Environmental Health Engineering and Management Journal 2022, 9(2), 189-200
http://ehemj.com
doi 10.34172/EHEM.2022.20

Environmental Health Engineering and Management Journal

Original Article

Environmental Health Engineering and Management

Open Access Publish Free

Potential Roles of Circular RNAs and Environmental and Clinical Factors in Intervertebral Disc Degeneration

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Abstract

Background: Intervertebral disc degeneration (IDD) is a common disability in the working-age population. The underlying pathogenesis of IDD needs elucidation. This study aimed to determine differentially expressed circular RNAs (circRNAs) in IDD by bioinformatics. Additionally, the environmental and clinical factors involved in IDD pathogenesis were reviewed.

Methods: The circRNA array profiling of patients with IDD and healthy individuals (GSE67566) was acquired from Gene Expression Omnibus (GEO). GEO2R was employed to analyze the expression profiles of the circRNAs. Functional *in silico* analysis was done on circRNAs with the highest differential expression. Environmental and clinical factors were reviewed through PubMed and Google Scholar.

Results: Twenty-five circRNAs were differentially expressed in IDD. Two circRNAs (hsa_circRNA_101645 and hsa_circRNA_101852) exhibited the most downregulated and upregulated expressions. The functional *in silico* analysis showed that the aforementioned circRNAs harbored target sites for AGO2 and EIF4A3 and several microRNAs. The upshots indicated that these 2 circular circRNAs might sponge hsa-miR-330-3p, hsa-miR-502-5p, hsa-miR-662, hsa-miR-874, and hsa-miR-646 and regulate *PSD3*, *SIK2*, *PCYT1B*, *ARID5B*, *MTMR3*, and *HIPK2* expressions, which play significant roles in autophagy and cellular senescence. Temperature, heavy metal exposure, age, overweight, occupation, exercise, hypertension, and smoking were the environmental and clinical factors associated with IDD progression.

Conclusion: Although the results need confirmation by experimental analysis, they reflect the possible role of particular circRNAs in IDD pathogenesis. The controversy concerning the association between IDD and environmental and clinical factors necessitates in-depth population research. Investigating novel molecular regulatory markers like circRNAs could clarify the underlying molecular mechanisms of IDD.

Keywords: Intervertebral disc degeneration, Circular RNAs, Environmental factors

Citation: Malakootian M, Gholipour A, Bagheri Moghaddam M, Arabian M, Oveisee M. Potential roles of circular rnas and environmental and clinical factors in intervertebral disc degeneration. Environmental Health Engineering and Management Journal 2022; 9(2): 189-200. doi: 10.34172/EHEM.2022.20.

Article History:
Received: 21 April 2022
Accepted: 24 May 2022
ePublished: 18 June 2022

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Introduction

Intervertebral disc degeneration (IDD) is the common cause of low back pain in about 70% of individuals worldwide. IDD can start early, so that 20% of people have mild disc degeneration in their teens, and it becomes severe with aging (1-3). Low back pain undermines the quality of life of its sufferers and imposes tremendous economic burdens comparable to or even worse than those of coronary heart disease and other major diseases such as diabetes, Alzheimer's, and renal diseases (4). Similar to other multifactorial diseases, the etiology of IDD is complicated, with the exact mechanism of IDD pathogenesis needing elucidation. Nonetheless, it is widely recognized that the contributors to IDD progression are not only genetic predispositions and molecular markers but also aging and environmental factors, including temperature, heavy metal pollution, work-related biomechanical factors (e.g., physical workload, hard work, working periods exceeding 8 hours, and work-related stress) (5-9). Interestingly, tall individuals are at greater risk of IDD development (10).

Several studies have determined that mutations in the *COL9A1*, *COL9A2*, and *COL9A3* genes encode collagen type IX and are, thus, associated with disc degeneration (11, 12). Aggrecan, also termed "cartilage-specific proteoglycan core protein" or "chondroitin sulfate

proteoglycan 1", contains many chondroitin sulfate chains and builds the nucleus pulposus. Changes in the length of the variable-number tandem repeat in the *ACAN* gene, which encodes aggrecan, result in differences in aggrecan properties and predispose to multilevel disc degeneration (13). Other genes such as matrix metalloproteinase-2 (*MMP-2*), matrix metalloproteinase-9 (*MMP-9*), TIMP metallopeptidase inhibitor-1 (*TIMP-1*), cyclooxygenase-2 (*COX-2*), and R-spondin-3 (*RSPO-3*) can participate in the pathophysiology of disc disease as well (14-16). Additionally, nucleotide variations in collagen IX (*COL9A3)* are linked to persistent obesity and can, therefore, affect the production of lumbar discs (17). The expression of human high-temperature requirement protein-1 (HTRA-1) is increased in arthritis and IDD, suggesting a link between HTRA-1 and disease progression. HTRA-1 also regulates a broad range of physiological processes by its proteolytic activity (18). Food ingredients such as vitamins are deemed among the environmental factors capable of affecting IDD. Pabalan et al demonstrated an association between FokI and ApaI polymorphisms in the *Vitamin D receptor* gene and IDD (19).

IDD is a chronic progression resulting in the structural failure of the intervertebral disc, with enhanced signs of aging (20). The nucleus pulposus, a central structural constituent of the intervertebral disc, consists of the extracellular matrix and nucleus pulposus cells (21-25). The deregulated function of nucleus pulposus cells and extracellular matrix degradation/synthesis can contribute to IDD development (26-30). Nevertheless, atypical stimuli such as neutrophil proteases can upregulate inflammatory cytokines, diminish the steadiness of nucleus pulposus cells through MMPs and type II collagen, and accelerate IDD (31, 32).

An emerging approach to IDD molecular mechanism exploration is studying different RNA types, including noncoding RNAs (ncRNAs). Composing a large family of RNAs without a coding function, ncRNAs include microRNAs (miRNAs), circular RNAs (circRNAs), and long noncoding RNAs (lncRNAs). Microarray and sequencing analyses show significant ncRNA differential expression patterns between IDD samples and normal ones, hence, the probable role of ncRNAs in IDD development (33-38).

CircRNAs are newly introduced as regulatory and single-stranded ncRNAs with loop structures produced by non-canonical back-splicing events (39-43). Some circRNAs mediate their function by interacting with proteins or obstructing mature messenger RNA (mRNA) formation. This type of RNA plays a crucial role in IDD pathogenesis. In this regard, the upregulation of circ-4099 has been shown to inhibit IDD development (44-46).

In the present study, it was aimed to determine differentially expressed circRNAs in IDD through a bioinformatics analysis of array profiling data from the NCBI Gene Expression Omnibus (GEO) database with a view to elucidating the underlying molecular mechanisms of IDD. In addition, the environmental and clinical factors involved in IDD pathogenesis were reviewed.

Materials and Methods

Data sets

The data set regarding the circRNA expression profile (GSE67566) was downloaded from the GEO database. The GSE67566 data set consists of the ncRNA array profiling of 5 non-degenerative (controls) and 5 degenerative nucleus pulposus cells.

Quality control

The samples, 10 in total, were divided into non-degenerative and degenerative groups. All 10 samples were subjected to quality control in the R Program using the ggplot2 package, which performed the principal component analysis and drew box plot curves.

Differential expression analysis

The profiles of the differentially expressed circRNAs between the normal and degenerative samples were investigated using GEO2R for all the data sets. Then, a volcano plot was drawn to show the differentially expressed circRNAs.

Moreover, circRNAs with a log fold change (logFC) score #2.5 and an adjusted P value <0.05 were selected as the top differentially expressed circRNAs. A box plot analysis, a standard method for displaying data distribution, was performed to identify the most upregulated and downregulated circRNAs.

Functional in silico analysis of 2 highly differentially expressed circRNAs

Following the selection of the most upregulated and downregulated circRNAs, the IDs of the circRNAs were identified by Circ2Disease (http://bioinformatics.zju.edu.cn/Circ2Disease/circRNAgroup.html), and RNA-binding protein sites and miRNA target sites matching each circRNA were investigated by CircInteractome (https://circinteractome.nia.nih.gov/index.html). Subsequently, the common miRNA target sites that matched both circRNAs were separated by Venny 2.1 (https://bioinfogp.cnb.csic.es/tools/venny/index.html). In the next stage, the target genes of each miRNA were analyzed by TargetScan. Afterward, the common target genes were separated by Venny 2.1 to study their possible effects on IDD. Finally, the signaling pathways of target genes were studied by Enrichr (https://maayanlab.cloud/Enrichr/enrich#).

