Emerging roles of long non-coding RNAs in spinal cord injury

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Abstract
Spinal cord injury (SCI) is the most serious complication of spinal injury and often leads to severe dysfunction of the limb below the injured segment. SCI causes not only serious physical and psychological harm to the patients, but imposes an enormous economic burden on the whole society. Great efforts have been made to improve the functional outcomes of patients with SCI; however, therapeutic advances have far been limited. Long non-coding RNA (lncRNA) is an important regulator of gene expression and has recently been characterized as a key regulator of central nervous system stabilization. Emerging evidence suggested that lncRNAs are significantly dysregulated and play a key role in the development of SCI. Our review summarizes current researches regarding the roles of deregulated lncRNAs in modulating apoptosis, inflammatory response, neuronal behavior in SCI. These studies suggest that specific regulation of lncRNA or its downstream targets may provide a new therapeutic approach for this desperate disease.

Keywords
astrocytes, lncRNA, microglia, neurons, spinal cord injury

Introducation
Spinal cord injury (SCI) is a serious neurological disease characterized by loss of nervous tissue and the consequent deficit of sensory and motor functions. Traumatic SCI can have several devastating consequences, such as chronic pain and paralysis. Bone loss caused by SCI is refractory to interventions.¹ The prevention, treatment and rehabilitation of spinal cord injury have become a major issue in the medical field due to the socio-economic loss caused by it. The pathogenesis of SCI is complex and involves two stages including primary and secondary injury. After primary injury caused by fractures, compression, etc., a series of pathophysiological changes such as inflammatory reaction, tissue hypoxia, neuronal necrosis and apoptosis, and local inhibitory microenvironment further aggravate SCI and lead to serious sensory and motor dysfunction.²,³ Clinically, a significant portion of SCI patients also experience continuously symptoms despite of many treatments.⁴ Therefore, it is crucial to identify novel molecular targets for developing mechanism-driven therapeutics which can effectively curb this disease.

Long noncoding RNAs (IncRNAs) are little or no protein-coding transcripts that contain more than 200 nucleotides and exert their physiological and pathological functions through interactions with genomic DNA, miRNAs, mRNAs and proteins.⁵,⁶ Due to their long length than other RNAs, IncRNAs possess special ability to combine varieties of complex secondary and tertiary structures, enabling them to modulate gene expression through multiple mechanisms, such as epigenetics, alternative splicing, small RNA sponging as well as transcriptional and translational regulation. An increasing number of studies have demonstrated IncRNAs are involved in the regulation of...
inflammation, angiogenesis and neuronal behavior after SCI, including nuclear factor-kB, cytokine expression, as well as the cell proliferation of neurons, astrocytes, oligodendrocytes and microglia. Importantly, increasing evidence has indicated that lncRNAs have critical roles in all biological processes, including central nervous system development and diseases.

As described above, SCI induces a complex series of pathophysiological changes in which the pathologic process consists of two intertwined stages: primary injury and secondary injury. After the primary injury, a series of molecular cascade reactions will be triggered, known as secondary injury. Pathological processes of secondary injury mainly include neuronal apoptosis, activation of microglia to release proinflammatory factors, and activation of astrocytes to form glial scar to limit neuroplasticity. Previous studies have shown that several lncRNAs are involved in the above pathological processes, among which XIST, ANRIL, SOX2OT, BDNF-AS and DGCR5 regulate neuronal apoptosis. MALAT1, TUG1, TUSC7 and SNHG5 regulate microglial activation and inflammatory response. SCIR1 and SNHG5 regulate glial scarring by regulating astrocyte activation.

However, studies focused on lncRNAs under SCI yet are still in their infancy. In this review article, we summarize the current knowledge regarding the deregulation of lncRNAs in SCI not only about their effects on cell proliferation, apoptosis but inflammation and cell differentiation. The potential clinical utilization of lncRNAs as therapeutic targets for the management of SCI is also discussed.

