**INTRODUCTION**

Low back pain is an extremely common condition with an estimated global point prevalence of ~10%, causing distress and economic losses due to pain, activity limitation and work absence. It also ranks highest in terms of overall disability among 291 conditions studied in the Global Burden of Disease 2010 Study. Intervertebral disc degeneration (IDD) of the lumbar spine, which is characterized by progressive structural failure and advanced signs of ageing of the intervertebral disc, is strongly associated with an increased risk for low back pain. Both genetic (e.g., polymorphisms in genes encoding collagen I, IX, XI and aggrecan) and lifestyle (e.g., the lack of sports activities and night shift work) factors have been linked to IDD development. However, the exact cellular and molecular mechanisms underlying IDD remain largely elusive. Anatomically, the intervertebral disc consists of a gelatinous core known as nucleus pulposus (NP) surrounded by a lamella of fibrous cartilage termed annulus fibrosus. NP cells play a crucial role in maintaining the...
TABLE 1  CircRNA expression profiles in intervertebral disc degeneration

| No. | Methods       | Samples            | Microarray filtering criteria | Upregulated | Downregulated | References |
|-----|---------------|--------------------|-------------------------------|-------------|---------------|------------|
| 1   | Microarray RT-PCR | degenerate disc tissues | fold change more than two and P values < .05 | 354 circRNAs | 282 circRNAs | 31         |
| 2   | Microarray RT-PCR | degenerate disc tissues | P < .05; Fold change >2      | 3724 circRNAs | 3570 circRNAs | 34         |
| 3   | Microarray RT-PCR | degenerate disc tissues | P < .05; Fold change >1.5    | 428 circRNAs | 364 circRNAs | 35         |
| 4   | Microarray RT-PCR | degenerate disc tissues | Fold change >2              | 51 circRNAs | 21 circRNAs | 36         |

This review serves to provide an overview of the current understanding regarding the functional roles of deregulated circRNAs in modulating phenotypes that are pertinent to IDD pathogenesis, including NP cell proliferation, apoptosis, ECM synthesis/degradation and pro-inflammatory cytokine production. We also discussed the translational value of circRNAs in terms of their clinical utilities as therapeutic targets for the management of IDD.

Circular RNAs expression profiling with transcriptome sequencing, microarray or PCR array followed by validation with reverse transcription-quantitative PCR (RT-qPCR) is the most frequently adopted approach to identify and confirm the differential expression of circRNAs in specific disease states. Profiling studies of circRNAs in IDD are listed in Table 1.

Microarray-based profiling of circRNAs deregulated in IDD was first performed by Liu and colleagues in which they conducted a comprehensive profiling of mRNAs, miRNAs, lncRNAs and circRNAs in five normal discs from cadaveric donors versus five degenerative discs from patients with IDD. The microarray data were then deposited in a public database. Among 2894 annotated circRNAs, 636 circRNAs were found to be differentially expressed (354 upregulated and 282 downregulated) in the degenerated discs as compared with normal control discs. Notably, a single miRNA was predicted to interact with a multitude of circRNAs. The upregulation of circRNA-101852 and downregulation of circRNA-101645 were confirmed by RT-qPCR. Zou and colleagues also utilized the same microarray datasets and identified a total of 76 pairs of differentially expressed circRNAs and their host genes in IDD. Pathway analysis revealed that host genes that encode the upregulated and downregulated circRNAs in IDD are involved in signalling pathways such as Wnt and integrin signalling. Significant upregulation of circ_0008305 and downregulation of circ_0041946 were confirmed in an independent set of human lumbar NP specimens by RT-qPCR. Another follow-up bioinformatic study by Zhang and colleagues using these deposited datasets identified 568 mRNAs, 55 miRNAs, 765 lncRNAs and 586 circRNAs that were significantly differentially expressed in degenerative discs than in normal discs. The authors then reconstructed the ceRNA networks in which three circRNAs, namely circ_0005139, circ_0037858 and circ_0087890, were predicted to be key regulators in IDD progression. The differentially expressed circRNA circ_0000189 was also predicted to play a central role and have crosstalk with miR-486-5p, the lnRNA DANCNR and 6 mRNAs—PYCR2 (Pyrroline-5-Carboxylate Reductase 2), TOB1 (Transducer of ERBB2-1), ARHGAP5 (Rho...
3 | FUNCTIONAL ROLES OF SPECIFIC CIRC RNAS IN IDD

