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A bioinformatic analysis of microRNAs role in osteoarthritis

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SUMMARY

Objective: To evaluate the underlying function of microRNAs (miRNAs) in osteoarthritis (OA).
Design: A bioinformatic analysis of miRNAs-OA studies was completed in multiple databases. All identified articles were assessed using specific inclusion and exclusion criteria (Eligible case–control studies for the present study included those which investigated miRNAs differential expression in cartilage tissues and cells of OA and controls. Abstracts, case reports, conference presentations, editorials, and expert opinions were excluded.). We performed bioinformatic analysis and assessed which miRNAs are commonly elevated or decreased in cartilage of OA, and assessed putative targets of these miRNAs using TargetScan, Database for Annotation, Visualization and Integrated Discovery (DAVID), FunRich and String.
Results: Fifty seven studies were included in this study. Our current review has identified 46 differentially expressed miRNAs involved in autophagy, inflammation, chondrocyte apoptosis, chondrocyte differentiation & homeostasis, chondrocyte metabolism and degradation of the extracellular matrix (ECM). Additionally, our literature search identified a wide range of miRNAs that have been shown to be differentially expressed in OA. The function of up-regulated miRNAs primarily target nucleus, whereas the function of down-regulated miRNAs primarily target transcription.
Conclusions: Comprehensive analysis of all miRNAs studies reveals cooperation in miRNA signatures and suggests that there may be two biologically synergic classes of miRNAs that are associated with OA. This finding suggests that miRNAs may be useful as diagnostic biomarkers and/or may provide new therapeutic targets in OA. Furthermore, a better understanding of the targets of these miRNAs will accelerate biomedical discoveries and improve clinical care based on new knowledge of OA-related disease mechanisms.

Introduction

Osteoarthritis (OA) is a common musculoskeletal problem that causes significant pain and stiffness in the joints, constituting serious socioeconomic costs and significantly impairing quality of life[1,2]. The etiology of OA remains enigmatic, however genetic predisposition is believed to be a principal etiological factor of OA. Conversely, environmental factors such as mechanical loading, trauma, obesity, gender, and aging are responsible for a smaller portion of cases. Several key pathological mechanisms of OA at a cellular and molecular level have been documented. These include chondrocyte apoptosis, degeneration of the cartilage, inflammation, and remodeling of the subchondral bone. This eventually leads to breakdown of the articular cartilage, and synovitis[3]. Current treatment options include conservative therapies such as physiotherapy and NSAIDs, as well as surgical procedures. Whilst these procedures may alleviate the clinical symptoms of OA, they may be unable to prevent the underlying disease process. The underlying molecular mechanisms of disease pathogenesis are still under investigation. An area that is currently subject of investigation is the role of microRNAs (miRNAs) in the development and progression of OA.

MiRNAs are naturally occurring, non-coding RNA molecules which post-transcriptionally regulate gene expression by binding to target mRNA molecules and preventing or repressing their translation. A single miRNA molecule is only 20–22 nucleotides in length, yet they are each capable of repressing the expression of multiple target genes[4,5]. Over recent years, studies have shed light on the wide variety of processes regulated by miRNAs, including

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cell growth, differentiation, apoptosis and extracellular matrix (ECM) regulation as well as hemostasis, bone metabolism and organ development[6–8]. On the other hand, abnormal expression of miRNAs is involved in a number of pathologies, such as cancers, cardiovascular disease and OA[2,9–12]. Multiple miRNAs-OA studies have been performed, but often suffered with small sample size, heterogeneous designs, and conflicting outcomes. Therefore, the role of miRNAs in OA is poorly described. Specifically, the objective of this study was to evaluate the current literature regarding the underlying function of miRNAs in OA.

Materials and methods

The study protocol was finalized in advance of any data collection, which defined objectives, search strategy, inclusion/exclusion criteria, data extraction, outcomes of interest, and analytical approaches. The present study complied with the Preferred Reporting Items for Systematic Reviews statement[13].

Search strategy

The databases used for the search were Ovid MEDLINE, Ovid EMBASE, PubMed, Cochrane Central Register of Controlled Trials (CCTR), Cochrane Database of Systematic Reviews (CDSR), ACP Journal Club, Database of Abstracts of Review of Effectiveness (DARE) and Scopus and Web of Science, from inception to October 1, 2016. A combination of controlled vocabulary and text words were used. MEDLINE uses a single term, MicroRNAs, but EMBASE and others uses the term MicroRNA, but includes more specific terms for individual miRNAs. In order to be as inclusive as possible, the search also included text words: mir, mirna*, microrna*. The same approach was used for OA: OA is used by MEDLINE, but EMBASE and others use arthritis, with more specific terms including cartilage or chondrocyte. There are similar differences for chondrolysis, remodeling of the subchondral bone vs degeneration of the cartilage. Text words were also used to be inclusive. The results were downloaded into EndNote (EndNote X7, Bld 7072, Thomson Research Soft, Stamford), and duplicates removed. The reference lists of all retrieved articles without language restrictions were reviewed for further identification of potentially relevant studies.

Selection criteria

All identified articles were assessed using the inclusion and exclusion criteria. Two investigators independently reviewed all titles and abstracts. To avoid outcomes distorted by language bias, we considered all articles without language restrictions, including papers in Mandarin. The full text of articles, which were considered potentially eligible based on abstract review, were then evaluated by two investigators. Disagreement about eligibility was resolved by discussion, or if necessary, by further discussion with another our co-authors. Eligible case–control studies for the present study included those which investigated miRNAs differential expression in cartilage tissues and cells of OA patients and/or control group participants. The miRNAs were identified through microarray analysis. Abstracts, case reports, conference presentations, editorials, and expert opinions were excluded. Two reviewers independently assessed the risk of bias of the trials. In a subsequent meeting, the reviewers tried to reach consensus on each criterion that they initially disagreed on.

