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Review Article

Role of miRNA in the Development and Progression of Osteoarthritis and Osteoporosis- A Review Article

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Abstract

Osteoporosis [OP] and Osteoarthritis [OA] have many things in common despite distinct pathophysiologies. Both Osteoporosis and Osteoarthritis are the most common musculoskeletal disorders, in the elderly, commonly affecting females. The incidence of both is increasing with a very high prevalence in an aging population. Both the diseases are multifactorial and remain largely understudied at the molecular level. Genetic and environmental factors have been recognized to have a major role in both. Several candidate genes have been found to have an association with both diseases and interestingly some of these are common having a role in both although they have not been studied in those suffering from both osteoporosis and osteoarthritis. Recent interest has been sought on the role of small ribonucleic acid such as microRNAs (miRNA) on various metabolic and genetic diseases. Numerous studies showed that these miRNAs are small non-coding sequences, nearly around 20-22 nucleotide base pairs that control the expressions of several genes whose altered expressions may lead to the bone formation imbalance and destruction.

Background

Purpose of Review: Osteoporosis and Osteoarthritis are a form of bone disease that most commonly affects the middle-aged and elderly. Besides many treatments and advancements in medical sciences, these diseases are still difficult to treat. Hence, there is a need to find some more specified and advanced treatment modalities to prevent the progression as well as treatment of both diseases. In our review, the role of miRNA is being discussed in both diseases which may provide more evidence to understand the diseases such as OP and OA.

Findings: The articles were searched and included from PubMed, MEDLINE, and DOAJ Databases in the English language. We implemented the research terminologies ["Osteoporosis" "Osteoarthritis"] and ["miRNA in OS and OP"] and identified publications that directly discussed survival factors in subjects with both diseases. Since OS and OP can present itself in various ways, comprising radiography and biochemical profile to diagnose the disease and opt the appropriate treatment, comparison of comprehensive radiographic and microscopic findings is important.

Inclusion criteria: Only those articles focused on the role of miRNA in OP and OA.

Exclusion criteria:

- Repetitive published literature.
- No relevant information was given in the literature.
- The articles without a control group.

Keywords: Osteoarthritis; Osteoporosis; miRNAs; Prognostic Marker

Introduction

The bone remodeling occurs throughout a person's life as it is a metabolically active tissue. In a normal skeletal structure, under normal circumstances, the bone-forming osteoblasts and bone-resorbing osteoclasts act in a well-ordered and rigorously regulated way, guaranteeing bone tissue regeneration. Remodeling also protects bones from harm with modification in the structure and strength to contextual loading needs [1]. Predisposing factors and aging can cause imbalances in bone remodeling. As a result of a bone resorption imbalance, bone resorption increases without being compensated by bone formation, resulting in reduced bone mass, strength, and skeleton microarchitecture degradation. These degenerative factors cause bone fragility, which increases the chance of fractures, which most usually affect the forearm, vertebral bodies, and hip. The imbalances between the new cells' formation and the old cells' destruction may cause diseases like Osteoporosis, Paget's disease, Osteoarthritis, and Rheumatoid arthritis. In this review, we have focused on the development and progression of Osteoporosis and Osteoarthritis.

Osteoporosis (OP)

Osteoporosis is considered as a severe public health issue, affecting around 200 million individuals globally. In the United States, osteoporosis had been diagnosed in around 10 million people, and another 34 million had osteopenia, making them susceptible for osteoporosis. Several studies have found that the prevalence of osteoporosis in Indian women of various ages ranges from 8% to 62 percent. Every year, osteoporosis causes around 1.5 million fractures, the most prevalent sites are hip, spine, and wrist. Osteoporosis has a significant impact on individual morbidity and mortality and global public health. Sir Astley Cooper first observed bone loss and hip fracture in 1822, and German and French physicians coined the name "osteoporosis" after studying the histology of osteoporotic bone in the 19th century. The Consensus Development Conference defined osteoporosis as a "systemic skeletal disorder characterized by low bone mass and microarchitectural deterioration of bone tissue, with a consequent increase in bone fragility and susceptibility to fracture". The current definition of osteoporosis is given by the National Osteoporosis Foundation (NOF) as "a disease characterized by low bone mass and structural deterioration of bone tissue, leading to bone fragility and an increased risk of fracture" [2].