3.1 | CircSEMA4B

CircSEMA4B (circRNA Semaphorin 4B) was the top downregulated circRNA in human lumbar IDD specimens in Liu et al’s\textsuperscript{30} circRNA microarray dataset. Wang et al\textsuperscript{36} further verified circSEMA4B downregulation in an independent set of lumbar IDD samples and correlated its levels with pro-inflammatory cytokine and ECM component expression. They found that circSEMA4B levels were negatively correlated with interleukin (IL)-1β and tumour necrosis factor (TNF)-α but positively with aggrecan and collagen II mRNA expression. In cultured NP cells, IL-1β downregulated circSEMA4B expression, suggesting that IL-1β was upstream of circSEMA4B. Functionally, IL-1β lowered collagen II and aggrecan expression, reduced the viability and induced the senescence of NP cells, all of which were reversed by the enforced expression of circSEMA4B. Consistently, knockdown of circSEMA4B aggravated these pathogenic phenotypes in the presence of IL-1β. Mechanistically, the protective phenotypes produced by enforced expression of circSEMA4B disappeared upon pharmacological Wnt signalling activation. Concordantly, circSEMA4B was found to function as a molecular sponge for reducing the availability of miR-431 to derepress the expression of glycogen synthase kinase (GSK)-3β and secreted frizzled-related protein 1 (SFRP1),\textsuperscript{26} both of which are known upstream repressors of the Wnt pathway.\textsuperscript{30,41} These findings collectively indicate that circSEMA4B could antagonize the pro-IDD Wnt signalling through the miR-431-GSK-3β/SFRP1 axis in NP cells. Restoring circSEMA4B expression or antagonizing miR-431 might thus represent a novel therapeutic approach for rectifying IL-1β-triggered degenerative processes.

3.2 | CircRNA_104670

Song et al\textsuperscript{35} performed circRNA microarray and identified circRNA_104670 as one of the highest upregulated circRNA in degenerative NP tissues. Bioinformatic reconstruction of the regulatory network of circRNA_104670 revealed that this circRNA might act through the miR-17-3p-matrix metalloproteinase (MMP)-2 axis in NP cells. Consistently, upregulation of circRNA_104670 and downregulation of miR-17-3p in degenerative NP tissues were confirmed by RT-qPCR and were found to be associated with Pfirrmann scores (a magnetic resonance imaging-based disc degeneration grading system). Luciferase and EGF/RFP reporter assays further verified circRNA_104670 as a sponge for miR-17-3p to upregulate MMP-2 (a collagen-degrading MMP). Functionally, circRNA_104670 promoted apoptosis and inhibited proliferation of NP cells and repressed collagen II expression. Importantly, the pro-IDD effect of circRNA_104670 was confirmed in vivo in which mice injected with small interfering-RNA (siRNA) against circRNA_104670 delivered via adeno-associated virus exhibited lower IDD grades. The in vivo protective action of circRNA_104670 knockdown was also attenuated upon miR-17-3p inhibition.\textsuperscript{35} These findings suggest that circRNA_104670 contributes to IDD development through impairing NP cell survival and shifting the balance towards ECM degradation via sponging miR-17-3p.
3.3 | Circ-4099

Circ-4099, located on exonic chromosome 11 and aligned in the sense direction of the protein-coding gene DENND5A (DENN domain containing 5A), was one of the top upregulated circRNAs in Wang et al’s microarray study. RT-qPCR and luciferase reporter assay further revealed that circ-4099 transcription could be induced by TNF-α in human NP cells, where such induction was blocked by mitogen-activated protein kinase (MAPK) and nuclear factor (NF)-κB pathway inhibitors. Enforced expression of circ-4099 also enhanced aggrecan and collagen II but reduced TNF-α and IL-1β expression in NP cells. Mechanistically, circ-4099 serves as a sponge for miR-616-5p as confirmed by immunoprecipitation for AGO2 (Argonaute RNA-Induced Silencing Complex [RISC] Catalytic Component 2) and RNA–RNA pull-down assay. The upregulation of aggrecan and collagen II and downregulation of TNF-α and IL-1β were also reversed by miR-616-5p mimics. Sox9 (SRY-Box 9), a transcription factor that promotes the expression of chondrocyte-specific genes including aggrecan and collagen II, was confirmed to be the direct target of miR-616-5p. These findings suggest that upregulation of circ-4099 in NP cells could act as an inflammation-responsive autoregulatory protective mechanism against IDD development via modulating the miR-616-5p-Sox9 pathway.

