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Review

Advances in studies of ncRNAs in the pathophysiology and treatment of spinal disease

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Abstract. Symptoms of spinal disease frequently accompany altered or damaged spine and vertebral structures resulting from endogenous and exogenous factors. Back braces, therapeutic agents, and surgery remain the main treatments for spinal diseases. However, the efficacy of currently available therapeutic agents is limited due to their side effects, whereas back braces and surgeries are less effective for certain patients. The significant effect of spinal disease on patients' morbidity and mortality emphasizes the necessity to develop novel and more effective therapeutic agents that mitigate the consequences of spinal disease. Accumulating research acknowledges that non-coding RNAs (ncRNAs), including miRNAs, lncRNAs, circRNAs, etc., are involved in the pathogenesis of spinal disease, their pronounced therapeutic potential and significant regulatory functions in spinal diseases. Hence, this review focuses on summarizing the latest advances in studies of ncRNAs in the progression and recovery of spinal diseases, as well as highlighting the collaboration of ncRNA networks in treating spinal disease.

Key words: ncRNA — miRNA — lncRNA — Osteoporosis — Spinal cord injury

Abbreviations: BMSCs, bone marrow-derived MSCs; CatK, cathepsin K; ceRNA, competing endogenous RNA; circRNAs, circular RNAs; eNOS, endothelial nitric oxide synthase; ERK1/2, extracellular signal-regulated kinase ½; lncRNAs, long non-coding RNAs; MALAT1, metastasis-associated lung adenocarcinoma transcript 1; MAPK, mitogen-activated protein kinase; miRNAs, microRNAs; mRNAs, messenger RNAs; MSCs, mesenchymal stem cells; ncRNAs, non-coding RNAs; OA, osteoarthritis; PDGF, platelet-derived growth factor; PI3K/AKT, phosphatidylinositol-3 kinase/protein kinase B; piRNA, piwi-interacting RNA; POP, postmenopausal osteoporosis; SCI, spinal cord injury; siRNA, small interfering RNA; snoRNAs, small nucleolar RNAs; snRNA, small nuclear RNAs; TNF, tumor necrosis factor; VEGF, vascular endothelial growth factor.

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Introduction

Spinal disease is a condition of backbone impairment that is caused by internal factors (such as ageing and degeneration) or external factors (such as trauma and injury) (Gallucci et al. 2007). Symptoms of spinal disease can manifest at any part of the vertebral column, for example, in the cervical spine and lumbar region (Patel et al. 2001; Pannell et al. 2015). Patients with spinal diseases suffer from pain in various parts of the body, such as in the back, leg, neck area, or even in the arms, which challenges the diagnosis and treatment of spinal diseases (Zou et al. 2020). A range of spinal diseases have impacted health globally, as they inflict heavy economic burdens on society and high death rates in developed and developing countries, respectively (Lee et al. 2014). Despite the enormous efforts to improve the quality of life of patients with spinal disease, the main treatment strategies (which include back braces, therapeutic agents, and surgery) are still not producing optimistic results (Meyer et al. 2008). The current therapeutic agents for osteoporosis significantly suppress bone resorption or promote bone formation; however, the adverse effects resulting from their prolonged use have reduced patient compliance and ultimately decreased their clinical efficacy (Tabatabaei-Malazy et al. 2017). Therefore, increasing emphasis has been placed on developing novel therapeutic strategies to address this major global health condition.

Over 98% of the human genome is comprised of non-coding RNAs (ncRNAs), a type of RNA that neither codes nor translates proteins and is involved in regulating the expression of various genes, biochemical pathways, DNA repair, cellular functions, and genome integrity (Shahrouki and Larsson 2012). Thousands of ncRNAs modulate the expression of genes that are critical for disease development, making them an ideal target for developing drugs with minimal side effects. The subtypes of ncRNAs include microRNAs (miRNAs), long non-coding RNAs (lncRNAs), and circular RNAs (circRNAs) (Sandhu et al. 2016). Although the functions of many newly identified ncRNAs have yet to be validated, their aberrant expression or functions have been closely associated with a broad spectrum of diseases, ranging from infectious diseases (such as hepatitis C) and solid tumours and haematological malignancies to organ disorders (e.g., nephropathy) (Matsui and Corey 2017). Concordantly, enhancing or silencing certain ncRNAs highlights the therapeutic potential of ncRNAs to modify gene expression without altering the underlying DNA sequence. For example, circ-HIPK2 knockdown blocks the activation of astrocytes, thus suppressing the formation of neuroinflammatory lesions (Zhang and Du 2016; Ding et al. 2018; Chen et al. 2019a). The roles and interactions of different ncRNAs in the development of certain spinal diseases have been extensively elaborated in recent review articles (Hao et al. 2017; Yang et al. 2020). However, a review that comprehensively covers the regulatory roles of ncRNAs in the progression and treatment of spine-related diseases is still lacking. Therefore, this review focuses on the current advances in studies of ncRNA-mediated targeted regulation in treating several spinal diseases (Table 1),