**Deregulated lncRNAs in spinal cord injury**

In order to clarify the changes in the expression level of lncRNAs after SCI and its role in spinal cord injury, genome-wide lncRNAs profiling by microarray or RNA-Sequencing (RNA-Seq) followed by bioinformatics analysis to examine the function of these deregulate lncRNAs and validation of individual candidates with quantitative reverse transcription PCR (qRT-PCR) are the most common approaches to identify differentially expressed lncRNAs in this disease. Owing to ethical concerns in obtaining human spinal cord tissues for lncRNA profiling, the samples analyzed in SCI studies are usually restricted to animal tissues.

Spinal cord contusion is a common experimental approach to induce SCI model. Ding et al. are among the first to use a microarray to profile differentially expressed lncRNA (DE lncRNA) in the lesion spinal cord of mouse. Few changes in LncRNA expression levels were noted 1 day after injury. However, The differential changes in LncRNA expression peaked 1 week after SCI and subsequently declined until 3 weeks after injury. KEGG pathway and lncRNA-mRNA co-expression network analyses showed that the neuroactive ligand-receptor interaction, the PI3K-Akt signaling pathway, and focal adhesions were potentially implicated in SCI pathology.

The pathology of SCI is composed of primary and secondary damage. The primary damage refers to the focal destruction of neural tissue caused by direct mechanical forces, then secondary injury which contains a series of complex pathophysiological damages in the cellular level, causing significant degeneration and consequent functional loss. Secondary injury process can be usually divided temporally into multiple contiguous phases: the immediate, acute, intermediate, and chronic stages of SCI. The immediate phase refers to the initial 1–2 h post SCI. Zhou et al. used microarray analysis to determine lncRNA and mRNA levels in the spinal cord at the immediate phase after contusion SCI. The lncRNAs and mRNAs with a p-value < 0.05 and a fold change > 2 were selected. A total of 772 lncRNAs (528 upregulated and 244 downregulated) and 992 mRNAs were shown to be differentially expressed between normal and SCI samples. Moreover, Bioinformatic analysis revealed differentially expressed lncRNA-mRNA pairs. These lncRNA-mRNA pairs were involved in pathways, such as toll-like receptor signaling pathway, p53 signaling pathway, MAPK signaling pathway and Jak–STAT signaling pathway. The results may establish the potential utility of lncRNAs and mRNAs as treatment targets for the immediate phase of SCI.

The early acute phase of SCI can be considered to last from 2 to 48 h following SCI, and it may play a major part in the development of the secondary injury. Shi et al. performed microarrays in mice undergone SCI to identify deregulated lncRNAs in the spinal cord. Using the criteria of p-value < 0.05 and fold change > 2, 3,193 DE lncRNAs (1,332 upregulated and 1,861 downregulated) and 4,308 DE mRNAs were detected at 2 days after SCI compared with control group. GO and KEGG analysis was then performed, illustrating the potential involvements of steroid biosynthesis, leukocyte transendothelial migration, NOD-like receptor signaling pathway, toll-like receptor (TLR) signaling pathway, and p53 signaling pathway in the pathogenesis of SCI. This study shed new light on lncRNA deregulation and their potential downstream pathways in the acute phase of SCI.

The majority of SCI patients are in the chronic phase of SCI because of the lack of effective therapeutic solutions. Zhang et al. performed microarray assay to identify deregulated lncRNAs in C5 lesion spinal cord isolated from chronic SCI (CSCI) rats. Compared to the control group, 1,266 lncRNAs (738 upregulated and 528 downregulated) and 847 mRNAs were shown to be differentially expressed in the CSCI group with the criteria of p-value ≤ 0.05 and fold change ≥ 1.1. KEGG pathway analysis for these target genes revealed that lncRNAs were involved in regulation of steroid biosynthesis, sphingolipid metabolism, Toll-like receptor signaling pathway and NOD-like receptor signaling pathway.
Compared to microarrays, RNA-Sequencing (RNA-Seq) possesses several advantages, such as a larger dynamic range of detection, higher sensitivity and specificity, and an enhanced ability to interrogate any location in the genome.\(^{21}\)

