Andrés MC, Hashimoto K, Pitt D, Itoi E, Goldring MB, Roach HI, Oreffo RO. The epigenetic effect of glucosamine and a nuclear factor-kappa B (NF-kB) inhibitor on primary human chondrocytes--implications for osteoarthritis. *Biochem Biophys Res Commun* 2011; 405(3): 362-7.
- [32] Sokolove J, Lepus CM. Role of inflammation in the pathogenesis of osteoarthritis: latest findings and interpretations. *Ther Adv Musculoskelet Dis* 2013; 5(2): 77-94.
- [33] Gordeladze JO, Drevon CA, Syversen U, Reseland JE. Leptin stimulates human osteoblastic cell proliferation, de novo collagen synthesis, and mineralization: impact on differentiation markers, apoptosis, and osteoclastic signaling. *J Cell Biochem* 2002; 85(4): 825-36.
- [34] Iliopoulos D, Malizos KN, Tsezou A. Epigenetic regulation of leptin affects MMP-13 expression in osteoarthritic chondrocytes: possible molecular target for osteoarthritis therapeutic intervention. *Ann Rheum Dis* 2007; 66(12): 1616-21.
- [35] Hashimoto K, Oreffo RO, Gibson MB, et al. DNA demethylation at specific CpG sites in the IL1B promoter in response to inflammatory cytokines in human articular chondrocytes. *Arthritis Rheum*. 2009; 60(11): 3303-13.
- [36] de Andrés MC, Imagawa K, Hashimoto K, Gonzalez A, Goldring MB, Roach HI, Oreffo RO. Suppressors of cytokine signalling (SOCS) are reduced in osteoarthritis. *Biochem Biophys Res Commun* 2011; 407(1): 54-9.

- [37] Chubinskaya S, Otten L, Soeder S, Borgia JA, Aigner T, Rueger DC, Loeser RF. Regulation of chondrocyte gene expression by osteogenic protein-1. *Arthritis Res Ther*. 2011; 13(2): R55.
- [38] Loeser RF, Im HJ, Richardson B, Lu Q, Chubinskaya S. Methylation of the OP-1 promoter: potential role in the age-related decline in OP-1 expression in cartilage. *Osteoarthritis Cartilage* 2009; 17(4): 513-7.
- [39] Buxton P, Edwards C, Archer CW, Francis-West P. Growth/differentiation factor-5 (GDF-5) and skeletal development. *J Bone Joint Surg Am*. 2001; 83-A Suppl 1(Pt 1): S23-30.
- [40] Miyamoto Y, Mabuchi A, Shi D, Kubo T, Takatori Y, Saito S, Fujioka M, Sudo A, Uchida A, Yamamoto S, Ozaki K, Takigawa M, Tanaka T, Nakamura Y, Jiang Q, Ikegawa S. A functional polymorphism in the 5' UTR of GDF5 is associated with susceptibility to osteoarthritis. *Nat Genet* 2007; 39(4): 529-33.
- [41] Chapman K, Takahashi A, Meulenbelt I, Watson C, Rodriguez-Lopez J, Egli R, Tsezou A, Malizos KN, Kloppenburg M, Shi D, Southam L, van der Breggen R, Donn R, Qin J, Doherty M, Slagboom PE, Wallis G, Kamatani N, Jiang Q, Gonzalez A, Loughlin J, Ikegawa S. A meta-analysis of European and Asian cohorts reveals a global role of a functional SNP in the 5' UTR of GDF5 with osteoarthritis susceptibility *Hum Mol Genet* 2008; 17(10): 1497-504.
- [42] Egli RJ, Southam L, Wilkins JM, Lorenzen I, Pombo-Suarez M, Gonzalez A, Carr A, Chapman K, Loughlin J. Functional analysis of the osteoarthritis susceptibility-associated GDF5 regulatory polymorphism. *Arthritis Rheum* 2009; 60(7): 2055-64.
- [43] Reynard LN, Bui C, Canty-Laird EG, Young DA, Loughlin J. Expression of the osteoarthritis-associated gene GDF5 is modulated epigenetically by DNA methylation. *Hum Mol Genet* 2011; 20(17): 3450-60.