Environmental and clinical determinants of IDD

PubMed (https://pubmed.ncbi.nlm.nih.gov/) and Google Scholar (https://scholar.google.com/) were drawn upon to

find all the environmental and clinical factors associated with the pathogenesis of IDD. The search identified 50 articles related to the effects of environmental factors, including temperature, heavy metal pollution, lifestyle, occupation, prolonged working hours, driving, strenuous physical activity and exercise, as well as clinical factors including hypertension, smoking, pregnancy, and diabetes mellitus.

Results

Quality control of the data set

The results of the principal component and box plot analyses demonstrated that all the non-degenerative and degenerative nucleus pulposus sample cells possessed sufficient quality and could be used for further analysis. The principal component analysis diagram of the studied samples showed that the samples of each group could both be individually categorized by themselves and be separated correctly according to their group (Figure 1A). The box plot analysis revealed that the non-degenerative and degenerative samples did not differ significantly from each other (Figure 1B), indicating their suitability for further expression analysis. The remote data of the samples are not shown in Figure 1A-B.

Differential expression of hsa_circRNA_101645 and hsa_circRNA_101852 in the degenerative samples

The upshot of the differential expression analysis demonstrated that 2893 circRNAs were differentially expressed (upregulated or downregulated) between the control and degenerative samples (Table S1, Supplementary file 1). The results of the volcano plot showed that 1187 circRNAs were upregulated and 1506 circRNAs were downregulated (Figure 2A). The red dots in the plot indicate the upregulated circRNAs, the blue dots show the downregulated circRNAs, and the black dots show the insignificant circRNAs.

Among the differentially expressed circRNAs, 25 circRNAs were upregulated and 14 circRNAs were downregulated based on the application of a logFC#2.5 and an adjusted *P* value <0.05 (Table 1). The most upregulated and downregulated circRNAs in the degenerative samples were circRNA_101645 and hsa_circRNA_101852, respectively (Figure 2B).

RNA-binding protein sites and miRNA target sites of hsa_circRNA_101645 and hsa_circRNA_101852

The possible function of hsa_circRNA_101645 (hsa_circ_0036763) and hsa_circRNA_101852 (hsa_circ_0040039) was analyzed using the CircInteractome database. The results showed that AGO2 and EIF4A3 were RNA-binding protein sites for hsa_circRNA_101645 and AGO2, while EIF4A3, FMRP, HuR, and IGF2BP2 were RNA-binding protein sites for hsa_circRNA_101852 (Table 2). Several miRNA target sites were found for each circRNA, with hsa-miR-330-3p, hsa-miR-502-5p, hsa-miR-662, hsa-miR-874, and hsa-miR-646 sharing target sites on both circRNAs (Table 2). The target genes of each miRNA were separated by TargetScan (Tables S2–S6, Supplementary file 1). The upshots demonstrated that *PSD3*, *SIK2*, *PCYT1B*, *ARID5B*, *MTMR3*, and *HIPK2* were common target genes among all 5 mentioned miRNAs. The signaling pathway analysis showed the involvement of these genes in phosphonate and phosphinate metabolism, glycerophospholipid metabolism, autophagy, and cellular senescence in cells (Figure 3). Therefore, it seems that the competing endogenous mRNAs (ceRNA) regulatory network of these circRNAs might be linked to IDD by regulating the pathways involved in its progression.

Environmental and clinical determinants of IDD

Numerous studies have reported environmental and clinical factors involved in the development of IDD, including temperature, heavy metal pollution, driving, work schedule, prolonged working hours, strenuous physical activity, age, sex, body weight, body mass index (BMI), overweight, obesity, pregnancy, diabetes mellitus,

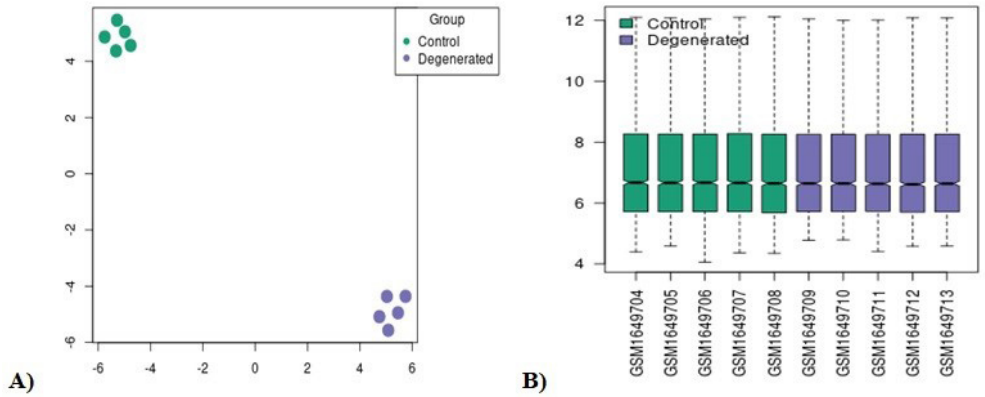

Figure 1. The results of the quality control data analysis. (A) The principal component analysis graph shows that all the samples in each group were separated correctly according to their group. B) The box plot demonstrates that the degenerative and non-degenerative intervertebral disc samples did not differ significantly. All the samples were appropriate for further expression analysis.

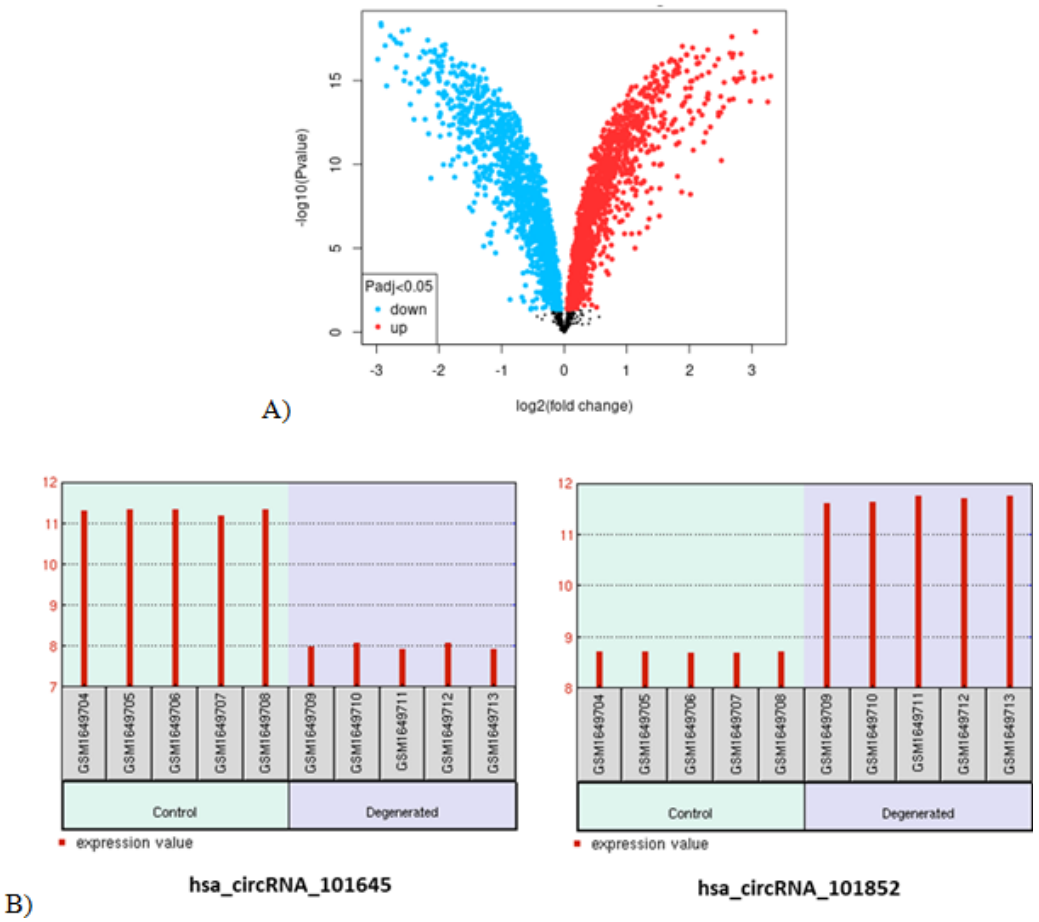

Figure 2. (A) The volcano plot for the differentially expressed circular RNAs (circRNAs). The red and blue dots reflect upregulated and downregulated circRNAs, respectively, and the black dots represent insignificant circRNAs. (B) In intervertebral disc degeneration samples, the highest differential expression was observed in hsa_circRNA_101645 (downregulated) and hsa_circRNA_101852 (upregulated).