Data extraction

Data was extracted from the text, tables, and figures of the included studies using a standardized datasheet. Expression profiles of miRNAs in cartilage tissues and cells of OA patients and/or control group participants were recorded, including the specific miRNAs, upregulation vs downregulation expression, the experimental models, and samples used. Experimentally verified miRNAs involving OA were also recorded, including miRNAs, their targets, functions, and sample used.

Applied bioinformatic analysis of miRNAs linked to IDD

We assessed which miRNAs are commonly elevated or decreased in the OA, including the cartilage tissues and cells from OA patients and/or control group participants using Venn diagrams (Venny; http://bioinfogp.cnb.csic.es/tools/venny/) and hierarchical clustering (GENE-E; https://software.broadinstitute.org/GENE-E/). Heatmap analysis was used to identify studies with common sets of IDD-related miRNAs and to investigate the relatedness of molecular findings on miRNAs in different studies. To facilitate comparisons, we generated matrix in which multiple studies and miRNAs were represented. Detection of a miRNA was assigned a value of 1 if the miRNA was detected, while a value of 0 was assigned for miRNAs that were not reported. Hierarchical clustering was also used to determine whether there are studies that detect the same set of miRNAs. Putative targets of different sets of miRNAs were determined using Target Scan (TargetScan Human Prediction of microRNA targets; http://www.targetscan.org/vert_71/). The relatedness of these gene targets in cellular networks was investigated by gene ontology (GO) analysis using DAVID (The Database for Annotation, Visualization and Integrated Discovery; https://david.ncifcrf.gov/), Functional Enrichment analysis tool (FunRich; http://www.funrich.org/), and String (http://string-db.org/).

Results

Study characteristics

For a comprehensive evaluation of the current literature on the disease-related roles of miRNAs in OA, we identified eligible studies by filtering for different inclusion and exclusion criteria (Fig. 1). After initial screening, 1456 references were removed that examined miRNAs but that did not focus on OA. Upon further evaluation of titles and abstracts, 452 additional references were omitted (Fig. 1). Of the remaining 65 candidate studies, we excluded eight more studies, because our assessment of the full text versions revealed that these studies were either reviews or unsuitable controls (Fig. 1). Finally, 57 studies were included in this study[14–70] (Tables I and II)

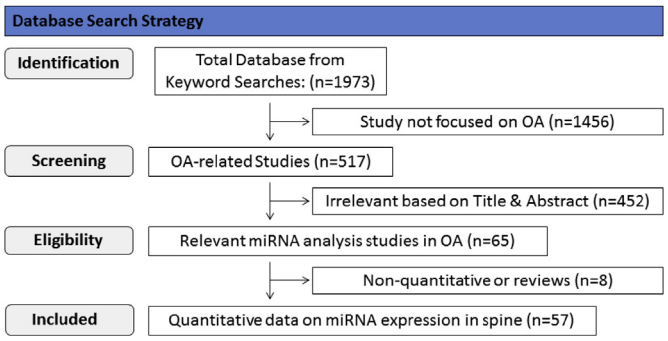

Fig. 1. Flow diagram of the search strategy. The figure shows the search strategy for identification of studies with quantitative miRNA expression data. All references obtained with selected keywords relevant to OA and miRNAs ($n = 1973$) were filtered using multiple selection criteria to obtain a final set of papers with a primary focus on OA that present quantitative miRNA expression data ($n = 57$).

Outcomes

A number of differentially expressed miRNAs have been experimentally verified as contributors to OA (Table I). As the body of research grows, the number over miRNAs implicated in OA has increased. Our current study has identified 46 differentially expressed miRNAs involved in autophagy, inflammation, chondrocyte apoptosis, chondrocyte differentiation and homeostasis, chondrocyte metabolism and degradation of ECM.

Our literature search identified a wide range of miRNAs that have been shown to be differentially expressed in OA (Table I). For the included studies, the implicated miRNAs were identified through microarray analysis of OA specimens, with comparison to control specimens such as healthy donors or trauma patients. Xu *et al.*[14] identified eight upregulated miRNAs and six downregulated miRNAs in OA synovial specimens, utilising non-inflamed synovial specimens as controls. More recently, Akhtar *et al.*[65] were able to identify a significant number of miRNAs both upregulated and down regulated in OA tissues compared to trauma patients, which a total of two miRNAs were upregulated, with 42 downregulated. Jones *et al.*[69] compared the expression of miRNA between OA patients and healthy donors. A total of 18 miRNAs were found to have a higher expression, whilst three had a lower expression. Furthermore, a number of both up and downregulated miRNAs have been identified through comparing OA patients to healthy donors or trauma patients[24,35,44,53,54,61,67,70]. These studies have for the first time shown overlapping expression profiles of miRNAs in different samples (Table II). With attempts to collate the data, significant problems arise. Several miRNAs are reported as upregulating or downregulating target genes in OA, however, none of the studies report miRNAs from the same class (Fig. 2).

