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Identificación in sílico *de miRNA y sus genes diana implicados en el desarrollo de osteoartritis*

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ABSTRACT

Introduction: osteoarthritis is considered the main cause of joint pain in older people, affecting four core tissues: cartilage, bone, joint capsule, and joint apparatus. In recent years, microRNAs have been described to play a vital role in the development of bone metabolism diseases, including osteoarthritis, since they can have an inhibitory effect or a promoting effect on disease progression.

Objective: through microarray analysis and bioinformatics tools, miRNAs and their potential target genes involved in signaling pathways associated with the development of osteoarthritis are identified.

Methods: the microRNAs were selected through microarray expression analysis from the “Gene Expression Omnibus” database, and through a literature search, their target genes were obtained by integrating different databases. This set of genes was compared with a set of differentially expressed genes from expression microarray analysis of samples from osteoarthritis patients. The shared gene set was subjected to signaling pathway enrichment analysis.

Results: four miRNAs were identified, miR-485, miR-940, miR-107, and miR-142-5p, that regulate 185 genes involved in 9 signaling pathways in

which *CSF1*, *CXCL3*, *FOS*, *IL6*, *IL6R*, *NFATC1*, *NFKB1*, *NFKB2*, *PPARG*, *THBS1* and *TNF* genes play a crucial role in bone and immune system-associated processes and their deregulation may favor the progression of osteoarthritis.

Conclusions: the microRNAs identified in this study could be used as biomarkers for the timely diagnosis and monitoring of osteoarthritis treatment.

Keywords: Osteoarthritis. Bone metabolism. MicroRNAs. Bone mineral density. Bioinformatics. Microarrays.

RESUMEN

Introducción: la osteoartritis se considera la principal causa de dolor articular en personas mayores, afectando a cuatro tejidos fundamentales: cartílago, hueso, cápsula articular y aparato articular. En los últimos años se ha descrito que los microRNA juegan un papel importante en el desarrollo de enfermedades del metabolismo óseo, incluida la osteoartritis, ya que pueden tener un efecto inhibidor o promotor de la progresión de la enfermedad.

Objetivo: a través del análisis de *microarrays* y herramientas bioinformáticas, se identificaron microRNA y sus potenciales genes diana involucrados en vías de señalización asociadas al desarrollo de la osteoartritis.

Métodos: los microRNA fueron seleccionados a través de un análisis de expresión de microarreglos de la base de datos “Gene Expression Omnibus”, y posteriormente se obtuvieron sus genes diana mediante la integración de diferentes bases de datos. Este conjunto de genes se comparó con otro grupo de genes diferencialmente expresados a partir del análisis de microarreglos procedentes de muestras de pacientes con osteoartritis. El conjunto de genes compartidos se sometió a un análisis de enriquecimiento de vías de señalización.

Resultados: se identificaron cuatro miRNA, miR-485, miR-940, miR-107 y miR-142-5p, que regulan 185 genes involucrados en 9 vías de señalización en las que los genes CSF1, CXCL3, FOS, IL6, IL6R, NFATC1, NFKB1, NFKB2, PPARG, THBS1 y TNF juegan un papel crucial en procesos asociados al sistema óseo e inmune y su desregulación puede favorecer la progresión de la osteoartritis.
Conclusiones: los microRNA identificados en este estudio podrían ser considerados como potenciales biomarcadores para el diagnóstico oportuno y seguimiento del tratamiento de la osteoartritis.

Palabras clave: Osteoartritis. Metabolismo óseo. MicroRNA. Densidad mineral ósea. Bioinformática. *Microarrays.*

INTRODUCTION

Osteoarthritis (OA) is the most prevalent chronic joint disease worldwide. It affects all joint tissues, causes complete joint dysfunction, and causes progressive loss of articular cartilage, which generates damage to other joint structures, such as the subchondral bone and the membrane synovium, leading to chronic disability and decreased quality of life (1). Changes in cartilage can be induced by aging. However, cartilage degeneration can occur in response to inappropriate mechanical stress and low-grade systemic inflammation associated with trauma, obesity, and genetic predisposition, which subserve the risk of development and progression of OA (2).

The complex interactions among cartilage, synovium, and subchondral bone significantly influence in cartilage function, making it challenging to pinpoint the onset and location of pathological changes. Consequently, it has been suggested that biological factors may trigger temporal and spatial alterations in chondrocytes and cellular components of cartilage, that potentially leading to a pathological state (3). Chondrocytes are derived from mesenchymal progenitors and its

function is to synthesize the extracellular matrix and form anlagen cartilage for bone development (4). Chondrogenesis occurs because of the condensation of mesenchymal cells expressing collagens I, III, and V and the differentiation of chondroprogenitor cells with expression of cartilage-specific collagens II, IX, and XI. During limb development, resting chondrocytes can form cartilage at the ends of opposing bones with intermediate interzones formed during cavitation, increase, and then proceed to terminal differentiation towards hypertrophy and apoptosis to allow endochondral ossification so the calcified hypertrophic cartilage is resorbed and replaced by bone (5). Proliferating chondrocytes are under the control of the parathyroid hormone/Indian hedgehog (PTHrP/Ihh) axis and express collagen VI and matrilin 1 (MATN1). The hypertrophic zone is characterized by collagen of vascular endothelial growth (VEGF) and VEGF receptors whose interaction allows non-vascularized and hypoxic tissue to be converted into bone through the activity of osteoclasts (bone-retaining cells) and osteoblasts (bone-forming cells). A similar sequence of events occurs in the postnatal growth plate, leading to rapid skeletal growth (6). These processes depend on a complex regulation through the interaction of transforming growth factor β (TGF-β), bone morphogenic protein (BMP), and the WNT signaling pathway. So, the alterations of these signaling pathways could lead to the development of OA (7). Recent studies have shown that microRNAs (miRNAs) play an essential role in the appearance and development of different diseases: multiple types of cancer, cardiovascular, metabolic, immune, kidney and bone metabolism diseases (8).