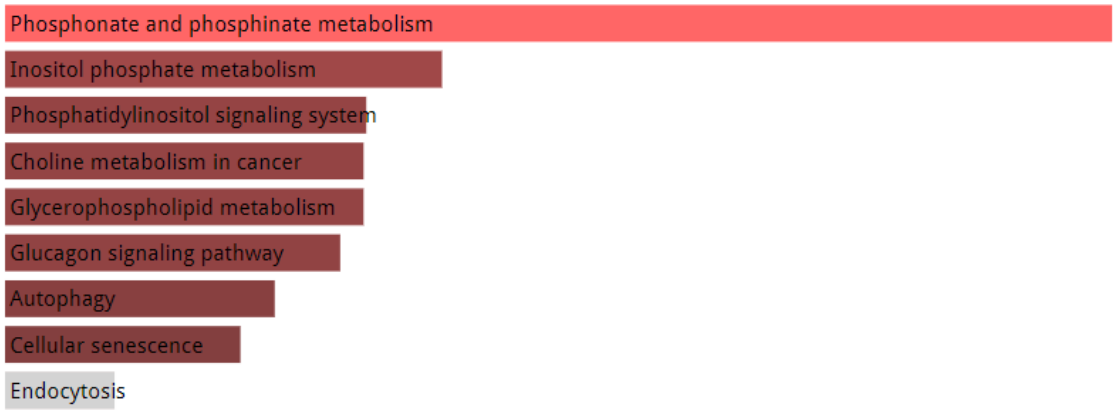

Figure 3. The important signaling pathways in which *PSD3, SIK2, PCYT1B, ARID5B, MTMR3* and *HIPK2* genes are involved.

hypertension, and smoking (47-49). Still, the reports abound with controversy vis-à-vis the precise role of environmental and clinical factors in IDD progression. Table 3 presents the clinical and environmental determinants of IDD reported by various investigations.

A previous study reported an association between lower temperatures and increased risks of muscular pain and injury, which could be associated with higher occurrence rates of IDD (50).

Evidence suggests that heavy metal pollution could interfere with bone homeostasis. Indeed, exposure to heavy metals promotes degenerative bone diseases including degenerative disk disease (51). Earlier studies have also reported that cadmium levels are negatively correlated with zinc levels in the intervertebral discs of patients with degenerative changes. In this group of patients, lead levels are positively associated with the levels of magnesium, zinc, and aluminum and negatively

Table 1. The upregulated and downregulated circular RNAs (circRNAs) based on a logFC score #2.5 and an adjusted P-value below 0.05 among degenerative and non-degenerative intervertebral disc samples

CircRNAs	Adjusted P-value	P-value	logFC	Significance
hsa_circRNA_101645	1/60E-14	5/49E-16	- 3/300365	Down
hsa_circRNA_104508	2/19E-13	1/87E-14	-3/255699	Down
hsa_circRNA_102116	1/92E-14	7/37E-16	-3/175986	Down
hsa_circRNA_103838	6/98E-16	1/21E-18	-3/057012	Down
hsa_circRNA_101557	1/96E-14	8/06E-16	-3/048583	Down
hsa_circRNA_101709	1/18E-14	3/40E-16	-3/039137	Down
hsa_circRNA_104630	2/41E-14	1/07E-15	-3/037861	Down
hsa_circRNA_104019	2/04E-13	1/72E-14	-2/974758	Down
hsa_circRNA_101370	1/82E-14	6/52E-16	-2/859529	Down
hsa_circRNA_105031	1/18E-14	3/24E-16	-2/827131	Down
hsa_circRNA_103486	2/84E-15	2/55E-17	-2/826916	Down
hsa_circRNA_101558	1/92E-14	7/77E-16	-2/797888	Down
hsa_circRNA_100427	2/50E-14	1/15E-15	-2/75521	Down
hsa_circRNA_102618	1/92E-14	7/77E-16	-2/719943	Down
hsa_circRNA_103198	2/84E-15	2/52E-17	-2/703984	Down
hsa_circRNA_103139	1/60E-13	1/27E-14	-2/703897	Down
hsa_circRNA_100772	3/39E-15	4/08E-17	-2/687741	Down
hsa_circRNA_102492	1/00E-15	2/42E-18	-2/681225	Down
hsa_circRNA_103634	2/82E-15	2/34E-17	-2/651395	Down
hsa_circRNA_102126	1/77E-13	1/46E-14	-2/639859	Down
hsa_circRNA_104952	2/73E-14	1/29E-15	-2/557336	Down
hsa_circRNA_000943	3/89E-13	3/87E-14	-2/545568	Down
hsa_circRNA_101369	2/12E-10	5/88E-11	-2/513249	Down
hsa_circRNA_103675	7/06E-13	8/01E-14	-2/502746	Down
hsa_circRNA_102367	1/19E-13	8/90E-15	-2/501381	Down
hsa_circRNA_101525	3/03E-15	3/14E-17	2/521327	Up
hsa_circRNA_103801	1/18E-14	3/35E-16	2/542272	Up
hsa_circRNA_000200	2/32E-14	9/78E-16	2/559996	Up
hsa_circRNA_103410	6/98E-16	1/15E-18	2/588725	Up
hsa_circRNA_100018	1/67E-15	6/33E-18	2/613127	Up
hsa_circRNA_100604	1/57E-15	5/42E-18	2/677874	Up
hsa_circRNA_104600	7/51E-15	1/69E-16	2/683763	Up
hsa_circRNA_104703	1/24E-15	3/47E-18	2/718491	Up
hsa_circRNA_102324	1/00E-15	2/18E-18	2/777566	Up
hsa_circRNA_400019	3/87E-14	2/07E-15	2/839063	Up
hsa_circRNA_103890	1/72E-15	8/20E-18	2/862396	Up
hsa_circRNA_101139	6/98E-16	5/39E-19	2/924426	Up
hsa_circRNA_101853	6/98E-16	3/80E-19	2/928536	Up
hsa_circRNA_101852	3/92E-15	5/40E-17	2/980678	Up

associated with molybdenum levels and IDD (48, 49).

In this regard, a prior study reported that smoking at least 4 packs a year was associated with the lumbar degenerative disease in men but not in women, even though this finding was not statistically significant (52). Another study on 83 individuals aged 13 to 20 in southern China found that overweight or obesity, based on BMI, was associated with IDD, while no significant association was found with smoking (53). A study in Japan on 308 student-athletes and 70 student-non-athletes showed that swimming and competitive baseball were associated with IDD (54). Evidence suggests that strenuous physical activity, heavy work, and smoking could be notable factors affecting IDD onset (10, 55). Nonetheless, whereas a study conducted in 2008 showed that lifting and smoking were associated with disc height (56), Yasuoka et al and Samartzis et al reported no association between smoking and IDD progression (53, 57).

Table 2. RNA-binding proteins and microRNAs (miRNAs) with target sites on hsa_circRNA_101645 and hsa_circRNA_101852

circRNA/circRNA IDs	RNA-binding Proteins	miRNAs
hsa_circRNA_101645/ hsa_circ_0036763	AGO2, EIF4A3	hsa-miR-1227, hsa-miR-1270 hsa-miR-127-5p, hsa-miR-330-3p hsa-miR-502-5p, hsa-miR-516a-5p, hsa-miR-518a-5p, hsa-miR-527 hsa-miR-576-3p, hsa-miR-583 hsa-miR-620, hsa-miR-646 hsa-miR-662, hsa-miR-767-3p hsa-miR-874, hsa-miR-938 hsa-miR-942
hsa_circRNA_101852/ hsa_circ_0040039	AGO2, EIF4A, FMRP, HuR IGF2BP2	hsa-miR-1203, hsa-miR-1248 hsa-miR-136, hsa-miR-146b-3p hsa-miR-188-3p, hsa-miR-330-3p hsa-miR-369-5p, hsa-miR-370 hsa-miR-383, hsa-miR-502-5p hsa-miR-503, hsa-miR-526b hsa-miR-545, hsa-miR-548p hsa-miR-574-5p, hsa-miR-626 hsa-miR-637, hsa-miR-638 hsa-miR-646, hsa-miR-648 hsa-miR-662, hsa-miR-665, hsa-miR-874

Obesity and overweight constitute the most studied factors associated with IDD, although the results reported from different countries are discordant. Investigations from the Netherlands, Canada, the United States, and Japan have found no association between BMI and disc narrowing. In the meantime, some studies on Japanese, Finnish, and British individuals have demonstrated an association between obesity and IDD, while some other investigations on the same populations have reported no such relationship (56, 58-78) (Table 3).

Various age-related studies have demonstrated that the severity of IDD increases steadily with age, indicating the natural progression of disc degeneration *in vivo* and the role of aging as a significant risk factor for IDD. In contrast, some studies from Switzerland, the United States, Japan, and the United Kingdom have shown no association between age and IDD (63-65, 67-77).