To understand the relationship between the various studies we used hierarchical clustering to generate a heatmap that visualizes which studies are producing consistent results for a related set of miRNAs. For example, we compared the findings of 11 distinct studies that examined miRNAs. To facilitate comparisons, we performed heatmap analysis using a matrix in which all 11 studies and miRNAs were represented. It is at present unclear what the

Table I
Experimentally verified miRNAs involving IDD

References	miRNAs	Target gene(s)	Functions	
Song 2014[39]	miR-21	GAS5, GDF5	↓	Autophagy
Zhang 2015[20]	miR-146a	Bcl2	↑	Autophagy
Kurowska 2011[64]	miR-155	SHIP1	↑	Inflammation
Qi 2013[49]	miR-483	BMP7, TGFβ, IL-1β, MMP13	↑	Inflammation
Wang 2013[45]	miR-146a	IRAK1, TRAF6	↑	Inflammation
Park 2013[51]	miR-558	COX-2	↓	Inflammation
Li 2015[28]	miR-130a	TNF-α	↓	Inflammation
Santini 2014[40]	miR-149	TNFα, IL-1, IL-6	↓	Inflammation
Xie 2015[22]	miR-26a	NF-κB	↓	Inflammation
Akhtar 2012[63]	miR-199a	COX-2	↓	Inflammation
Zhang 2015[21]	miR-210	DR6 and NF-κB	↓	Inflammation, chondrocyte apoptosis
Makki 2015[27]	miR-139	MCPIP1	↑	Chondrocyte apoptosis
Song J2013[48]	miR-9	Protogenin	↓	Chondrocyte apoptosis
Li 2012[60]	miR-146a	VEGF, Smad4	↑	Chondrocyte apoptosis
Jin 2014[42]	miR-146a	VEGF, Smad4 and TGF-β	↑	Chondrocyte apoptosis
Bai 2015[34]	miR-195	HIF-1α	↑	Chondrocyte apoptosis
Abouheif 2010[66]	miR-34a	Col2α1, iNOS	↑	Chondrocyte apoptosis
Li 2012[59]	miR-223	NF1A, MCSFR	↑	Chondrocyte apoptosis
Philipot 2014[41]	miR-24	p16INK4a	↓	Chondrocyte differentiation and apoptosis
Zhong 2012[52]	miR-337	TGF-βR2	↓	Chondrocyte differentiation and homeostasis
Etich 2015[32]	miR-26a	Cd200, Col10a1, Col9a1 and Ctgf	↓	Modulate ECM homeostasis
Ham 2014[43]	miR-23b	PRKACB	↑	Chondrocyte differentiation and homeostasis
Swingler 2012[55]	miR-455-3p	TGF-β	↑	Cartilage homeostasis
Kostopoulou 2015[30]	miR-33a	ABCA1, ApoA1	↑	Chondrocyte metabolism
Steck 2012[56]	miR-675	Col2α1	↑	Chondrocyte metabolism
Seidl 2016[15]	miR-138	Sp1 and HIF-2α	↓	Chondrocyte differentiation
Zhang 2014[36]	miR-21	GDF5	↑	Chondrocyte differentiation and homeostasis
Martinez 2012[57]	miR-145	Sox9	↑	Chondrocyte differentiation and homeostasis
Dai 2012[62]	miR-101	Sox9	↑	Chondrocyte differentiation and homeostasis
Hou 2015[31]	miR-193b	TGF-β2, TGF-βR3, SOX9, Col2	↑	Chondrocyte differentiation and homeostasis
Li 2015[29]	miR-16-5p	Smad3	↑	Chondrocyte differentiation and homeostasis
Le 2016[18]	miR-29	Smad, NF-κB, WNT	↑	Chondrocyte differentiation and homeostasis
Ji 2016[19]	miR-105	Runx2, ADAMTS4, 5, 7, 12	↓	Chondrocyte differentiation and matrix degradation
Lu 2015[17]	miR-15a	ADAMTS5	↓	Matrix degradation
Miyaki 2009[68]	miR-140	ADAMTS5	↓	Matrix degradation
Vonk 2014[38]	miR-148a	MMP13, ADAMTS5, Col-X	↓	Matrix degradation
Liang 2012[58]	miR-140	ADAMTS5, MMP13, TIMP1, SP1	↓	Matrix degradation
Park S 2013[50]	miR-127-5p	MMP13	↓	Matrix degradation
Song 2013[46]	miR-181b	MMP13	↑	ECM degradation
Meng 2016[16]	miR-320	MMP13	↓	Matrix degradation
Wang 2015[23]	miR-411	MMP13	↓	Matrix degradation
Makki 2015[26]	miR-9	MMP13, MCPIP1	↑	Matrix degradation
Song 2013[47]	miR-488	MMP-13, ZIP8	↓	Matrix degradation
Song 2015[25]	miR-222	MMP13, HDAC4	↓	Cartilage degradation
Yang 2014[37]	miR-145	Smad3	↑	ECM degradation
Dai 2015[33]	miR-101	Sox9	↑	Cartilage degradation