MiRNAs are a class of endogenous, small (19–25 nt), non-coding RNAs that negatively regulate gene expression and basic physiological processes such as cell differentiation, growth, proliferation, metabolism, and apoptosis. The miRNA-mediated target gene regulation process begins with the recognition of the pre-miRNA duplex chain through the

DICER protein, which is an RNAse III responsible for the elimination of the terminal loop of the pre-miRNA, which together with the argonaute protein (AGO) form part of the RNA-induced silencing complex (RISC). The chains derived from the mature duplex miRNA are loaded into AGO in humans and are ATP-dependent. In general, the strand with the lowest stability in the 5' position or 5' uracil is preferably loaded into AGO and will be considered as the guide strand. The selection of this chain depends on the union of the first 6-8 nucleotides with the 3'UTR region of the target mRNA (seed region) and the type of AGO protein that is present in the RISC. It has been shown that miRNAs bind to specific sequences, and the base complementarity between the miRNA and its target gene determines the fate of the mRNA. The interaction between the miRNA seed region (2-8 nt) and the 3'UTR of the mRNA is of great importance since perfect complementarity allows the AGO2 protein with exonuclease function to cleave the mRNA at RNA processing proteins, which associate with AGO and function as mRNA storage sites (P bodies). On the other hand, when the binding of the miRNA to the seed region of the mRNA is imperfect, a hairpin is formed between the miRNA and its target gene between the ninth and tenth nucleotide of the miRNA, which induces translation suppression (9). To date, few studies have investigated circulating miRNAs in OA, and their findings lack consistency, with the diagnostic value of these miRNAs yet to be clearly established. Therefore, this work aims to identify miRNAs and their potential target genes involved in signaling pathways whose deregulation can lead to the development of OA, through search of existing literature and bioinformatics tools.

MATERIAL AND METHODS

Selection of miRNAs

To select a set of miRNAs involved in the development of OA, microarray files in CEL format were first obtained from studies where changes in

miRNA expression profiles in patients with OA were analyzed, which were selected through searching in different databases: PubMed (https://pubmed.ncbi.nlm.nih.gov/) and Gene Expression Omnibus (GEO) (https://www.ncbi.nlm.nih.gov/geo/). Files in ".txt" format were obtained from a study where miRNAs differentially expressed in primary osteoblasts from patients with hip replacement for osteoporosis or OA were identified using the miRCURY LNA microRNA Array, 7th Generation technology (QIAGEN, San Diego, USA) with access number GSE74209 (10). In another study using high-throughput seqRNA (DNBSEQ [BGI-Shenzhen, China]), changes in the expression profiles of ncRNAs from synovial tissue samples of anterior cruciate ligation tears were analyzed, from which the analyzed data of differentially expressed miRNAs were obtained (11). Finally, through a literature search, a set of miRNAs associated with OA was compiled, which are summarized in a review and bioinformatics analysis carried out by Cong et al. 2017 (12). The group of miRNAs selected for this study was selected through a comparative analysis represented in a Venn diagram using the "Bioinformatics & Evolutionary Genomics" tool (https://bioinformatics.psb.ugent.be/webtools/Venn/).

Prediction of potential miRNA target genes

To identify the target genes of the selected miRNAs, a search was performed in different databases that use computational algorithms to determine the nucleotide pairing between the 3'UTR region of a target mRNA and the 5' "Seed" region (2-7 nucleotides) of a miRNA. The databases used were: miRWalk (http://mirwalk.umm.uni-heidelberg.de/), miRDB (https://mirdb.org/), TargetScan (https://www.targetscan.org/vert_80/), Tools4miRs (https://tools4mirs.org/software/), and miRTarBase (https://mirtarbase.cuhk.edu.cn/). The target RNAs for each miRNA were

selected if they were present in at least three of the five databases used (13).

Candidate genes selection

To select candidate genes, a search was performed in PubMed and GEO, looking for studies that employed genome-wide analysis technologies to identify differentially expressed genes (DEG) associated with OA. Files were obtained from a survey that identified differentially expressed genes in a sample of 79 individuals divided into three groups that included 20 healthy controls, 26 OA patients, and 33 rheumatoid arthritis (RA) patients through expression microarrays on the GeneChip platform. Human Genome U133A/B from Affymetrix. Files were obtained in CEL format and corresponding to the control group and the OA group. The original files in CEL format were processed to expression values using the Robust Multiarray Averange (RMA) method in the R-BiocMananger environment. Probe-level data were transformed into expression values, followed by background correction and data normalization. The cut-off criteria used to select differentially expressed genes were that they had expression change values < -0.5 and > 0.5 because the change rate is expressed in Log2, which represents that a gene is at least twice as expressed in one condition compared to another. A false discovery rate (FDR) < 0.05 was also shown as a cut-off criterion to control the false positive rate. The selection of candidate genes was carried out through a comparative analysis between the genes predicted for each miRNA and the DEG from the microarray analysis. This set of genes was represented through a Venn diagram, ensuring that the shared genes were targets of the miRNAs and were involved in OA.

Interaction network between miRNAs and target genes

Once the list of genes involved in the signaling pathways of interest was available, an interaction network between miRNAs and target genes was developed using the Cytoscape v3.7.2 software. In Cytoscape, the default damping criterion for setting the dissipation coefficient is the probability of termination (dissipation). This requires a value between 0 and 1, which sets the dissipation directly on average. So, in this study, we used a local clustering index of 0.592, set as an optimal probability value by the same software. These interactions allow the identification of potential miRNAs and candidate genes whose changes in their expression profiles could affect bone metabolism.

RESULTS

Identification of miRNAs involved in the pathogenesis of OA

Through the search for miRNA expression data in different databases, three groups composed of 453 differentially expressed miRNAs were identified corresponding to the work where the miRCURY LNA microRNA Array, 7th generation (QIAGEN, San Diego, USA) technology was used) (10), 211 differentially expressed miRNAs where high-throughput Seq-RNA technology was used through the DNBSEQ platform (BGI-Shenzhen, China) (11) and 136 miRNAs from a literature review (12) (Fig. 1A).