Duran et al.\(^{22}\) investigated the molecular mechanisms of the chronic SCI in rat models by examining the changes in expression of both protein-coding and long non-coding genes at 1 month, 3 months, and 6 months after injury, respectively. This filtering process yielded 13,847 expressed protein-coding genes and 555 expressed lncRNA genes. 2,055 DE genes overlapped among all three time points. The most enriched pathways throughout the chronic stages include fibrosis, immune responses, and inflammatory responses. Their results demonstrated that a high level of transcriptional disturbance persists during the chronic injury phases, with many genes enriched in pathways such as immune and inflammatory responses, as well as gliosis. A major barrier to the treatment of chronic SCI is the increased astrogliosis that inhibits axonal growth and regeneration.\(^ {23}\) These two studies implied that genes related to gliosis are among the highly upregulated DE genes in the chronic phases of SCI.

The above mentioned studies have unequivocally demonstrated that lncRNA expression is significantly deregulated in all phases of the SCI and strongly suggested that lncRNAs might be functionally involved in SCI pathogenesis (Table 1).

### Functional roles of specific deregulated lncRNAs in SCI

| LncRNAs | Expression | Functional role | Related gene | References |
|---------|------------|----------------|--------------|------------|
| XIST    | Up         | Enhanced neuronal apoptosis | miR-494/PTEN/AKT | 24 |
| ANRIL   | Up         | Inhibited neuronal apoptosis | miR-125a/MCL-1/ERK/MAPK | 25 |
| Sox2ot  | Up         | Enhanced neuronal apoptosis | miR-211/MCL-1 isoform2/AKT | 26 |
| BDNF-AS | Up         | Enhanced neuronal apoptosis | miR-130b-5p/PRDM5 | 27 |
| DGC5    | Down       | Inhibited neuronal apoptosis | PRDM5 | 28 |
| MALAT1  | Up         | Activated microglia; increased pro-inflammatory cytokines | miR-199b1/IKK/iNF-κB | 29 |
| TUG1    | Up         | Activated microglia; increased pro-inflammatory cytokines | TRIL/TLR4/NF-κB | 30 |
| TUSC7   | Down       | Inhibited microglia activation; reduced pro-inflammatory | miR-449a/PPAR-γ | 31 |
| SCIR1   | Down       | Inhibited astrocytes proliferation and migration; alleviated the gial inhibition | Wnt/Bmp | 32 |
| SNHG5   | Up         | Activated microglia and astrocytes | KLF4/eNOS | 33 |

### Functional roles of lncRNAs in spinal cord injury

In the past years, studies showed that lncRNAs have played important roles in the occurrence and progression of several neurological disorders. Some well demonstrated examples suggested that lncRNAs can regulate gene expression in cis or trans way through recruitment of proteins or molecular complexes to specific loci, serve as scaffolds to form nuclear or cytoplasmic complexes, and pair with other RNAs to trigger posttranscriptional regulation. The diverse modes of action for lncRNAs make them suitable to orchestrate multiple genes expression after SCI. The functional characterization of differentially expressed lncRNAs in SCI is just emerging. Herein, specific lncRNAs with their functions and molecular mechanisms characterized in SCI are discussed in detail (Table 2).