There is also controversy on the association between IDD and different occupations and sports (Table 3) (54, 55). Studies have demonstrated that occupational lifting and leisure time resistance training have moderate additional effects on disc degeneration (56, 62). The position on the field in American football has also been

Table 3. The list of the environmental and clinical determinants of intervertebral disc degeneration (IDD) progression

Clinical or environmental determinant	Country	Reference	Association With IDD	
			No	Yes
Temperature	-	(18, 50)		Y
Heavy metals	-	(48, 49, 51)		Y
Age	Switzerland	(63)	N	
	USA	(53, 63, 64, 67)	N	Y
	Japan	(74, 75)	N	
	Washington	(76)	N	
	UK	(65, 68)	N	Y
	Portugal	(69)		Y
Sex	Switzerland	(63)	N	
	USA	(77)	N	
	Washington	(76)	N	
	Japan	(75)		Y
Body weight/BMI/ Overweight/Obesity	Canada	(52, 62)	N	
	Switzerland	(63)	N	
	USA	(64)	N	
	UK	(58, 65)	N	Y
	China	(60, 73)	N	Y
	Finland	(61, 77)	N	Y
	Japan	(74, 75, 78)	N	
Pregnancy	Japan	(74)	N	
Diabetes mellitus	Japan	(75)	N	
Hypertension	Japan	(75)	N	
	China	(70, 72)		Y
	USA	(71)		Y
Back injuries	Finland	(77)	N	
Smoking	Finland	(77)	N	
	Japan	(74, 75)	N	
	Canada	(56, 62)	N	Y
	Finland	(77)	N	
Car driving	Canada	(62)	N	
	Japan	(62)	N	
Occupation/Work schedule	Finland	(77)	N	
	Japan	(74)	N	
	Switzerland	(63)		Y
Weight lifting at work	Canada	(56)		Y
Sports activities	Sweden	(10)	N	
Recreational activities at leisure time	Canada	(56, 62)	N	
Resistance training	Canada	(56, 62)	N	Y
Lifting weight	Japan	(75)	N	
	Canada	(56, 62)	N	Y
American football position	Japan	(78)		Y
American football playing career	Japan	(78)	N	
Fast bowling	Western Australia	(79)		Y

related to the creation and development of IDD in players (78).

Numerous studies have investigated the effect of hypertension on IDD (70, 71). Sun et al found that the tissue renin-angiotensin system, a potential mechanism for hypertension, might contribute to oxidative stress and inflammatory response to IDD. Another study showed that age was an incontrovertible risk factor for IDD and hypertension (70, 72, 75, 79).

In sum, although research has indicated possible associations between IDD development and progression and many environmental and clinical factors, none of them alone can cause IDD. It appears that IDD progression is influenced by several mechanisms including environmental, biomechanical (61), inflammatory (66), and clinical factors.

Discussion

IDD, characterized by the progressive structural failure of intervertebral discs, is strongly associated with an increased risk for low back pain. Genetic and lifestyle factors have been associated with IDD development (11, 63, 80). The precise molecular mechanisms underlying IDD have, however, remained largely indefinable.

In the present study, deregulated circRNAs in the nucleus pulposus cells of IDD samples were identified and their probable role in IDD pathogenesis was assessed. The results revealed 2 circRNAs, hsa_circRNA_101645 and hsa_circRNA_101852, with differential expression levels. Of all the circRNAs evaluated, the 2 aforementioned ones had the highest involvement in IDD. In the present study, the previously postulated environmental and clinical determinants of IDD progression were also reviewed and it was found that for all the nominated factors, IDD development had no uniquely single determinant.

The intervertebral disc contains a gelatinous core known as "the nucleus pulposus", which plays a crucial role in maintaining its integrity. Studies have suggested that aberrant nucleus pulposus cell function is a key to IDD pathogenesis (81-85). More recent research has shed light on the involvement of circRNAs in the development of IDD (46). Meng et al showed that hsa_circ_0001658 was significantly upregulated in the nucleus pulposus tissues of patients with IDD, and it inhibited IDD development by regulating hsa-miR-181c-5p/FAS (86). Tang et al reported that hsa-circ-0040039 had the highest logFC score. They also concluded that 2 members of the RAS oncogene family, namely RAB1A and RAB1B, and multiple coagulation factor deficiency (MCFD2), as the related network of hsa-circ-0040039, might play significant roles in IDD (87). Wang et al detected significant circ-4099 expression upregulation in nucleus pulposus cells after treatment with tumor necrosis factor alpha (44).

The GEO2R analysis revealed that 25 circRNAs were highly differentially expressed in the nucleus pulposus cells of patients with IDD, with hsa_circRNA_101645

and hsa_circRNA_101852 exhibiting the most marked difference in expression. These findings are consistent with those reported by Lan et al, who confirmed the upregulation of circRNA-101852 and the downregulation of circRNA-101645 using real-time quantitative polymerase chain reaction (RT-qPCR) and applying the Arraystar human array analysis (88).

The analyses in the present study identified another circRNA, hsa_circRNA_100772, which was significantly downregulated in IDD samples. Wang et al used qRT–PCR and found a significant decline in the expression of hsa_circRNA_100772 (circBase ID: hsa_circ_0021535, also termed "circKIF18A") in the IDD group compared with the control group (89). However, the existing literature features no experimental analyses on the other circRNAs introduced in the present study.

Notably, ceRNAs are referred to as "miRNA sponges" as they directly bind to miRNAs to counteract post-transcriptional repression (90, 91). Some circRNAs are enriched with miRNA-binding sites and act as ceRNAs to interact with miRNAs and regulate the expression of mRNA targets (90, 92-95). Cheng et al showed that CircVMA21 could improve inflammatory cytokines-induced nucleus pulposus cell apoptosis and extracellular matrix through the miR-200c-XIAP pathway in IDD (96). Song et al concluded that circRNA_0000253 could be used as a ceRNA to combine with miRNA-141-5p with the aim of promoting the secretion of interleukin-1 beta (IL-1β), which stimulates oxidative stress response and apoptotic protein expression (97).

In the present study, the functional bioinformatics analysis revealed several miRNA target sites harbored in both hsa_circRNA_101645 and hsa_circRNA_101852. It was found that these circRNAs could act as miRNA sponges (hsa-miR-330-3p, hsa-miR-502-5p, hsa-miR-662, hsa-miR-874, and hsa-miR-646) and affect IDD pathogenesis by targeting *PSD3*, *SIK2*, *PCYT1B*, *ARID5B*, *MTMR3,* and *HIPK2*. The results of this study demonstrated that these genes were involved in various signaling pathways in cells such as autophagy and cellular senescence. Previous studies have reported the role of autophagy in IDD and indicated that the abnormal regulation of autophagy levels could be a significant mechanism leading to IDD. Moreover, *Beclin-1*, *Atg8*, *Atg12*, *Cathepsin B*, *Presenilin-1*, and *p62* are autophagy-related genes significantly upregulated in degenerated disc tissues compared with healthy disc tissues (98-101). The findings of the present study concerning the involvement of these genes in autophagy and cellular senescence are consistent with the results of the aforementioned investigations. These circRNAs could affect the pathogenesis of IDD by acting as sponges and regulating the expression of genes involved in autophagy and cellular senescence. This postulation needs experimental confirmation.

In this study, functional *in silico* analysis identified AGO2 as an RNA-binding protein with a target site on both hsa_circRNA_101645 and hsa_circRNA_101852. Studies have demonstrated that miRNAs can be loaded into AGOs to induce the translational inhibition or exonucleolytic mRNA decay of specific transcripts. In mammals, AGOs have been mainly described for their cytoplasmic role in the biogenesis of small RNAs (smRNAs), which function as the key components of the RNA-induced silencing complex (102, 103). Evidence indicates the role of the ceRNA regulatory network of these circRNAs in the pathogenesis of IDD (44, 96, 97, 104). The immunoprecipitation of AGO2 shows that circ-4099 can sponge miR-616-5p, and the upregulation of circ-4099 in nucleus pulposus cells could act as a protective mechanism against inflammation by modulating the miR-616-5p-Sox9 pathway (44, 104). These circRNAs might regulate miRNA production by harboring the AGO2 protein, affecting IDD pathogenesis. This theory needs to be confirmed by further experiments.

Although many environmental and clinical factors have been nominated as the determinants of IDD development and progression, the existing controversy precludes their introduction as independent risk factors for this degenerative disease. For instance, whereas some investigators have confirmed age progression as an independent risk factor and a strong predictor of IDD (69), others have found no such association (63, 74-76).