MiRNA target gene prediction

The prediction of the potential target genes of the miRNAs (mRNA) was carried out based on their presence in at least three of the five databases used for the analysis, identifying 723 target genes for miR-485, 1030 genes for miR-940, 821 genes for miR-107 and 1133 genes for miR-142-5p, which were unified into a single list, eliminating repeats (Fig. 1B).

Candidate gene selection

Data from GEOs with accession number GSE55235 were analyzed to analyze GDE associated with OA. Data were retrieved in CEL format from the GeneChip Human Genome U133A/B expression microarray. Differential expression analysis showed 199 downregulated genes and 2123 upregulated genes that met the < -0.5 and > 0.5-Fold-Change cutoff criteria with a *p*-value < 0.05 (Fig. 1C). The list of the GDE from the microarray was compared with the unified list of target genes of the miRNAs through a Venn diagram where it is observed that 379 genes involved in OA are shared and that they are targets of the selected miRNAs (Fig. 1D). The genes recovered from this analysis were used to identify the signaling pathways involved in the development of OA.

Signaling pathways in OA

The genes shared between microarrays and target genes were subjected to a signaling pathway analysis through the KEGG tool in the ShinyGO software, this tool allows to identify to which route or signaling pathway a set of genes analyzed in an online database of genomes, enzymatic pathways and biological chemicals within cells and specific variants of them in particular organisms belong, where 9 signaling pathways related to the development of OA were identified (Table I), an interaction network between these signaling pathways was generated (Fig. 2), and the genes involved in these signaling pathways were subjected to an enrichment analysis where 20 OA-related diseases were identified (Fig. 3).

Interaction network between target genes and miRNAs

From the 185 genes identified in the signaling pathways associated with OA, an interaction network was generated together with the four selected miRNAs (Fig. 4). From this interaction network, 12 genes were selected that play an essential role in bone metabolism and that, according to a review in the literature, are associated with the

development of OA. Colony Stimulating Factor 1 (CSF1), C-X-C Motif Chemokine Ligand 3 (CXCL3), Fos Proto-Oncogene, AP-1 Transcription Factor Subunit (FOS), Interleukin 6 (IL6), Interleukin 6 Receptor (IL6R), KRAS Proto-Oncogene, GTPase (KRAS), Nuclear Factor Of Activated T Cells 1 (NFATC1), Nuclear Factor Kappa B Subunit 1 (NFKB1), Nuclear Factor Kappa B Subunit 2 (NFKB2), Peroxisome Proliferator Activated Receptor Gamma (PPARG), Thrombospondin 1 (THBS1), and Tumor Necrosis Factor (TNF). The expression profile of this set of genes was represented through a heat map showing the downregulated and upregulated genes in OA (Fig. 5).

DISCUSSION

Our study presents an in-silico analysis focused on evaluating the expression signatures of human miRNAs involved in the regulation of genes that participate in different signaling pathways whose alterations can lead to the development of OA. According to the bioinformatics search, four miRNAs involved in OA were identified: miR-485/miR-142 is down-regulated, and miR-940/miR-107 is up-regulated. MiR-485 has been associated with the development of OA through the inhibition of the Notch2 and NF-kB signaling pathways, promoting chondrocyte proliferation in OA and inhibiting apoptosis (14). MiR-142 has a protective effect against OA by competing with the lncRNA XIST that regulates chondrocyte growth and apoptosis (15). MiR-940 regulates the expression of genes such as MyD88, which induces a level of inflammation and simultaneously stimulates the NF-kB signaling pathway mechanism (16). MiR-107 affects cartilage matrix degradation in the pathogenesis of OA through the regulation of caspase 1, positively regulating chondrocyte proliferation (17). However, although these miRNAs have been linked to the development of OA, their role as potential biomarkers in bone metabolism and related diseases has yet to be explored.

Our analysis revealed nine signaling pathways associated with bone metabolism whose dysregulation is associated with the development of OA. PI3K-Akt signaling pathway involves different molecules that regulate diverse biological processes. In cartilage, it regulates synovial inflammation, subchondral bone sclerosis, extracellular matrix homeostasis, chondrocyte proliferation, apoptosis, autophagy, and inflammation (18). MAPK pathway transmits extracellular signals to cells through a cascade reaction involving kinases in articular chondrocytes and inducing phosphorylation cascades. These stimuli include inflammatory factors, cytokines in the joint fluid, changes in osmotic pressure, and changes in biological stress (19). TNF signaling is tightly regulated by post-translational ubiquitination, an essential mechanism for the regulation of many biological processes. The role of inflammatory factors such as IL-1, TNF, and caspase-8/3 are involved in chondrocyte apoptosis, leading to further degenerative changes in cartilage (20). FOX signaling pathway is related to cell fate and promotes chondrocyte homeostasis (21). Osteoclast differentiation is a biological process responsible for the resorption of bone tissue, its role is well established in average bone turnover. However, osteoclasts play essential roles in other diseases, such as progressive joint destruction. It has been reported that the degradation of the cartilage and osteochondral junction compartments of the joint is carried out by the action of osteoclast-derived metalloproteinases (MMPs) so that alterations in the differentiation pathway of these cells could be constitutively activated, leading to the resorption of cartilage tissue, and favoring the development of OA (22). JAK-STAT signaling pathway is responsible for regulating cellular responses to cytokines such as IL-6 and epidermal growth factor (EGF) and biological processes such as cell proliferation, cell differentiation, and apoptosis. One study suggests that *CXCL8* and *CXCL11* may be involved in apoptosis and inhibit primary chondrocyte proliferation by regulating the expression of phosphorylated *STAT3*,

leading to the development of OA (23). Rheumatoid arthritis is a disease that affects the joints and induces inflammation, which causes thickening of the tissues surrounding the joints, resulting in joint failure and pain (24). The TNF-kappa B signaling pathway regulates the expression of proinflammatory genes. This signaling pathway has been reported to regulate the activation of osteoclast differentiation, activate the inflammatory response, and promote the expression of catabolic factors such as MMPs that induce the destruction of articular cartilage (25). The AMPK signaling pathway plays a role in regulating growth and reprogramming metabolism. AMPK proteins are essential mediators of AMPK signaling activities and could provide energy for the inflammatory reactions that promote the development of OA (26).