Table 1. Functions and mechanisms of ncRNAs in spinal diseases

Spinal diseases	ncRNAs	Functions and mechanisms	References
SCI	miRNA	Knockdown of miR-21 deteriorates the functional and morphological preservation of spinal cord tissue.	Hu et al. 2013
		miR-210 accelerates angiogenesis, promotes astrogliosis, maintains axons and myelin, and improves the functional recovery of the spinal cord.	Ujigo et al. 2014
		BMSCs transduced with AAV-as-miR-383 expand the area of the intact spinal tissue, reduce the cavity volume of the spinal cord, and restore locomotor activity.	Wei et al. 2017
		miR-34a mediates SCI progression by inducing apoptosis.	Fan et al. 2012
		miR-31 maintains neuronal viability and minimizes secondary SCI by blocking apoptosis.	Wang et al. 2019c
	lncRNA	Upregulated lncRNA SNHG5 intensifies SCI by promoting the survival of astrocytes and microglia.	Jiang et al. 2018
		Overexpressed lncRNA DGCR5 ameliorates acute SCI by inhibiting neuronal apoptosis.	Zhang et al. 2018a
		The lncRNA Neat1 promotes neuronal differentiation and migration, suppresses the apoptosis of spinal cord progenitor cells, and promotes the recovery of locomotor function following SCI.	Cui et al. 2019
		The lncRNA-F630028O10Rik/miR-1231-5p/Col1a1 ceRNA network triggers microglial pyroptosis through the PI3K/AKT pathway, which then aggravates SCI.	Xu et al. 2020
	circRNA	The circRNA circ-HIPK3 sponges miR-558 to increase the expression of DPYSL5 and inhibit neuronal cell apoptosis.	Zhao et al. 2020
	siRNA	MSC-exosomes loaded with PTEN-siRNA promote axonal growth and neovascularisation, inhibit microgliosis and astrogliosis, improve the structural and electrophysiological functions of the spinal cord, and accelerate the functional recovery of SCI.	Guo et al. 2019

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Table 1. (continued)

Spinal diseases	ncRNAs	Functions and mechanisms	References
axSpA	miRNA	The expression of miR-17-5p, miR-27a, miR-29a and miR-126-3p is upregulated in peripheral blood mononuclear cells from patients with axSpA.	Li et al. 2019
		The expression of miR-16-1-3p, miR-28-5p, miR-199a-5p and miR-126-3p is upregulated in both monocytes and CD4+ T cells from patients with axSpA.	Fogel et al. 2019
Osteoporosis	miRNA	miR-124 suppresses osteoblast differentiation and inhibits bone formation.	Qadir et al. 2015, Xu and Zhu 2020
		The dual effects of miR-542-3p on osteoblast differentiation depend on the specific pathophysiological state of osteoporosis. miR-542-3p activates osteoblast differentiation and increases bone formation by abrogating the inhibitory action of SFRP1 on the WNT signalling pathway. In contrast, miR-542-3p inhibits osteogenic differentiation and induces osteoblast apoptosis by suppressing the BMP-7/PI3K pathway.	Kureel et al. 2014, Zhang et al. 2018b
	lncRNA	lncRNA-Jak3 activates Nfatc1, increases CatK expression, and promotes osteoclastogenesis.	Lee et al. 2019
		The lncRNA AK023948 decreases phospho-PI3K, phospho-Akt, and phospho-PDK1 levels in the bone tissue of postmenopausal osteoporotic rats. AK023948 also inhibits osteoblast apoptosis and promotes osteoblast proliferation through the PI3K/AKT signalling pathway.	Wang et al. 2020b
		The lncRNA MEG3 suppresses the osteogenic differentiation of BMSCs by inducing miR-133a-3p expression.	Wang et al. 2017
		The upregulation of the lncRNA CASC11 in patients with POP is positively correlated with TNF-α levels. The level of the lncRNA CASC1 1 is also positively correlated with a high recurrence rate of POP.	Yu et al. 2019
		The lncRNA MALAT1 blocks the osteogenic differentiation of BMSCs and accelerates osteoporosis progression by activating the MAPK pathway.	Zheng et al. 2019
		The lncRNA ZBTB40-IT1 blocks osteogenesis and induces osteoclastogenesis by downregulating the expression of WNT4 and other related genes, and decreases the ratio of RANKL/OPG.	Mei et al. 2019
	circRNA	circRNA-28313 abrogates the inhibitory effect of miR-195-a on M-CSF1, which then promotes the differentiation of BMM cells into osteoclasts and induces bone resorption.	Chen et al. 2019b
		circIGSF11 reduces miR-199b-5p expression, decreases osteoblast differentiation, and disrupts the osteoblast differentiation from BMSCs.	Zhang et al. 2019
		Notably, circRNA_0016624 sponges miR-98, which mediates osteogenic differentiation by downregulating BMP2. The levels of both circRNA_0016624 and BMP2 are low in patients with osteoporosis; overexpressed circRNA_0016624 upregulates BMP2 expression and impedes osteoporosis.	Yu and Liu 2019
	siRNA	Nanosized PLGA capsules loaded with the PEI-RANK siRNA complex reduce the RANK mRNA levels and suppress osteoclast differentiation and osteoclastic activity.	Sezlev Bilecen et al. 2019
SMA	lncRNA	A novel strategy to treat SMA by inhibiting the interaction between the lncRNA SMN-AS1 and PRC2 blocks PRC2 recruitment and upregulates the transcription of SMN2 in fibroblasts and neurons.	Woo et al. 2017
Bone cancer pain	siRNA	PDGF siRNA relives bone cancer pain by suppressing the AKT-ERK signalling pathway. It downregulates the expression of GFAP, SP, CGRP and phosphorylation of ERK and AKT.	Xu et al. 2016
Osteoarthritis	siRNA	YAP siRNA impairs the progression of osteoarthritis by downregulating IL-1β-induced catabolic gene expression, reducing cartilage damage, and inhibiting chondrocyte apoptosis and aberrant subchondral bone formation.	Gong et al. 2019