Table II
Expression profiles of miRNAs in IDD tissues and cells

Reference	miRNAs	Experimental models and Sample size
Xu 2016[14] Up	miR-125b, miR-21, miR-155, miR-421, miR-106p, miR-146, miR-34c, miR-577	Five OA synovial specimens vs four non-inflamed synovial specimens
Xu 2016[14] Down	miR-221-3p, miR-940, miR-25, miR-127, miR-100, miR-222	
Ukai 2012[54]Up	miR199a-3p, miR-193b	17 OA patients vs three ACL injury patients
Ukai 2012[54] Down	miR-320c	
Diaz 2012[61] Up	miR-483-5p	Six OA patients vs four healthy donors
Diaz 2012[61] Down	miR-149*, miR-582-3p, miR-1227, miR-634, miR-576-5p, miR-641	
Akhtar 2010[65] Up	miR-491-3p, miR-146	20 OA patients vs three trauma patients
Akhtar 2010[65] Down	miR-187, miR-219-5p, miR-127-5p, miR-518-3p, miR-520d-3p, miR-550, miR-544, miR-502-5p, miR-638, miR-643, miR-603, miR-602, miR-563, miR-558, miR-575, miR-181c, miR-637, miR-564, miR-337-5p, miR-298, miR-622, miR-659, miR-208, miR-142-5p, miR-29b, miR-144, miR-19b, miR-32, miR-141, miR-27b, miR-302a, miR-423-3p, miR-503, miR-130b, miR-34a, miR-33a, miR-301b, miR-138, miR-497, miR-545, miR-372, miR-610	
Jones 2009[69] Up	miR-9, miR-25, miR-98, miR-211, miR-200a, miR-299, miR-34b, miR-104, miR-105, miR-122a, miR-135a, miR-135b, miR-139, miR-144, miR-147, miR-148, miR-149, miR-302d	Three OA patients vs four healthy donors
Jones 2009[69] Down	miR-107, miR-146, miR-149,	
Iliopoulos 2008[70] Up	miR-483, miR-22, miR-377, miR-103, miR-16, miR-223, miR-30b, miR-23b, miR-509	33 OA patients vs 10 healthy donors
Iliopoulos 2008[70] Down	miR-29a, miR-140, miR-25, miR-337, miR-210, miR-26a, miR-373	
Zhang 2012[53] Up	miR-193b, miR-199a-3p, miR-199b-3p, miR-455-3p, miR-210, miR-381, miR-92a, miR-320c, miR-136	N/A
Zhang 2012[53] Down	miR-490-5p, miR-4287, miR-miR-BART8, miR-US25-1	
Borgonio 2014[44] Up	miR-16, miR-20b, miR-29c, miR-30b, miR-93, miR-126, miR-146a, miR-184, miR-186, miR-195, miR-345, miR-885-5p	27 OA patients vs 27 healthy donors
Akhtar 2015[35] Up	miR-602, miR-608	46 OA patients
Song 2015[24] Down	miR-370, miR-373	N/A
Tardif 2009[67] Down	miR-140, miR-27a	N/A

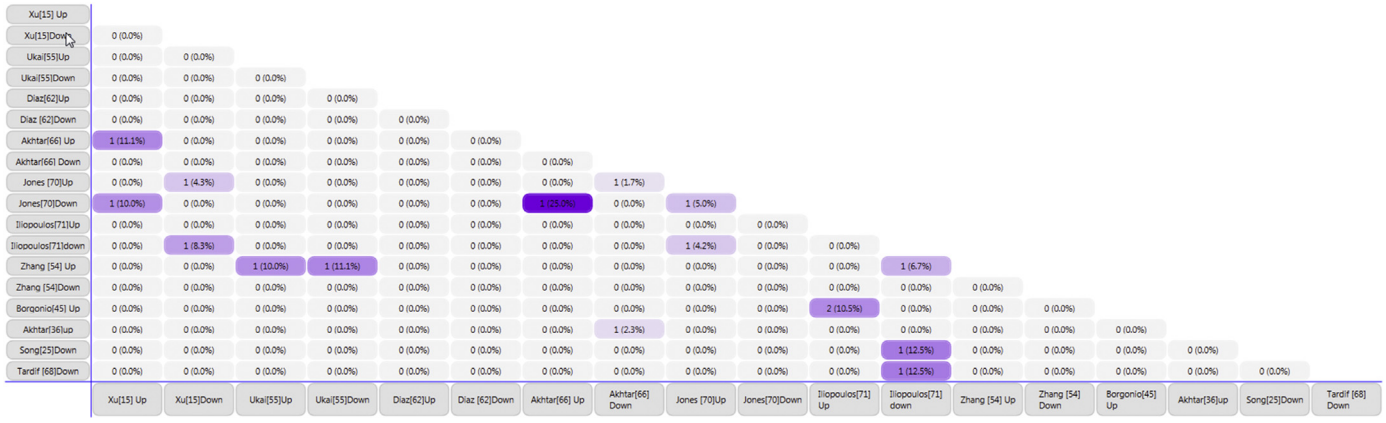

Fig. 2. Tabular Venn diagram analysis. Cross-tables show the number and percentage of miRNAs that are in common among studies examining miRNA expression by microarray analysis. The number of miRNAs in common to each of the groups is rather.

biological and clinical distinctions are in the two groups of studies (Up & Down). However, considering the robustness of miRNAs as biomarkers, it is clear that a re-evaluation of the medical records of the two groups in these studies is warranted. We asserted that groups of miRNAs that are coordinately modulated with the disease state (either up- or down-regulated) have common functions. Therefore, we assessed the predicted targets of each set of miRNAs using TargetScan and tabulated the number of mRNAs that match to a specific miRNA. The resulting table was used for hierarchical clustering analysis to define which mRNA targets are in common with different sets of miRNAs.

Preliminary Venny analysis by TargetScan and FunRich tools shows common targets of “Up miRNAs (miR-146, miR-193b, miR-16 and miR-30b)” and “Down miRNAs (miR-373, miR-25, miR-149 and miR-140)” (Fig. 3). Strikingly, heatmap analysis showed several classes of miRNAs that derived from the 11 studies (Fig. 4). We next performed GO analysis to understand which biological programs are controlled by coordinately regulated miRNAs with common targets. Therefore, we selected subsets of mRNAs that were targeted by three or more miRNAs and defined the function of the corresponding target genes using several GO analysis tools, including DAVID, FunRich and String. GO terms for groups of common target genes and their putative functions were visualized using bar graphs (Fig. 5). Remarkably, our findings indicate that the putative of function of up-regulated miRNAs is to target primarily nucleus, while down-regulated miRNAs primarily target transcription factors (TFs) (Fig. 5). These results suggest that two biologically synergic groups of miRNAs target the same molecular mechanisms to affect OA.