- [44] Delgado-Calle J, Fernandez AF, Sainz J, Zarrabeitia MT, Sañudo C, García-Renedo R, Pérez-Núñez MI, García-Ibarbia C, Fraga MF, Riancho JA.. Genome-wide profiling of bone reveals differentially methylated regions in osteoporosis and osteoarthritis. *Arthritis Rheum*. 2013; 65(1): 197-205.
- [45] Furumatsu T, Tsuda M, Yoshida K, Taniguchi N, Ito T, Hashimoto M, Ito T, Asahara H. Sox9 and p300 cooperatively regulate chromatin-mediated transcription. *J Biol Chem* 2005; 280(42): 35203-8.
- [46] Young DA, Lakey RL, Pennington CJ, Jones D, Kevorkian L, Edwards DR, Cawston TE, Clark IM. Histone deacetylase inhibitors modulate metalloproteinase gene expression in chondrocytes and block cartilage resorption. *Arthritis Res Ther*. 2005; 7(3): R503-12.



- [47] Wang X, Song Y, Jacobi JL, Tuan RS. Inhibition of histone deacetylases antagonized FGF2 and IL-1 $\beta$  effects on MMP expression in human articular chondrocytes. *Growth Factors* 2009; 27(1): 40-9.
- [48] Chabane N, Zayed N, Afif H, Mfuna-Endam L, Benderdour M, Boileau C, Martel-Pelletier J, Pelletier JP, Duval N, Fahmi H. Histone deacetylase inhibitors suppress interleukin-1 $\beta$ -induced nitric oxide and prostaglandin E2 production in human chondrocytes. *Osteoarthritis Cartilage*. 2008 Oct; 16(10): 1267-74.
- [49] Vega RB, Matsuda K, Oh J, Barbosa AC, Yang X, Meadows E, McAnally J, Pomajzl C, Shelton JM, Richardson JA, Karsenty G, Olson EN. Histone deacetylase 4 controls chondrocyte hypertrophy during skeletogenesis. *Cell* 2004; 119(4): 555-66.
- [50] Hong S, Derfoul A, Pereira-Mouries L, Hall DJ. A novel domain in histone deacetylase 1 and 2 mediates repression of cartilage-specific genes in human chondrocytes. *FASEB J*. 2009; 23(10): 3539-52.
- [51] Higashiyama R, Miyaki S, Yamashita S, Yoshitaka T, Lindman G, Ito Y, Sasho T, Takahashi K, Lotz M, Asahara H. Correlation between MMP-13 and HDAC7 expression in human knee osteoarthritis. *Mod Rheumatol* 2010; 20(1): 11-7
- [52] Huh YH, Ryu JH, Chun JS. Regulation of type II collagen expression by histone deacetylase in articular chondrocytes. *J Biol Chem* 2007; 282(23): 17123-31.
- [53] Saito T, Nishida K, Furumatsu T, Yoshida A, Ozawa M, Ozaki T. Histone deacetylase inhibitors suppress mechanical stress-induced expression of RUNX-2 and ADAMTS-5 through the inhibition of the MAPK signaling pathway in cultured human chondrocytes. *Osteoarthritis Cartilage* 2013; 21(1): 165-74.
- [54] Komori T, Yagi H, Nomura S, Yamaguchi A, Sasaki K, Deguchi K, Shimizu Y, Bronson RT, Gao YH, Inada M, Sato M, Okamoto R, Kitamura Y, Yoshiki S, Kishimoto T. Targeted disruption of Cbfa1 results in a complete lack of bone formation owing to maturational arrest of osteoblasts. *Cell* 1997; 89: 755-64.
- [55] Kamekura S, Kawasaki Y, Hoshi K, Shimoaka T, Chikuda H, Maruyama Z, Komori T, Sato S, Takeda S, Karsenty G, Nakamura K, Chung UI, Kawaguchi H.. Contribution of runt-related transcription factor 2 to the pathogenesis of osteoarthritis in mice after induction of knee joint instability. *Arthritis Rheum* 2006; 54(8): 2462-70
- [56] Franceschi RT, Xiao G. Regulation of the osteoblast-specific transcription factor, Runx2: responsiveness to multiple signal transduction pathways. *J Cell Biochem* 2003; 88(3): 446-54.