Osteoporosis is caused by an imbalance in bone remodeling, which results in a decrease in bone strength. Due to a disruption in the structure, bones become more fragile, increasing fracture risk. Bone loss typically takes years to occur and its etiology might present without warning symptoms. The bone turnover gets disrupted in Osteoporosis causing an imbalance between bone resorption and production processes. Osteogenesis is a process that occurs fast during childhood and results in the denser and longer bones, culminating in growth of the skeleton. The density of adult bone reflects modifiable conditions during adolescence, with the maximal bone mass reaching at the age of 30 years. As a result, this phase is considered as a good predictor of osteoporosis development later on. Osteoporosis is currently a substantial human and economic burden which will continue to increase as the population ages, resulting in reduced bone mass and increased fracture risk. Hip is the commonest fracture site following osteoporosis as the rate of, life expectancy rises 1–3% annually worldwide, especially in postmenopausal females. In Western countries, attention has been increased towards the diagnosis, treatment, and research on osteoporosis; but, in the Middle East and Africa, this disease receives less attention. In spite of osteoporosis being a global problem, very few countries have recommendations for this disease.

Osteoarthritis (OA)

Osteoarthritis (OA) is a deteriorating joint problem of multifactorial origin. Depending on the age, sex, and disease classification, the estimated population prevalence ranges from 4 to 30%. Obesity, past knee injury, specific physical activity, and history of OA in the family are also risk factors. Of all the recognized risk factors of Knee Osteoarthritis (KOA), obesity has the strongest connection to the development and progression of knee OA [3]. Subcommittee on Osteoarthritis of the American College of Rheumatology Diagnostic and Therapeutic Criteria Committee defined osteoarthritis (OA) as "a heterogeneous group of conditions that lead to joint symptoms and signs which are associated with defective integrity of articular cartilage, in addition to related changes in the underlying bone at the joint margins". The gradual degeneration of articular cartilage is the characteristic feature and is one of the primary causes of impairment in the elderly. The epidemiological profile of OA in India is not well known, although it is believed that more than 30-40% of our population over the age of 50 has osteoarthritis [4]. Hip and knee OA, in addition to being the leading cause of joint replacements, is a tremendous healthcare burden for society and a personal hardship for those who are impacted by the condition. In India, more than 56.6 percent of the population over the age of 65 suffers with OA Knee OA was found to be prevalent in 28.7% of the population. Among various forms of arthritis, OA is the most common, particularly in the knees. The knee is made up of osseous bones (proximal tibia, distal femur and patella), cartilage (hyaline cartilage and meniscus), ligaments, and a synovial membrane, and is the biggest human synovial joint. The latter is in charge of making synovial fluid, which lubricates and nourishes the avascular cartilage. This joint is a typical site for painful illnesses such as OA because of the significant use and stress exerted on it.

Multiple causes have been identified as contributing to this condition, including biomechanical, genetic, and environmental stress. Because of the growth in life expectancy and the aging population in society, the number of patients with OA is growing. The most potent risk factor for OA pathogenesis is advanced age. Knee OA (KOA) was found to be most common in people aged 60 to 65 years old. But the present scenario of KOA occurrence has changed as the patients of <30 years of age are also reported to be affected by the disease. Gender may also be one of the factors responsible for KOA. Males are more often affected as compared to females in the age <45 years, whereas females of age >55 years are affected more frequently than males of the same age group. Obesity, genetic predisposition, and nutritional factors are also found to be involved in the commencement of KOA. OA is classified into two broad categories which are: Primary and Secondary OA. These are basically two types of Osteoarthritis.

Primary OA is an idiopathic phenomenon that results from an unknown cause. Although the pathogenesis of this condition is not completely understood, various environmental, metabolic, and genetic components are responsible for the disease initiation and advancement.

Secondary OA is caused by known predisposing conditions/factors, however the changes in pathology are similar to that for primary OA. The main causes of secondary OA are Accidental injury to joints, Inflammatory diseases (such as Perthes disease, Lyme disease), and all chronic forms of arthritis (e.g., gout, pseudogout, and rheumatoid arthritis). In gout, uric acid crystals cause cartilage degradation at a faster pace, Healed infection of the joints, Sports injuries, and Congenital and developmental disorders such as developmental dysplasia of the hip, abnormally formed joint, and ligamentous hyperlaxity syndrome.