#### XIST

The X-inactive-specific transcript (XIST) is one of the first lncRNAs discovered in mammals owing to its heavy involvement in X inactivation. Several studies have demonstrated that XIST expression was upregulated in the spinal cord of SCI rats, in which knockdown of this lncRNA improved hindlimb locomotor activity, attenuated tissue

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**Table 1.** Long non-coding RNA expression profiles in SCI.

| No. | Methods | Sample | Phase of SCI | Filtering criteria | Upregulated | Downregulated | References |
|-----|---------|--------|--------------|-------------------|-------------|---------------|------------|
| 1   | Microarray qRT-PCR Bioinformatics analysis | T10 | Immediate phase | Fold change > 2; \( P < 0.05 \) | 528 lncRNAs | 244 lncRNAs | 17 |
| 2   | Microarray qRT-PCR Bioinformatics analysis | T10 | Acute phase | Fold change > 2; \( P < 0.05 \) | 1332 lncRNAs | 1861 lncRNAs | 18 |
| 3   | Microarray qRT-PCR Bioinformatics analysis | C5 | Chronic phase | Fold change > 1.1; \( P < 0.05 \) | 738 lncRNAs | 528 lncRNAs | 19 |
| 4   | RNA-sequencing | T9 | Chronic phase | Fold change > 2; \( P < 0.05 \) | 407 lncRNAs | 148 lncRNAs | 22 |

**Table 2.** Functional roles of specific deregulated lncRNAs in SCI.

| LncRNAs | Expression | Functional role | Related gene | References |
|---------|------------|----------------|--------------|------------|
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damage, and inhibited apoptosis in rats following SCI. The pro-apoptotic action of XIST was found to be mediated through mopping up anti-apoptotic miRNA, namely miR-494, leading to derepression of its pro-apoptotic target. Moreover, miR-494 was found to target phosphate and tension homology deleted on chromosome ten (PTEN), which is a pro-apoptotic gene by negatively regulating the PI3K/AKT signaling pathway. In this regards, the PI3K/AKT signaling pathway is a major determinant in the control of diverse cellular processes.\textsuperscript{35,36} Further study demonstrated that antagoniR-494 could reverse the protective effects of XIST knockdown on SCI rats through blocking the PTEN/PI3K/AKT signaling pathway. These results suggested that XIST knockdown may play an important role in limiting neuronal apoptosis in rats following SCI, and that the observed protective effects of XIST knockdown might have been mediated by its regulation on the phosphorylation of AKT by competitively binding miR-494.\textsuperscript{24} These findings have revealed, for the first time, the importance of the XIST/miR-494/PTEN/AKT signaling axis in the pathogenesis of SCI and suggest that XIST may be a promising molecular target for SCI therapy.

ANRIL

MCL-1 is an anti-apoptotic member of the Bcl-2 family and is identified as a key regulator to modulate both apoptotic and autophagic neuronal cell death.\textsuperscript{37} Besides, MCL-1 isoform1 is shown to promote cell survival by inhibiting apoptosis while isoform2 is death-inducing, which can induce cell apoptosis. Antisense non-coding RNA in the INK4 locus (ANRIL) is a newly discovered non-coding RNA lying on the strongest genetic susceptibility in the chromosome 9p21 region and has been identified in several physiological and pathological processes in central nervous system (CNS).\textsuperscript{38} Li et al.\textsuperscript{25} investigated the functional role of ANRIL in SCI, they found that the expression of ANRIL in lesion spinal cord was progressively upregulated compared with normal samples. Besides, Suppression of ANRIL was identified to promote H$_2$O$_2$-induced PC-12 cell injury by decreasing cell viability, migration, invasion and promoting apoptosis in PC-12cells. Further results showed that ANRIL acted as a sponge of miR-125a and its suppression aggravated H$_2$O$_2$-induced PC-12 cell injury through upregulating miR-125a. Moreover, we found MCL-1 was a target of miR-125a and MCL-1 level was negatively correlated with miR-125a level. In addition, MCL-1 ameliorated H$_2$O$_2$-induced PC-12 cell injury by activating MAPK/ERK pathway. These data suggested that ANRIL might inhibit SCI development by reducing apoptosis in an MAPK/ERK-dependent manner.