Interestingly, we have observed that the miRNAs identified in this study, as well as their potential target genes involved in the described signaling pathways, have a key role in the activation and differentiation of osteoclasts. The CSF1 gene encodes an essential cytokine for osteoclastogenesis that promotes the proliferation, survival, and differentiation of monocytes/macrophages and is regulated by miR-485, miR-940, and miR-107. Its negative regulation inhibits the formation of mature osteoclasts. However, when miR-485 is deregulated, it could allow the expression of CSF1 and, therefore, the differentiation of osteoclasts (27,28). On the other hand, the CXCL3 gene can recruit and activate various immune cells such as monocytes/macrophages, neutrophils, T cells, natural killer (NK) cells, fibroblasts, and endothelial cells that participate in the pathogenesis of OA (29). This gene is regulated by miR-485 and miR-940; these miRNAs could play a vital role in the recruitment of cells such as monocytes, which have a fundamental role in the progression of OA, given their participation in inflammatory responses and their ability to differentiate into osteoclasts (30). The *NFkB1*/*NFkB2* genes are precursors of NF-κB, which, together with FOS, are transcription factors that are activated in immune cells and activated

in osteoclast precursors. These genes are regulated by miR-485, while the FOS gene is regulated by miR-107, so these miRNAs could play a key role in regulating the differentiation of osteoclasts capable of degrading cartilage in OA. Another cytokine involved is IL6, which is present in elevated levels of synovial fluid of individuals with a confirmed clinical diagnosis of OA, and its mechanism of action has been shown to involve its ability to interact with its receptor IL6R. This interaction significantly suppresses the synthesis of neutrophil gelatinase-associated lipocalin (NGAL) in the immortalized human chondrocyte line, C28/I2 (31). Keeping this in mind, here, we report that NGAL regulates the activity of matrix metalloproteinase-9 (MMP-9), whose activity is crucial in OA for the destruction of articular cartilage (32). MiR-485, miR-940, and miR-107 could regulate the expression of *IL6*, while *IL6R* is targeted by miR-485, miR-940, miR-107, and miR-142-5p so that these miRNAs could play a vital role in the secretion of MMPs by osteoclasts in individuals with OA. *KRAS* gene is a small GTPase that functions as a signal transducer from cell surface receptors activated by extracellular stimuli to various well-regulated cytoplasmic signaling networks, such as mitogen-activated protein kinase (MAPK) and phosphoinositide 3-kinase (PI3K). Although the role of KRAS in bone metabolism is unclear, studies in mice have shown that mutations in this gene are associated with an increase in the number of osteoclasts and, therefore, in bone resorption (33). KRAS is targeted by miR-485, miR-940, miR-107, and miR-142-5p so that these miRNAs could be involved in the activation of osteoclast differentiation. The NFATC1 gene plays the role of the master regulator of osteoclast differentiation transcription. Its activation allows the differentiation of cells of the monocyte/macrophage lineage after stimulation by the two essential cytokines, CSF1 and RANKL. This gene is the target of miR-485, so deregulation of this miRNA could promote osteoclast differentiation and increase cartilage and bone tissue resorption. PPARG is a gene that regulates chondrocyte apoptosis in

individuals with OA through the caspase-3-dependent mitochondrial pathway, and PPARG-mediated autophagy activation alleviates inflammation in rheumatoid arthritis (34). MiR-485, miR-940, and miR-107 regulate this gene, and these miRNAs might play a role in regulating chondrocyte cell death. The THBS1 gene is involved in chondrogenesis; its primary known function is its antiangiogenic and anti-inflammatory effect in several models, mainly in cancers and heart diseases. THBS1 exerts an antiproliferative role in T lymphocytes, exerting an anti-inflammatory effect, so this gene has a chondroprotective effect (35). This gene is targeted by miR-485, miR-940, miR-107, and miR-142-5p, so the regulation mediated by these miRNAs could be associated with the development of OA. TNF is a proinflammatory cytokine and, together with other cytokines, is a catabolic factor for cartilage; this cytokine promotes the release of matrix metalloproteinases (MMPs) from synovial fibroblasts, resulting in cartilage destruction, and inhibits chondrogenesis through the nuclear factor-kB (NF-kB) pathway by downregulating SOX production (36). MiR-485, miR-940, and miR-107 regulate a TNF, so the function of these miRNAs could be related to cartilage formation and maintenance.

Based on bioinformatics analysis and a literature search on the role of miRNAs and their potential target genes in the development of OA, we propose a model that represents the role of the genes involved in the identified signaling pathways and their miRNA-induced regulation (Figure 6). On the other hand, the alterations in the expression profiles of miRNAs and target genes identified in this study are also related to other diseases that may be risk factors that favor the development of OA. Recent studies from Finnish population suggest that periodontitis and osteoarthritis are related in a bidirectional pattern (37). Other studies have analyzed the relationship between osteoporosis and OA, where the role of common and divergent factors has been identified, leading to new findings on the role of BMD. It has been reported that the

relationship between BMD and OA depends on the stage, definition, location, and way in which BMD is measured, suggesting that OA should be further specified in terms of bone involvement. Therefore, the osteoporotic and erosive phenotypes would be candidates for bone-targeting drugs. At the same time, the bone-forming subtype, which refers to bone-forming tumors that can be benign or malignant and are characterized by abnormal proliferation of bone cells, could be studied (38).

