Cell Proliferation

Cell Prolif., 2015, **48**, 278–283 doi: 10.1111/cpr.12180

MicroRNA in intervertebral disc degeneration

Zheng Li*, Xin Yu*, Jianxiong Shen*, Matthew T.V. Chan† and William Ka Kei Wu†·‡

**Department of Orthopaedic Surgery, Peking Union Medical College Hospital, Peking Union Medical College, Beijing 100007, China, †Department of Anaesthesia and Intensive Care, The Chinese University of Hong Kong, Hong Kong 999077, China and ‡State-Key Laboratory of Digestive Disease and Institute of Digestive Disease, LKS Institute of Health Science, The Chinese University of Hong Kong, Hong Kong 999077, China*

Received 23 September 2014; revision accepted 22 November 2014

Abstract

Aetiology of intervertebral disc degeneration (IDD) is complex, with genetic, developmental, biochemical and biomechanical factors contributing to the disease process. It is becoming obvious that epigenetic processes influence evolution of IDD as strongly as the genetic background. Deregulated phenotypes of nucleus pulposus cells, including differentiation, migration, proliferation and apoptosis, are involved in all stages of progression of human IDD. Non-coding RNAs, including microRNAs, have recently been recognized as important regulators of gene expression. Research into roles of microRNAs in IDD has been very active over the past 5 years. Our review summarizes current research enlightenment towards understanding roles of microRNAs in regulating nucleus pulposus cell functions in IDD. These exciting findings support the notion that specific modulation of microRNAs may represent an attractive approach for management of IDD.

Introduction

Intervertebral disc degeneration (IDD) is one of the major causes of low back pain, which compose a global burden with severe health care and socioeconomic costs (1–3). Even though IDD treatment has advanced remarkably over the last decade, many patients still do not achieve sustained remission, constituting a major and unmet clinical need (4). Aetiology of IDD is multifactorial, including genetic predisposition, lifestyles (for example, type of occupation, smoking, alcohol consumption) and aging (1,5–7). Underlying IDD molecular and cellular mechanisms are still largely unknown. Thus, increasing numbers of studies support the observation that nucleus pulposus (NP) cells are important in maintaining integrity of intervertebral discs (IVD) by their roles in producing type II collagen, aggrecan and other components of the extracellularmatrix (ECM) (8–10). There is a growing body of evidence to support that microRNA (miRNA) can regulate many, if not all, aspects of cell activity, from differentiation and proliferation to apoptosis, to mediate their effects on a great range of physiological and pathological processes (11–14). In this review, we summarize the current knowledge on roles of miRNAs in regulation of NP cell functions, including differentiation, proliferation, ECM synthesis, apoptosis, and the possible implications for IDD.

Correspondence: Jianxiong Shen, Department of Orthopaedic Surgery, Peking Union Medical College Hospital, Peking Union Medical College, Beijing 100007, China. Tel.: 86-010-69152812; Fax: 86-010-69152812; E-mail: shenjianxiong@medmail.com.cn
Xin Yu and Zheng Li contributed equally to this work.

MicroRNA biogenesis and function

Over the last decade it has become better recognized that small ribonucleic acids (RNAs) are important components of gene regulatory networks (15–17). Among these, miRNAs are a class of small molecules and non-coding single-stranded RNAs of 18–22 nucleotides, that act as post-transcriptional gene regulatory elements (18–20). Currently, miRNAs have been found in virtually all species of animals, plants and viruses examined; there are over 2042 mature human miRNA sequences listed in the miRNA registry (21,22). It is estimated that there are approximately 1500 predicted miRNAs in the human genome that have the potential to regulate at least 20–30% of all human genes (23,24).

MiRNAs are transcribed from their respective gene loci as primary miRNAs (pri-miRNA) (25) and their biogenesis consists of a series of maturation steps (26). They can be derived from two sources: (i) Pri-miRNAs can be transcribed from specific miRNA-encoding

regions of the genome (intergenic miRNA) by RNA polymerase II (27,28). These pri-miRNAs are then cleaved in the nucleus by a multiprotein complex, which contains an anchor protein DGCR8 (DiGeorge syndrome critical region gene 8), giving rise to 70-nucleotide pre-miRNAs (29,30). (ii) Pri-miRNAs can be derived from mRNA intronic sequences (mirtrons), whose maturation does not require Drosha/DGCR8 processing. These miRNAs are debranched and spliced by lariat debranching enzyme (Ldbr) and are co-transcribed with host protein-coding genes, forming pre-miRNA hairpins as above (31,32). Intriguingly, these intronic miRNAs are usually involved in the same biological pathways as host protein-coding genes (33).