Sox2ot

LncRNAsox2 overlapping transcript (Sox2ot), localized on human chromosome 3q26. 33, has been demonstrated to play a key role in tumorigenesis of several cancers.\textsuperscript{39,40} Moreover, knockdown of Sox2ot is also found to alleviate diabetes mellitus-induced retinal ganglion cell injury.\textsuperscript{41} Therefore, Yin et al.\textsuperscript{26} are the first to investigate the effects and possible mechanisms of Sox2ot on cell injury in SCI. They confirmed that Sox2ot expression was significantly increased in H$_2$O$_2$-induced PC-12 cell and suppression of Sox2ot inhibited cell injury by increasing cell viability, migration, invasion, and decreasing apoptosis and autophagy. Moreover, miR-211 expression was upregulated in PC-12 cells after suppression of Sox2ot. Furthermore, MCL-1 isoform2 was identified as a direct target of miR-211 and could be negatively regulated by miR-211. Suppression of miR-211 aggravated H$_2$O$_2$-induced cell injury by regulation of MCL-1 isoform2. Further studies showed that inhibition of miR-211 suppressed the activation of the Akt/m TOR/p70S6K signaling pathway in H$_2$O$_2$-treated PC-12 cells, which was reversed after knockdown of MCL-1 isoform2 at the same time. Mechanistically, Akt/m TOR/p70S6K is an important pathway for regulating cell growth, survival, and differentiation, and its activation plays key roles in improving SCI-induced motor function defects.\textsuperscript{42} In conclusion, these findings indicate that the downregulation of Sox2ot protect PC-12 cells from H$_2$O$_2$-induced injury in SCI via targeting the miR-211/MCL-1 isoform2 axis. MCL-1 isoform2 further regulate the activation of Akt/m TOR/p70S6K pathway to mediate H$_2$O$_2$-induced injury. Hence, the Sox2ot/miR-211/MCL-1 isoform2 axis may be a promising therapeutic strategy for SCI.

BDNF-AS

PR (PRDI-BF1 and RIZ) domain protein 5 (PRDM5) is a subfamily of the kruppel-like zinc finger gene products that modulate cellular processes such as cell differentiation, growth and apoptosis. Recently, accumulating evidence has strongly implied that the expression of PRDM5 was significantly increased in neurons in rat model of ASCI.\textsuperscript{43} Zhang et al.\textsuperscript{27} investigated the functional role of lncRNA brain-derived neurotrophic factor antisense (BDNF-AS), which has been declared that BDNF-AS knockdown was a novel method to prevent neurotoxicity in mouse embryonic neural stem cell (ESC)-derived neurons in SCI.\textsuperscript{44} The authors demonstrated that the expressions of BDNF-AS and PRDM5 were significantly upregulated, whereas, miR-130b-5p expression was decreased in the spinal cord tissues of SCI model rats. Further experiments showed that knockdown of BDNF-AS improved hindlimb locomotor activity in rats following SCI and inhibited neuronal cell apoptosis. Mechanistically, BDNF-AS inhibition exerted an anti-apoptotic effect by targeting miR-130b-5p, thereby negatively regulating an important pro-apoptotic gene, PRDM5 in SCI rats. These findings suggested that the lncRNA BDNF-AS played a pivotal role in SCI development through inducing aberrant neuronal apoptosis.
DGCR5