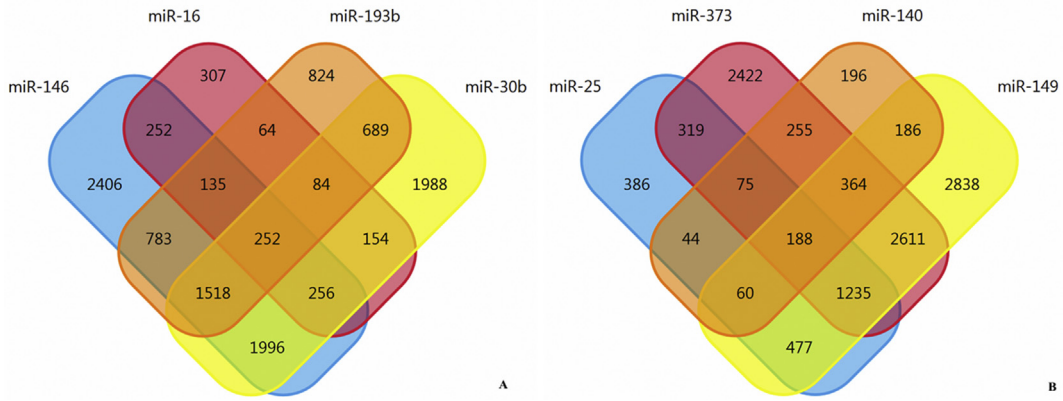

Fig. 3. Tabular Venn diagram analysis. The cross-table in Panel A is based on all miRNAs that are identified in two or more studies depicted in Fig. 2, and shows the similarities in predicted target genes of up-regulated miRNAs. Panel B shows the same as Panel A for putative target genes of down-regulated miRNAs identified in Fig. 2 as having been observed in two or more studies.

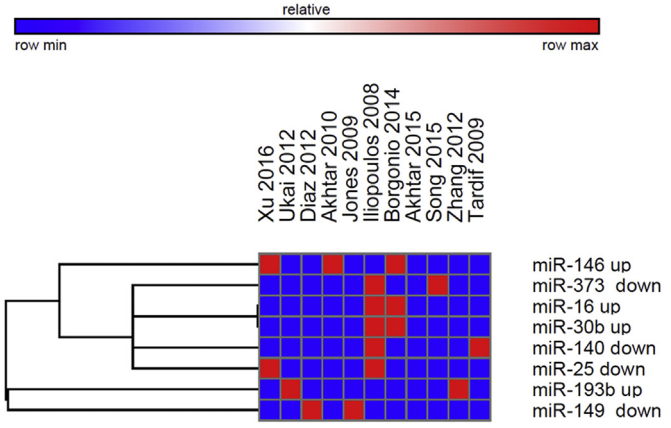

Fig. 4. Identification of common miRNAs and their targets in OA. The figure shows heatmap analyses of the relatedness between studies examining miRNA expression, miRNAs identified as differentially expressed in OA, and the predicted gene targets of these miRNAs.

Discussion

MiRNAs and chondrocyte autophagy

Autophagy is a conserved lysosomal degradation process essential for cell physiology and human health. Recent evidence has indicated that autophagy may plays the important role in the pathogenesis of OA[71–73]. In addition, enhanced autophagy correlates with decreased apoptosis in arthritis. Several miRNAs target molecules that are involved in autophagy. Song *et al.*'s[39] study showed that the expression level of miR-21 was significantly reduced in OA patients, and the ectopic expression of GAS5 is capable of suppressing miR-21 induction. Human GAS5 is up-regulated in OA patients and that this up-regulated GAS5 is involved in autophagy by the indirect regulation of miR-21. Furthermore, in Zhang *et al.*'s[20] study, they have shown that miR-146a was induced during hypoxia by HIF-1α, and miR-146a promoted autophagy by decreasing Bcl-2 expression, an autophagy inhibitor.

MiRNAs and inflammation

Inflammation is a crucial aspect of OA, triggered in part by the products of ECM breakdown. As described earlier, miR-155 and miR-483 upregulation may play a role in promoting an inflammatory response[49,64]. Wang *et al.*[45] suggested that MiR-146a exerts negative control on inflammatory responses by suppressing cytokine-induced expression of interleukin-1 receptor-associated kinase-1 (IRAK1) and tumor necrosis factor receptor-associated factor 6 (TRAF6) by impairing nuclear factor-kappa B (NF-κB) activity and inhibiting the expression of target genes. Furthermore, it was also found that miR-558 functions as a negative regulator of IL-1b-mediated catabolic responses by repressing COX-2 expression and catabolic signaling pathways in human chondrocytes[51]. Similar to the miR-149, is down-regulated in OA chondrocytes, and this decrease seems to be correlated to increased expression of pro-inflammatory cytokines such as tumor necrosis factor-α (TNF-α), interleukin 1 beta (IL-1β) and IL-6[40]. Additionally, miR-130a[28], miR-26a[22] and miR-199a[63] are downregulated in inflammation of OA, both of which target TNF-α, NF-κB and COX-2, respectively. These miRNAs may be the important regulator of human cartilage inflammation and a new target for OA therapy.