3.4 | Circ-GRB10

Guo and colleagues reanalysed Liu et al’s microarray datasets and showed that circ-GRB10 (Growth Factor Receptor-Bound Protein...
10) and miR-328-5p were one of the most significantly negatively correlated circRNA-miRNA pairs.\(^4\) By RT-qPCR, the investigators verified the significant downregulation of circ-GRB10 and upregulation of miR-328-5p as well as their negative correlation in an independent set of IDD NP samples. Functionally, enforced expression of circ-GRB10 suppressed NP cell apoptosis whereas knockdown of circ-GRB10 produced the opposite the effect, suggesting that the aberrant downregulation of circ-GRB10 in NP tissues might contribute to IDD through impairing NP cell survival. Mechanistically, reduced circ-GRB10 levels were found to increase the availability of miR-328-5p and thereby repressing ERBB2 (v-erb-b2 erythroblastic leukaemia viral oncogene homolog 2; a target of miR-328-5p). These data suggested that deregulation of circ-GRB10/miR-328-5p/ERBB2 pathway was involved in IDD development.\(^4\) Restoring the expression of circ-GRB10 or enhancing the ERBB2 signalling might thus serve a new therapeutic approach for the IDD.

### 3.5 | CircVMA21

Cheng et al.\(^45\) showed that miR-200c was overexpressed in human IDD NP samples and inducible by TNF-\(\alpha\) plus IL-1\(\beta\) in cultured NP cells where enforced expression of miR-200c promoted apoptosis and shifted ECM homeostasis from anabolism towards catabolism (ie reduced aggrecan and collagen II but enhanced ADAMTS (A Disintegrin and Metalloproteinase with Thrombospondin motifs)-4, ADAMTS-5, MMP-13 and MMP-3 expression). miR-200c mediated these biological functions in NP cells via suppressing the expression of X linked inhibitor of apoptosis protein (XIAP). In this regard, circVMA21 (circRNA Vacuolar ATPase Assembly Factor VMA21) was found to be downregulated in IDD NP tissues, where it served as a sponge for miR-200c and restrained apoptosis and ECM anabolism/catabolism imbalance induced by TNF-\(\alpha\) and IL-1\(\beta\). Importantly, the authors showed that intradiscal injection of adenoviral circVMA21 alleviated IDD in rats. These data demonstrated that aberrant downregulation of circVMA21 contributed to IDD phenotypes via the miR-200c/XIAP axis. Restoring circVMA21 expression might thus be a viable approach for attenuating NP cell apoptosis and reversing ECM anabolism/catabolism imbalance against IDD development.

### 4 | CONCLUSION AND FUTURE PERSPECTIVES

Intervertebral disc degeneration is a common cause of low back pain which is a serious public health issue. Previous studies have shown that aberrant NP cell functions, including proliferation, apoptosis, senescence, ECM deposition/degradation and cytokine secretion, are causally involved in IDD pathogenesis. Recently, it was shown that two classes of ncRNA, namely miRNAs and IncRNAs,\(^11,12\) play key roles in modulating NP cell phenotypes during IDD development. Emerging evidence, as summarized by our review, also indicated that deregulation of circRNAs, a newly reported class of ncRNA, was involved in IDD development (Table 2). Deregulation of these circRNAs (eg circSEMA4B, circRNA_104670, circ-4099, circ-GRB10 and circVMA21) modulates the aforementioned NP cell phenotypes through sponging their target miRNAs and thereby repressing or derepressing the corresponding downstream mRNAs (Figure 1). Treatment for IDD might thus be achieved through restoring the expression of downregulated circRNAs or silencing of the aberrantly upregulated circRNAs. Nevertheless, the best way to achieve NP cell-specific delivery of circRNA-based therapeutics remains undefined. The relative importance of different classes of ncRNAs (ie miRNAs, IncRNAs and circRNAs) in IDD development is also unclear, rendering prioritization and selection of therapeutic targets difficult. Aside from therapy, early detection and prognostication of IDD are clinically challenging. Due to the tissue- and development stage-specific expression pattern of circRNAs, their use as biomarkers has been promulgated. In this connection, the use of circulating or tissue circRNAs for diagnosing or prognosticating diseases, including cancers, cardiovascular diseases, diabetes, autoimmune diseases and infections, has been demonstrated.\(^46\) However, such endeavours have not been attempted in IDD. More translational work, for example, systematic identification and multi-cohort validation of circulating circRNAs associated with disease status, clinical outcomes and treatment responses in IDD patients, is thus required to maximize the clinical utilities of circRNAs for the management of IDD.

