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MicroRNAs in Intervertebral Disc Degeneration: A Comprehensive Literature Review and In-Silico Analysis of Therapeutic Potential

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Research Article

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ABSTRACT

MicroRNAs (miRNAs), which are non-coding RNAs that range from 19 to 23 nucleotides in length, play a crucial role in various biological processes and diseases. Intervertebral disc degeneration, frequently leading to low back pain, is linked to such factors as mechanical stress, aging, and inflammation. MicroRNAs are involved in numerous biological processes related to the pathogenesis of IVDD by regulating gene expression in cells. The potential of miRNAs in IVDD is crucial for both diagnosis and treatment. Identifying miRNAs associated with IVDD may assist developing future therapeutic strategies. In this study, in-silico approaches were employed to investigate miRNAs that regulate the expression of genes involved in intervertebral disc degeneration, leading to the identification of potential biomarker miRNAs. For the analysis, differentially expressed mRNAs and miRNAs were examined using datasets GSE167199 and GSE186542, as well as GSE63492 and GSE19943 from the NCBI GEO Datasets database. Enrichment analyses were conducted for all genes and miRNAs, and the results were visualized. Potential gene targets and binding sites for miRNAs were analyzed through bioinformatics databases. The findings indicate that the *RAB11FIP4* gene has a significant interaction with miR-486-5p, with miRNA expression decreasing in degenerated cells, and RAB11FIP4 expression increasing in degenerated nucleus pulposus cells. The results of the in-silico analyses suggest that miR-486-5p may serve as a marker miRNA in intervertebral disc degeneration, and targeting this miRNA also promises success in reversing the degeneration process.

1. Introduction

MicroRNAs, which are short RNA molecules, bind to the 3'-UTR regions of target mRNAs and facilitate biological processes associated with the inhibition of gene expression. Research has shown that miRNAs act as epigenetic regulators in essential biological events such as differentiation, cellular development, apoptosis, and homeostasis. Consequently, miRNAs hold significant importance for both fundamental scientific research and clinical applications.

Intervertebral disc degeneration (IVDD) is a prevalent condition affecting millions globally, diminishing quality of life and imposing a considerable burden on healthcare systems. The molecular pathogenesis of IVDD is highly intricate, and the role of gene expression regulators, particularly miRNAs, in this process is gaining increasing interest. It has been proposed that miRNAs may serve as vital biomarkers and therapeutic targets for diagnostic and treatment strategies by regulating biological processes associated with IVDD [1].

This study aims to explore the effects of miRNAs on IVDD and to identify potential biomarker miRNAs through bioinformatic analyses. The findings of the study may help in developing miRNA-based therapeutic strategies by offering new insights into the molecular mechanisms of IVDD.

1.1. MicroRNA

MicroRNAs, which are short RNA molecules, bind to the 3'-UTR regions of target mRNAs and facilitate biological processes associated with the inhibition of gene expression and mRNA degradation. Discovered in the 1990s through studies on C. elegans, miRNAs constitute only a small fraction of the mammalian genome but they play a crucial role in gene regulation [2]. These molecules are involved in the regulation of fundamental biological processes making them highly significant for both basic scientific research and clinical applications [3,4,5].

1.1.1. MicroRNA Biogenesis

The formation of miRNA occurs in multiple stages, beginning in the nucleus and concluding in the cytoplasm. In the nucleus, a 3-4 kb long, 5' cap and polyA-containing primary microRNA (pri-miRNA) is synthesized by RNA polymerase II enzymes from miRNA genes. This pri-miRNA is then converted into pre-miRNA, featuring a monophosphate at the 5' end and a hydroxyl extension at the 3' end, by Drosha, which acts as a catalytic unit by cleaving the molecule within the double-stranded hairpin structure of the RNA III member and the DGCR8 protein complex that recognizes the RNA structure. The pre-miRNAs are shielded from degradation by exportin-5

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and transported to the cytoplasm. Following transport, mature miRNA is produced with the RNase III enzyme Dicer, and the duplex miRNA is unwound to expose the complementary strand. While one strand is degraded, the other strand associates with a ribonucleoprotein complex comprising RNA binding protein, PKR activating protein, and Argonaute-2 proteins, which are responsible for transactivation to form the RISC complex [5]. The miRISC complex binds to the 3' UTR region of the target mRNA and induces post-transcriptional changes [7,8].