- [57] Chen WP, Bao JP, Hu PF, Feng J, Wu LD. Alleviation of osteoarthritis by trichostatin A, a histone deacetylase inhibitor, in experimental osteoarthritis. *Mol Biol Rep* 2010; 37(8): 3967-72.
- [58] El Mansouri FE, Chabane N, Zayed N, Kapoor M, Benderdour M, Martel-Pelletier J, Pelletier JP, Duval N, Fahmi H.I. Contribution of H3K4 methylation by SET-1A to in-

terleukin-1-induced cyclooxygenase 2 and inducible nitric oxide synthase expression in human osteoarthritis chondrocytes. *Arthritis Rheum* 2011; 63(1): 168e79.

- [59] Rodova, M. Lu Q, Li Y, Woodbury BG, Crist JD, Gardner BM, Yost JG, Zhong XB, Anderson HC, Wang J. Nfat1 regulates adult articular chondrocyte function through its age-dependent expression mediated by epigenetic histone methylation. *J Bone Miner Res.* 2011; 26(8): 1974-86.
- [60] Michan S, Sinclair D. Sirtuins in mammals: insights into their biological function. *Biochem J* 2007; 404(1): 1-13.
- [61] Haigis MC, Sinclair DA. Mammalian sirtuins: biological insights and disease relevance. *Annu Rev Pathol* 2010; 5: 253-95.
- [62] Dvir-Ginzberg M, Gagarina V, Lee EJ, Hall DJ. Regulation of cartilage-specific gene expression in human chondrocytes by SirT1 and nicotinamide phosphoribosyltransferase. *J Biol Chem.* 2008; 283(52): 36300-10.
- [63] Takayama K, Ishida K, Matsushita T, Fujita N, Hayashi S, Sasaki K, Tei K, Kubo S, Matsumoto T, Fujioka H, Kurosaka M, Kuroda R. SIRT1 regulation of apoptosis of human chondrocytes. *Arthritis Rheum* 2009; 60(9): 2731-40.
- [64] Gagarina V, Gabay O, Dvir-Ginzberg M, Lee EJ, Brady JK, Quon MJ, Hall DJ. SirT1 enhances survival of human osteoarthritic chondrocytes by repressing protein tyrosine phosphatase 1B and activating the insulin-like growth factor receptor pathway. *Arthritis Rheum* 2010; 62(5): 1383-92.
- [65] Fujita N, Matsushita T, Ishida K, Kubo S, Matsumoto T, Takayama K, Kurosaka M, Kuroda R. Potential involvement of SIRT1 in the pathogenesis of osteoarthritis through the modulation of chondrocyte gene expressions. *J Orthop Res* 2011; 29(4): 511-5.
- [66] Dvir-Ginzberg M, Gagarina V, Lee EJ, Booth R, Gabay O, Hall DJ. Tumor necrosis factor  $\alpha$ -mediated cleavage and inactivation of sirT1 in human osteoarthritic chondrocytes. *Arthritis Rheum* 2011; 63(8): 2363-73.
- [67] Gabay O, Oppenheimer H, Meir H, Zaal K, Sanchez C, Dvir-Ginzberg M. Increased apoptotic chondrocytes in articular cartilage from adult heterozygous SirT1 mice. *Ann Rheum Dis.* 2012; 71(4): 613-6.
- [68] Matsushita T, Sasaki H, Takayama K, Ishida K, Matsumoto T, Kubo S, Matsuzaki T, Nishida K, Kurosaka M, Kuroda R. The overexpression of SIRT1 inhibited osteoarthritic gene expression changes induced by interleukin-1 $\beta$  in human chondrocytes. *J Orthop Res* 2013; 31(4): 531-7.
- [69] Moon MH, Jeong JK, Lee YJ, Seol JW, Jackson CJ, Park SY. SIRT1, a class III histone deacetylase, regulates TNF- $\alpha$ -induced inflammation in human chondrocytes. *Osteoarthritis Cartilage* 2013; 21(3): 470-80.