MiRNAs and chondrocyte apoptosis

Previous studies have shown that chondrocyte apoptosis is stimulated in aging and OA[74,75]. Several miRNAs target apoptotic genes in chondrocytes. Zhang *et al.*[21] found that miR-210 can target 3′-UTR of DR6 to inhibit its expression. MiR-210 mimic and DR6 siRNA inhibit the activation of NF-κB and cell apoptosis of chondrocytes. However, Song *et al.*[48] suggested reduction of miR-9 induction, which results in increased PRTG levels in OA pathogenesis, may be responsible for chondrocyte apoptosis. MiR-146a is involved in human chondrocyte apoptosis in response to mechanical injury, and may contribute to the mechanical injury sustained by chondrocytes and the pathogenesis of OA by increasing the levels of VEGF and damaging the transforming growth factor beta (TGF-β) signaling pathway through the targeted inhibition of Smad4 in human chondrocytes[42,60]. Philipot *et al.*[41] identified miR-24 as a negative regulator of p16INK4a. Accordingly, p16INK4a expression increased while miR-24 level was repressed upon IL-1β addition, in OA cartilage and during *in vitro* terminal chondrogenesis. Then they disclosed that deregulation of miR-24 has been associated with chondrocyte apoptosis. Furthermore, upregulation of a number of miRNAs are also associated with chondrocyte apoptosis in OA. These miRNAs include miR-139[27], miR-195[34], miRNA-34a[66] and miR-223[59], both of which target MCPIP1, HIF-1α, Col2α1&iNOS and NF1A&MCSFR, respectively.

MiRNAs and ECM homeostasis

Degradation of the ECM is a crucial process in the degeneration of cartilage. Generation and degradation of the ECM are constantly