MiRNAs inhibit gene expression by selectively binding to complementary 3′untranslated regions (3′UTRs) of target mRNAs through complementary base pairing (34,35). Most identified miRNAs are highly conserved among species (36). Expression of miRNAs has both spatial and temporal specificity, together with tissue and cell type specificity (37). They play crucial roles in diverse pathological conditions, such as in cancer, neurodegeneration and aging (38–40). Recent findings have revealed that miRNAs can be involved in cell proliferation, differentiation and apoptosis, and are thus involved in broader processes, such as animal development, homeostasis and bone metabolism (41–45). Recent progress in biology has shown that miRNAs are dysregulated in different cancer types, including gastric cancer, breast cancer, osteosarcoma, lung cancer and hepatocellular carcinoma (46–50). Thus, miRNAs have considerable potential to become a research focus for prevention and treatment of IDD, specially for targeting IDD-related cell processes, such as NP cell proliferation and apoptosis.

MiRNAs in intervertebral disc degeneration

MiRNAs and NP cell apoptosis

NP cell death mediated by apoptosis is involved in various deleterious consequences of IDD, such as inflammation, degeneration and ECM degradation (51). NP cell apoptosis may have dual effects on IDD depending on the extent of the cell death (4,52). On the one hand, NP cell apoptosis can offset effects of aberrant NP cell proliferation during IDD (53). On the other hand, apoptosis can be harmful as NP cells produce interstitial collagen fibres, which are critical for maintaining tensile strength of the fibrous cap (54). Thus, apoptosis of NP cells plays an important role in determining their stability.

MiR-155 is one of the well-documented miRNAs involved in regulation of apoptotic pathways and immunological responses (55). Its aberrant expression has been found to be associated with various diseases, such as lung, gastric, pancreatic and colon cancers (56–59). Wang *et al.* have shown that 29 miRNAs exhibit significantly differential expression in degenerative NP cells, of which miR-155 was found to be one of the most down-regulated (60). Overexpression of miR-155 can inhibit NP cell apoptosis by repressing FADD and caspase-3 expression. *In situ* hybridization and immunohistochemistry have further revealed that miR-155, when expressed in cytoplasm of human NP cells is inversely correlated with FADD and caspase-3.

MiR-27a is a further well-studied miRNA expressed in diverse tissue types. Its aberrant expression is found to be associated with several diseases, including (once again) colorectal, bladder and gastric cancers (61–63). Moreover, previous studies have shown that miR-27a is involved in cell proliferation, apoptosis and tumourigenesis (62,64). It has been found that it affects apoptotic signalling pathways in initiation and progression of gliomas. Using real-time RT-PCR, Liu *et al.* (65) discovered that expression of miR-27a is high in degenerative NP cells. Furthermore, its enforced expression inhibits phosphoinositide-3 kinases (PI3K) expression by directly targeting its 3′-UTR, and this inhibition is abolished by mutation of miR-27a binding sites. In short, up-regulation of miR-27a initiates apoptosis of NP cells by targeting PI3K.

MiRNAs and NP cell proliferation

Increasing numbers of studies have demonstrated that formation of NP cell clusters and aberrant NP cell proliferation play a crucial role in IDD (8). More evidence has shown that miRNAs play an important role in control of NP cell proliferation by post-transcriptional regulation of a number of genes (66).

Previous investigations revealed that miR-10b is involved in regulation of cell proliferation in various cell types, especially in cancer cells, such as breast, liver, pancreatic and gastric cancers (67–70). It is also deregulated in these cancers and its levels are closely associated with tumour progression and pathological grade (71,72). In addition, our previous results have shown that miR-10b is significantly down-regulated in gastric cancer cell lines and tissues as demonstrated by quantitative real-time PCR. Overexpression of miR-10b in MGC-803 and HGC-27 cells dramatically suppressed cell proliferation, migration and invasion and induced apoptosis (11). Similar to its roles in cancer, miR-10b is up-regulated in degenerative NP tissues and is significantly associated with disc degeneration grade. Moreover, overexpression of miR-10b significantly increased

NP cell proliferation. In addition, miR-10b promoted proliferation of NP cells by directly targeting HOXD10. MiR-10b also induces Akt phosphorylation in a RhoC-dependent manner (73).