The cases of osteoarticular manifestations are frequently present in patients with systemic sclerosis and have a significant impact on the patient's quality of life (39). In another study, the risk of mortality and cardiovascular morbidity in patients with OA was analyzed. The authors compared the incidence and prevalence of arterial hypertension between rheumatoid arthritis and OA. Their results showed no differences in the incidence or prevalence of hypertension between the research groups. Only the patients with rheumatoid arthritis participants with long-term remission had a marginally lower prevalence of hypertension (40). In obesity, OA is related to excessive joint loading with altered biomechanical patterns along with hormonal and cytokine deregulation. Weight loss in OA can bring clinically significant improvements in pain and delay the progression of structural joint damage. On the other hand, the coexistence of diabetes mellitus type 2 in patients with OA has been related to the development and progression of the disease. Furthermore, DM is associated with a higher degree of osteoarthritic pain. Numerous risk factors are common to both DM and OA, for example, obesity, hypertension, and dyslipidemia (41). Finally, this work presents strengths and weaknesses. It is important to note that the identification of new therapeutic targets and signaling pathways involved in joint metabolism is essential to elucidate the mechanisms that lead to the development of OA and thus propose new molecules that can be used as potential biomarkers for drug monitoring

or early detection of the disease. The use of standardized methods for identifying miRNA target genes and conducting microarray analysis enhances the reproducibility of the results. Additionally, by utilizing data from patient samples analyzed through various technologies, the study ensures a robust association of the selected miRNAs with OA. These methodological strengths support the reliability and validity of the findings, providing a solid foundation for future research. However, the study also has limitations. The results may not be generalizable due to potential variability in the samples analyzed, influenced by factors such as diet, lifestyle, environmental conditions, and genetic differences among populations. Additionally, while bioinformatics methods are consistent across reports, variations in the number of samples, platforms used, and specific analysis techniques can lead to differing outcomes. Therefore, biological validation assays are necessary to confirm the bioinformatics predictions. Furthermore, we consider that the expression of these miRNAs could be analyzed in different biological fluids, such as plasma, serum, urine, and saliva, to better support their use as potential noninvasive biomarkers for the early detection of OA.

CONCLUSIONS

MiRNAs play an essential role in the pathogenesis of OA. Deregulation of miR-485/miR-142, as well as upregulation of miR-940/miR-107, affects different pathways involved in the pathogenesis of this disease, increasing the expression of enzymes that degrade the cartilage of articular chondrocytes, decreasing the production of matrix components or facilitating the apoptosis of these cells. In addition, miRNAs also participate in the production of proinflammatory cytokines and the induction of joint inflammation, as well as in pathways related to the progression of OA. Given the critical role of miRNAs in the development of this disease, these molecules could be proposed as potential biomarkers for the early detection of OA. However, further studies are

needed to validate the specificity and sensitivity of these molecules in different populations.

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695 **Table**

Table I. Signaling pathways associated with osteoarthritis

Pathways	**nGenes**	**Total pathway genes**	**Enrichment FDR**
PI3K-Akt signaling pathway	70	354	3.81E-14
MAPK signaling pathway	57	294	1.45E-11
TNF signaling pathway	40	212	1.20E-16
FoxO signaling pathway	35	131	3.05E-11
Osteoclast differentiation	32	200	9.18E-10
JAK-STAT signaling pathway	28	232	1.84E-05
Rheumatoid arthritis	27	231	8.65E-10
NF-kappa B signaling pathway	25	126	1.79E-07
AMPK signaling pathway	23	156	2.18E-05

FDR: false discovery rate. nGenes: number of genes.

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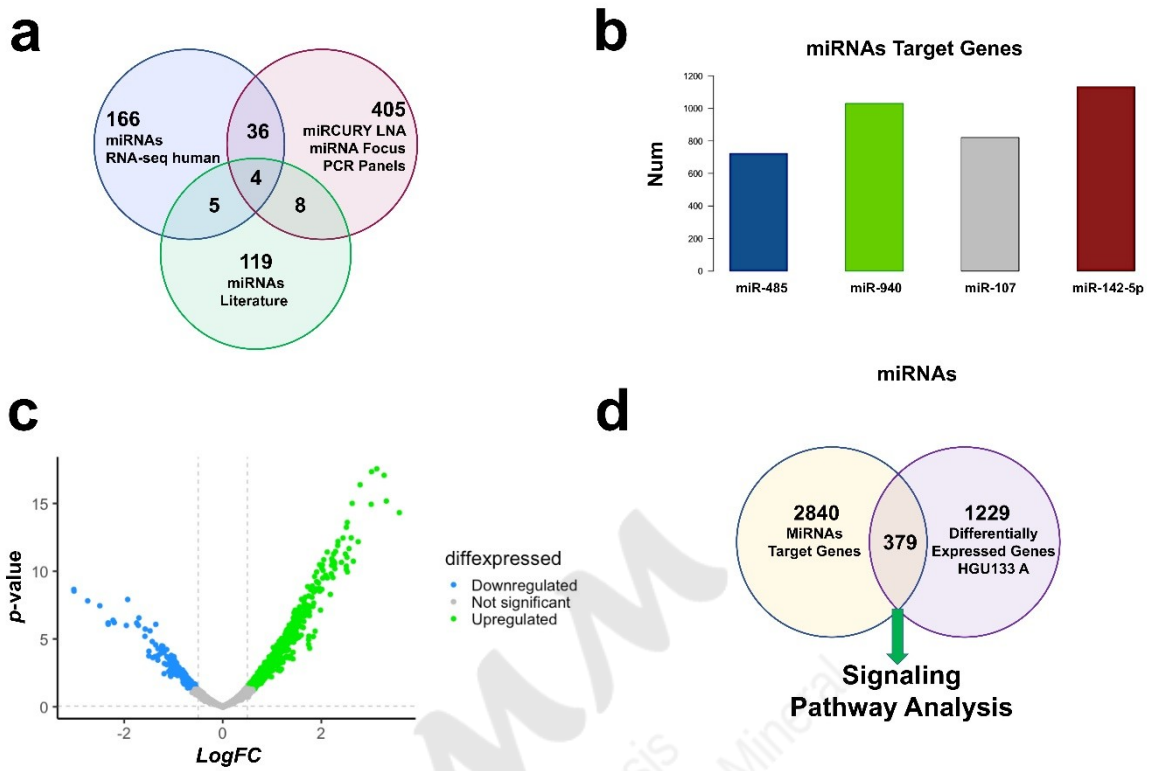

Figure 1. Analysis of miRNA selection and potential target genes. A. Venn diagram between groups of miRNAs from seqRNA, microarrays, and systematic literature review. B. Number of target genes present in at least three databases identified for each miRNA. C. Volcano diagram of differentially expressed genes from the HGU133A/B expression microarray analysis. D. Venn diagram between the group of target genes of each miRNA and the differentially expressed genes from the HGU133A/B microarray analysis.

