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Review

Research Progress of MicroRNAs in Spinal Cord Injury

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Abstract

Spinal cord injury is a serious and devastating condition. Recently, research into microRNAs (miRNAs) has become increasingly exhaustive and it has been determined that they are closely related to the pathophysiological processes of spinal cord injury. They participate in the regulation of the inflammatory response of spinal cord injury, the death of neuronal cells, and the repair of neural functions, which are related to the recovery of spinal cord injury. This review focuses on the relationship between miRNA and spinal cord injury, lists miRNA-324-5p, miRNA-221 and miRNA-124, which are helpful for the repair of spinal cord injury, and finally summarizes the current research progress of miRNA-based therapies, so as to provide a foundational reference for clinical and scientific researchers.

Keywords: spinal cord injury; miRNA; miRNA therapy

1. Introduction

Spinal cord injury (SCI) is a serious and debilitating condition that currently is one of the most challenging of medical problems, as it exhibits a high rate of disability and death [1]. Globally, there are over 27 million people living with SCI, with approximately one million new cases each year. Among them, falls and car accidents are the main causes [2]. Recently, the trend globally for SCI is for it to affect younger people, mostly under 30 years of age [3]. The occurrence of irreversible motor and sensory impairment following SCI has implications for the whole person [4]. Many studies have also shown that SCI patients are more likely to suffer from depression or anxiety than normal people [5–7]. Additionally, the financial cost per SCI patient is generally higher [8]. It is evident that SCI can place a serious burden on the patient physically, psychologically, and financially. However, currently the pathological mechanisms of spinal cord injury are known to be complex and undefined, and clinical diagnosis relies mainly on physical examination, imaging, and relevant biochemical indicators [9]. Most importantly, the treatment of SCI is limited, currently focused on surgical relief of compression, and reduction of secondary pathologies, as well as the use of hormonal drugs to reduce inflammation and swelling [10]. Therefore, it is of great importance to identify and study the pathogenesis of SCI and develop new treatment methods and tools.

miRNAs are RNAs of 21–25 amino acids in length that do not encode proteins [11,12]. Intracellularly they regulate gene expression by binding to the 3'-untranslated region (UTR) of messenger RNA (mRNA) to either inhibit translation or induce degradation of the target mRNA [13]. Further, it has been shown that miRNAs influence both various physiological processes during development and tissue homeostasis by regulating the expression of approximately 90% of human genes [14]. Recently miRNAs have been widely studied for their role in various human diseases including tumours [15], haematological diseases [16], cardiovascular diseases [17], and central nervous system diseases [18]. Currently, studies in animal models [19] and bioinformatics [20] analyses have preliminarily demonstrated that alterations in miRNA expression have an impact on key processes in the pathophysiology of SCI. In-depth studies of miRNAs may also generate novel approaches to the treatment of SCI.

In this review, initially the close relationship between miRNAs and SCI is summarized and their potential to treat SCI through multiple pathways briefly outlined. Three miRNAs are then described that are more closely related to SCI, have been studied more frequently, and have potential applications for SCI treatment. Finally, current approaches to miRNA-based drug therapy for SCI and current issues of clinical translation are discussed and future directions for miRNA research in SCI are examined.

2. Characterization of miRNA Expression after Spinal Cord Injury and Its Therapeutic Potential

Expression of miRNAs in the rat spinal cord is exceptionally abundant, with one study showing that approximately 77% of identified mature rat miRNAs are expressed there [21]. Tang *et al.* [22] identified a total of 3361 miRNAs expressed in the spinal cord of adult rats. Additionally, the spatial distribution of miRNAs in the spinal cord varied. For example, miRNA-9 is more highly expressed in the dorsal sacral medulla, whereas, miRNA-124a/125b is more highly expressed in the ventral aspect [23]. SCI models can be classified according to the mech-