- [70] Bernstein E, Kim SY, Carmell MA, Murchison EP, Alcorn H, Li MZ, Mills AA, Elledge SJ, Anderson KV, Hannon GJ. Dicer is essential for mouse development. *Nat Genet* 2003; 35(3): 215-7.
- [71] Harfe BD, McManus MT, Mansfield JH, Hornstein E, Tabin CJ. The RNaseIII enzyme Dicer is required for morphogenesis but not patterning of the vertebrate limb. *Proc Natl Acad Sci U S A* 2005; 102(31): 10898-903.
- [72] Kobayashi T, Lu J, Cobb BS, Rodda SJ, McMahon AP, Schipani E, Merkerschlager M, Kronenberg HM. Dicer-dependent pathways regulate chondrocyte proliferation and differentiation. *Proc Natl Acad Sci U S A*. 2008; 105(6): 1949-54.
- [73] Wienholds E, Kloosterman WP, Miska E, Alvarez-Saavedra E, Berezikov E, de Bruijn E, Horvitz HR, Kauppinen S, Plasterk RH. MicroRNA expression in zebrafish embryonic development. *Science* 2005; 309(5732): 310-1.
- [74] Tuddenham L, Wheeler G, Ntounia-Fousara S, Waters J, Hajihosseini MK, Clark I, Dalmay T. The cartilage specific microRNA-140 targets histone deacetylase 4 in mouse cells. *FEBS Lett* 2006; 580(17): 4214-7.
- [75] Miyaki S, Sato T, Inoue A, Otsuki S, Ito Y, Yokoyama S, Kato Y, Takemoto F, Nakasa T, Yamashita S, Takada S, Lotz MK, Ueno-Kudo H, Asahara H. MicroRNA-140 plays dual roles in both cartilage development and homeostasis. *Genes Dev* 2010; 24(11): 1173-1185
- [76] Nakamura Y, Inloes JB, Katagiri T, Kobayashi T. Chondrocyte-specific microRNA-140 regulates endochondral bone development and targets dnpep to modulate bone morphogenetic protein signaling. *Mol Cell Biol* 2011; 31(14): 3019-28.
- [77] Nakamura Y, He X, Kobayashi T, Yan YL, Postlethwait JH, Warman ML. Unique roles of microRNA140 and its host gene WWP2 in cartilage biology. *J Musculoskeletal Neuronal Interact*. 2008; 8: 321-2.
- [78] Yang J, Qin S, Yi C, Ma G, Zhu H, Zhou W, Xiong Y, Zhu X, Wang Y, He L, Guo X. MiR-140 is co-expressed with Wwp2-C transcript and activated by Sox9 to target Sp1 in maintaining the chondrocyte proliferation. *FEBS Lett* 2011; 585(19): 2992-7.
- [79] Miyaki S, Nakasa T, Otsuki S, Grogan SP, Higashiyama R, Inoue A, Kato Y, Sato T, Lotz MK, Asahara H. MicroRNA-140 is expressed in differentiated human articular chondrocytes and modulates interleukin-1 responses. *Arthritis Rheum* 2009; 60(9): 2723-2730
- [80] Nicolas FE, Pais H, Schwach F, Lindow M, Kauppinen S, Moulton V, Dalmay T. Experimental identification of microRNA- 140 targets by silencing and overexpressing miR-140. *RNA* 2008; 14(12): 2513-20.
- [81] Pais H, Nicolas FE, Soond SM, Swingler TE, Clark IM, Chantry A, Moulton V, Dalmay T. Analyzing mRNA expression identifies Smad3 as a microRNA-140 target regulated only at protein level. *RNA* 2010; 16(3): 489-94.

- [82] Swingle TE, Wheeler G, Carmont V, Elliott HR, Barter MJ, Abu-Elmagd M, Donell ST, Boot-Handford RP, Hajihosseini MK, Münsterberg A, Dalmay T, Young DA, Clark IM. The expression and function of microRNAs in chondrogenesis and osteoarthritis. *Arthritis Rheum* 2012; 64(6): 1909-19.
- [83] Suomi S, Taipaleenmäki H, Seppänen A, Ripatti T, Väänänen K, Hentunen T, Säämänen AM, Laitala-Leinonen T. MicroRNAs regulate osteogenesis and chondrogenesis of mouse bone marrow stromal cells. *Gene Regul Syst Bio* 2008; 2: 177-91.