Environmental factors like temperature can affect the expression of circRNAs (105, 106). Notably, circRNAs are expressed at very low levels, and they accumulate in the tissue due to their high stability at room temperature (106). Pan et al demonstrated that heat stress could induce the expression and accumulation of circRNAs remarkably in plant response (105). Nonetheless, no study is available on the relationship between the expression levels of circRNAs and temperature changes in humans. Given the earlier findings in plants, this hypothesis needs further in-depth research in humans.

Heavy metals, recognized as environmental pollutants, could accumulate in the human body and cause genetic damage by inhibiting critical proteins from different DNA repair pathways. Consequently, heavy metal exposure could lead to human diseases such as IDD (48, 49, 51, 107). Despite the existing evidence indicating the contribution of some heavy metals to IDD progression, more experimental investigations are required to elucidate the effects of heavy metal exposure on the expression of circRNAs and the exact mechanisms thereof.

Conclusion

Overall, investigating molecular regulatory pathways and novel molecular regulatory markers like circRNAs can enhance our understanding of the exact molecular mechanisms underlying the emergence and progression of IDD. The results of the present study, albeit in need of confirmation by experimental analyses, indicate the possible role of some circRNAs within a ceRNA regulatory

network in IDD pathogenesis. Still, with respect to the relationship between IDD and environmental and clinical factors, more in-depth population research, not least in Iran, is required. Expanding our knowledge of the molecular mechanisms underlying IDD pathogenesis will confer earlier detection and more effective treatments.

Acknowledgments

This work was supported by research grants to Dr. Oveisee and Dr. Malakootian from Bam University of Medical Sciences and the Research Deputyship of Rajaie Cardiovascular Medical and Research Center (Ethical code: IR.MUBAM.REC.1401.003).

Ethical Approval

The study protocol was approved by the Ethics Committee of Bam University of Medical Sciences.

Competing interests

The authors declare that there is no conflicts of interests.

Authors' contributions

MM and MO contributed to the design, supervision, and finalization of the draft. MM, AGh, MBM and MA participated in bioinformatics analysis, statistical analysis, and literature review. MM, AGh, MBM, MA and MO contributed to the writing and submission of the manuscript. All the authors participated in the finalization of the manuscript and approved the final draft. MM and AGh have equally contributed to this work.

Supplementary files

Supplementary file 1 contains Tables S1-S6.

References

1. Frymoyer JW, Cats-Baril WL. An overview of the incidences and costs of low back pain. Orthop Clin North Am 1991; 22(2): 263-71.
2. Nakamura M, Nishiwaki Y, Ushida T, Toyama Y. Prevalence and characteristics of chronic musculoskeletal pain in Japan: a second survey of people with or without chronic pain. J Orthop Sci 2014; 19(2): 339-50. doi: 10.1007/s00776-013-0525-8.
3. Nakamura M, Nishiwaki Y, Ushida T, Toyama Y. Prevalence and characteristics of chronic musculoskeletal pain in Japan. J Orthop Sci 2011; 16(4): 424-32. doi: 10.1007/s00776-011-0102-y.
4. Maetzel A, Li L. The economic burden of low back pain: a review of studies published between 1996 and 2001. Best Pract Res Clin Rheumatol 2002; 16(1): 23-30. doi: 10.1053/berh.2001.0204.
5. Urban JP, Roberts S. Degeneration of the intervertebral disc. Arthritis Res Ther 2003; 5(3): 1-11. doi: 10.1186/ar629.
6. Boos N, Weissbach S, Rohrbach H, Weiler C, Spratt KF, Nerlich AG. Classification of age-related changes in lumbar intervertebral discs: 2002 Volvo Award in basic science. Spine (Phila Pa 1976) 2002; 27(23): 2631-44. doi: 10.1097/00007632-200212010-00002.
7. Miller JA, Schmatz C, Schultz AB. Lumbar disc degeneration: correlation with age, sex, and spine level in 600 autopsy specimens. Spine (Phila Pa 1976) 1988; 13(2): 173-8.
8. Yan C, Wang X, Xiang C, Wang Y, Pu C, Chen L, et al. Applications of Functionalized Hydrogels in the Regeneration of the Intervertebral Disc. Biomed Res Int 2021; 2021:2818624. doi: 10.1155/2021/2818624.
9. Jang TW, Ahn YS, Byun J, Lee JI, Kim KH, Kim Y, et al. Lumbar intervertebral disc degeneration and related factors in Korean firefighters. BMJ Open 2016; 6(6): e011587. doi: 10.1136/bmjopen-2016-011587.
10. Wahlström J, Burström L, Nilsson T, Järvholm B. Risk factors for hospitalization due to lumbar disc disease. Spine (Phila Pa 1976) 2012; 37(15): 1334-9. doi: 10.1097/BRS.0b013e31824b5464.
11. Feng Y, Egan B, Wang J. Genetic factors in intervertebral disc degeneration. Genes Dis 2016; 3(3): 178-85. doi: 10.1016/j.gendis.2016.04.005.
12. Chan D, Song Y, Sham P, Cheung KM. Genetics of disc degeneration. Eur Spine J 2006; 15 Suppl 3(Suppl 3): S317-25. doi: 10.1007/s00586-006-0171-3.
13. Kawaguchi Y, Osada R, Kanamori M, Ishihara H, Ohmori K, Matsui H, et al. Association between an aggrecan gene polymorphism and lumbar disc degeneration. Spine (Phila Pa 1976) 1999; 24(23): 2456-60. doi: 10.1097/00007632-199912010-00006.
14. Dong DM, Yao M, Liu B, Sun CY, Jiang YQ, Wang YS. Association between the-1306C/T polymorphism of matrix metalloproteinase-2 gene and lumbar disc disease in Chinese young adults. Eur Spine J 2007; 16(11): 1958-61. doi: 10.1007/s00586-007-0454-3.
15. Sun Zm, Miao L, Zhang YG, Ming L. Association between the-1562 C/T polymorphism of matrix metalloproteinase-9 gene and lumbar disc disease in the young adult population in North China. Connect Tissue Res 2009; 50(3): 181-5. doi: 10.1080/03008200802585630.
16. Valdes AM, Hassett G, Hart DJ, Spector TD. Radiographic progression of lumbar spine disc degeneration is influenced by variation at inflammatory genes: a candidate SNP association study in the Chingford cohort. Spine (Phila Pa 1976) 2005; 30(21): 2445-51. doi: 10.1097/01.brs.0000184369.79744.a5.
17. Solovieva S, Lohiniva J, Leino-Arjas P, Raininko R, Luoma K, Ala-Kokko L, et al. COL9A3 gene polymorphism and obesity in intervertebral disc degeneration of the lumbar spine: evidence of gene-environment interaction. Spine (Phila Pa 1976) 2002; 27(23): 2691-6. doi: 10.1097/00007632-200212010-00008.
18. Li Y, Yuan J, Rothzerg E, Wu X, Xu H, Zhu S, et al. Molecular structure and the role of high-temperature requirement protein 1 in skeletal disorders and cancers. Cell Prolif 2020; 53(2): e12746. doi: 10.1111/cpr.12746.
19. Pabalan N, Tabangay L, Jarjanazi H, Vieira LA, Dos Santos AA, Barbosa CP, et al. Association between the FokI and ApaI polymorphisms in the vitamin D receptor gene and intervertebral disc degeneration: a systematic review and meta-analysis. Genet Test Mol Biomarkers 2017; 21(1): 24-32. doi: 10.1089/gtmb.2016.0054.