It has been also found that miRNAs can be produced independently of Drosha/DGCR8. This pathway involves the generation of miRNAs from the intron of a protein-coding gene via the pre-mRNA splicing machinery, known as miRtrons. The expression of these miRNAs correlates with host gene expression, depending on their location within introns or splice site junctions. MiRtrons are continuously transported into the cytoplasm and processed by Dicer [9]. MicroRNAs can be tissue-specific and are present in body fluids, allowing them to migrate to different parts of the body and communicate between cells. Extracellularly secreted miRNAs can be packaged into exosomes, loaded into high-density lipoproteins, or bound by Ago2 to ensure their stability and protection from RNAses in body fluids. Approximately 90% of circulating miRNAs are known to associate with proteins such as NPM1 (nucleophosmin 1), Ago2, and high-density lipoprotein. Encased in exosomes, miRNAs can be recognized through endocytic uptake or receptors on the cell surface, allowing them to enter neighboring cells and influence mRNA targets outside their original tissue. In this manner, they can act as ligands related to the immune system by impacting both intracellular and extracellular targets [10].

1.1.2. Potential Role of miRNAs in Diagnosis and Prognosis of Diseases

MicroRNAs are regarded as significant biomarkers for various diseases in body fluids and tissues due to their ease of detection, sensitivity to disease, tissue specificity, and stability. For instance, miRNA-122-5p is highly expressed in the liver, whereas miRNA-140 serves as a selective miRNA for cartilage [11]. These characteristic positions miRNAs as crucial biomarkers for identifying the onset and progression of diseases. With these advantages, miRNAs play a vital role in accurate diagnosis, monitoring disease prognosis, guiding treatment, and evaluating response to therapy [12].

Two fundamental strategies are employed in the application of microRNAs for disease treatment. The first involves replacing a miRNA with reduced expression using a synthetic oligonucleotide, similar to healthy controls. The second strategy is miRNA inhibition therapy, where the overexpression of a miRNA with elevated levels is suppressed by antagonists [13,14].

1.2. Intervertebral Disc Degeneration

Intervertebral disc degeneration is recognized as a global health issue, placing a significant burden on healthcare systems due to an aging population; it is also the primary cause of low back pain. Intervertebral disc tissue comprises the nucleus pulposus (NP), annulus fibrosus (AF), and end plates that connect to the upper and lower vertebrae. AF and NP are two essential cell types that work together within the intervertebral disc tissue to alleviate pressure on the spine and support this load [15]. The pathogenesis of IVDD is intricate, with numerous factors that can accelerate degeneration, particularly genetics, age, occupational influences, and unhealthy lifestyle choices such as smoking and alcohol consumption. Currently, there is no effective treatment strategy to reverse IVDD, aside from non-surgical and symptomatic relief. Extensive research in recent years has underscored the significant role of degeneration-related genes in the IVDD process. During IVDD, the intervertebral disc experiences various molecular and biochemical changes. Some of these changes include the conversion of type II collagen (*COL II*) to type I collagen (*COL I*), a reduction in proteoglycan content, and alterations in matrix composition leading to a decrease in NP cells [16]. As these conditions progress, structural damage occurs in AF and NP tissues. Other intracellular events, such as apoptosis, increased autophagy, the release of inflammatory factors, and the degradation of the extracellular matrix (ECM) also contribute to the degenerative process. Recent studies indicate that miRNAs regulating gene expression may play a role in the development of intervertebral disc degeneration, similar to many other diseases, and targeting these miRNAs could be crucial for treatment and diagnosis [15,16].

1.2.1. miRNAs Involved in IVDD

Studies have shown that miRNAs are differentially expressed in intervertebral disc degeneration, indicating that miRNAs may play a role in the development of IVDD [15, 16]. NP cells are responsible for forming the extracellular matrix in the intervertebral disc, and the ECM structure is disrupted due to the apoptosis and necrosis of these cells. Consequently, the formation of the extracellular matrix leads to decreased water retention in the disc tissue, impairing its supportive function. While normal apoptosis is crucial for maintaining homeostasis, excessive apoptosis reduces the density of NP cells, disrupts disc function, and contributes to the degeneration process [17]. Identifying microRNAs that are significant in the degeneration process and determining the processes they influence are regarded as vital strategies to reverse IVD degeneration. In the healthy tissue, NP cells are concentrated in specific regions and present in smaller numbers, whereas in the early stages of degeneration, abnormal proliferation and clustering of NP cells occur. This clustering is particularly evident in damaged areas. Studies have also indicated that miR-185, miR-4478, miR-142-3p, miR-20a, miR-221, miR-106a-5p, miR-623, miR-532, miR-200c, and miR-660 may be linked to IVDD apoptosis.