The Di George syndrome-associated noncoding RNA, DGCR5, has been considered as a significant neural lncRNA that closely related to neurological disorders. Upregulation of REST contributed to neuronal death induced by the neurotoxicant PCB-95, as a REST target, DGCR5 is inhibited by REST through a proximal upstream binding site, implying its potential neuroprotective effect. Zhang et al.28 are the first to report that DGCR5 is a crucial anti-apoptotic transcript in SCI through binding with PRDM5 protein directly, unlike its previous mechanism of action with miRNAs. In their study, PRDM5 was identified as the target of lncRNA DGCR5 for the first time, devoting itself to modulating neuronal apoptosis in SCI. DGCR5 was downregulated in SCI model rats and in neurons treated with hypoxia. Over-expression of DGCR5 inhibited neuronal apoptosis. However, the protective effect can be reversed by pcDNA-PRDM5 transfection, which means over-expressed DGCR5 inhibited neuronal apoptosis and ameliorated SCI in rat through negatively regulating PRDM5. These data suggested DGCR5 suppresses neuronal apoptosis through directly binding and negatively regulating PRDM5, and thereby ameliorating SCI. This study clarifies the targeting relationship between DGCR5 and PRDM5 protein, providing novel targets and theoretic foundation for SCI prevention and treatment.

MALAT1

Metastasis associated lung adenocarcinoma transcript 1 (MALAT1), also known as nuclear-enriched abundant transcript 2 (NEAT2), is a lncRNA with physiological functions in different cellular processes, including alternative splicing, nuclear organization and epigenetic modulation of gene expression. Although MALAT1 expression level was significantly increased in the spinal cord of the rat contusion epicenter accompanied by activation of IKKβ/NF-κB signaling pathway and an increase in the level of pro-inflammatory cytokines TNF-α and IL-1β. Moreover, MALAT1 expression dramatically increased in the microglia in vitro, but knockdown of MALAT1 attenuated LPS-induced activation of MGs and TNF-α and IL-1β production. Furthermore, they confirmed that MALAT1-activated IKKβ/NF-κB signaling pathway and promoted the production of pro-inflammatory cytokines through downregulating miR-199b. Functionally, MALAT1 knockdown gradually improved the hindlimb locomotor activity of SCI rats, as well as inhibited TNF-α, IL-1β levels and Iba-1 protein, the marker of activated microglia in injured spinal cords. As we all know, microglia cells (MGs), as a kind of phagocytes in central nervous system (CNS), widely distributed in CNS, playing an important role in the nutrition, protection and restoration of neuron. Although MGs are able to decompose and engulf diseased neurons early after SCI, which is conducive to maintain cell microenvironment, continuing activation of MGs induces neuronal degeneration and death. As SCI progresses, MGs release a variety of harmful cytokines involved in the formation of oxygen free radicals, excitotoxicity and inflammatory responses, aggravating neuronal injury. Therefore, knocking down MALAT1 may reduce the activation of MGs through MALAT1/miR-199b/IKKβ/NF-κB axis in SCI, thereby reducing inflammation and improving the microenvironment.

TUG1

Toll-like receptor 4 (TLR4) and TLR4 interactor with leucine-rich repeats (TRIL) play a crucial role in the inflammatory response of SCI which leads to MGs activation and increases blood-spinal cord barrier (BSCB) permeability. The taurine upregulated gene 1 (TUG1) is a newly identified lncRNA frequently upregulated in human malignancies and mechanistically linked to TLR4 signaling pathway activation. Jia et al.30 investigated the expression of TUG1 in lesion spinal cord tissues and its functional role in SCI in relation to TLR4 signaling. The authors collected lesion samples from SCI rats and normal tissues from control subjects. The expressions of TRIL and TLR4 as well as TUG1 were consistently increased and were accompanied by increased expressions of inflammatory cytokines after SCI, whereas these were synchronously downregulated after TUG1 knockdown. Similar to the studies on MALAT1, knockdown of TRIL reduced TLR4 and its mediated NF-κB pathway and inflammatory cytokine IL-1β expressions were reduced, suggesting that TRIL may regulate the TLR4-mediated NF-κB pathway after SCI. Concordantly, downregulation of TUG1 inhibited the TLR4-mediated NF-κB inflammatory pathway and attenuated SCI-induced inflammatory damage to BSCB and neurological function by inhibiting TRIL expression. Collectively, these results suggest that TUG1 and TRIL knockdown may act as an alternative strategy for the treatment of spinal cord injury.