Epigenetics of OP and OA

Epigenetics investigates the alterations in the transcriptional activity of genes induced due to the causes other than alterations in DNA sequences, as opposed to genetics, which investigates hereditary variation of DNA sequences. DNA methylation and histone protein modifications are two examples of traditional epigenetic covalent changes (ubiquitination, methylation, sumoylation, acetylation, and phosphorylation). Non-coding RNAs (ncRNAs), which exhibit epigenetic features in gene expression control, have recently been identified as one of the epigenetic processes [5]. The thorough evaluation of the number of transcriptional molecules, including protein-coding messenger RNA (mRNA) and ncRNA, is currently a fast-increasing topic in biomedical research for common illnesses such as osteoarthritis, thanks to the employment of high throughput technology (OA). In the middle-aged and older population, osteoarthritis is the commonest form of arthritis and the primary reason for persistent impairment.

MicroRNAs (miRNAs) are tiny ~ 22 nucleotide long non-coding RNA molecules. These are key post-transcriptional regulators in cells that regulate gene expression by inhibiting the translation of particular mRNAs or causing the destruction of specific mRNAs. Because miRNAs target a collection of functionally similar genes and may be modified to target whole pathways rather than changing individual genes, they have become important therapeutic targets.

The miRNAs are expected to play an important role in regulating a variety of biological processes, including cell differentiation, proliferation, death, immunity, and metabolism, and may also be responsible for determining the physiology of the cell in many circumstances [6]. As a result, improper miRNA expression should have an impact on these key processes, and it has been linked to a number of illnesses in humans, such as cancer, viral infections, nervous system problems, cardiovascular, skeletomuscular, and diabetic diseases [7]. This means that using abnormally generated miRNAs as disease biomarkers is not only an important diagnostic tool, but it also suggests these non-coding RNAs are interesting therapy targets [6]. OA and OP each have their own set of miRNAs that play a role.

Diagnosis of osteoporosis

For many years, dual-energy X-ray absorptiometry measurement of bone mineral density (BMD) has been the most extensively used method for diagnosing osteoporosis and predicting fracture risk. As BMD decreases, the likelihood of fragility fractures increases. However, various additional components of bone strength have been found that alter either the structural or material qualities of bone and are not always represented by BMD measurements. Clinical practice also often uses markers of bone synthesis such as serum procollagen type I N-terminal propeptide (s-PINP) and bone resorption markers such as serum C-terminal telopeptide type I collagen (s-CTX) and urine N-telopeptide (NTX). They have assisted doctors in identifying patients at high risk for fractures and monitoring therapy success by providing a non-invasive evaluation of bone turnover across a variety of skeletal disorders. Clinical practice often utilizes bone synthesis markers such as serum procollagen type I N-terminal propeptide (s-PINP) and bone resorption markers such as serum C-terminal telopeptide type I collagen (s-CTX) and urine N-telopeptide (NTX). These markers were developed to provide a noninvasive assessment of bone turnover in a range of skeletal disorders, and they've proven to be useful in identifying patients at high risk for fractures and tracking therapy success. Despite advances in diagnostics, bone loss is gradual

and asymptomatic, therefore osteoporosis is frequently identified after the first clinical fracture, resulting in diminished autonomy and higher mortality. Furthermore, these individuals frequently require hospitalization, which raises the risk of complications. In this context, both clinical practice and translational research would benefit from the identification of novel potential biomarkers that can be used alone or along with the existing markers for timely and accurate diagnosis prior to the occurrence of fractures, as well as for evaluation of the patient's response to therapy. Osteoporosis is a complicated and multidimensional disease with a known hereditary component, despite the fact that genetic variants have a little effect on gene expression and explicate only a small percentage of the disease etiology [8]. As a result, investigating novel epigenetic variables linked to this disease may help us learn more about its pathophysiology and epidemiology.