AAV, adeno-associated virus; axSpA, axial spondyloarthritis; BMM, bone marrow monocyte/macrophage; BMP, bone morphogenetic protein; BMSCs, bone marrow mesenchymal stem cells; CASC11, cancer susceptibility 11; CatK, cathepsin K; CGRP, calcitonin gene-related peptide; GFAP, glial fibrillary acidic protein; MALAT1, metastasis-associated lung adenocarcinoma transcript 1; MAPK, mitogen-activated protein kinase; M-CSF1, macrophage colony-stimulating factor; MSC, mesenchymal stem cells; Nfatc1, nuclear factor of activated T-cells; PDGF, platelet-derived growth factor; PDK1, phosphoinositide-dependent kinase-1; PI3K, phosphaditylinositol-3 kinase; POP, postmenopausal osteoporosis; PTEN, phosphatase and tensin homologue; RANK, receptor activator of nuclear factor kappa B; SCI, spinal cord injury; SFRP1, secreted frizzled-related protein-1; SMA, spinal muscular atrophy; SMN, survival motor neuron 1; SP, substance P; TNF-α, tumour necrosis factor-α; YAP, Yes-associated protein.

highlights the role of ncRNA networks, and discusses the future perspectives of different types of ncRNAs.

Potential treatments for spinal diseases

Many types of spinal diseases have been identified, and they have a high global economic burden and costs; however, current major treatment strategies, mainly therapeutic agents and surgery, are still not producing optimistic results. For example, spinal cord injury (SCI) is a disabling and irreversible spinal disease mainly caused by trauma and is also caused by tumours, infections and other stimuli (Van Middendorp et al. 2012). SCI progresses progressively in three phases: acute SCI, secondary SCI and chronic SCI (Jiang and Zhang 2018). One of the most commonly used therapeutic approaches is surgical techniques, including spinal alignment, nerve decompression and stabilization of the spine. However, only approximately 2% of patients with SCI can recover sufficiently to walk after attempted surgical treatments (Janssen and Hausebout 1989). Rapidly improved implant materials, such as nanoscaffolds and polyethylene glycol, have facilitated the stabilization of unstable fractures in reconstructive surgeries, subsequently preventing further neurological injury and allowing earlier rehabilitation of patients (Van Middendorp et al. 2012; Kong et al. 2017). Other potential treatment methods for SCI, including central nervous system cell transplantation (Cristante et al. 2009), hyperbaric oxygen therapy (Kelly et al. 1972), and pharmacological therapies such as corticosteroid and ganglioside administration (Schwab and Bartholdi 1996), have also facilitated the rapid development of clinical work in the field of SCI regeneration, but their efficacies are still limited.

Osteoporosis, a disease in which bone weakening increases the risk of a broken bone, is one of the most common and deadly chronic diseases worldwide. Osteoporosis is a disease literally describing porous bone, and this prevalent bone disorder is characterized by rapid reductions in bone mass and bone mineral density (Wu et al. 2013). Currently, two main strategies are available for treating osteoporosis. Treatments designed to increase bone formation are called anabolic therapies. Treatments aiming to reduce bone resorption are called antiresorptive therapies. Antiresorptive therapy is considered by most clinicians the first-line treatment for osteoporosis because it enhances the strength and quality of bone and increases the bone mineral density. Currently, five main classes of antiresorptive drugs exist, including bisphosphonates, oestrogens, selective estrogen receptor modulators (SERMs), calcitonin and monoclonal antibodies such as denosumab (Chen and Sambrook 2012). However, all these Food and Drug Administration (FDA)-approved antiresorptive drugs have their own prevalence of adverse events (<10%). For example, women treated with oestrogens have increased risks of breast cancer and coronary heart diseases (Investigators et al. 2002). Currently, bisphosphonates and denosumab are still recommended as first-line therapies for patients with osteoporosis. Bisphosphonates and denosumab have the same problem: they suppress both bone resorption and bone formation, which leads to a low bone turnover state that would be less able to repair microdamage or even cause bone fracture (Ng 2012). Since the suppression of cathepsin K (CatK) activity prevents bone resorption without perturbing bone formation, it has become an attractive target for antiresorptive drug development (Dai et al. 2020). To date, odanacatib (ODN), developed by Merck & Co., is the only CatK inhibitor candidate showing high therapeutic efficacy in patients with postmenopausal osteoporosis in Phase III clinical trials (Bone et al. 2015). Unfortunately, due to the undesirable adverse effects on nonbone tissues, i.e., higher incidence of cardio-cerebrovascular events than the placebo group, the development of ODN was finally stopped. Therefore, a desirable alternative strategy would be to design and develop a novel 'smart' CatK inhibitor chemically conjugated with a bone-targeted aptamer (Zhang et al. 2012; Liu et al. 2015), which would facilitate the CatK inhibitor targeting to bone to reduce its exposure in nonbone sites and prevent potential adverse effects on nonbone tissues.

For other spinal diseases, such as axial spondyloarthritis (axSpA) and osteoarthritis (OA), optional treatment methods are limited when conventional treatment fails. Tumor necrosis factor (TNF) blockers remain the most promising replacement therapy for patients with axSpA; however, they are not able to prevent new bone formation in advanced cases of axSpA (Sieper and Poddubnyy 2014). In addition, anti-IL-17, IL-23 and IL-12 therapies have shown some level of efficacy in axSpA models, and larger studies are required to confirm and determine the underlying molecular mechanisms (Sieper 2016). Consistently, these small molecules also show therapeutic potential in OA (Van Spil et al. 2019). Other studies have revealed that mesenchymal stem cells and mitochondria are promising targets for OA (Shariatzadeh et al. 2019; Mao et al. 2020). Nevertheless, these new WNT discoveries are still in their infant state. To date, only supportive care is provided to patients with spinal diseases in the clinic, and further advances are expected in cellular engineering and gene therapy. For instance, emerging evidence has revealed the roles and interactions of various ncRNAs in the development of certain spinal diseases (Hao et al. 2017; Yang et al. 2020).