TUSC7

Peroxisome proliferator-activated receptor gamma (PPAR-γ) is a class of nuclear transcription factors activated by ligands, which can inhibit inflammation after activation. Bioinformatics software predicts that miR-449a, a member of miR-449 family, which can increase the expressions of inflammatory factors in the spinal cord of SCI rats and reduce the protective effect of electro-acupuncture on neurons, has binding sites with PPAR 3' UTR. Additionally, it has showed that miR-449 is a targeting gene of lncRNA tumor suppressor candidate 7 (TUSC7), which has low expression in glioma cells and over-expression of TUSC7 can inhibit the proliferation of glioma cells. Because microglia activation can lead to excessive production of inflammatory factors, Yu et al.31 conducted a study to...
investigated the mechanisms of TUSC7/miR-449/PPAR-\(\gamma\) axis on the inflammation caused by microglia activation following SCI. In the spinal cord tissue of SCI rats and HAPI cells induced by LPS, TUSC7 expression was reduced and miR-449a expression was increased. Overexpression of TUSC7 inhibited microglia activation and the expression of inflammatory factors (TNF-\(\alpha\) and IL-1\(\beta\)). Moreover, they found a targeting regulatory relationship between TUSC7 and miR-449a through RIP and RNA pull-down assay, and a negative regulatory relationship between miR-449a and PPAR-\(\gamma\). Mechanistically, TUSC7 could regulate PPAR-\(\gamma\) through miR-449a, and overexpression of TUSC7 inhibited microglia activation and the expression of inflammatory factors through miR-449a. These findings suggested overexpression of TUSC7 inhibited microglia activation and the expression of inflammatory factors in microglia cells by regulating miR-449a/PPAR-\(\gamma\). Thus, restored expression of TUSC7 or inhibition of miR-449a might provide a potential therapeutic target for SCI and expect to move threat to neuron regeneration and tissue degeneration after SCI.

**SCIR1**

Long non-coding spinal cord injury related 1 (lncSCIR1) was first named by Wang et al. because it was significantly downregulated after the spinal cord contusion in rats.\(^{32}\) A study using RNA-Seq technique by Wang showed that the expression level of IncRNA SCIR1 decreased at the lesion site after contusion SCI in rats, whose knockdown with small interference RNA (siRNA) induced a significant increase of cell migration in cultured astrocytes. These results suggested that the dramatic decrease of SCIR1 might be involved in the induction of gliosis and astrocytes migration post SCI. Then they detected that the bone morphogenetic protein 7 (Bmp7) mRNAs were significantly upregulated, whereas wingless-type MMTV integration site family, member 3(Wnt3) significantly downregulated both in vivo and vitro. Previous studies indicated that the decrease of the growth promoting Wnt3 and upregulation of BMP signals have detrimental effects on functional recovery, because external supply of Wnt3 and inhibition of BMPs can promote axon regeneration and meanwhile alleviate the glial inhibition.\(^{55-57}\) The strong correlation between SCIR1 downregulation and the decrease/increase of Wnt3/BMP7 expression respectively, together with the promoting effects of SCIR1 downregulation on astrocytes proliferation and migration, suggested that SCIR1 serve as a beneficial factor for SCI. Local overexpression of SCIR1, thereby, might help neutralize the inhibitory environment around the lesion site and promote functional recovery.