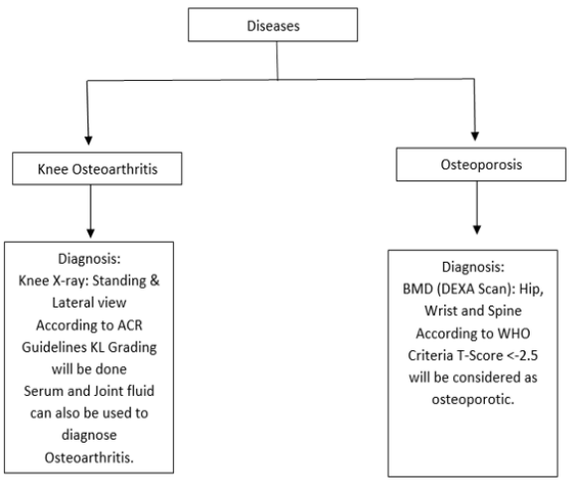
Diagnostic biomarkers of osteoporosis

Evidence indicates that miRNAs play an important role in regulating a wide range of biological processes, including development, cell differentiation, proliferation, death, immunity, and metabolism. They define the physiological constitution of cells in a variety of situations. In addition, abnormal miRNA expression may affect those key processes, and has been associated with cancer, viral infections, neurological disorders, cardiovascular and muscle diseases, and diabetes [9]. This means that using these abnormally produced miRNAs as biomarkers for disorders is not only a useful diagnostic method, but it also makes these ncRNAs promising targets for future therapeutic development. In conjunction with this, high-throughput technologies like as miRNA microarrays, Real-Time PCR TaqMan Array microfluid cards, LNA-based high-throughput PCR, and next-generation sequencing (NGS) have made analyzing circulating miRNA expression patterns easier. These techniques have largely replaced low-throughput analysis (Northern blotting and cloning), which has drawbacks such as poor quantification output, more time consumption-, and low sensitivity, and is widely used in initial screening of circulating miRNA and signature generation from body fluid in a variety of diseases. The plausibility of identifying miRNAs as diagnostic markers for osteoporosis is certainly appealing in this context, and it has led to increased number of researches that aim to understand role of miRNAs in bone cells and also to explore, their potential as circulating biomarkers and identify candidates of interest over the years. While some of these findings are encouraging, there is a lack of a unified method to analysis, resulting in a complicated and disorganized picture. There are substantial discrepancies in the kind of samples used (e.g., plasma, serum, or whole blood) and the populations used as control groups among studies (e.g., healthy, osteopenic, and osteoarthritic subjects). The analysis is also done in a variety of methods, depending on the number of miRNAs assessed, the platforms employed, and the reference genes used to standardize the results. Despite these limitations, many investigations produced somewhat consistent findings, identifying miRNAs that appear to be differently expressed in osteoporosis and have distinct targets and actions on bone turnover, as revealed by experimental analysis. We discuss the most recent discoveries on circulating miRNAs as diagnostic biomarkers in osteoporosis, attempting to elucidate their roles and targets at the bone level where possible. Differential expression of miR-21 and miR-148a in the serum of patients can successfully differentiate osteoporotic and nonosteoporotic individuals, according to various studies. Seeliger., *et al.* for example, revealed 9 elevated circulating miRNAs (miR-21, miR-23a, miR-24, miR-93, miR-100, miR-122a, miR-124a, miR-125b, and miR-148a) that could discriminate between blood samples of osteoporotic and non-osteoporotic broken individuals in a cohort of 30 people per group. Two of these miRNAs, in particular miR-21 and miR-148a, are known to have specialized functions in bone homeostasis at the cellular level. Both osteoclasts and osteoblasts are affected by miR-21. This miRNA promotes the development of murine BMMs by downregulating PDCD4 (an OC differentiation repressor) and the survival of adult osteoclasts by downregulating FasL (a pro-apoptotic protein implicated in the Fas/FasL pathway) [10]. Estrogen signaling likely inhibits miR-21 biogenesis and promotes osteoclast apoptosis, at least in part, through this mechanism. The importance of miR-21 in these cells is highlighted by this discovery. In MC3T3-E1 cells, miR-21 promotes differentiation and mineralization by targeting Smad7, a repressor of osteoblast proliferation, differentiation, and mineralization. Likewise, miR-148a stimulates osteoclast development by directly targeting MAFB, a RANKL inhibitor. It was also shown to impede ST2 cell development toward the osteogenic lineage by directly targeting lysine-specific demethylase 6b (Kdm6b), an osteoblast differentiation regulator, in recent research. In later research, both of these miRNAs were highly unregulated in the plasma of osteoporotic women. However, miR-21 exhibited the opposite effect, and miR-148a is also a powerful osteosarcoma diagnostic marker, raising doubts about the specificity of such miRNAs for osteoporosis. miR-31 and miR-194 are two microRNAs. Other miRNAs (miR-130b-3p, miR-151a-3p, miR-151b, miR194-5p, miR-590-5p, and miR-66) levels were upregulated in the blood of osteoporotic women compared to osteopenic women, and interestingly, expression levels of miR-151b and miR-194-5p were also negatively correlated with femoral neck T-scores are also potential candidates [12]. In clinical blood samples from osteoporosis vs. non-osteoporosis individuals, miR122-5p was dramatically downregulated. In separate research, miR1225p was shown to be the most prevalent miRNA in a pooled sample of osteoporotic patients, with a 15.79-fold increase over a pooled sample of non-osteoporotic patients [11]. In research done, a similar finding was obtained between osteoporotic and osteopenic patients. In osteo-

porotic osteoblasts and osteoporotic osteoclasts, miR-93-5p is up-regulated [13]. In osteoporotic rats, miR-140* and miR-214 were notably elevated [14].