ncRNAs in spinal diseases

miRNAs

MicroRNAs (miRNAs, miRs), a class of endogenous and single-stranded small non-coding RNAs that consist of ap-

proximately 22 nucleotides, were first discovered in 1993. To date, approximately 28,000 types of miRNAs have been discovered in plants, animals, and viruses. Importantly, miRNAs regulate gene expression at the posttranscriptional level through an interaction with specific messenger RNAs (mRNAs), the induction of mRNA degradation, or suppression of mRNA translation (Bartel 2004). Notably, each miRNA targets multiple genes, and several miRNAs may jointly regulate the same gene, suggesting diverse possibilities of targeting miRNAs for treatment. Moreover, most of the miRNA target genes are located at the binding sites of various pathways associated with bone metabolism, including ERK/MAPK signalling, oestrogen receptor signalling, and the Wnt/β-catenin signalling pathway (Hu et al. 2020). Considering the significant effect of miRNAs on the progression of spinal disease, the roles of miRNAs in the pathogenesis, diagnosis, and treatment of spinal diseases have been attracting attention globally.

miRNAs are involved in SCI, as evidenced by the pronounced changes in the expression of 97 miRNAs following SCI (Liu et al. 2009). Antagomir-21, which knocks down miR-21, increased neuronal cell apoptosis, disrupted the recovery of hindlimb motor function, and exacerbated damage to spinal cord tissue in the rat contusion SCI model. Antagomir-21 retarded recovery from SCI by increasing the translation of the proapoptotic genes Fas ligand (FasL) and phosphatase and tensin homologue (PTEN), indicating the protective role of miR-21 in restraining SCI progression through the control of proapoptotic gene expression (Hu et al. 2013). In addition, miRNAs also contribute to spinal cord regeneration, as shown by Ujigo and colleagues who injected miR-210 into the lesion of spinal cords of SCI mice. The administration of miR-210 triggered angiogenesis and astrogliosis, preserved axons and myelin, accelerated functional recovery, and inhibited apoptosis in the spinal cord through the downregulation of protein tyrosine phosphate 1B (Ptp1b) and ephrin-A3 (EFNA3) levels (Ujigo et al. 2014). The therapeutic potential of transplanting bone marrow-derived MSCs (BMSCs) for SCI has been documented in preclinical studies. BMSCs were first transduced with AAV-as-miR-383 (adeno-associated virus carrying an antisense sequence for miR-383) and then transplanted into a nude rat SCI model. Suppression of miR-383 increased glial cell line-derived neurotrophic factor (GDNF) protein levels, which then expanded the area of intact spinal tissue, reduced the cavity volume, and promoted locomotor recovery in SCI rats (Wei et al. 2017). In addition to the regenerative roles of miRNAs, miRNA-induced apoptosis has been recently emphasized for SCI treatment. For example, miR-34a supports SCI progression by inducing apoptosis through the inhibition of two antiapoptotic proteins, BCL-2 and XIAP (Fan et al. 2012). On the other hand, miR-31 facilitates the recovery of SCI and minimizes secondary SCI by repressing neuronal apoptosis *via* the phosphatidylinositol-3 kinase/protein kinase B (PI3K/AKT) pathway (Wang et al. 2019c). These findings corroborate the therapeutic potential of utilizing miRNAs to treat spinal diseases, although the underlying molecular mechanism of miRNA-mediated apoptosis in SCI progression remains unclear.

Circulating miRNAs are highly stable in body fluid samples; however, abnormal expression of these miRNAs is frequently discovered in patients with axSpA, suggesting their potential use as diagnostic biomarkers for axSpA (Mitchell et al. 2008). A pilot study by Magrey and colleagues compared the miRNA expression profile in the plasma of 15 patients with axSpA to 5 healthy volunteers. They reported the abnormal expression of certain miRNAs: miR-32 and miR-34a were upregulated, while miR-10b, miR-16, miR-30a, miR-150 and miR-154 were downregulated (Magrey et al. 2016). Other potential biomarkers of axSpA include the expression of miR-17-5p, miR-27a, miR-29a and miR-126-3p, which are markedly elevated in peripheral blood mononuclear cells (PBMCs) from patients with axSpA compared to healthy volunteers (Li et al. 2019). Additionally, Fogel and colleagues observed the significant upregulation of miR-16-1-3p, miR-28-5p, miR-126-3p, and miR-199a-5p in $CD4^+$ T cells from 81 patients with axSpA compared with healthy volunteers, and the levels of miR-146a-5p were negatively correlated with the severity of inflammation in patients with axSpA (Fogel et al. 2019). Another clinical study thoroughly analysed the plasma levels of 760 circulating miRNAs in 68 patients with axSpA. Interestingly, among a series of miRNAs that were expressed at lower levels, the reduced levels of miR-29a-3p, miR-146a-5p, miR-222-3p and miR-625-3p signified a more advanced stage of axSpA and spinal involvement (Prajzlerová et al. 2017). Overall, these results suggest a regulatory role for circulating miRNAs in the pathogenesis of axSpA, highlighting their potential as biomarkers for diagnosing axSpA.