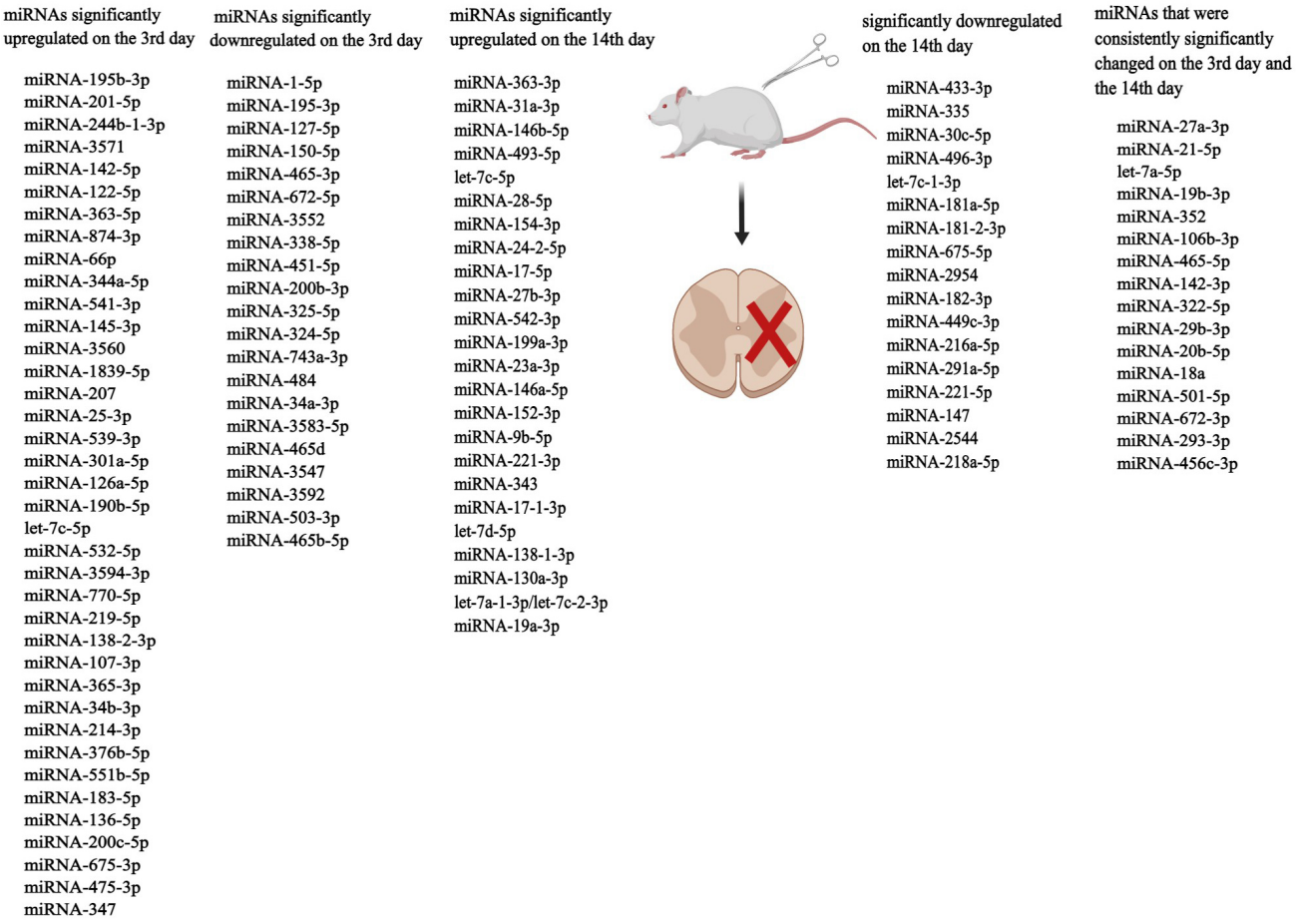

Fig. 1. Map of miRNA changes over time after unilateral and contralateral spinal cord injury in rats.