Diagnosis of osteoarthritis

The American College of Rheumatology has produced osteoarthritis diagnostic and categorization recommendations based on clinical and radiographic indicators for the most commonly affected joints (knee, hip, and hand). Excellent, new "high-tech" biochemical and imaging approaches, as well as recommendations for clinical evaluation of the illness and its treatment, have recently been created and validated. As a result, we have consistent and reliable procedures for assessing the established condition, but they are limited in their ability to provide an early and accurate diagnosis for the various kinds of osteoarthritis. Both the diagnostic procedure and differential diagnosis include obtaining a thorough history and analyzing the symptoms (particularly pain), as well as a thorough physical examination that includes looking for the cause of pain and soreness. Even though the patient is of 'osteoarthritis age' and the joint exhibits 'osteoarthritis signs' on the x-ray, not all deformities and discomfort in and around the joints are indications and symptoms of osteoarthritis. On the contrary, even if the underlying illness is osteoarthritis, the patient's complaints may be due to diseases secondary to the fundamental disease (e.g., enthesopathy or tendinopathy), therefore physiotherapy and local injections might treat the patient's problems more readily. In circumstances where cross-sectional diagnostic measurements fail to give diagnostic clues, the course of the disease and diligent follow-up provide diagnostic hints. Serum, joint fluid, and x-ray films can all be used to diagnose, and other imaging techniques (ultrasound, radioisotope scanning, computerized tomography, magnetic resonance imaging, and arthroscopy) can also be helpful. Differential diagnosis isn't just useful in theory; misinterpretation of osteoarthritis results in missing or inappropriate therapy, as well as psychological stress for the patient.



Flowchart of diagnosis of Osteoarthritis and Osteoporosis.

Diagnostic biomarkers of osteoarthritis

In clinical practice, the accepted procedures to diagnose OA are X-ray and MRI (magnetic resonance imaging). However, specialized blood tests to assist in the diagnosis and monitoring of OA progression are currently being developed.

Clinicians and scientists are looking for a new molecule or molecules that may be utilized as a biomarker to detect early OA and track its development. MiRNAs might be good blood-based biomarkers for OA, given their high frequency of expression in OA and their impressively stable form. More research is needed, however, to discover the OA-specific miRNAs that are highly sensitive to OA alterations.

Role of miRNA in cartilage function and its involvement in OA and OP

Although the specific functions of miRNA in cartilage and chondrocytes is unknown, its relevance has been proven. Dicer is required for appropriate skeletal development and is required for miRNA biogenesis [15]. Dicer loss in chondrocytes causes a decrease in proliferating chondrocytes following two independent processes: lower proliferation and rapid differentiation into post-mitotic hypertrophic chondrocyte. Kobayashi., *et al*. [8] has established that miRNAs have a role in cartilage function. Dicer-deficient chondrocytes caused skeletal growth abnormalities and untimely mortality in Dicer-null animals in that study. Because Dicer is a critical component of miRNA production, our findings suggested that miRNA may have an indirect function in chondrocyte biology. Simultaneously, Iliopoulos., *et al*. [16,17] studied the expression of 365 miRNAs in articular cartilage from patients with OA and total knee arthroplasty, as well as healthy persons who had never had a joint problem. They detected 16 differently expressed miRNAs in osteoarthritic cartilage compared to normal cartilage that can be used to differentiate osteoarthritic and normal chondrocytes. As a result, mounting data shows that miRNA dysregulation may have a role in OA, as well as obesity and inflammation [18]. Jones., *et al*. [19] also looked at the expression of 157 miRNAs in human subjects and found a few differently expressed miRNAs in OA cartilage and bone compared to normal tissue. Some common miRNAs involved in defining the complicated gene expression patterns of OA chondrocytes are described here, as well as their functions in transcription control and putative demethylation processes relevant to OA. On murine chromosome 8 and the little arm of human chromosome 16, the miR-140 gene is positioned between exons 16 and 17 of the E3 ubiquitin-protein ligase gene Wwp2 [20]. Tuddenham., *et al*. [21] found that miR-140 was expressed particularly in cartilage tissues of mouse embryos throughout long and flat bone growth, and that this miRNA downregulates histone deacetylase 4. Gene expression profiles were compared in human articular chondrocytes