Regulatory roles for miRNAs in postmenopausal osteoporosis (POP) by mediating osteoblast and osteoclast differentiation have also been identified (Mandourah et al. 2018). Among the miRNAs, miR-124 and miR-542-3p deserve special attention because they exert dual effects on osteoblast differentiation, depending on the specific pathophysiological state of osteoporosis. Briefly, miR-124 promotes osteoblast differentiation *via* the BMP/TGF-β pathway and in contrast inhibits bone formation by suppressing regulators of osteogenic differentiation, such as Dlx 2, Dlx 3 and Dlx 5 (Qadir et al. 2015; Xu and Zhu 2020). Similarly, miR-542-3p also exerts dual effects on osteoblast differentiation. Notably, miR-542-3p induces osteoblast differentiation by inhibiting the expression of a negative regulator of the WNT signalling pathway, i.e., the secreted frizzled-related protein-1 (SFRP1). Overexpression of miR-542-3p, however, induces osteoblast apoptosis to inhibit osteogenic differentiation through the

suppression of the BMP-7/PI3K pathway (Kureel et al. 2014; Zhang et al. 2018b). The dual effects of these miRNAs suggest that they represent flexible targets for different stages of osteoporosis and serve as potential biomarkers to predict and prevent osteoporosis.

lncRNAs

Long non-coding RNAs (lncRNAs) are another type of ncRNA with lengths exceeding 200 nucleotides. Emerging evidence supports the hypothesis that lncRNAs are closely involved in various biological processes, including apoptosis, autophagy, cell pluripotency and proliferation. Abnormally expressed lncRNAs were previously found to be mainly enriched in the central nervous system and are extensively involved in the pathogenesis of cardiovascular and cerebrovascular diseases, such as heart failure and neurological diseases (Shi et al. 2013; Chi et al. 2019). However, the exact functional roles of lncRNAs in these diseases remain largely unknown. lncRNAs are crucial in regulating BMSCs, particularly in bone formation, and dysfunctional BMSCs affect the pathogenesis of various age-related spinal diseases (Baker et al. 2015). More importantly, the regulatory role of lncRNAs in bone metabolism is usually associated with modulating miRNA activity *via* various signalling pathways (Jia et al. 2019; Wang et al. 2019a). An understanding of the underlying molecular mechanisms of lncRNAs in bone metabolism will provide promising therapeutic strategies for treating spinal diseases.

Similar to miRNAs, hundreds of lncRNAs are differentially expressed in the contusion SCI mouse model compared with mice without SCI. In addition, the number of upregulated and downregulated lncRNAs fluctuates from the initial injury until 3 weeks post-SCI, suggesting potential roles for lncRNAs throughout the pathogenesis of SCI (Ding et al. 2016). For example, the upregulated levels of the lncRNA SNHG5 (small nucleolar RNA host gene 5) in spinal cord tissue of SCI rats increased the expression of Krüppel-like factor 4 (KLF4) and endothelial nitric oxide synthase (eNOS), thus promoting the viability of astrocytes and microglia and ultimately exacerbating neuroinflammation and neuronal damage after SCI (Jiang and Zhang 2018). These findings partially explain the pathological mechanism of SCI and provide a basis for targeting lncRNAs to prevent and diagnose SCI. Consistently, numerous studies have described the therapeutic roles of lncRNAs in halting SCI progression. For instance, overexpression of a DiGeorge syndrome-associated ncRNA (DGCR5), an essential lncRNA that is predominantly expressed in the brain, alleviates acute SCI by inhibiting neuronal apoptosis through the downregulation of PRDI-BF1 and RIZ domain proteins (PRDM5) in an acute SCI mouse model (Zhang et al. 2018a). Cui and colleagues observed that the lncRNA nuclear-enriched abundant transcript 1 (Neat1) promoted the differentiation and migration of neural stem cells partially by activating Wnt/β-catenin signalling in SCI mice (Cui et al. 2019). Notably, Neat1 expression is increased upon the overexpression of miR-124, suggesting that the lncRNA-miRNA network restores spinal cord function in patients with SCI. In addition to the lncRNA Neat1/miR-124 network, the lncRNA-F630028O10Rik/miR-1231-5p/Col1a1 ceRNA network induces microglial pyroptosis *via* the activation of the PI3K/AKT pathway. Suppressing microglial pyroptosis promotes locomotor recovery after SCI, thus providing a novel therapeutic strategy for neuronal repair and regeneration in patients with SCI (Xu et al. 2020).

As osteoclastogenesis and impaired osteogenesis are hallmarks of spinal disease, lncRNAs are involved in the main pathogenic mechanism of osteoperosis through the disruption of the dynamic balance between osteoblast-regulated bone formation and osteoclast-mediated bone resorption (Hassan et al. 2015). Overexpression of the lncRNA AK023948 in osteoblasts of POP model rats increases Akt phosphorylation and activates the PI3K/AKT signalling pathway, thus increasing osteoblast proliferation (Wang et al. 2020b) and bone formation. Parathyroid hormone, the sole FDA-approved anabolic treatment for osteoporosis, significantly reduces the expression of the lncRNA ZBTB40-IT1 in the U-2OS human osteosarcoma cell line. Downregulation of the lncRNA ZBTB40-IT1 promotes osteogenesis and suppresses osteoclastogenesis by decreasing WNT4, RUNX2, OSX, ALP, and COLIA1 gene expression and the RANKL/OPG ratio (Mei et al. 2019). In contrast, overexpression of the lncRNA Janus kinase 3 (lncRNA-Jak3) in osteoclasts upregulates the expression of the CTSK gene, which subsequently promotes osteoclastogenesis (Lee et al. 2019).