20. Xu D, Ma X, Sun C, Han J, Zhou C, Wong SH, et al. Circular RNAs in Intervertebral Disc Degeneration: An Updated Review. Front Mol Biosci 2021; 8: 781424. doi: 10.3389/fmolb.2021.781424.
21. Jiang C, Guo Q, Jin Y, Xu JJ, Sun ZM, Zhu DC, et al. Inhibition of EZH2 ameliorates cartilage endplate degeneration and attenuates the progression of intervertebral disc degeneration via demethylation of Sox-9. EBioMedicine 2019; 48: 619-29. doi: 10.1016/j.ebiom.2019.10.006.
22. Bach FC, de Rooij KM, Riemers FM, Snuggs JW, de Jong WA, Zhang Y, et al. Hedgehog proteins and parathyroid hormone-related protein are involved in intervertebral disc maturation, degeneration, and calcification. JOR Spine 2019; 2(4): e1071. doi: 10.1002/jsp2.1071.
23. Kang L, Xiang Q, Zhan S, Song Y, Wang K, Zhao K, et al. Restoration of autophagic flux rescues oxidative damage and mitochondrial dysfunction to protect against intervertebral disc degeneration. Oxid Med Cell Longev 2019; 2019: 7810320. doi: 10.1155/2019/7810320.
24. Ohnishi T, Yamada K, Iwasaki K, Tsujimoto T, Higashi H, Kimura T, et al. Caspase-3 knockout inhibits intervertebral disc degeneration related to injury but accelerates degeneration related to aging. Sci Rep 2019; 9(1): 1-15. doi: 10.1038/s41598-019-55709-3.
25. Hanaei S, Abdollahzade S, Sadr M, Mirbolouk MH, Fattahi E, Khoshnevisan A, et al. The role of interleukin 4 and IL-4RA in intervertebral disc degeneration: investigation of single nucleotide polymorphisms in genes and a systematic review & meta-analysis of IL-4 expression level. Br J Neurosurg 2020; 34(1): 66-71. doi: 10.1080/02688697.2019.1698010.
26. Li Z, Shao Z, Chen S, Huang D, Peng Y, Chen S, et al. TIGAR impedes compression-induced intervertebral disc degeneration by suppressing nucleus pulposus cell apoptosis and autophagy. J Cell Physiol 2020; 235(2): 1780-94. doi: 10.1002/jcp.29097.
27. Li Y, Zhang T, Tian W, Hu H, Xin Z, Ma X, et al. Loss of TIMP3 expression induces inflammation, matrix degradation, and vascular ingrowth in nucleus pulposus: A new mechanism of intervertebral disc degeneration. FASEB J 2020; 34(4): 5483-98. doi: 10.1096/fj.201902364RR.
28. Gao D, Hao L, Zhao Z. Long non-coding RNA PART1 promotes intervertebral disc degeneration through regulating the miR-93/MMP2 pathway in nucleus pulposus cells. Int J Mol Med 2020; 46(1): 289-99. doi: 10.3892/ijmm.2020.4580.
29. He D, Zhou M, Bai Z, Wen Y, Shen J, Hu Z. Propionibacterium acnes induces intervertebral disc degeneration by promoting nucleus pulposus cell pyroptosis via NLRP3-dependent pathway. Biochem Biophys Res Commun 2020; 526(3): 772-9. doi: 10.1016/j.bbrc.2020.03.161.
30. Zhao K, An R, Xiang Q, Li G, Wang K, Song Y, et al. Acid-sensing ion channels regulate nucleus pulposus cell inflammation and pyroptosis via the NLRP3 inflammasome in intervertebral disc degeneration. Cell Prolif 2021; 54(1): e12941. doi: 10.1111/cpr.12941.
31. Scheele J, de Schepper EI, Van Meurs JB, Hofman A, Koes BW, Luijsterburg PA, et al. Association between spinal morning stiffness and lumbar disc degeneration: the Rotterdam Study. Osteoarthritis Cartilage 2012; 20(9): 982-7. doi: 10.1016/j.joca.2012.05.011.
32. Dudek M, Yang N, Ruckshanthi JP, Williams J, Borysiewicz E, Wang P, et al. The intervertebral disc contains intrinsic circadian clocks that are regulated by age and cytokines and linked to degeneration. Ann Rheum Dis 2017; 76(3): 576-84. doi: 10.1136/annrheumdis-2016-209428.
33. Zhu J, Zhang X, Gao W, Hu H, Wang X, Hao D. lncRNA/circRNA-miRNA-mRNA ceRNA network in lumbar intervertebral disc degeneration. Mol Med Rep 2019; 20(4): 3160-74. doi: 10.3892/mmr.2019.10569.
34. Wan ZY, Song F, Sun Z, Chen YF, Zhang WL, Samartzis D, et al. Aberrantly expressed long noncoding RNAs in human intervertebral disc degeneration: a microarray related study. Arthritis Res Ther 2014; 16(5): 465. doi: 10.1186/s13075-014-0465-5.
35. Chen Y, Ni H, Zhao Y, Chen K, Li M, Li C, et al. Potential role of lncRNAs in contributing to pathogenesis of intervertebral disc degeneration based on microarray data. Med Sci Monit 2015; 21: 3449-58. doi: 10.12659/msm.894638.
36. Li Z, Chen X, Xu D, Li S, Chan MT, Wu WK. Circular RNAs in nucleus pulposus cell function and intervertebral disc degeneration. Cell Prolif 2019; 52(6): e12704. doi: 10.1111/cpr.12704.
37. Wang C, Wang WJ, Yan YG, Xiang YX, Zhang J, Tang ZH, et al. MicroRNAs: New players in intervertebral disc degeneration. Clin Chim Acta 2015; 450: 333-41. doi: 10.1016/j.cca.2015.09.011.
38. Wang S, Sun J, Yang H, Zou W, Zheng B, Chen Y, et al. Profiling and bioinformatics analysis of differentially expressed circular RNAs in human intervertebral disc degeneration. Acta Biochim Biophys Sin (Shanghai) 2019; 51(6): 571-9. doi: 10.1093/abbs/gmz036.
39. Li Z, Ma J, Shen J, Chan MT, Wu WK, Wu Z. Differentially expressed circular RNAs in air pollution–exposed rat embryos. Environ Sci Pollut Res Int 2019; 26(33): 34421-9. doi: 10.1007/s11356-019-06489-w.
40. Liu R, Liu X, Bai X, Xiao C, Dong Y. Identification and characterization of circRNA in longissimus dorsi of different breeds of cattle. Front Genet 2020; 11: 565085. doi: 10.3389/fgene.2020.565085.
41. Ma C, Shi T, Qu Z, Zhang A, Wu Z, Zhao H, et al. CircRNA_ACAP2 suppresses EMT in head and neck squamous cell carcinoma by targeting the miR-21-5p/STAT3 signaling axis. Front Oncol 2020; 10: 583682. doi: 10.3389/fonc.2020.583682.
42. Li X, Gao F, Fan Y, Xie S, Li C, Meng L, et al. A novel identified circ-ANKHD1 targets the miR-27a-3p/SFRP1 signaling pathway and modulates the apoptosis of granulosa cells. Environ Sci Pollut Res Int 2021; 28(41): 57459-69. doi: 10.1007/s11356-021-14699-4.
43. Li S, Jiang C, Tan J, Zhou Q, Yin J, He Y. Sodium arsenite-mediated upregulation of circDHX34 promotes apoptosis in hormone-independent breast cancer cells by regulating apoptotic genes. Environ Sci Pollut Res Int 2022; 29(2): 2728-36. doi: 10.1007/s11356-021-15891-2.
44. Wang H, He P, Pan H, Long J, Wang J, Li Z, et al. Circular RNA circ-4099 is induced by TNF-α and regulates ECM synthesis by blocking miR-616-5p inhibition of Sox9 in intervertebral disc degeneration. Exp Mol Med 2018; 50(4): 1-14. doi: 10.1038/s12276-018-0056-7.
45. Song J, Wang HL, Song KH, Ding ZW, Wang HL, Ma XS, et al.