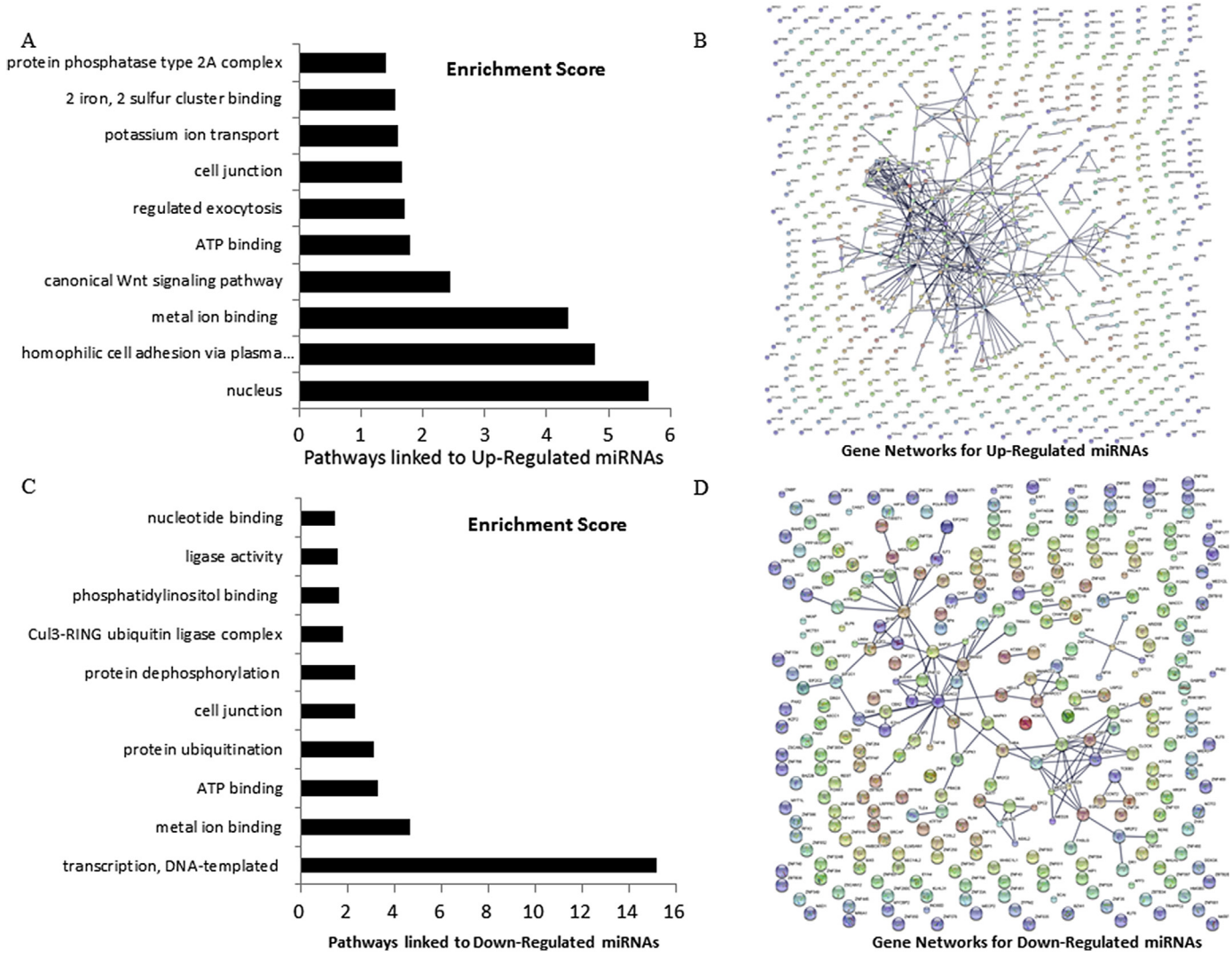

Fig. 5. Common targets of OA related miRNAs are linked to distinct biological processes. Different classes of common miRNAs associated with OA collectively target distinct gene networks. Based on the principle that coordinately regulated miRNAs together control a common biological process, we examined GO terms for groups of common target genes and their putative functions. Groups of genes that have similar GO terms (i.e., functional annotation clusters) were rank ordered based on their enrichment scores (DAVID analysis) to reveal which pathways are controlled by up-regulated miRNAs (Panel A) have principal gene targets that are generally associated with 'Nucleus'. In addition, miRNAs that are down-regulated (Panel C) have principal gene targets that are generally associated with 'Transcription'. We analyzed the major functions of the gene targets of common miRNAs using network analysis (String) that connects genes with similar molecular, cellular or biological properties. Networks formed by the gene targets of miRNAs that are either up-regulated (Panels B) or down-regulated (Panels D). It is evident from the analyses that the larger the group of targets for a given subset of miRNAs, the larger the network that can be formed. More importantly, the figure shows extensive predicted gene networks for each miRNA subset, consistent with the concept that miRNAs control biological processes by targeting multiple related genes.

occurring and are in a state of equilibrium in a healthy cartilage. Etich *et al.*[32] found that miR-26a directly inhibits the expression of PH/H marker genes Col10α1 and Cd200 and of the ECM adaptor gene Col9α1 in PECs, which cause a significant reduction in matrilin-3, COMP and proteolytic fragments of CTGF to destabilize the chondrocyte matrix. A number of miRNAs that are upregulated in OA Chondrocyte differentiation and homeostasis are also important regulators of Chondrocyte metabolism. These miRNAs include miRNA-337[52], miRNA-23b[43], miRNA-455-3p[55], miRNA-33a[30], miRNA-675[56], miR-138[15] and miR-21[36], both of which target TGF-βR2, PRKACB, TGF-β, ABCA1&ApoA1, Col2α1, Sp1& HIF-2α and GDF5, respectively. Through the target gene Sox9, miR-101[62], miR-145[57] and miR-101[33] are involved in down-regulation of collagen type II and aggrecan, and their inhibition can prevent chondrocyte ECM degradation. Furthermore, it is also important to recall here that the TGF-β2 and TGF-βR3 pathway is suppressed by over-expression of miR-193b[31]. The data from the Li *et al.*'s[29] and Yang *et al.*'s[37] study indicated that miR-16-5p and miR-145 are important regulator of SMAD3 expression in human chondrocytes and may contribute to the development of OA. Similar to the miR-29[18] negatively regulated Smad, NF-κB, and canonical WNT signaling pathways in OA. Many matrix metalloproteinases (MMPs) and metalloprotease with thrombospondin motifs (ADAMTSs) are highly expressed in OA and are proportional to the grade of degeneration. And expectantly, many miRNAs are involved in the regulation of MMPs and ADAMTSs within cartilage[16,17,19,23,25,26,38,46,47,50,58,68].

Bioinformatic analysis of quantitative expression data for OA-related miRNAs

A variety of miRNAs have been identified as over- or under-expressed in OA compared to controls. While investigating the role of miRNAs in OA, Venny diagram analysis of studies reported

before 2016 showed that none of these previous studies identified the same class of miRNAs. However, more recently published studies have yielded better convergence of the data, possibly due to improvements in detection methods for miRNAs.

Because we detected two groups of miRNAs (Fig. 5), it is of interest to know whether these miRNAs have similar predicted targets. Therefore, we used miRNA target prediction applications (e.g., Target Scan) and tabulated all predicted mRNAs for all miRNAs that were detected in each study. This analysis reveals that four miRNAs that are reported as up-regulated in OA in these studies (i.e., miR-146, miR-193, miR-16 and miR-30b) have many targets in common (Fig. 3). Similarly, four down-regulated miRNAs observed in these studies (i.e., miR-373, miR-25, miR-149 and miR-140) also have a shared set of targets (Fig. 4). Taken together, it appears that groups of miRNAs are coordinately up- or down-regulated in OA specimens to suppress a common set of genes. Bioinformatics analysis that compares GO terms was used to evaluate whether the common targets of representative coordinately regulated miRNA sets are components of larger biological programs and regulatory networks. This analysis resulted in the striking finding that shared miRNAs detected in Up-regulated group primarily target nuclear proteins, the majority of which are known TFs or other proteins supporting transcription. Furthermore, the miRNAs detected in Down-regulated group are also associated with TFs. The cooperation between the respective targets and broader biological programs controlled by distinct common sets of mRNAs indicates that OA may be guided by effects of proteins required for gene expression.

The observation that TFs are targeted by OA-related miRNA, which are either up- or down-regulated, is consistent with a relatively simple molecular framework for miRNA-mediated spine degeneration. This model clarifies that all miRNAs can only suppress their cognate TFs, and that miRNAs, which are derived from OA specimens, are modulated to provoke progression of OA, regardless as to whether miRNAs are up- or down-regulated. Stimulation of any process by miRNAs can be achieved by a 'double-negative principle'[76]. For example, up regulated miR-146, miR-193, miR-16 and miR-30b could higher expression of TFs or other positive proteins and down-regulate expression of negative proteins, and then lead to high gene expression which may be protective of the cartilage material. Furthermore, down-regulated miR-373, miR-25, miR-149 and miR-140 could lower expression of positive TFs, and then lead to cartilage degenerative gene over-expression. Additionally, down regulated these miRNAs could up-regulate expression of negative TFs, and then lead to cartilage protective gene under-expression. While the exact mechanisms remain to be determined, because the results from multiple studies we evaluated report miRNAs that may directly affect TFs, it is clear that perturbations in miRNA-TF regulatory networks may lead to broad ranging pleiotropic effects during successive stages of OA, not unlike a molecular chain reaction (i.e., positive feed-forward loops). The possibility arises that TFs targeted by the miRNAs function in the same regulatory network to control biological processes associated with OA.