The effects of lncRNAs on BMSC activities play a central role in POP pathogenesis. For instance, increased expression of the lncRNA MEG3 suppresses the osteogenic differentiation of BMSCs by upregulating miR-133a-3p expression, eventually leading to POP (Wang et al. 2017). Interestingly, Yu and colleagues reported that the increased plasma levels of the lncRNA cancer susceptibility 11 (CASC11) and TNF-α in patients with POP are decreased after treatment. Additionally, a high level of CASC11 is positively correlated with a high recurrence rate of POP (Yu and Liu 2019). In addition to MEG3, the lncRNA metastasis-associated lung adenocarcinoma transcript 1 (MALAT1) also impairs the osteogenic differentiation of BMSCs through the activation of the mitogen-activated protein kinase (MAPK) signalling pathway. In lncRNA MALAT1 knockdown mice, mRNA levels of osteogenic differentiation-related genes (OCN, Collagen I and RUNX2) and alkaline phosphatase (ALP) activity were significantly decreased, while the levels of MAPK signalling pathway-related proteins (such as ex-

tracellular signal-regulated kinase 1/2 (ERK1/2) and P38) were markedly higher than those in the control group. These changes in protein and mRNA expression were indicative of lncRNA MALAT1-mediated suppression that aggravated the progression of osteoporosis through the MAPK pathway (Zheng et al. 2019). Collectively, these findings provide insights into the effects of lncRNAs on the proliferation, differentiation, and survival of bone cells through different signalling pathways, providing evidence for the therapeutic potential of applying the regulatory activities of lncRNAs in osteoporosis pathophysiology.

Regarding spinal muscular atrophy (SMA), a neurodegenerative disease resulting from mutated survival motor neuron 1 (SMN1) and the SMN2 gene, Woo and colleagues proposed a novel therapeutic strategy using chemically modified oligonucleotides. The oligonucleotides prevented the lncRNA SMN-AS1 from recruiting Polycomb Repressive Complex 2 (PRC2), resulting in upregulation of SMN2 transcription levels in fibroblasts and neuronal cultures (Woo et al. 2017). The action of the transcriptional repressive complex that silences the target gene has caused a range of diseases. The treatment for these diseases may potentially benefit from the lncRNA-associated gene-upregulation strategy, due to its ability to increase target gene expression.

circRNAs

In contrast to the linear RNAs described above, the 3' and 5' ends of circular RNAs (circRNAs) are covalently joined together to form a closed continuous loop. circRNAs are less susceptible to exoribonuclease degradation, and this unique feature highlights their crucial roles in modulating various cell biological behaviours (Enuka et al. 2016; Zhang et al. 2017). hsa-circ 0074834, a circRNA that is expressed at low levels in BMSCs from patients with bone fractures, is an example of a circRNA that regulates the osteogenic differentiation and adipogenesis of BMSCs. Upregulation of hsa-circ0074834 mediates the osteogenesis-angiogenesis coupling process by controlling the expression of vascular endothelial growth factor (VEGF) and zinc finger E-box binding homeobox 1 (ZEB1). Following this interaction, BMSC osteogenic differentiation and bone defect restoration are promoted (Ouyang et al. 2019). circRNAs are similar to lncRNAs in terms of their essential regulatory roles in bone metabolism through their effects on BMSCs, osteoclasts and osteoblasts (Patil et al. 2020).

A screen of circRNA expression patterns in a traumatic SCI rat model revealed five circRNAs (rno_circRNA_005342, rno_circRNA_015513, rno_circRNA_002948, rno_circRNA_006096, and rno_circRNA_013017) that were distinctly expressed (Qin et al. 2019), similar to the findings reported by Zhou et al. (2019) and Kou et al. (2020). Their microarray and bioinformatics analyses revealed altered expression of numerous circRNAs in rat models of different stages of SCI and ankylosing spondylitis. Although these dysregulated circRNAs are distributed on all chromosomes, they are mainly located on chromosomes 1 to 10 (Liu et al. 2020). Another crucial action of circRNAs is sponging miRNAs, and the interaction between these two types of ncRNAs is closely associated with different pathological processes, such as heart failure, cancers (Deng et al. 2019; Lu et al. 2020) and spinal disease (Magrey et al. 2016; Xu et al. 2020a). Notably, circ-HIPK3 sponges miR-558 to upregulate the expression of DPYSL5, resulting in reduced neuronal cell apoptosis in a mouse SCI model, suggesting a protective role of circ-HIPK3 in SCI progression (Zhao et al. 2020). More importantly, microarray data mining and comprehensive bioinformatics analyses have been applied to further explore the circRNAs that regulate the pathogenesis and treatment of SCI. Based on the findings of differentially expressed circRNAs, miRNAs and mRNAs, a competitive endogenous RNA (circRNA-miRNA-mRNA) network with regulatory roles in the pathology and treatment of SCI has been established (Peng et al. 2020). These findings provide strong evidence of circRNA involvement and potential collaboration of circRNAs with other types of ncRNAs in the physiological and pathological processes of SCI.

Various circRNAs are involved in osteoporosis pathogenesis by regulating osteogenesis and osteoclastogenesis in mesenchymal stem cells (MSCs) and monocyte/macrophage cells. Several studies have corroborated circRNAs as promising diagnostic biomarkers of osteoporosis (Huang et al. 2019; Wang et al. 2019b). Hundreds of differentially expressed circRNAs are involved in osteoporosis progression, and overexpression of circRNA_0016624, for instance, upregulates BMP2 expression and ultimately prevents osteoporosis, suggesting a therapeutic role for circRNA_0016624 in osteoporosis treatment (Yu and Liu 2019). In response to the osteoporosis process, the regulatory roles of circRNAs in osteogenesis and osteoclastogenesis are also closely associated with miRNAs and lncRNAs (He et al. 2020). For instance, circRNA_0016624, which is significantly downregulated in female patients with osteoporosis, regulates levels of miR-98 (Yu et al. 2019b). Furthermore, circRNA_28313, which functions as a competing endogenous RNA (ceRNA) of miR-195a, markedly suppresses miR-195a expression in M-CSF, resulting in the induction of bone resorption by promoting the differentiation of bone marrow monocyte/macrophage (BMM) cells into osteoclasts in ovariectomized (OVX) mice (Chen et al. 2019b). Knockdown of circIGSF11 significantly increases the expression of miR-199b-5p, facilitating the differentiation of BMSCs into osteoblasts (Zhang et al. 2019). Collectively, these findings have revealed the therapeutic potential of circRNAs in treating different spinal diseases.