CircularRNA_104670 plays a critical role in intervertebral disc degeneration by functioning as a ceRNA. Exp Mol Med 2018; 50(8): 1-12. doi: 10.1038/s12276-018-0125-y.
46. Wang X, Wang B, Zou M, Li J, Lü G, Zhang Q, et al. CircSEMA4B targets miR-431 modulating IL-1β-induced degradative changes in nucleus pulposus cells in intervertebral disc degeneration via Wnt pathway. Biochim Biophys Acta Mol Basis Dis 2018; 1864(11): 3754-68. doi: 10.1016/j.bbadis.2018.08.033.
47. Rustenburg CM, Faraj SS, Ket JC, Emanuel KS, Smit TH. Prognostic factors in the progression of intervertebral disc degeneration: Which patient should be targeted with regenerative therapies? JOR Spine 2019; 2(3): e1063. doi: 10.1002/jsp2.1063.
48. Kubaszewski Ł, Zioła-Frankowska A, Frankowski M, Nowakowski A, Czabak-Garbacz R, Kaczmarczyk J, et al. Atomic absorption spectrometry analysis of trace elements in degenerated intervertebral disc tissue. Med Sci Monit 2014; 20: 2157-64. doi: 10.12659/MSM.890654.
49. Nowakowski A, Kubaszewski L, Frankowski M, Wilk-Franczuk M, Ziola-Frankowska A, Czabak-Garbacz R, et al. Analysis of trace element in intervertebral disc by atomic absorption spectrometry techniques in degenerative disc disease in the Polish population. Ann Agric Environ Med 2015; 22(2): 362-7. doi: 10.5604/12321966.1152096.
50. Barandun MA, Bult S, Demierre S, Vidondo B, Forterre F. Colder ambient temperatures influence acute onset canine intervertebral disc extrusion. Front Vet Sci 2020; 7: 175. doi: 10.3389/fvets.2020.00175.
51. Zhang S, Sun L, Zhang J, Liu S, Han J, Liu Y. Adverse impact of heavy metals on bone cells and bone metabolism dependently and independently through anemia. Adv Sci (Weinh) 2020; 7(19): 2000383. doi: 10.1002/advs.202000383.
52. Takatalo J, Karppinen J, Taimela S, Niinimäki J, Laitinen J, Blanco Sequeiros R, et al. Body mass index is associated with lumbar disc degeneration in young Finnish males: subsample of Northern Finland birth cohort study 1986. BMC Musculoskelet Disord 2013; 14:87. doi: 10.1186/1471-2474-14-87.
53. Samartzis D, Karppinen J, Mok F, Fong DY, Luk KD, Cheung KM. A population-based study of juvenile disc degeneration and its association with overweight and obesity, low back pain, and diminished functional status. J Bone Joint Surg Am 2011; 93(7): 662-70. doi: 10.2106/JBJS.I.01568.
54. Chaiwanichsiri D, Jiamworakul A, Jitapunkul S. Lumbar disc degeneration in Thai elderly: a population-based study. J Med Assoc Thai 2007; 90(11): 2477-81.
55. Wu D, Zheng C, Wu J, Huang R, Chen X, Zhang T, et al. Molecular biological effects of weightlessness and hypergravity on intervertebral disc degeneration. Aerosp Med Hum Perform 2017; 88(12): 1123-8. doi: 10.3357/AMHP.4872.2017.
56. Videman T, Battié MC, Parent E, Gibbons LE, Vainio P, Kaprio J. Progression and determinants of quantitative magnetic resonance imaging measures of lumbar disc degeneration: a five-year follow-up of adult male monozygotic twins. Spine (Phila Pa 1976) 2008; 33(13): 1484-90. doi: 10.1097/BRS.0b013e3181753bb1.
57. Yasuoka H, Asazuma T, Nakanishi K, Yoshihara Y, Sugihara A, Tomiya M, et al. Effects of reloading after simulated microgravity on proteoglycan metabolism in the nucleus pulposus and anulus fibrosus of the lumbar intervertebral disc: an experimental study using a rat tail suspension model. Spine (Phila Pa 1976) 2007; 32(25): E734-E40. doi: 10.1097/BRS.0b013e31815b7e51.
58. de Schepper EI, Damen J, van Meurs JB, Ginai AZ, Popham M, Hofman A, et al. The association between lumbar disc degeneration and low back pain: the influence of age, gender, and individual radiographic features. Spine (Phila Pa 1976) 2010; 35(5): 531-6. doi: 10.1097/BRS.0b013e3181aa5b33.
59. Hassett G, Hart DJ, Manek NJ, Doyle DV, Spector TD. Risk factors for progression of lumbar spine disc degeneration: the Chingford Study. Arthritis Rheum 2003; 48(11): 3112-7. doi: 10.1002/art.11321.
60. Hangai M, Kaneoka K, Kuno S, Hinotsu S, Sakane M, Mamizuka N, et al. Factors associated with lumbar intervertebral disc degeneration in the elderly. Spine J 2008; 8(5): 732-40. doi: 10.1016/j.spinee.2007.07.392.
61. Liuke M, Solovieva S, Lamminen A, Luoma K, Leino-Arjas P, Luukkonen R, et al. Disc degeneration of the lumbar spine in relation to overweight. Int J Obes (Lond) 2005; 29(8): 903-8. doi: 10.1038/sj.ijo.0802974.
62. Videman T, Battié MC, Ripatti S, Gill K, Manninen H, Kaprio J. Determinants of the progression in lumbar degeneration: a 5-year follow-up study of adult male monozygotic twins. Spine (Phila Pa 1976) 2006; 31(6): 671-8. doi: 10.1097/01.brs.0000202558.86309.ea.
63. Elfering A, Semmer N, Birkhofer D, Zanetti M, Hodler J, Boos N. Young investigator award 2001 winner: Risk factors for lumbar disc degeneration: A 5-year prospective MRI study in asymptomatic individuals. Spine (Phila Pa 1976) 2002; 27(2): 125-34. doi: 10.1097/00007632-200201150-00002.
64. Farshad-Amacker NA, Hughes AP, Aichmair A, Herzog RJ, Farshad M. Is an annular tear a predictor for accelerated disc degeneration? Eur Spine J 2014; 23(9): 1825-9. doi: 10.1007/s00586-014-3260-8.
65. Williams FM, Popham M, Sambrook PN, Jones AF, Spector TD, MacGregor AJ. Progression of lumbar disc degeneration over a decade: a heritability study. Ann Rheum Dis 2011; 70(7): 1203-7. doi: 10.1136/ard.2010.146001.
66. Somovilla-Gómez F, Lostado-Lorza R, Corral-Bobadilla M, Escribano-García R. Improvement in determining the risk of damage to the human lumbar functional spinal unit considering age, height, weight and sex using a combination of FEM and RSM. Biomech Model Mechanobiol 2020; 19(1): 351-87. doi: 10.1007/s10237-019-01215-4.
67. Silva MJ, Holguin N. Aging aggravates intervertebral disc degeneration by regulating transcription factors toward chondrogenesis. FASEB J 2020; 34(2): 1970-82. doi: 10.1096/fj.201902109R.
68. Ganson NJ, Kelly SJ, Scarlett E, Sundy JS, Hershfield MS. Control of hyperuricemia in subjects with refractory gout, and induction of antibody against poly (ethylene glycol) (PEG), in a phase I trial of subcutaneous PEGylated urate oxidase. Arthritis Res Ther 2006; 8(1): R12. doi: 10.1186/ar1861.
69. Molinos M, Cunha C, Almeida CR, Gonçalves RM, Pereira