- [84] Yang B, Guo H, Zhang Y, Dong S, Ying D. The microRNA expression profiles of mouse mesenchymal stem cell during chondrogenic differentiation. *BMB Rep* 2011; 44(1): 28-33.
- [85] Yang B, Guo H, Zhang Y, Chen L, Ying D, Dong S. microRNA-145 regulates chondrogenic differentiation of mesenchymal stem cells by targeting Sox9. *PLoS One* 2011; 6(7): e21679.
- [86] Martinez-Sanchez A, Dudek KA, Murphy CL. Regulation of human chondrocyte function through direct inhibition of cartilage master-regulator SOX9 by miRNA-145. *J Biol Chem* 2012; 287(2): 916-24.
- [87] Dudek KA, Lafont JE, Martinez-Sanchez A, Murphy CL. Type II collagen expression is regulated by tissue-specific miR-675 in human articular chondrocytes. *J Biol Chem* 2010; 285(32): 24381-7.
- [88] Lin E, Kong L, Bai XH, Luan Y, Liu CJ. miR-199a, a bone morphogenic protein 2-responsive MicroRNA, regulates chondrogenesis via direct targeting to Smad1. *J Biol Chem* 2009; 284(17): 11326-35.
- [89] Lin L, Shen Q, Zhang C, Chen L, Yu C. Assessment of the profiling microRNA expression of differentiated and dedifferentiated human adult articular chondrocytes. *J. Orthop. Res* 2011; 29(10): 1578-1584
- [90] Karlson TA, Shahdadfar A, Brinckmann JE. Human primary articular chondrocytes, chondroblasts-like cells, and dedifferentiated chondrocytes: differences in gene, microRNA, and protein expression and phenotype. *Tissue Eng. Part C: Methods* 2011; 17(2): 219-227.
- [91] Xu J, Kang Y, Liao WM, Yu L. MiR-194 regulates chondrogenic differentiation of human adipose-derived stem cells by targeting Sox5. *PLoS One* 2012; 7(3): e31861.
- [92] Zhong N, Sun J, Min Z, Zhao W, Zhang R, Wang W, Tian J, Tian L, Ma J, Li D, Han Y, Lu S.. MicroRNA-337 is associated with chondrogenesis through regulating TGFBR2 expression. *Osteoarthritis Cartilage* 2012; 20(6): 593-602.
- [93] Kim D, Song J, Jin EJ. MicroRNA-221 regulates chondrogenic differentiation through promoting proteasomal degradation of Slug by targeting Mdm2. *J Biol Chem* 2010; 285(35): 26900-7.

- [94] Kim D, Song J, Kim S, Chun CH, Jin EJ. MicroRNA-34a regulates migration of chondroblast and IL-1 $\beta$ -induced degeneration of chondrocytes by targeting EphA5. *Biochem Biophys Res Commun* 2011; 415(4): 551-7.
- [95] Kim, D. J Song, S Kim, Park HM, Chun CH, Sonn J, Jin EJ. MicroRNA-34a modulates cytoskeletal dynamics through regulating RhoA/Rac1 crosstalk in chondroblasts. *J Biol Chem* 2012; 287(15): 12501-9.
- [96] Song J, Lee M, Kim D, Han J, Chun CH, Jin EJ. MicroRNA-181b regulates articular chondrocytes differentiation and cartilage integrity. *Biochem Biophys Res Commun*. 2013; 431(2): 210-4.
- [97] Iliopoulos D, Malizos KN, Oikonomou P, Tsezou A. Integrative microRNA and proteomic approaches identify novel osteoarthritis genes and their collaborative metabolic and inflammatory networks. *PLoS One*. 2008; 3(11): e3740.
- [98] Jones SW, Watkins G, Le Good N, Roberts S, Murphy CL, Brockbank SM, Needham MR, Read SJ, Newham P. The identification of differentially expressed microRNA in osteoarthritic tissue that modulate the production of TNF-alpha and MMP13. *Osteoarthritis Cartilage*. 2009; 17(4): 464-72.