Other ncRNAs

Small interfering RNA (siRNA), also known as silencing RNA, is another type of double-stranded ncRNA that degrade mRNAs after transcription and prevents translation through the RNA interference (RNAi) pathway. Genes can be knocked down or silenced by delivering a synthetic siRNA with a complementary sequence to the target site or by transfecting the cells with a specific siRNA, implying the importance of siRNAs in treating spinal diseases. For example, an intrathecal injection of a platelet-derived growth factor (PDGF) siRNA into the L4–6 spinal cord segments of animals with bone cancer pain markedly attenuated pain. Bone cancer pain was relieved by downregulating the expression of glial fibrillary acidic protein (GFAP), substance P (SP) and calcitonin gene-related peptide (CGRP), as well as reducing the phosphorylation of PDGF receptors and proteins in the ERK/AKT signalling pathway (Xu et al. 2016). In preventing osteoarthritis, a Yes-associated protein (YAP) siRNA downregulates the expression of IL-1β-induced catabolic genes, thus limiting cartilage damage, chondrocyte apoptosis and aberrant subchondral bone formation (Gong et al. 2019). Guo et al. intranasally administered MSC-derived exosomes (MSCs-Exos) packed with PTEN-siRNA to rats with complete SCI and proved their clinical value. Enhanced axonal growth and neovascularization, as well as reduced microgliosis and astrogliosis improved the structural and electrophysiological functions and accelerated the functional recovery of SCI rats (Guo et al. 2019). Similarly, nanosized poly(lactic acid-co-glycolic acid) (PLGA) capsules packed with a polyethyleneimine-receptor activator of nuclear factor kappa B (PEI-RANK) siRNA complex significantly suppress the mRNA expression of the RANK gene, osteoclast differentiation and osteoclastic activity, signifying the prospect of this systemic siRNA treatment in intensifying the recovery of osteoporosis (Sezlev Bilecen et al. 2019). Other approaches for bone site-specific delivery of siRNAs to the tissue or cellular level involve nanoparticulate siRNA carriers (Kanasty et al. 2013), bisphosphonates (Giger et al. 2013), oligopeptides (Zhang et al. 2015), and aptamers (Liang et al. 2015) and their therapeutic potential in several spinal diseases have cumulatively been documented (Liu 2016).

Piwi-interacting RNA (piRNA), the largest class of small ncRNAs in animal cells, silences transposable elements to preserve genome integrity *via* the interaction of RNA-induced silencing complexes (RISCs) with Piwi proteins. During the initial osteogenic and chondrogenic differentiation of bone marrow mesenchymal stromal cells, a series of piRNAs are differentially expressed in the respective phases, most notably the downregulation of piR-hsa-23209 and piR-hsa-2107 in the chondrogenic differentiation stage (Della Bella et al. 2020). Although the potential targets of these two piRNAs require further functional studies, they hinge on their potential roles in treating degenerative spinal diseases. Wang et al. (2020a) further confirmed that the higher expression of piRNAs in BMSC-derived exosomes regulates the p53 signalling pathway and is closely associated with osteogenic differentiation and apoptosis. Moreover, upregulated expression of piRNAs in stem cells from apical papilla (SCAP)-derived exosomes regulates multiple target genes in the MAPK signalling pathway, which are strongly related to bone tissue formation.

Other ncRNAs, such as small nuclear RNAs (snRNAs) and small nucleolar RNAs (snoRNAs), are less studied in the field of spinal disease. Serum levels of abundant snoRNAs are differentially expressed in ageing musculoskeletal and osteoarthritis models, providing evidence for the development of snoRNAs as diagnostic biomarkers and therapeutic targets for osteoarthritis (Steinbusch et al. 2017).

Conclusions and future perspectives

Currently, ncRNAs play essential roles in various diseases, especially spinal diseases. The exact number of ncRNAs in the human genome remains largely unknown, due to continuous discoveries of new members (Boivin et al. 2020). The functions of most ncRNAs have not been well validated; sometimes, they are even considered junk RNAs because they are the by-products of spurious transcription with no functional activities (Palazzo and Lee 2015). In this review, we focused on well-studied types of ncRNAs in the field of spinal disease (Figure 1). Many other types of ncRNAs (e.g., rRNA, tRNA, and snoRNAs) are not covered in this review either due to insufficient relevant research or the lack of effect on spinal diseases.

Similar to proteins, mutations and imbalances in ncRNA sequences are responsible for the pathogenesis of numerous spinal diseases. Among them, miRNAs are the most studied type of ncRNA and are involved in diverse biological processes, including cell differentiation, biological development, and the occurrence and progression of diseases (Bartel 2018). Notably, miRNAs have been extensively studied, plausibly due to their abilities to target over 60% of human and other mammalian genes, providing broad possibilities to develop miRNAs as biomarkers for disease diagnosis and targets for drugs (Friedman et al. 2009). More importantly, in terms of regulating disease progression, other types of ncRNAs, such as lncRNAs and circRNAs, sponge miRNAs to modulate miRNA expression (Peng et al. 2020). During the dynamic imbalance of bone metabolism, the interactions between different ncRNAs (often referred to as ceRNA networks) act by targeting major genes and signalling pathways, such as the Wnt signalling pathway and RANK/RANKL system. Moreover, these ncRNAs are closely associated with osteogenic differentiation of BMSCs, which are essential for regulating spinal disease, particularly POP (Yang et al. 2020).