### DATA AVAILABILITY STATEMENT

Research data are not shared.

### ORCID

Zheng Li [https://orcid.org/0000-0001-6024-0194](https://orcid.org/0000-0001-6024-0194)

Shuang Li [https://orcid.org/0000-0002-1737-9796](https://orcid.org/0000-0002-1737-9796)

### REFERENCES

1. Wynne-Jones G, Cowen J, Jordan JL, et al. Absence from work and return to work in people with back pain: a systematic review and meta-analysis. Occup Environ Med. 2014;71(6):448-456.

2. Croft PR, Papageorgiou AC, Ferry S, Thomas E, Jayson MI, Silman AJ. Psychologic distress and low back pain. Evidence from a prospective study in the general population. Spine. 1995;20(24):2731-2737.

3. Hoy D, March L, Brooks P, et al. The global burden of low back pain: estimates from the global burden of disease 2010 study. Ann Rheum Dis. 2014;73(6):968-974.

4. Luoma K, Riihimäki H, Luukkonen R, Raininko R, Viikari-Juntura E, Lamminen A. Low back pain in relation to lumbar disc degeneration. Spine. 2000;25(4):487-492.

5. Feng Y, Egan B, Wang J. Genetic factors in intervertebral disc degeneration. Genes Dis. 2016;3(3):178-185.

6. Elfering A, Semmer N, Birkhofer D, Zanetti M, Hodler J, Boos N. Risk factors for lumbar disc degeneration: a 5-year prospective MRI study in asymptomatic individuals. Spine. 2002;27(2):125-134.

7. Yasuoka H, Asazuma T, Nakanishi K, 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. 2007;32(25):E734-E740.

8. Setton LA, Chen J. Cell mechanics and mechanobiology in the intervertebral disc. Spine. 2004;29(23):2710-2723.

9. Roughley PJ. Biology of intervertebral disc aging and degeneration: involvement of the extracellular matrix. Spine. 2004;29(23):2691-2699.

10. 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):156-162.

11. Li Z, Yu X, Shen J, Chan MT, Wu WK. MicroRNA in intervertebral disc degeneration. Cell Prolif. 2015;48(3):278-283.

12. Li Z, Li X, Chen C, et al. Long non-coding RNAs in nucleus pulposus cell function and intervertebral disc degeneration. Cell Prolif. 2018;51(5):e12483.

13. Beermann J, Piccoli MT, Vierreck J, Thum T. Non-coding RNAs in development and disease: background, mechanisms, and therapeutic approaches. Physiol Rev. 2016;96(4):1297-1325.

14. Yao RW, Wang Y, Chen LL. Cellular functions of long noncoding RNAs. Nat Cell Biol. 2019;21(5):542-551.

15. Barrett SP, Salzman J. Circular RNAs: analysis, expression and potential functions. Development. 2016;143(11):1838-1847.

16. Arnaiz E, Sole C, Manterola L, Ipaaraguire L, Otaegui D, Lawrie CH. CircRNAs and cancer: biomarkers and master regulators. Semin Cancer Biol. 2019;58:90-99. https://doi.org/10.1016/j.semcancer.2018.12.002.

17. Xu S, Zhou L, Ponnsammy M, et al. A comprehensive review of circRNA: from purification and identification to disease marker potential. PeerJ. 2018;6:e5503.

18. Li Z, Huang C, Bao C, et al. Exon-intron circular RNAs regulate transcription in the nucleus. Nat Struct Mol Biol. 2015;22(3):256-264.

19. Yu CY, Kuo HC. The emerging roles and functions of circular RNAs and their generation. J Biomed Sci. 2019;26(1):29.

20. Vo JN, Cieslik M, Zhang Y, et al. The landscape of circular RNA in cancer. Cell. 2019;176(4):869-881.e13.

21. Akhter R. Circular RNA and Alzheimer’s disease. Adv Exp Med Biol. 2018;1087:239-243.

22. Chen X, Yang T, Wang W, et al. Circular RNAs in immune responses and immune diseases. Theranostics. 2019;9(2):588-607.

23. Chen C, Tan H, Bi J, et al. Identification of competing endogenous RNA regulatory networks in vitamin A deficiency-induced congenital scoliosis by transcriptome sequencing analysis. Cell Physiol Biochem. 2018;48(5):2134-2146.