anism of injury, such as avulsion of the spinal brachial plexus, contusion of the spinal cord, ischaemia and reperfusion of the spinal cord, compression of the spinal cord, dislocation, transection, or chemical injury [24]. As SCI models of three injury mechanisms—avulsion of the spinal brachial plexus, ischemia-reperfusion of the spinal cord, contusion of the spinal cord, and spinal cord transection—are currently well studied, the miRNA expression patterns of these four injury-dependent mechanisms are briefly outlined here. The first is the miRNA expression pattern after spinal brachial plexus nerve avulsion. After such unilateral nerve avulsions in adult rats, the injured side was compared to the contralateral side. It was found that on day three after injury, 55 miRNAs were up-regulated and 24 were down-regulated. More significant up-regulations included miRNA-201-5p and miRNA-142-5p, while more significant down-regulation included miRNA-34a-3p and miRNA-324-5p. Up-regulation of 36 miRNAs including miRNA-363-3p and down-regulation of 23 miRNAs including miRNA-147 were observed at day 14 after injury. Additionally, 11 miRNAs, including miRNA-21-5p, continued to increase in expression after SCI, while only miRNA-466c-3p continued to decrease in expression after SCI. In comparison, 16 miRNAs including miRNA-18a showed persistent and significant changes at both days three and fourteen after SCI [22] (Fig. 1). Thirteen miRNAs were aberrantly expressed 24 hours after spinal cord ischemia-reperfusion. while 12 miRNAs including miRNA-331-5p were upregulated and miRNA-3084b-5p was downregulated. Forty-eight hours after spinal cord ischemia-reperfusion, 105 miRNAs showed differential expression. This included the upregulation of 44 miRNAs including miRNA-140-5p and the downregulation of 61 miRNAs including miRNA-129-2-3p. Only miRNA-22-3p was significantly upregulated at both 24 and 48 hours following reperfusion [25]. Studies of significant miRNA dysregulation after spinal cord contusion have recently been extensively reported [26–28]. Liu *et al.* [21] published an earlier report on miRNA expression analysis after contusion SCI in rats. Of the 46 miRNAs examined, 30 miRNAs were found to be increased and 16 miRNAs were found to be decreased after spinal cord contusion. Additionally, miRNA-21 was found to be elevated in the serum of patients with spinal cord contusion immediately during the acute phase of injury and peaked on day seven following SCI, before decreasing to normal control levels [27]. He *et al.* [29] reported the miRNA expression profile of the rat spinal cord 3 days after transection. Compared with the sham-operated

group, the expression of 42 miRNAs, including miRNA-124, was down-regulated by 2-fold and the expression of 42 miRNAs, including miRNA-182, was up-regulated by more than 2-fold. In addition, miRNA-326, miRNA-30b-5p, miRNA-10a-5p and miRNA-127-3p were more than 4-fold down-regulated. Since the main function of miRNAs is the regulation of gene expression products, it is inferred that miRNA expression patterns after SCI may fall into three broad categories: (1) increased after injury, (2) decreased after injury, and (3) possibly bi-directional changes after injury. These expression patterns may also regulate gene expression products at different pathophysiological stages after SCI. This has illuminating implications for elucidation of the pathogenesis of SCI and identification of new targets for the treatment of SCI. The pathological process of SCI is currently divided into a primary injury phase and secondary injury phase. The primary injury phase is mainly the compression, contusion, and transection of the spinal cord, which are mechanical injuries. while the secondary injury phase is typically the period of biological effects such as inflammation, neuronal cell death, and destruction of neurological functions [30]. As miRNA is a molecule that typically regulates gene expression products, it may play a key role in the secondary damage phase of SCI. In recent years a number of miRNAs have been identified as important regulators of SCI, which can influence the pathophysiological processes of SCI through a variety of pathways. miRNA-21, miRNA-212-3p, and miRNA-26a inhibit neuronal apoptosis through the PTEN/AKT pathway thereby contributing to the recovery of motor function after SCI in rats [31–33]. miRNA-940, miRNA-182, miRNA-488, and miRNA-543-5p are involved in the NF-KB pathway to inhibit the release of pro-inflammatory factors such as tumour necrosis factor-α (TNF-α) and interleukin-1β (IL-1β) and subsequently regulate the inflammatory response after SCI in rats [34–37]. miRNA-411, miRNA-129-5p, miRNA-9-5p, and miRNA-7a inhibit apoptosis of neuronal cells after SCI in rats [38–41]. miRNA-466c-3p and miRNA-155 are involved in the regulation of mitochondrial function after SCI in rats [42,43]. miRNA-99a and miRNA-672-3p regulate oxidative stress induced after SCI in rats [44,45]. miRNA-125a and miRNA-216a-5p regulate M1/M2 polarization in microglia by targeting IRF5 (Recombinant Interferon Regulatory Factor 5) and TLR4 (Toll-Like Receptor 4), respectively, ultimately inhibiting the inflammatory response after SCI in rats [46,47]. Many of the basic experiments described above have shown that miRNAs are extensively involved in various repair pathways of SCI. However, miRNAs may not contribute to SCI recovery through a single pathway. Many studies are now highlighting the role of complex regulatory networks among various non-coding RNAs in human diseases [48–50]. miRNA regulation through a single pathway may only be part of a large network, which may point the way to future research on non-coding RNAs. Although most of the experimental studies are currently at the animal testing stage, the conclusions of further clinical trials are unclear and there are no detailed and complete reports on the analysis of abnormal miRNA expression profiles in SCI patients. However, the data from the aforementioned animal models of SCI may largely reflect the condition of SCI patients. It is thus clear that miRNAs have shown initial potential for the treatment of SCI.