and human mesenchymal stem cells, using miRNA microarrays and quantitative polymerase chain reaction. (MSCs). They discovered that miR-140 expression differed the most between chondrocytes and MSC [20]. Interleukin-1 (IL-1) has been shown to decrease miR-140 expression in chondrocytes in in-vitro research. IL-1-induced ADAMTS5 expression is inhibited by ds-miR140 transfection of chondrocytes, and IL-1-dependent suppression of aggrecan gene expression is rescued [22]. ADAMTS5 is involved in the formation of OA; data suggests that miR-140 controls cartilage growth and homeostasis, and reduction in its level may contribute to the development of age-related OA-like alterations. Tardif., *et al*. employed miR140 and miR-27a to alter two important variables in human OA chondrocytes: insulin-like growth factor-binding protein 5 (IGFBP-5) and MMP-13. They discovered that human chondrocytes had much lower levels of IGFBP-5 than OA chondrocytes [23]. Despite the fact that these findings imply that IGFBP-5 is a direct target of miR-140, miR-27a indirectly reduces MMP-13 and IGFBP-5. One such miRNA is miR-122-5p. Upregulation of miR-122-5p, miR-19b-3p, and miR-486-5p were found as the independent predictors for OA in research [27]. In osteoarthritis, miR-93-5p was downregulated [24]. In osteoarthritis patients, miR-21 is increased, and overexpression of miR-21 might slow down the chondrogenesis process [25].

- **miRNA 122-5p:** This 22-nucleotide miRNA sequence was discovered in Homo sapiens. Eight databases have contributed annotations to this document (miRBase, RefSeq, ENA, MirGeneDB, GeneCards, LncBase, MalaCards, TarBase). Seventy-four papers have been written about it. The hsa-miR-122-5p, hsa-miR-122, miR-122, MIR122, and miR-122-5p genes produce the hsa-miR-122-5p sequence in Homo sapiens (humans). The human reference genome contains this gene. 101F10.1, 12CC4, 1A1-3B, 2C18, 2PP2A, 30K, 39K2, 3D3, 3G2, 3pK interact with protein-coding genes. OA and OP each have their own set of miRNAs that play an important role. One such miRNA is miR-122-5p [26]. In clinical blood samples from osteoporosis vs. non-osteoporosis individuals, miR122-5p was dramatically downregulated. In separate research [13], miR1225p was shown to be the most prevalent miRNA in a pooled sample of osteoporosis subjects, with a 15.79-fold increase over a pooled sample of non-osteoporotic patients. In research done [27], a similar finding was obtained between osteoporotic and osteopenic patients.
- **miRNA 93-5p:** MiR-93-5p is observed up-regulated in osteoporotic osteoblasts and osteoporotic osteoclasts and belongs to the miR-106b25 cluster [13]. The lncRNA NTF3-5 has been demonstrated to promote osteogenic differentiation and bone regeneration by downregulating miR-93-3p [28]. According to one research, lncRNA-AK131850 can function as a sponge for miR-93-5p during osteoclastogenesis, enhancing vascular endothelial growth factor A (VEGF-A) transcription, expression, and secretion by lowering miR-93-5, consequently encouraging endothelial progenitor cell vasculogenesis [29]. This discovery was not directly connected to osteoporosis, but it was closely related to osteoclasts, therefore it might be valuable in the development of osteoporosis medicines. In OA, miR-93-5p is downregulated, and it can directly target CASC2 to prevent chondrocyte apoptosis triggered by LPS (YUN SUN). miR-93-5p was downregulated in osteoarthritis cartilage and IL-1-treated chondrocytes [24]. The role of miRNAs in OA has recently attracted a lot of interest. The rat model of OA was relieved by overexpression of miR93-5p, which increased chondrocyte activity and inhibited cartilage matrix breakdown and chondrocyte death. Furthermore, we discovered that TCF4 is a direct target of miR-93-5p, and that its expression in human OA-affected cartilage tissue samples was inversely linked with miR-93-5p expression. The increased TCF4 expression reduced the effect of miR-93-5p on chondrocyte apoptosis and the production of anabolic and catabolic factors. MiR-93-5p has been linked to inflammatory responses as well as ECM deposition and breakdown. [30]. Through c-Ski, Zhang., *et al*. found that miR-93-5p impacted TGF-1-induced fibroblast proliferation and ECM deposition [31]. MiR-93-5p also decreased the loss of collagen by inhibiting MMP3 [32]. As a result, we looked into the involvement of miR-93-5p in OA in our research. Because it has an important function to perform in the course of OA, IL-1 is a cytokine which may elicit a variety of pathogenic responses in chondrocytes. Overexpression of miR-93-5p inhibited IL-1–induced chondrocyte death substantially. In general, IL-1 increases the expression of matrix-degrading enzymes (MMP3 and MMP13), promotes inflammation of synovium and the generation of proinflammatory cytokines, and ultimately contributes to degradation of cartilage [33]. miR-93-5p over- expression rectifies the imbalance between anabolism and catabolism of articular cartilage in an articulating joint, according to different studies. In vitro and in vivo, overexpression of miR-93-5p reduces OA by suppressing cartilage matrix breakdown and chondrocyte death in part through regulating TCF4 expression. These findings add to our understanding of the role of miRNA in OA pathophysiology and should aid identification of new treatment options.
- **miRNA 21:** In an intergenic region, MicroRNA 21 is located. The Mir 21 gene is 3433 nucleotides long, according to reports. It has a 3' UTR overlap with the Transmembrane Protein 49 (TMEM 49) 3' UTR (also known as Human Vacuole Membrane Protein 1, VMP-1). In osteoarthritis patients, miR-21 is increased, and overexpression of miR-21 might slow down the