24. Jin D, Wu X, Yu H, et al. Systematic analysis of IncRNAs, mRNAs, circRNAs and miRNAs in patients with postmenopausal osteoporosis. Am J Transl Res. 2018;10(5):1499-1510.

25. Li HZ, Lin Z, Xu XH, Lin N, Lu HD. The potential roles of circRNAs in osteoarthritis: a coming journey to find a treasure. Biosci Rep. 2018;38(5):BRS20180542.

26. Wang X, Wang B, Zou M, 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):3754-3768.

27. Jeck WR. Sharpless NE. Detecting and characterizing circular RNAs. Nat Biotechnol. 2014;32(5):453-461.

28. Panda AC, Gorospe M. Detection and analysis of circular RNAs by RT-PCR. Bio Protec. 2018;8(6):e2775.

29. Zhang YH, Song J, Shen L, Shao J. Systematic identification of IncRNAs and circRNAs-associated ceRNA networks in human lumbar disc degeneration. Biotech Histochem. 2019;41-11.

30. Liu X, Che L, Xie YK, et al. Noncoding RNAs in human intervertebral disc degeneration: an integrated microarray study. Genom Data. 2015;5:80-81.

31. Lan PH, Liu ZH, Pei YJ, et al. Landscape of RNAs in human lumbar disc degeneration. Oncotarget. 2016;7(39):63166-63176.

32. Zou F, Ding Z, Jiang J, Lu F, Xia X, Ma X. Confirmation and preliminary analysis of circRNAs potentially involved in human intervertebral disc degeneration. Mol Med Rep. 2017;16(6):9173-9180.

33. Zhu J, Zhang X, Gao W, Hu H, Wang X, Hao D. IncRNA/circRNA-miRNA-mRNA ceRNA network in lumbar intervertebral disc degeneration. Mol Med Rep. 2019;20(4):3160-3174.

34. Wang S, Sun J, Yang H, et al. Profiling and bioinformatics analysis of differentially expressed circular RNAs in human intervertebral disc degeneration. Acta Biochim Biophys Sin. 2019;51(6):571-579.

35. Song J, Wang HL, Song KH, 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):27.

36. Wang H, He P, Pan H, 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):27.

37. Kulcheski FR, Christoff AP, Margis R. Circular RNAs are miRNA sponges and can be used as a new class of biomarker. J Biotechnol. 2016;238:42-51.

38. Salzman J, Chen RE, Olsen MN, Wang PL, Brown PO. Cell-type specific features of circular RNA expression. PLoS Genet. 2013;9(9):e1003777.

39. Zhou C, Molinie B, Daneshvar K, et al. Genome-wide maps of m6A circRNAs identify widespread and cell-type-specific methylation patterns that are distinct from mRNAs. Cell Rep. 2017;20(9):2262-2276.

40. Hedgepeth CM, Deardorff MA, Rankin K, Klein PS. Regulation of glycogen synthase kinase 3beta and downstream Wnt signaling by axin. Mol Cell Biol. 1999;19(10):7147-7157.

41. Svensson A, Norrby M, Libellius R, Tägerud S. Secreted frizzled related protein 1 (Sfrp1) and Wnt signaling in innervated and denervated skeletal muscle. J Mol Histol. 2008;39(3):329-337.

42. Sekiya I, Tsuji K, Koopman P, 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):10738-10744.

43. Zwickl H, Niculescu-Morgza E, Halbwirth F, et al. Correlation analysis of m6A circRNAs identify widespread and cell-type-specific methylation patterns that are distinct from mRNAs. Mol Med Rep. 2019;51(5):571-579.

44. Cheng X, Zhang L, Zhang K, et al. Circular RNA VMA21 promotes intervertebral disc degeneration. Cell Prolif. 2018;9(3):90-99.

45. Cheng X, Zhang L, Zhang K, et al. Circular RNA VMA21 promotes intervertebral disc degeneration. Cell Prolif. 2018;9(3):90-99.

46. Zhang Z, Yang T, Xiao J. Circular RNAs: promising biomarkers for human diseases. EBioMedicine. 2018;34:267-274.

How to cite this article: Li Z, Chen X, Xu D, Li S, Chan MTV, Wu WKK. Circular RNAs in nucleus pulposus cell function and intervertebral disc degeneration. Cell Prolif. 2019;52:e12704. https://doi.org/10.1111/cpr.12704.