3. The Following Three miRNAs have been Shown to be Closely Associated with SCI in Basic Experiments and may be Applied to SCI Therapy

3.1 miRNA-324-5p

MiRNA-324-5p is located on chromosome 17p13.1 [51]. and has been shown to be associated with central nervous system disorders such as epilepsy [52]. *In vitro* experiments have revealed that overexpression of miRNA-324-5p further inhibits the viability of oxygen-glucose deprivation (OGD)-treated neuronal cells and ultimately induces apoptosis [53]. In *in vivo* experiments, Wang *et al*. [54] found that miRNA-324-5p expression was significantly elevated in the acute phase after SCI in rats. Inhibition of endogenous miRNA-324-5p expression in rats with SCI reduced neuronal loss near the injury area and improved locomotor performance in rats with SCI. Additionally, knockdown of miRNA-324-5p inhibited the downregulation of brain-derived neurotrophic factor (BDNF) and glial cell-derived neurotrophic factor (GDNF) in SCI rats [54]. miRNA-324-5p directly targets NAD-dependent deacetylase sirtuin-1 (Sirt1) and chemokine ligand 5 (CCL5), and negatively regulates the levels of Sirt1 and CCL5 [54,55]. Both Sirt1 and CCL5 have been shown to be involved in the regulation of a large number of biological processes including the cell cycle, DNA repair, apoptosis and inflammation, autophagy and senescence [56,57]. It is not clear how miRNA-324-5p is expressed in SCI patients. And the regulation of Sirt1/CCL5 by miRNA-324-5p has not been reported in human cases. Experimental validation in human cell lines or non-human primates may be required in future studies. However, the successful experience of applying miRNA-324-5p inhibitors in animal models may demonstrate the great potential of its related inhibitors for application in the treatment of human SCI.

3.2 miRNA-221

MiRNA-221 is one of the most significantly and progressively increased miRNAs over seven days following spinal cord injury [21]. It has been found that miRNA-221 can inhibit apoptosis by regulating the apoptosis regulator p53 upregulated modulator of apoptosis (PUMA) and downstream c-Jun N-terminal kinase (JNK)/H2A histone family member X (H2AX) signalling [58]. On the one hand, miRNA-221 overexpression leads to inhibition of hippocampal neuronal proliferation and on the other hand

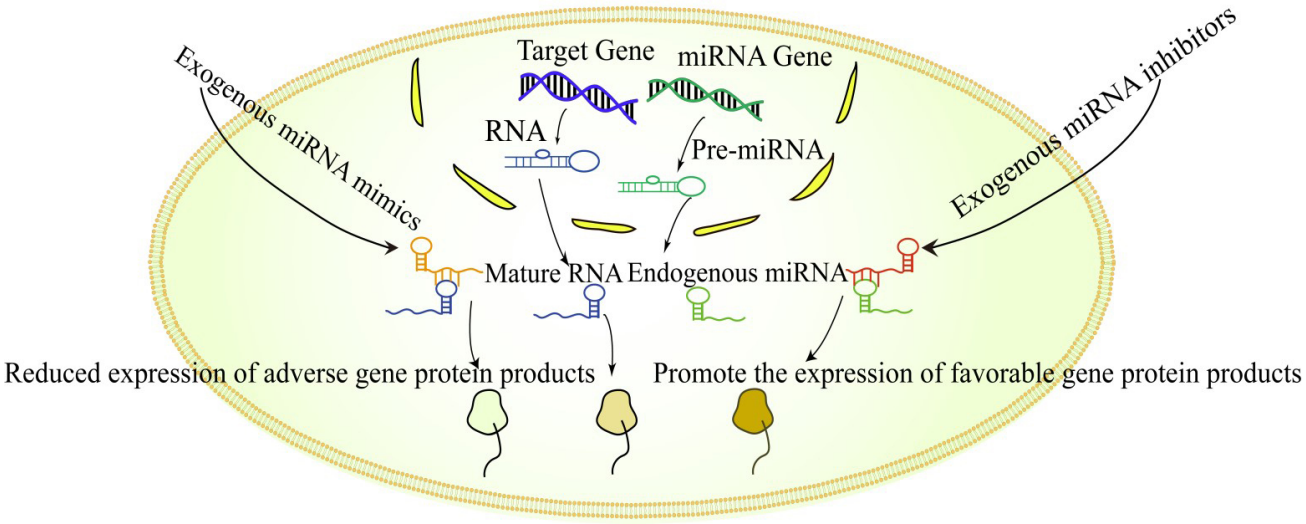

Fig. 2. Schematic diagram of miRNA-based therapy.