The excessive release of inflammatory factors plays a critical role in the IVDD process. Overexpression of inflammatory factors disrupts the ECM structure and accelerates degeneration. Interleukin (*IL*), tumor necrosis factor (*TNF*), prostaglandin E2 (*PGE2*), and nitric oxide are the primary factors involved in the inflammatory response within intervertebral disc tissue. Studies indicate that miRNAs are significant in all these processes and possess therapeutic potential. Studies have also indicated that miR-185, miR-4478, miR-142-3p, miR-20a, miR-221, miR-106a-5p, miR-623, miR-532, miR-200c, and miR-660 may be linked to IVDD apoptosis [18].

ECM is an environment composed of collagen, non-collagen elastic fibers, proteoglycans, glycoproteins, and water that supports cell growth and development. The primary components of the ECM are *COL II* and glycoproteins, which, along with water, provide mechanical support for the vertebrae of the IVD tissue. A reduction in collagen and proteoglycans within the IVD accelerates the onset of degeneration. Matrix metalloproteinases (*MMPs*) are zinc-dependent proteolytic enzymes that can degrade extracellular matrix components. MMPs and a disintegrin and metalloproteinase with a thrombospondin motif (*ADAMTSs*) are directly involved in degeneration processes by breaking down proteoglycans and collagens [19,20]. MiR-93, miR-377, miR-127-5p, and miR-1260b are implicated in ECM degradation [21,22]. MiR-100 is one of the miRNAs that facilitate ECM degradation [23].

2. Material and Method

2.1. Identification of Target Genes

In-silico studies have been performed in order to identify marker genes and microRNAs predicted to target these genes for the treatment of intervertebral disc degeneration.

Within the scope of this study, differentially expressed mRNAs and miRNAs between human nucleus pulposus cells and degenerated human nucleus pulposus cells, designated as control, were identified using the NCBI Geo Datasets database with the keyword "intervertebral disc degeneration." The GSE167199 and GSE186542 datasets were utilized to analyze the differentially expressed genes from human intervertebral disc tissue, categorized by control and patient groups. These datasets were examined using the GEO2R analysis tool from the NCBI database, revealing differentially expressed mRNAs in both healthy and degenerated nucleus pulposus cells. mRNAs selected for further analysis were based on LogFC and P values. The analysis continued with the top 250 mRNAs ($P<0.05$).

2.2. Identification of Target miRNAs

Differentially expressed miRNAs between control and degenerated nucleus pulposus cells were identified using the NCBI Geo Datasets database, specifically the GSE63492 and GSE19943 datasets. They were subsequently analyzed with the GEO2R NCBI analysis tool. MiRNAs were selected based on LogFC and P values, and the analysis continued with the top 250 miRNAs.

2.3. Identification of miRNA Targets

For the analyzed miRNAs, the Targetscan and Miranda databases were utilized to identify potential target genes. The effort here is to determine whether the mRNAs predicted which are expected to be targeted by the identified miRNAs are among those with differential expression in the previous datasets.

2.4. Enrichment Analysis of Identified Genes and miRNAs

In order to acquire a clearer understanding of the biological processes that potential targets of Mir-486-5p may be linked to within the cell, Gene Ontology and KEGG enrichment analyses were conducted using the David database and visualized through the ShinyGo database.

2.5. Determination of Protein Interaction Map

After identifying the potential target of the miRNA recognized as a biomarker, the interaction map of proteins which can engage and contribute to the degeneration process was established using the String database. In this context, the *RAB11FIP4* protein can interact with numerous essential pathways in the cell and play a role in the processes of intervertebral disc degeneration.

2.6. Statistical Analyses

Statistical analysis was performed with the NCBI GEO2R analysis tool. Benjamini & Hochberg (False discovery rate), $P<0.05$ was considered statistically significant. The genes to be analyzed in all datasets were selected in this way.

3. Results

3.1. Identification of Target mRNAs and miRNAs

Among the top 250 genes identified across all datasets analyzed by considering the LogFC value and P values, 12 mRNAs which are commonly different in both datasets, were identified. These genes include *L1CAM, SFRP2, MGAM, KCNK2, RAB11FIP4, CCN3, COL2A1, PCSK2, ANK1, CCL2, ABCA13,* and *DKK1*. The genes with expression changes common to the two separate datasets are illustrated in Fig. 1-2 using a Venn diagram, whereas the differentiated genes are represented in the heatmap.

After analyzing the GSE63492 and GSE19943 datasets, the Top 250 miRNAs with the most significant expression changes were selected based on the LogFC and P values, and the miRNAs with the most differential expression common to the two different miRNA datasets were determined. These miRNAs are ebv-miR-BART6-3p, hsa-miR-508-5p, hsa-miR-486-5p, and hsa-miR-518b. The miRNAs common to the two datasets are displayed in the Venn diagram, and the differentiated miRNAs are shown in the heatmap in Fig. 1-2.