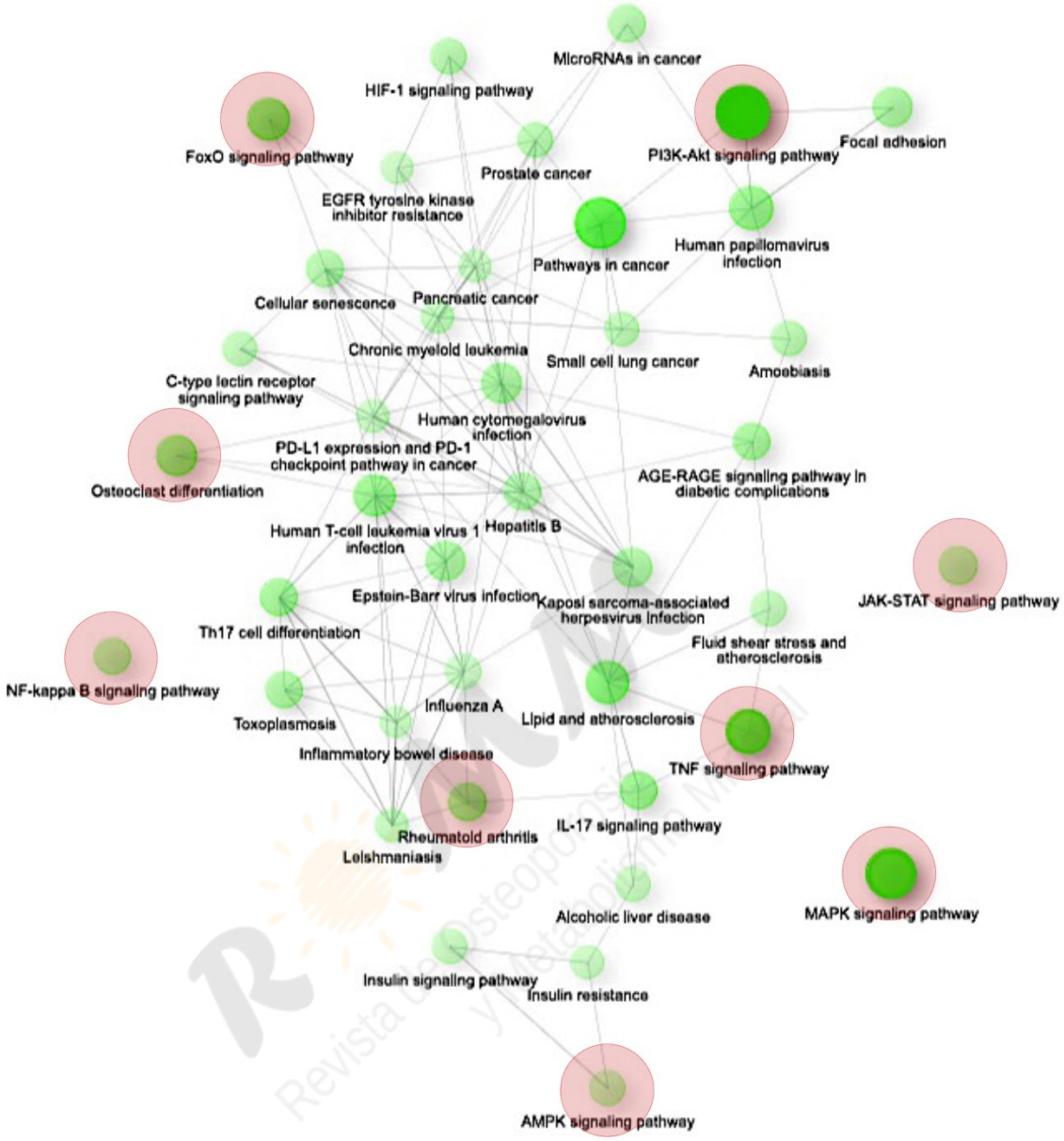

Figure 2. Analysis of interaction networks between the selected signaling pathways. Pathways involved in the development and progression of OA are highlighted in red. PI3K-Akt signaling pathway, MAPK pathway, TNF signaling, FOX signaling pathway, osteoclast differentiation, JAK-STAT signaling pathway, rheumatoid arthritis, NF-kappa B signaling pathway, and AMPK signaling pathway.

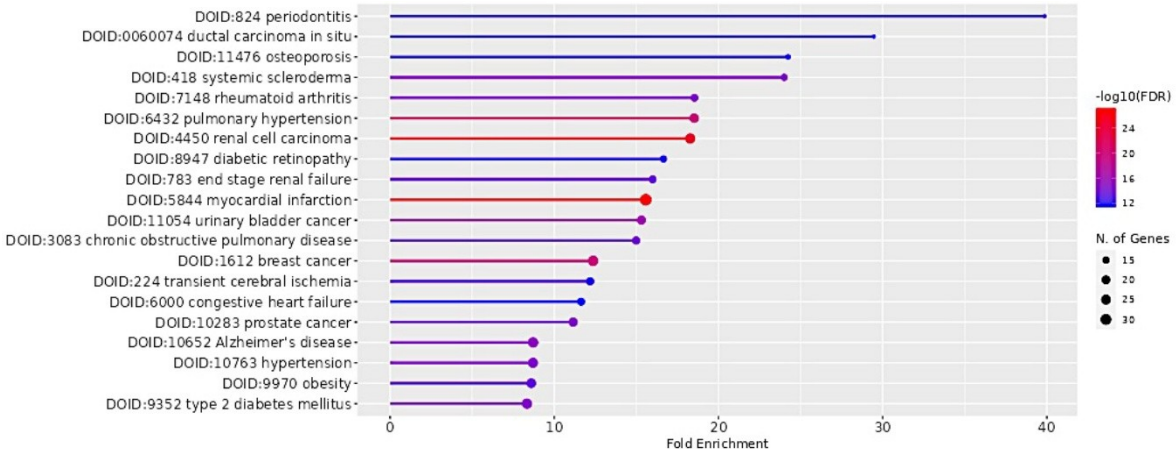

Figure 3. Chart of pathologies associated with OA. The different comorbidities associated with the development and progression of OA are shown.