- [99] Díaz-Prado S, Cicione C, Muiños-López E, Hermida-Gómez T, Oreiro N, Fernández-López C, Blanco FJ. Characterization of microRNA expression profiles in normal and osteoarthritic human chondrocytes. *BMC Musculoskelet Disord*. 2012; 13: 144.
- [100] Murata K, Yoshitomi H, Tanida S, Ishikawa M, Nishitani K, Ito H, Nakamura T. Plasma and synovial fluid microRNAs as potential biomarkers of rheumatoid arthritis and osteoarthritis. *Arthritis Res Ther*. 2010; 12(3): R86.
- [101] Borghonio Cuadra VM, González-Huerta NC, Romero-Córdoba S, Hidalgo-Miranda A, Miranda-Duarte A. Altered expression of circulating microRNA in plasma of patients with primary osteoarthritis and in silico analysis of their pathways. *PLoS One*. 2014; 9(6): e97690.
- [102] Tardif G, Hum D, Pelletier JP, Duval N, Martel-Pelletier J. Regulation of the IGFBP-5 and MMP-13 genes by the microRNAs miR-140 and miR-27a in human osteoarthritic chondrocytes. *BMC Musculoskelet. Disord* 2009; 10: 148.
- [103] Liang ZJ, Zhuang H, Wang GX, Li Z, Zhang HT, Yu TQ, Zhang BD. MiRNA-140 is a negative feedback regulator of MMP-13 in IL-1 $\beta$ -stimulated human articular chondrocyte C28/I2 cells. *Inflamm Res* 2012; 61(5): 503-9.
- [104] Akhtar N, Rasheed Z, Ramamurthy S, Anbazhagan AN, Voss FR, Haqqi TM. MicroRNA-27b regulates the expression of matrix metalloproteinase 13 in human osteoarthritis chondrocytes. *Arthritis Rheum* 2010; 62(5): 1361-71.
- [105] Raver-Shapira N, Marciano E, Meiri E, Spector Y, Rosenfeld N, Moskovits N, Bentwich Z, Oren M. Transcriptional activation of miR-34a contributes to p53-mediated apoptosis. *Mol Cell*. 2007; 26(5): 731-43.



- [106] Abouheif MM, Nakasa T, Shibuya H, Niimoto T, Kongcharoensombat W, Ochi M. Silencing microRNA-34a inhibits chondrocyte apoptosis in a rat osteoarthritis model in vitro. *Rheumatology (Oxford)* 2010; 49(11): 2054-60.
- [107] Nakasa T, Miyaki S, Okubo A, Hashimoto M, Nishida K, Ochi M, Asahara H.. Expression of microRNA-146 in rheumatoid arthritis synovial tissue. *Arthritis Rheum* 2008; 58(5): 1284-92
- [108] Yamasaki K, Nakasa T, Miyaki S, Ishikawa M, Deie M, Adachi N, Yasunaga Y, Asahara H, Ochi M. Expression of MicroRNA-146a in osteoarthritis cartilage. *Arthritis Rheum.* 2009; 60(4): 1035-41.
- [109] Li J, Huang J, Dai L, Yu D, Chen Q, Zhang X, Dai K. miR-146a, an IL-1 $\beta$  responsive miRNA, induces vascular endothelial growth factor and chondrocyte apoptosis by targeting Smad4. *Arthritis Res Ther* 2012; 14(2): R75.
- [110] Li X, Gibson G, Kim JS, Kroin J, Xu S, van Wijnen AJ, Im HJ. MicroRNA-146a is linked to pain-related pathophysiology of osteoarthritis. *Gene* 2011; 480(1-2): 34-41.
- [111] Guan YJ, Yang X, Wei L, Chen Q. MiR-365: a mechanosensitive microRNA stimulates chondrocyte differentiation through targeting histone deacetylase 4. *FASEB J* 2011; 25(12): 4457e66.
- [112] Song J, Kim D, Lee CH, Lee MS, Chun CH, Jin EJ. MicroRNA-488 regulates zinc transporter SLC39A8/ZIP8 during pathogenesis of osteoarthritis. *J Biomed Sci.* 2013;20:31.