3.2. Identification of miRNA Targets

Analysis using the TargetScan and Miranda databases revealed that mir-508-5p and mir-518b do not share a common target among dataset-compatible mRNAs, while mir-486-5p can target two genes common to the datasets. These common genes are *RAB11FIP4* and *ANK1*. The *ANK1* gene was excluded from further analysis due to differing gene expressions across the datasets examined. Consequently, the *RAB11FIP4* gene was identified as a potential target of mir-486-5p, and it was noted that its expression significantly increased in patients across all databases. Fig. 1-2 illustrates the miRNA targets and the biological processes associated with these targets in the cell.

3.3. Determination of Protein Interaction

In-silico analysis indicated that the *RAB11FIP4* gene may play a role in the degeneration process by being targeted by mir-486-5p. However, to ascertain which pathways and genes the *RAB11FIP4* gene interacts with in the cell and the pathways it influences, a protein interaction map was created. This map suggests that the *RAB11FIP4* gene may be involved in significant biological processes such as inflammation, abnormal ECM proliferation, and apoptosis, which contribute to degeneration through the WNT and AKT pathways. Many

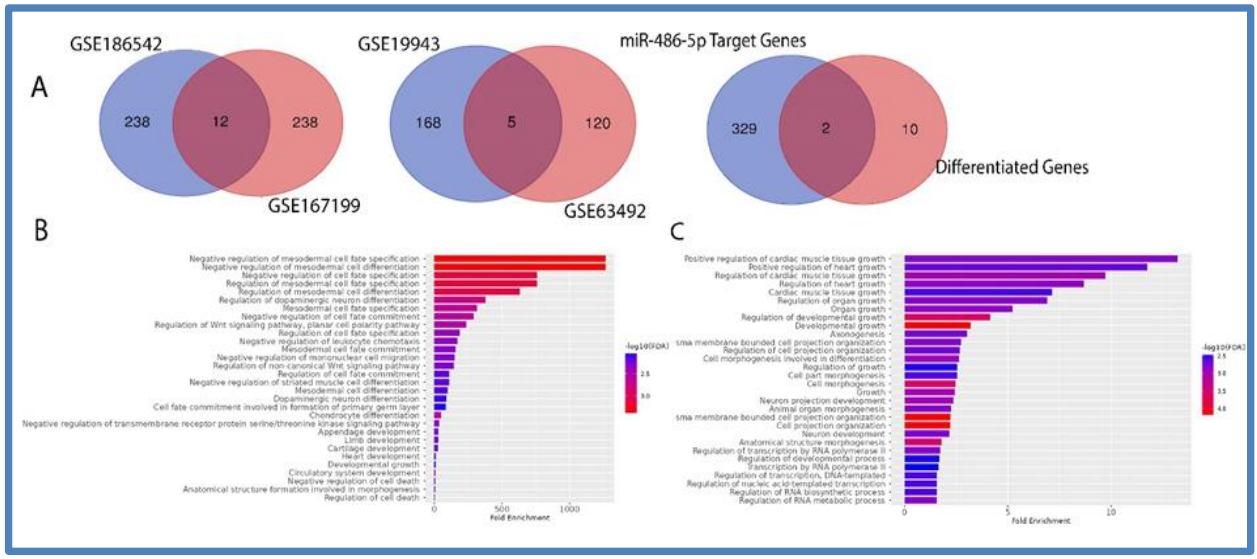

Fig. 1 Illustration of bioinformatic analyses. A: mRNA, miRNA, Venn diagram representation of common mRNAs and miRNAs in the dataset, B: representation of *RAB11FIP4* gene enrichment analysis, C: MiR-486-5p target genes enrichment analysis.