**SNHG5**

Krüppel-like factor 4 (KLF4) is a conserved zinc-finger-containing transcription factor and is regarded as a multi-effect regulator for diverse cellular processes, such as growth arrest, proliferation and apoptosis.\(^{58}\) These multiple regulatory roles of KLF4 represent its involvement in various pathophysiological processes in SCI.\(^{39}\) Small nuclear RNA host gene 5 (SNHG5) is for the first time to be mentioned in B-cell lymphoma and positively regulates KLF4 expression through sponging miR-32 which targets inhibition of KLF4 transcription.\(^{60}\) Jiang et al.\(^{33}\) reported that the levels of SNHG5, KLF4 and eNOS were upregulated in the lesion spinal cord of SCI rats. Interestingly, levels of SNHG5 were positively correlated with both GFAP, a specific protein for astrocytes, and Iba-1, a specific protein for microglia, suggesting an increase in the activation and density of astrocytes and microglia following SCI. Further research indicated that SNHG5 overexpression promoted cell viability of both astrocytes and microglia, but significantly reduced BBB scores of SCI rats. In contrary, knockdown of SNHG5 by siRNA in astrocytes and microglia effectively reduced the viability of cells. Moreover, expression level of KLF4 was enhanced by SNHG5 overexpression and attenuated by SNHG5 knockdown. Furthermore, the eNOS expression and the viability of astrocytes and microglia induced by SNHG5 overexpression was also attenuated by KLF4 knockdown indicating that SNHG5 regulates eNOS of activated microglia via KLF4. These findings collectively suggested that SNHG5 could mediate SCI through KLF4/eNOS signaling axis in inducible of astrocytes and microglia viability.

**Challenges and strategies**

SCI is a kind of fatal injury which leads to neurological damage, necrosis, and dysfunction in patients. As a severe traumatic injury, SCI occurs commonly in modern society.\(^{61}\) As shown above, several reports have suggested the dysregulation of IncRNAs in SCI, in which these regulatory molecules have crosstalk with astrocytes response, AKT signaling, and microglia activation that are pertinent to glial inflammation and infection.\(^{24,26,29-33}\) From the mechanistic point of view, LncRNAs might be involved in SCI progression through modulating neuronal proliferation, apoptosis. Deregulated IncRNAs might also alter the state of microglia or astrocytes and thereby contributing to inflammation or colloidal scar inhibition (Figure 1). Nevertheless, The current knowledge of IncRNAs in terms of their deregulation and mechanisms in SCI is far from complete. There are still many deficiencies that hinder the transformation to clinical treatment. For instance, most of the research on IncRNAs is based on rats, studies on the safety and efficacy of IncRNAs in non-human primates are warranted.\(^{17-19,22}\) Additionally, the application of IncRNAs requires a suitable delivery system. How to select the appropriate vector to ensure efficient and targeted delivery to the target is the future direction of research. Furthermore, systematic identification and validation of IncRNAs as therapeutic strategies in SCI are still lacking. More...
translational works are thus needed to maximize the clinical potentials of lncRNAs in SCI.

Over the recent years, a rapid expansion of technologies has greatly accelerated the discovery and functional characterization of disease-associated lncRNAs. Compared to miRNAs that can target multiple mRNAs, lncRNAs have higher tissue specificity, indicating their advantages as therapeutic targets. However, the clinical utilities of lncRNAs remain not fully established. Future investigations are therefore needed to clarify the upstream and downstream mechanisms as well as clinical implications of lncRNA deregulation in SCI. Further animal studies and subsequent clinical trials are also required to verify the clinical potentials of lncRNAs as therapeutics in SCI.

Conclusion

As we learn about the complexities of the pathological mechanisms of SCI, we cannot be surprised by the thorny nature of bioinformatics engineering and lncRNAs deregulation in SCI. Despite this review elaborates on the dysregulation of lncRNAs and the role of 10 common lncRNAs in the pathogenesis of SCI, it will be a huge challenge to translate these fundamental researches into clinical applications.

In future research, lncRNA will be the key to tackle this desperate disease. Treatment for SCI might thus be achieved through targeting deregulated lncRNAs, for example, using lncRNA-specific small interfering RNA delivered by nanoparticles or lipid-encapsulation as well as small-molecule inhibitors that perturb that interaction of particular lncRNA with its RNA or protein partners.

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Figure 1. Functional roles of specific deregulated lncRNAs in SCI.
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