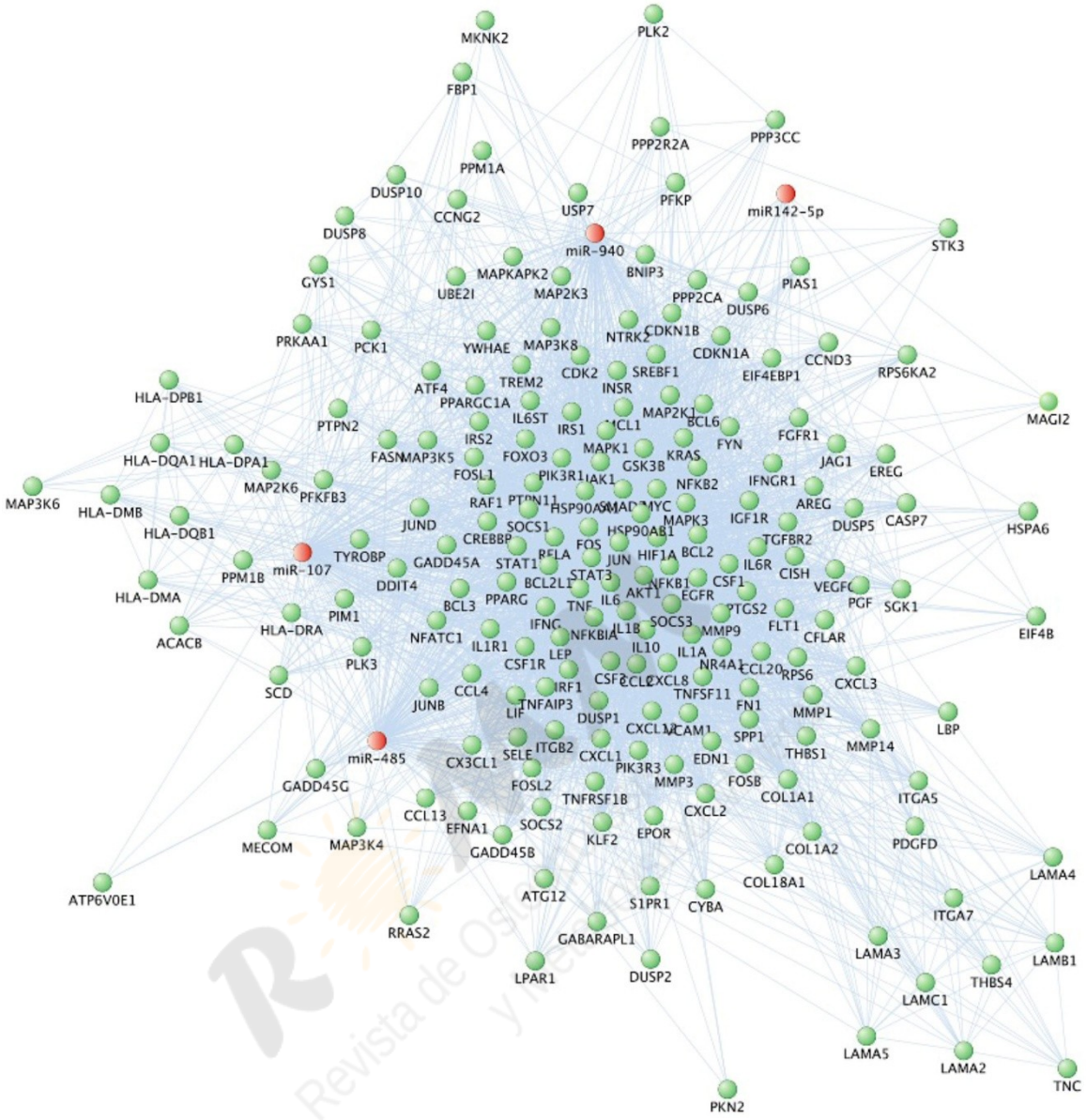

Figure 4. Interaction network between miRNA and target genes. Genes marked in red were selected for their participation in OA-related signaling pathways. They are targets of miRNAs miR-485, miR-940, miR-107, and miR-142-5p and present interaction with multiple signaling pathways.