P, Silva PS, et al. Age-correlated phenotypic alterations in cells isolated from human degenerated intervertebral discs with contained hernias. Spine (Phila Pa 1976) 2018; 43(5): E274-E84. doi: 10.1097/BRS.0000000000002311.
70. Sun K, Sun X, Sun J, Jiang Y, Lin F, Kong F, et al. Tissue renin-angiotensin system (tRAS) induce intervertebral disc degeneration by activating oxidative stress and inflammatory reaction. Oxid Med Cell Longev 2021; 2021: 3225439. doi: 10.1155/2021/3225439.
71. Lambrechts MJ, Pitchford C, Hogan D, Li J, Fogarty C, Rawat S, et al. Lumbar spine intervertebral disc desiccation is associated with medical comorbidities linked to systemic inflammation. Arch Orthop Trauma Surg 2021; 1-11. doi: 10.1007/s00402-021-04194-3.
72. Guo Y, Li C, Shen B, Zhu Z, Chen X, Hu T, et al. Is There Any Relationship between Plasma IL-6 and TNF-α Levels and Lumbar Disc Degeneration? A Retrospective Single-Center Study. Dis Markers 2022; 2022: 6842130. doi: 10.1155/2022/6842130.
73. Samartzis D, Karppinen J, Chan D, Luk KD, Cheung KM. The association of lumbar intervertebral disc degeneration on magnetic resonance imaging with body mass index in overweight and obese adults: a population-based study. Arthritis Rheum 2012; 64(5): 1488-96. doi: 10.1002/art.33462.
74. Makino H, Kawaguchi Y, Seki S, Nakano M, Yasuda T, Suzuki K, et al. Lumbar disc degeneration progression in young women in their 20's: a prospective ten-year follow up. J Orthop Sci 2017; 22(4): 635-40. doi: 10.1016/j.jos.2017.03.015.
75. Teraguchi M, Yoshimura N, Hashizume H, Yamada H, Oka H, Minamide A, et al. Progression, incidence, and risk factors for intervertebral disc degeneration in a longitudinal population-based cohort: the Wakayama Spine Study. Osteoarthritis Cartilage 2017; 25(7): 1122-31. doi: 10.1016/j.joca.2017.01.001.
76. Sharma A, Parsons M, Pilgram T. Temporal interactions of degenerative changes in individual components of the lumbar intervertebral discs: a sequential magnetic resonance imaging study in patients less than 40 years of age. Spine (Phila Pa 1976) 2011; 36(21): 1794-800. doi: 10.1097/BRS.0b013e31821590ad.
77. Farshad-Amacker NA, Hughes AP, Aichmair A, Herzog RJ, Farshad M. Determinants of evolution of endplate and disc degeneration in the lumbar spine: a multifactorial perspective. Eur Spine J 2014; 23(9): 1863-8. doi: 10.1007/s00586-014-3382-z.
78. Nagashima M, Abe H, Amaya K, Matsumoto H, Yanaihara H, Nishiwaki Y, et al. Risk factors for lumbar disc degeneration in high school American football players: a prospective 2-year follow-up study. Am J Sports Med 2013; 41(9): 2059-64. doi: 10.1177/0363546513495173.
79. Burnett AF, Khangure MS, Elliott BC, Foster DH, Marshall RN, Hardcastle PH. Thoracolumbar disc degeneration in young fast bowlers in cricket: a follow-up study. Clin Biomech (Bristol, Avon) 1996; 11(6): 305-10. doi: 10.1016/0268-0033(96)00007-1.
80. Luoma K, Riihimäki H, Luukkonen R, Raininko R, Viikari-Juntura E, Lamminen A. Low back pain in relation to lumbar disc degeneration. Spine (Phila Pa 1976) 2000; 25(4): 487-92. doi: 10.1097/00007632-200002150-00016.
81. Setton LA, Chen J. Cell mechanics and mechanobiology in the intervertebral disc. Spine (Phila Pa 1976) 2004; 29(23): 2710-23. doi: 10.1097/01.brs.0000146050.57722.2a.
82. Roughley PJ. Biology of intervertebral disc aging and degeneration: involvement of the extracellular matrix. Spine (Phila Pa 1976) 2004; 29(23): 2691-9. doi: 10.1097/01.brs.0000146101.53784.b1.
83. Loreto C, Musumeci G, Castorina A, Loreto C, Martinez G. Degenerative disc disease of herniated intervertebral discs is associated with extracellular matrix remodeling, vimentin-positive cells and cell death. Ann Anat 2011; 193(2): 156-62. doi: 10.1016/j.aanat.2010.12.001.
84. Li Z, Yu X, Shen J, Chan MT, Wu WKK. Micro RNA in intervertebral disc degeneration. Cell prolif 2015; 48(3): 278-83. doi: 10.1111/cpr.12180.
85. Li Z, Li X, Chen C, Li S, Shen J, Tse G, et al. Long non-coding RNA s in nucleus pulposus cell function and intervertebral disc degeneration. Cell prolif 2018; 51(5): e12483. doi: 10.1111/cpr.12468.
86. Meng GD, Xu BS. Circular RNA hsa_circ_0001658 Inhibits Intervertebral Disc Degeneration Development by Regulating hsa-miR-181c-5p/FAS. Comput Math Methods Med 2021; 2021: 7853335. doi: 10.1155/2021/7853335.
87. Tang J, Zhang C, Wang S, Chen J. A novel circRNA–miRNA–mRNA network reveals hsa-circ-0040039 as a biomarker for intervertebral disc degeneration. J Int Med Res 2021; 49(12): 0300060520960983. doi: 10.1177/0300060520960983.
88. Lan PH, Liu ZH, Pei YJ, Wu ZG, Yu Y, Yang YF, et al. Landscape of RNAs in human lumbar disc degeneration. Oncotarget 2016; 7(39): 63166-76. doi: 10.18632/oncotarget.11334.
89. Wang J, Xia D, Lin Y, Xu W, Wu Y, Chen J, et al. Oxidative stress-induced circKIF18A downregulation impairs MCM7-mediated anti-senescence in intervertebral disc degeneration. Exp Mol Med 2022; 54(3): 285-97. doi: 10.1038/s12276-022-00732-0.
90. Hansen TB, Jensen TI, Clausen BH, Bramsen JB, Finsen B, Damgaard CK, et al. Natural RNA circles function as efficient microRNA sponges. Nature 2013; 495(7441): 384-8. doi: 10.1038/nature11993.
91. Taheri Bajgan E, Gholipour A, Faghihi M, Mowla SJ, Malakootian M. Linc-ROR has a Potential ceRNA Activity for OCT4A by Sequestering miR-335-5p in the HEK293T Cell Line. Biochem Genet 2021; 60(3):1007-1024. doi: 10.1007/s10528-021-10140-0.
92. Piwecka M, Glažar P, Hernandez-Miranda LR, Memczak S, Wolf SA, Rybak-Wolf A, et al. Loss of a mammalian circular RNA locus causes miRNA deregulation and affects brain function. Science 2017; 357(6357): eaam8526. doi: 10.1126/science.aam8526.
93. Memczak S, Jens M, Elefsinioti A, Torti F, Krueger J, Rybak A, et al. Circular RNAs are a large class of animal RNAs with regulatory potency. Nature 2013; 495(7441): 333-8. doi: 10.1038/nature11928.
94. Zheng Q, Bao C, Guo W, Li S, Chen J, Chen B, et al. Circular RNA profiling reveals an abundant circHIPK3 that regulates cell growth by sponging multiple miRNAs. Nat Commun 2016; 7:11215. doi: 10.1038/ncomms11215.
95. Hansen TB, Kjems J, Damgaard CK. Circular RNA and

miR-7 in cancer. Cancer Res 2013; 73(18): 5609-12. doi: 10.1158/0008-5472.CAN-13-1568.
96. Cheng X, Zhang L, Zhang K, Zhang G, Hu Y, Sun X, et al. Circular RNA VMA21 protects against intervertebral disc degeneration through targeting miR-200c and X linked inhibitor-of-apoptosis protein. Ann Rheum Dis 2018; 77(5): 770-9. doi: 10.1136/annrheumdis-2017-212056.
97. Song J, Chen ZH, Zheng CJ, Song KH, Xu GY, Xu S, et al. Exosome-transported circRNA_0000253 competitively adsorbs microRNA-141-5p and increases IDD. Mol Ther Nucleic Acids 2020; 21:1087-99. doi: 10.1016/j.omtn.2020.07.039.
98. Kakiuchi Y, Yurube T, Kakutani K, Takada T, Ito M, Takeoka Y, et al. Pharmacological inhibition of mTORC1 but not mTORC2 protects against human disc cellular apoptosis, senescence, and extracellular matrix catabolism through Akt and autophagy induction. Osteoarthritis Cartilage 2019; 27(6): 965-76. doi: 10.1016/j.joca.2017.08.019.
99. Yurube T, Buchser WJ, Moon HJ, Hartman RA, Takayama K, Kawakami Y, et al. Serum and nutrient deprivation increase autophagic flux in intervertebral disc annulus fibrosus cells: an in vitro experimental study. Eur Spine J 2019; 28(5): 993-1004. doi: 10.1007/s00586-019-05910-9.
100. Zhang TW, Li ZF, Dong J, Jiang LB. The circadian rhythm in intervertebral disc degeneration: an autophagy connection. Exp Mol Med 2020; 52(1): 31-40. doi: 10.1038/s12276-019-0372-6.
101. Gruber HE, Hoelscher GL, Ingram JA, Bethea S, Hanley EN Jr. Autophagy in the Degenerating Human Intervertebral Disc: In Vivo: Molecular and Morphological Evidence, and Induction of Autophagy in Cultured Annulus Cells Exposed to Proinflammatory Cytokines—Implications for Disc Degeneration. Spine (Phila Pa 1976) 2015; 40(11): 773-82. doi: 10.1097/BRS.0000000000000865.
102. Bodak M, Cirera-Salinas D, Luitz J, Ciaudo C. The role of RNA interference in stem cell biology: beyond the mutant phenotypes. J Mol Biol 2017; 429(10): 1532-43. doi: 10.1016/j.jmb.2017.01.014.
103. Treiber T, Treiber N, Meister G. Regulation of microRNA biogenesis and its crosstalk with other cellular pathways. Nat Rev Mol Cell Biol 2019; 20(1): 5-20. doi: 10.1038/s41580-018-0059-1.
104. Sekiya I, Tsuji K, Koopman P, Watanabe H, Yamada Y, Shinomiya K, et al. SOX9 enhances aggrecan gene promoter/enhancer activity and is up-regulated by retinoic acid in a cartilage-derived cell line, TC6. J Biol Chem 2000; 275(15): 10738-44. doi: 10.1074/jbc.275.15.10738.
105. Pan T, Sun X, Liu Y, Li H, Deng G, Lin H, et al. Heat stress alters genome-wide profiles of circular RNAs in Arabidopsis. Plant Mol Biol 2018; 96(3): 217-29. doi: 10.1007/s11103-017-0684-7.
106. Braicu C, Zimta AA, Gulei D, Olariu A, Berindan-Neagoe I. Comprehensive analysis of circular RNAs in pathological states: biogenesis, cellular regulation, and therapeutic relevance. Cell Mol Life Sci 2019; 76(8): 1559-77. doi: 10.1007/s00018-019-03016-5.
107. Morales ME, Derbes RS, Ade CM, Ortego JC, Stark J, Deininger PL, et al. Heavy metal exposure influences double strand break DNA repair outcomes. PloS One 2016; 11(3): e0151367. doi: 10.1371/journal.pone.0151367.