chondrogenesis process. According to certain research, miR-21 might be used as a biomarker for various forms of arthritis and as a candidate miRNA for bone-related inflammatory diseases. Only a few articles have shown the significance of miR-21 in arthritis and associated bone problems, and investigations on distinct forms of arthritis are few. MicroRNA-21 induction was demonstrated to be suppressed by the non-coding RNA GAS5, restoring normal autophagic flow. It has been suggested that microRNA-21 might be a promising target for the development of OA treatments. The expression level of miR-21 was dramatically lowered in OA patients, according to Song., *et al*. and ectopic GAS5 production can restrict induction of miR-21. Human GAS5 is up-regulated in OA subjects and is engaged in autophagy via miR-21-mediated indirect regulation [34]. Apoptotic genes in chondrocytes are targeted by many miRNAs. MiR-210 can reduce DR6 expression by targeting its 30 -UTR, according to [33]. First, RANKL may enhance osteoclastogenesis and bone resorption via targeting Pten to activate the PI3K/Akt signaling pathway or targeted down-regulation of programmed cell death protein 4. (PDCD4) [35]. Nine miRNAs were discovered to be elevated in osteoporosis patients, six of which were also substantially enhanced in the corresponding hip bone tissue [miR-21, miR23a, miR-24, miR-25, miR-100, miR-125b [37]. Panach L., *et al*. 2015 [36] found 12 distinct differentially expressed miRNAs that were in subjects with fractures in comparison to osteoarthritis subjects, and verified that miRNA-122, miRNA-125, and miRNA-21 were elevated in an independent validation sample. Seeliger., *et al*. [37] also discovered these miRNAs, and miRNA-21 levels were found to be substantially linked with the bone resorption marker CTX.

- **miRNA 140:** The miRNA-140 gene is located between exons 16 and 17 of the E3 ubiquitin-protein ligase gene Wwp2, on murine chromosome 8 and the small arm of chromosome 16 in humans. This miRNA was originally shown to be highly expressed in cartilage during long and flat bone formation [38]. HDAC4 was shown to be down-regulated by miR-140 in the first investigation employing this miRNA [35]. Chemoresistance of osteosarcoma tumor xenografts was shown to be mediated in part by miR-140-dependent inhibition of HDAC4, as well as enhanced expression of p52 and p21 in subsequent investigations [39]. In gliomas, on the other hand, miR-140 overexpression was linked to tumor malignancy development [40].