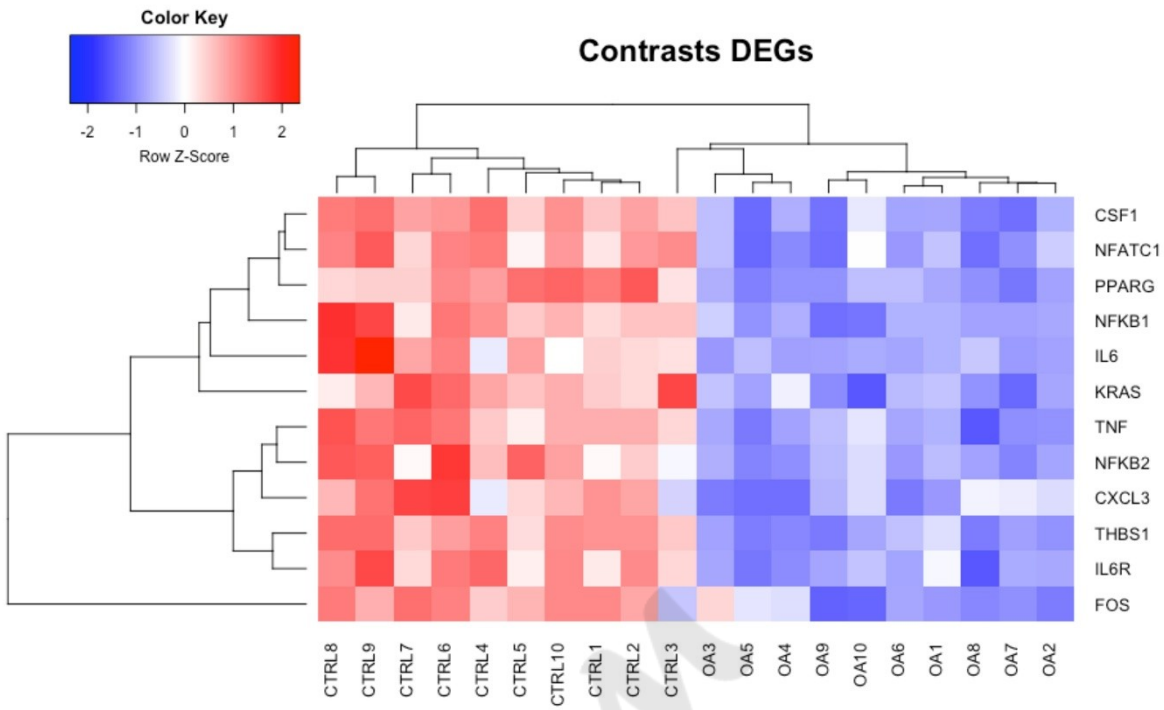

Figure 5. Differential expression analysis. Heatmap showing the expression profiles of genes involved in the development and progression of OA. Down-regulated genes are shown in blue, and up-regulated genes in red.

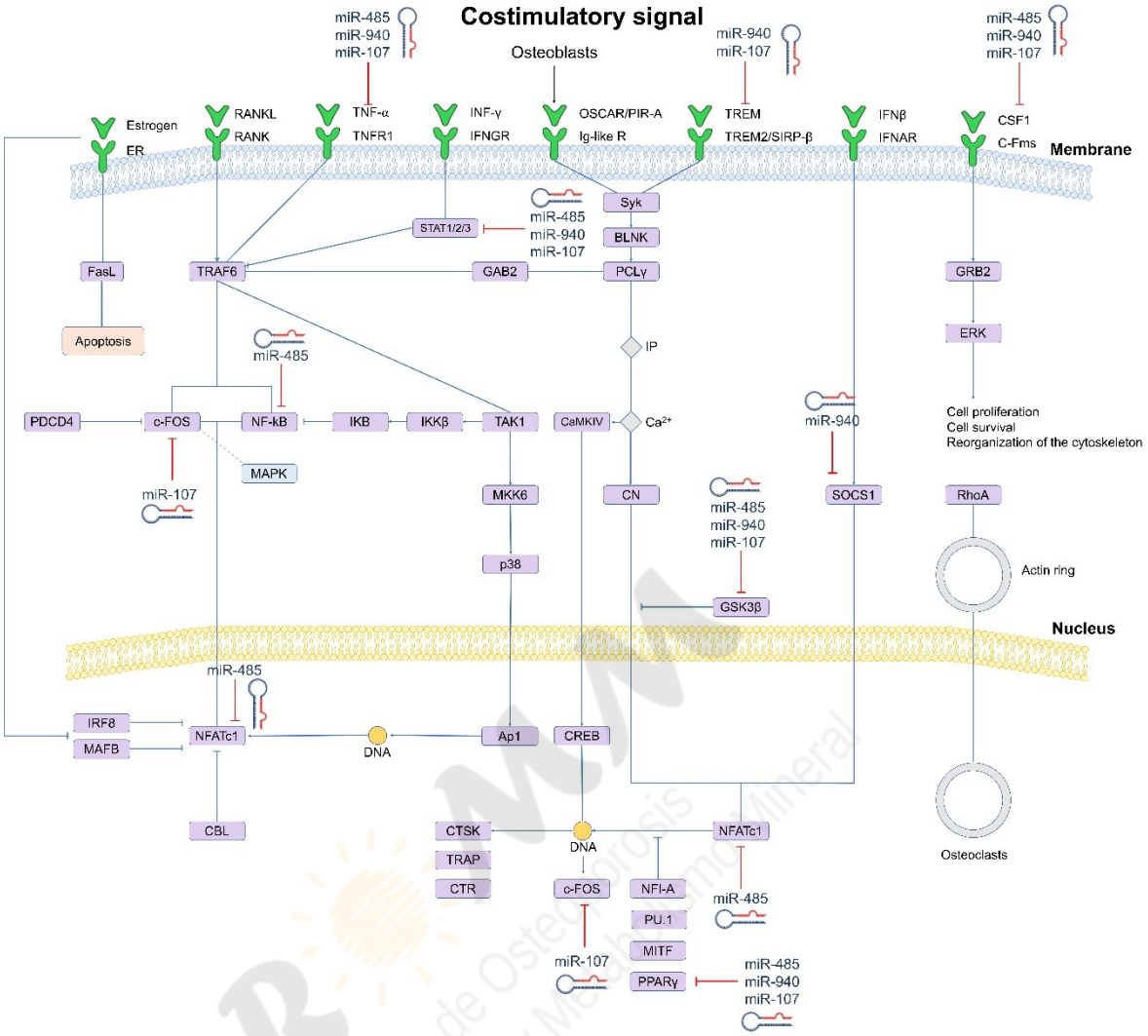

Figure 6. Schematic of the signaling networks involved in OA development and their miRNA-induced regulation. It is shown that miRNAs directly (solid lines) or indirectly (dashed lines) inhibit vital genes and transcription factors in osteoclast differentiation.