The development of OA remains largely enigmatic as it is multi-factorial, with a range of processes compromised. However, it is well established that a large number of miRNAs pathways are implicated. These over and under-expressed miRNAs control transcription, ion binding and ATP binding kinase-related signaling pathways. In addition they can lead to autophagy, inflammation, chondrocyte apoptosis, chondrocyte differentiation and degradation of ECM. OA-miRNAs screening is an innovative approach to OA prevention and treatment that takes into account individual differences in people's genes. It gives clinicians tools to better understand the complex mechanisms underlying a patient's health, OA disease, or condition, and to better predict which treatments will be most effective. What is more, given that the costs and disability associated with symptomatic OA is high, early diagnosis or primary prevention of OA seems more important than complex clinical treatments. The OA-miRNAs screening not indicate who will acquire OA, but rather it indicates the possibility of an individual developing OA easier than would otherwise have occurred. The risk miRNAs assessment of OA applied pronounced clinical implications. Our study comprehensive reviewed the relationship between miRNAs and OA, and it can be used to screen individuals at risk, and these patients can be advised on how their lifestyle may impact potential OA. Moreover, it will give a further clinical direction on which patients will benefit from surgical interventions. It may provide the opportunity for OA patients to assess their outcomes from the interventions. A linkage will also necessitate that donors of allogenic tissue or cells used for cartilage repair be screened for OA-miRNAs, as there seems to be little point in repairing a lesion with a tissue that is itself at risk for OA. A variety of possible miRNA therapeutic targets have been identified by the existing research. However, the therapeutic benefits of such treatments have been largely examined *in vitro*, with a distinct lack of animal studies. These are required to determine the safety and efficacy of such drugs. Therefore, there remains a large body of work to develop miRNAs therapies for OA and provide an alternative to conservative or surgical techniques.

Advantages and limitations of this study

The major strength of performing this study is that we conducted a comprehensive search of multiple databases, selected and appraised studies by independent pairs of reviewers, and followed a priori planned protocol that included several hypotheses for the role of miRNAs in OA. Our study suffers several important limitations. First, unpublished researches with negative results cannot be identified. Therefore, publication bias may exist, which could result in the overestimation of the role of miRNAs in OA. Second, other biological processes which have not yet been studied, but may also be affected by miRNAs in OA. Therefore, our results provide justification for further evaluation about the role of miRNAs in OA.

Conclusion

In this study, we summarized roles of miRNAs in OA. While there has been a much progress over the past decade, there remain a number of challenges. Currently, only 46 miRNAs have been reported to be involved in the pathology of OA, even though around 100 miRNAs have been shown to be differentially expressed. Comprehensive analysis of all miRNAs studies reveals cooperation in miRNA signatures and suggests that there may be two biologically synergic classes of miRNAs that are associated with OA. One set of studies converges on unique miRNA signatures that are involved in regulation of TFs that directly control gene expression, while the other studies indicate different miRNAs that control nucleus functions. These results provide clarity among papers on miRNA expression in OA. Our analysis of the current data suggests that miRNAs may be useful as diagnostic biomarkers in discriminating different molecular manifestations of OA. There are also a range of degenerative pathways regulated by miRNAs which may provide therapeutic targets in OA. Furthermore, a better understanding of the targets of these miRNAs will accelerate biomedical discoveries and improve clinical care based on new knowledge of OA-related disease mechanisms.

Author contributions

- Conception and design: LC
- Analysis and interpretation of the data: LC, YZ
- Drafting of the article: LC, GJT
- Critical revision of the article for important intellectual content: All authors
- Final approval of the article: All authors
- Statistical expertise: LC, GJT
- Obtaining of funding: LC
- Collection and assembly of data: LC, YZ

Competing interest statement

There are no conflicts of interest.

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The example of the Pubmed search strategy

Identification

1. microRNAs and OA. Search results: 142.
2. miRNA and OA. Search results: 169.
3. mir* and OA. Search results: 146.
4. microRNAs and osteoarthritis. Search results: 124.
5. miRNA and osteoarthritis. Search results: 192.
6. mir* and osteoarthritis. Search results: 137.
7. microRNAs and chondrocyte. Search results: 170.
8. miRNA and chondrocyte. Search results: 140.
9. mir* and chondrocyte. Search results: 130.
10. microRNAs and cartilage. Search results: 197.
11. miRNA and cartilage. Search results: 194.
12. mir* and cartilage. Search results: 185.
13. microRNAs and chondrolysis. Search results: 1.
14. miRNA and chondrolysis. Search results: 1.
15. mir* and chondrolysis. Search results: 2.

Pubmed Total: 1930

Screening

After initial screening, 1413 references were removed that examined miRNAs but that did not focus on OA. Upon further evaluation of titles and abstracts, 452 additional references were omitted. Of the remaining 65 candidate studies, we build up an endnote database to assess the full text versions. The remaining 65 candidate studies were selected for comprehensive evaluation. We excluded eight more studies after evaluating the full text versions of the remaining articles due to unsuitable controls and replication of data. Finally, 57 studies were selected from Pubmed.

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