  Asahara and colleagues [41] previously demonstrated the expression of miR-140 in articular cartilage of normal humans and significantly reduced expression in OA tissue miR-140 expression suppressed with in vitro treatment of chondrocytes with IL-1, Chondrocytes transfection with miR-140, on the other hand, suppresses IL-1-induced ADAMTS5 expression [41].

  In this issue of Genes and Development, the same group finds that miR-140 plays a significant role in the pathogenesis of OA through a mechanism that may entail, at least in part, the control of ADAMTS5. Akhtar N., *et al*. 2010 [42] demonstrate with a series of elegant in vivo experiments and cutting-edge mouse genetics that, while miR-140 is not required for the formation of articular surface cartilage, universal knockout of miR-140 predisposes to age-related OA changes and, conversely, overexpression of miR-140 in chondrocytes protects against OA. The research is significant for a variety of reasons. It is the first time that miRNAs are shown to affect articular cartilage homeostasis in vivo; as a result, it offers new insight into the processes that govern articular cartilage regeneration and may have substantial therapeutic implications. More broadly, it adds to our knowledge of the role of miRNAs in healthy and pathological tissue homeostasis. Finally, it provides solid experimental evidence that ADAMTS5 mRNA is a miR-140 target, revealing previously unknown mechanisms that control the expression of this aggrecanase. Researchers now have the task of demonstrating definitively that miR-140-dependent regulation of ADAMTS5 mRNA in an articular surface chondrocyte is the major molecular mechanism mediating miR-140's crucial involvement in the pathogenesis of OA. The study [43] does not go beyond correlation in this aspect, but it is nevertheless a significant and elegant contribution to the subject.

In osteoporotic rats, miR-140* and miR-214 were particularly upregulated (Li a, 2015). Similarly, research found that miR140 expression in chondrocytes was greater in tandem with SOX9 and COL2A1 expression than in hMSCs, suggesting that miR140 may enhance chondrogenic differentiation of hMSCs by targeting SOX9 and COL2A1 [44]. Wa Q., *et al*. 2017 [45] discovered that miRNA140 reduced the growth of C3H10T1/2 MSCs by targeting the CXC motif chemokine ligand 12. Kilpinen., *et al*. (2016) investigated expansion-induced miRNA and mRNA expression alterations in BMMSCs obtained from old and young donors using microarray profiling (OP). In this study, a specially modified miRNA agonist, miRNA-140 agomir, was administered intra-articularly to OA rats, and the miRNA-140 levels were significantly upregulated in cartilage for approximately 7 weeks post- injection, without any complications, indicating that miRNA agomir could be a viable option for intra-articular treatments. Our findings imply that intra-articular injection of miRNA-140 agomir can slow the course of OA in rats through regulating ECM homeostasis. Intra-articular delivery of miRNAs might be a viable treatment for OA, however further studies are required to determine the therapeutic potential of miRNAs as OA therapies. Many microRNAs are induced by Sox9, with miR-140 being particularly sensitive [46]. This is due to its ability to target Sp1 and disrupt the cell cycle [47]. During hMSC chon-

drogenesis in vitro, several additional targets for miR-140-5p were discovered, including RALA and FZD6 [48]. MiR455 is significantly Sox9 inducible and co-regulated with miR-140 in both ATDC5 and hMSC chondrogenesis models [49]. TGF inhibits miR-140 production [50], while Smad3 has been identified as a direct target of miR-140-5p [51].

Conclusion

The nature of miRNAs, which targets multiple mRNAs and therefore genes, presents difficulties in the study of miRNA biomarkers. These difficulties include a lack of reference expression levels in adults, a lack of standardisation for normalisation methods, a wide range of experimental variation. Combining miRNAs might be more efficient than using just one marker. In this review, we concluded that some miRNA like microRNA 122-5p, miRNA 93-5p, miRNA 21, and miRNA 140 as a common biomarker can be used to determine susceptibility to Osteoarthritis knee and Osteoporosis in the same subjects. Therefore, miRNA 122-5p, miRNA 93-5p, miRNA 21, and miRNA 140 may become a common biomarker to determine susceptibility to Osteoarthritis knee and Osteoporosis in the same subjects.

We advise adding the effect of age itself as a covariate to future studies of single and multiple diseases because it can be difficult to distinguish between ageing itself and the connection between age-related diseases like OA and OP.

Last but not least, we advise studies to use well-phenotype population samples (including co-morbid age-related diseases whenever possible) and test people for a variety of miRNAs (preferably at multiple time points) in order to replicate and validate their findings.

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