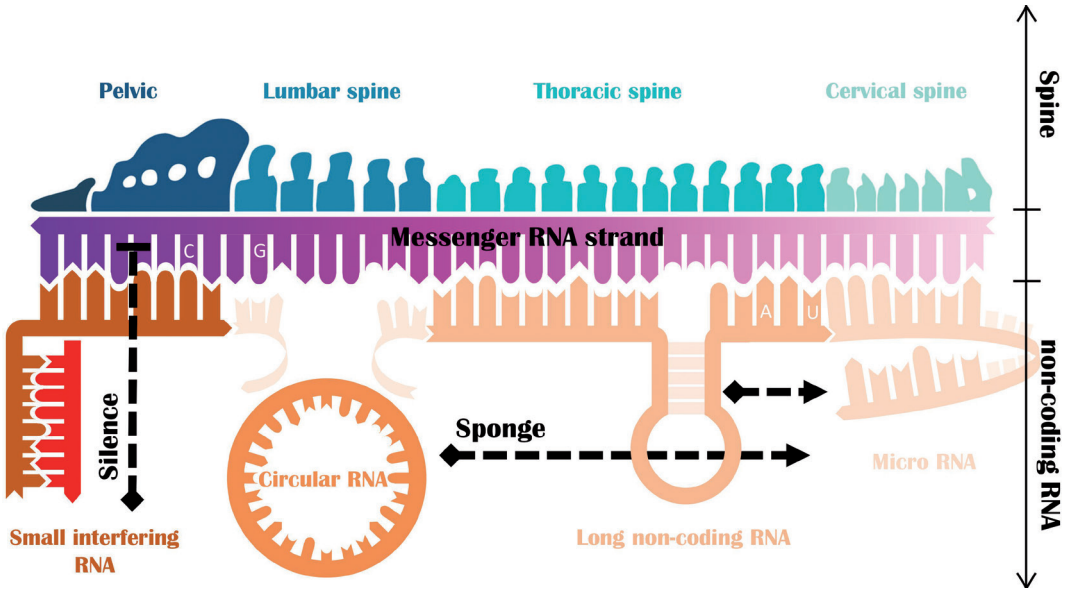

Figure 1. Types of non-coding RNAs (ncRNAs) that are involved in the field of spinal diseases include microRNAs (miRNAs), long non-coding RNAs (lncRNAs), circular RNAs (circRNAs) and small interfering RNAs (siRNAs). The target genes of miRNAs are predominantly located at the binding sites of various pathways involved in bone metabolism, including ERK/MAPK signalling, oestrogen receptor signalling, and the Wnt/β-catenin signalling pathway. lncRNAs and circRNAs similarly regulate bone metabolism through effects on BMSCs, osteoclasts and osteoblasts. Although the regulatory role of lncRNAs in bone metabolism is related to their modulation of miRNA activity, the effects of circRNAs on sponging miRNAs contribute to the pathogenesis of spinal disease. An siRNA silences specific genes by interfering with mRNA translation or degrading mRNA.

Despite the considerable preclinical research on the ncRNA-mediated pathophysiology of spinal disease, clinical applications or translational research of ncRNAs for spinal disease is limited. Existing studies generally described the differential expression of ncRNAs in disease development and proposed the application of different ncRNAs as diagnostic biomarkers. The identification of differentially expressed ncRNAs in biofluids as promising candidate biomarkers is a goal to determine the severity of spinal diseases before regular diagnosis, due to their stability in fluids and tissue specificity. However, the therapeutic usage of ncRNAs for specific spinal diseases has been less explored. The currently limited utilization of miRNAs as treatment tools in this field is due to their unclear underlying molecular mechanisms, delivery methods, and active form *in vivo*. Recent advances in miRNA-based gene therapies include restoring disease-suppressed miRNA expression, blocking overexpressed miRNA activities, and various types of miRNA delivery systems. These novel approaches provide promising therapeutic potential for the treatment of osteoporosis and related fractures. However, less efficient on-target action *in vivo*, the lack of selective biodistribution at the target site, and occurrence of adverse effects remain the main hurdles in the clinical application of miRNA-based gene therapies for spinal disease (Sun et al. 2019). Cutting-edge technologies, such as miRNA chips (Wei et al. 2019), high-throughput sequencing technologies (Huang et al. 2021) and nanotechnologies, may aid in elucidating the mechanisms of ncRNAs and the relationship between ncRNAs and spinal diseases in depth. More importantly, as discussed above, the advances in spinal cord tissue-targeted siRNA administration (using exosomes or nanostructured materials) have revealed the prominent therapeutic potential of these strategies for spinal disease. Patients are expected to benefit from targeted approaches, due to reduced off-target side effects and disease recurrence rates.

Conflicts of interest. The authors declare no conflict of interest.

Authors' contributions. Conceptualization, JW and WW; validation, JW and WW; investigation, JW and WW; resources, YJ and XX; writing – original draft preparation, JW, WW and YJJ; writing – review and editing, JW, WW and YJJ; visualization, YJJ; supervision, YJ and XX; project administration, YJ and XX. All authors have read and agreed to the published version of the manuscript.

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