MiR-21, a well-known miRNA, is most frequently dysregulated in different types of human cancer, such as those of the breast, lung, liver and stomach, and has been shown to be implicated in multiple cell processes, including migration, differentiation, proliferation, apoptosis and invasion (74–77). Liu *et al.* reported that miR-21 was up-regulated in human degenerative NP tissues compared to normal NP (78). Moreover, enforced expression of miR-21 promotes NP cell proliferation. In this regard, overexpression of miR-21 led to increased phosphorylation of Akt by directly targeting PTEN. Furthermore, effects of miR-21 on NP cell proliferation and cyclin D1 induction in human NP cells can be blocked by Ly294002, an AKT inhibitor.

miRNAs and ECM remodelling of NP cells

The disc is a complex structure highly specialized for mechanical functions such as spine connectivity, flexure, rotation and extension (79). Currently available evidence implicates loss of IVD ECM upon IDD, as a major cause of low back pain (80). During degeneration, IVD matrix undergoes structural, mechanical and molecular changes, which result in loss of demarcation between the outer annulus fibrosus and inner NP tissues (81,82). ECM is constantly synthesized and degraded by disc cells, during which rates of the processes are normally in balance (83). However, this balance becomes shifted towards degradation in IDD, with alterations in collagen type and reduction in proteoglycan content, leading to loss of tissue integrity (84). Understanding which factors affect ECM changes is important to fully comprehend the possible implications for IDD.

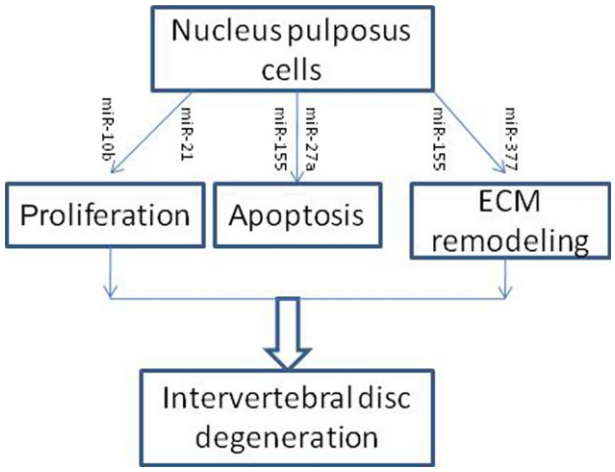

Figure 1. MiRNAs in intervertebral disc degeneration. MiR-10b and miR-21 induce nucleus pulposus (NP) cell proliferation; miR-155 and miR-27a inhibit NP cell apoptosis; miR-155 and miR-377 are involved in NP cell ECM remodelling. ECM, extracellularmatrix.

Table 1. The expression and function of miRNAs in intervertebral disc degeneration

miRNA	Expression	Function	Target	References
miR-155	Decrease	Apoptosis	FADD, caspase-3	(60)
miR-27a	Increase	Apoptosis	PI3K	(65)
miR-10b	Increase	Proliferation	HOXD10	(73)
miR-21	Increase	Proliferation	PTEN	(78)
miR-155	Decrease	ECM remodelling	Collagen I and III	(85)
miR-377	Increase	ECM remodelling	ADAMTS5	(86)

ECM, extracellularmatrix.

Chen *et al.* found that ligamentum flavum (LF) thickness and expression of collagens I and III, as well as miR-155, were higher in LF from lumbar spinal stenosis (LSS) patients than from lumbar disc herniation (LDH) patients (85). To this end, expression level of miR-155 positively correlated with LF thickness and levels of types I and III collagen. Overexpression of miR-155 increased mRNA and protein expression of collagens I and III in fibroblasts isolated from LF, while down-regulated expression of miR-155 had opposite effects.

Protein kinase C (PKC) signalling, a major regulator of chondrogenic differentiation, has also been implicated in pathological ECM remodelling. Tsirimonaki *et al.* demonstrated that PKCε activation induced up-regulation of miR-377, which was coupled to reductions in ADAMTS5 and cleaved aggrecan (86).

Conclusion

In this review, we summarized roles of miRNAs in function of NP cells and their contributions to IDD (Fig. 1 and Table 1); miRNAs critically affect them. As the aetiology of IDD is multifactorial, these data should improve our insights into pathogenesis of IDD and inform relationships between genetic predisposition and risk factor exposure. Taken together, miRNAs definitively represent an emerging area of research that will provide new insight into IDD pathogenesis with the hope of bringing about novel drug candidates and biomarkers.

Acknowledgements

Funding: This work was supported by the National Natural Science Foundation of China (NSFC) (Grant Numbers: 81401847, 81272053 and 81330044).

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