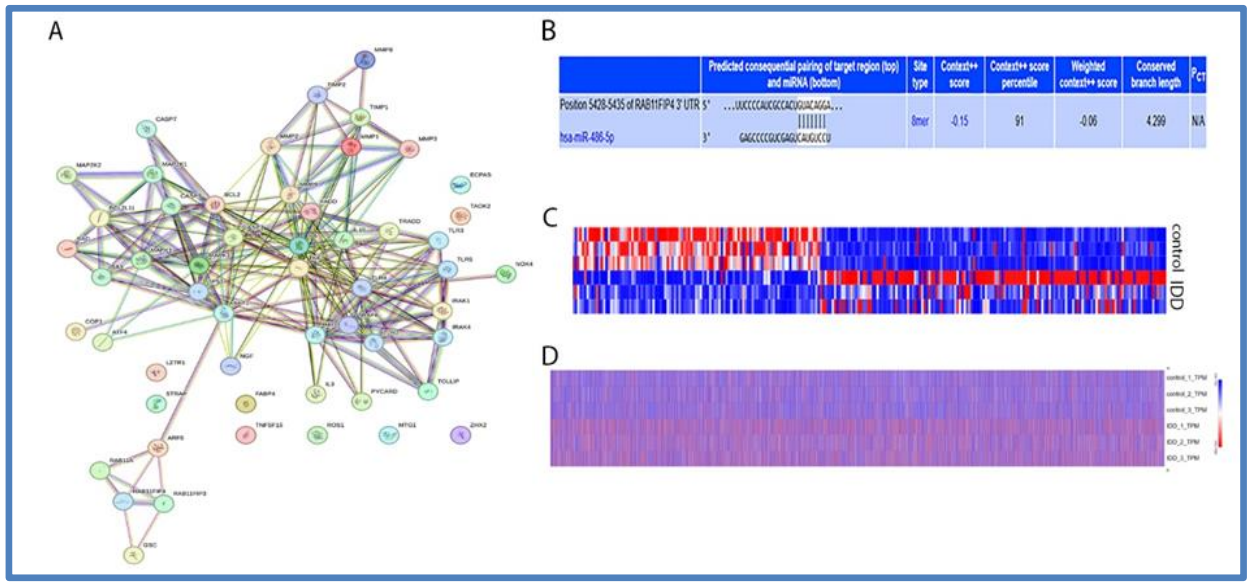

Fig. 2 Illustration of bioinformatic analyses. A: *RAB11FIP4* String database protein-protein network B: TargetScan programme output for *RAB11FIP4*-miR-486-5P target nucleotide C Heatmap representation of miRNA expression profile, D: Heatmap representation of mRNA expression profile.

crucial pathways can be involved in the processes of intervertebral disc degeneration. Figure 2 illustrates the genes that interact with the *RAB11FIP4* gene and the predicted cellular pathways involved.

4. Discussion

miRNAs function by binding to the 3'-UTR regions of target mRNA, thereby repressing gene expression or leading to mRNA degradation. Additionally, miRNAs are recognized for their crucial regulatory roles in biological processes such as cell differentiation, apoptosis, inflammation, and the cell cycle. These characteristics render miRNAs as significant biomarkers and therapeutic targets in various disease processes.

This study presents a vital advancement in identifying genetic factors that contribute to intervertebral disc degeneration and the miRNAs that target these factors. miR-486-5p was found to exhibit a significant interaction with the *RAB11FIP4* gene, suggesting that this miRNA could serve as a potential biomarker in the IVDD process. Elevating the expression of miR-486-5p, a potential biomarker, in cells through microRNA mimics may aid the degeneration process by reducing *RAB11FIP4* expression in degenerating cells.

By integrating miRNA and mRNA profiling, our findings detailed the genetic alterations at both transcriptional and post-transcriptional levels. Moreover,

the *RAB11FIP4* gene was shown to be involved in critical biological processes such as the WNT and AKT pathways, offering an important clue for targeted approaches in IVDD treatment. This discovery enhances our understanding of the molecular mechanisms underlying IVDD.

Our results line up with similar findings previously reported in the literature. For instance, miR-486-5p has been shown to be involved in inflammation and cell cycle mechanisms. However, this study makes a unique contribution by revealing for the first time in detail the specific effects of miR-486-5p on IVDD through its interaction with *RAB11FIP4.*

An important aspect of the study is that it illuminates cellular signaling pathways such as WNT and AKT pathways in determining the role of *RAB11FIP4* in IVDD. While WNT pathways are critical for metabolic balance and ECM homeostasis of disc cells, AKT pathways may be implicated in IVDD-related processes such as inflammation and apoptosis. These findings underscore the significance of miRNA-mRNA interactions for clinical and therapeutic strategies.

Although in-silico tests provide a valuable approach to generating biological hypotheses, these hypotheses require experimental validation. Confirming the effect of miR-486-5p on *RAB11FIP4* expression using in-vitro or in-vivo models will more accurately reveal the pathological role of this miRNA in the IVDD process and its therapeutic potential.

In conclusion, this study provides that miRNA-mRNA interactions provide important clues to the molecular mechanisms of IVDD. However, experimental support of these findings will be a critical step for the development of miRNA-based therapies and clinical management of IVDD.

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