neuronal apoptosis increases [59], silencing miRNA-221 increases the expression of BDNF by neuronal cells [60]. *In vitro* experiments with oxygen-glucose deprivation-treated (OGD) human neural precursor cells (AGE1.HN) and human neuroblastoma cells (SY-SH-5Y), miRNA-221 was significantly downregulated. This resulted in elevated tumour necrosis factor-α (TNF-α) and interleukin 6 (IL-6) and an increased percentage of apoptotic cells [61]. In addition, miRNA-221 has been shown to directly target tumour necrosis factor alpha induced protein 2 (TNFAIP2) to regulate the inflammatory response and apoptosis of neuronal cells [62]. *In vivo* experiments revealed that the inflammatory markers TNF-α and IL-6 and the index of oxidative stress were significantly upregulated in mice after SCI and overexpression of miRNA-221 significantly inhibited the expression of these factors [63]. miRNA-221 also directly targets suppressor of cytokine signalling-1 (SOCS-1), which is associated with inflammation [64]. A growing number of studies now show that the immune inflammatory response after SCI plays a crucial role in the injury and recovery process [65–67]. This also shows the important role that miRNA-221 plays in the recovery from SCI, especially through inflammation suppression pathways.

3.3 miRNA-124

One study [68] found that miRNA-124 concentrations in the mouse central nervous system (CNS) were more than 100-fold higher than in other systems and that miRNA-124 expression varied within the CNS, with expression ratios of 60.7% in the cerebellum and 35.4% in the spinal cord. miRNA-124 expression was significantly reduced from one to seven days after SCI in mice [69]. Overexpression of miRNA-124 promotes the differentiation of bone marrow mesenchymal stem cells (BMSCS) towards neurons and it inhibits the expression of proteins with anti-neuronal activity, including repressor element-1 silencing transcription factor (REST), small c-terminal domain phosphatase 1 (SCP1), and Recombinant Sex Determining Region Y Box 9 (Sox9) Protein [70]. In *in vitro* experiments with the mouse macrophage cell line RAW264.7 and human HEK293 cells, miRNA-124 was shown to reduce IL-6 and TNF-α production via the Recombinant Signal Transducer and Activator of Transcription 3 (STAT3) Tumour necrosis factor-α-converting enzyme (TACE) production and subsequently inhibit TNF-α release to regulate Lipopolysaccharide (LPS)-induced pro-inflammatory cytokine production [71]. Chitosan is a natural non-toxic degradable complex. One study [71] piggybacked miRNA-124 in chitosan and then transfected it into rat microglia and found that transfection of miRNA-124 reduced the expression of major histocompatibility complex-II (MHC-II), TNF-α, and the expression of Reactive oxygen species (ROS) was found to reduce the inflammatory response after SCI. It also prevented the development of secondary neuronal damage induced by activated microglia/macrophage secretory proteins after SCI. More relevant studies have now shown that the downregulation of circRNA-2960, its target miRNA-124, by molecular sponge action after SCI attenuates the inflammatory response and inhibits apoptosis at the site of the lesion [72]. The application of miRNA-124 mimics at the appropriate stage as well as at the lesion site based on the significant changes in miRNA-124 in the early stages of SCI and differential expression in the CNS may provide a new approach to promote recovery in SCI patients.

4. Research Progress of miRNA-Based Therapies

Although the effect of miRNAs in treating human diseases, including SCI, remains to be elucidated, there is growing evidence that miRNAs represent a new class of drug targets.

4.1 Currently, Two Types of miRNA-Based Therapies have been Developed

(1) miRNA mimics and (2) miRNA inhibitors. The former is an exogenous synthetic miRNA mimic that acts specifically on its target RNA to silence the endogenous RNA and thereby attenuate the protein expression product of the unfavourable gene. The latter is a synthetic inhibitor of a miRNA that binds to the endogenous miRNA through specific targeting and weakens the silencing effect of the endogenous miRNA on the favourable gene and promotes the expression of the protein product of the favourable gene [73] (Fig. 2).

4.2 Challenges for miRNA-Based Clinical Therapies

4.2.1 Mode of Administration

(1) Intrathecal drug delivery. Intrathecal administration in the subarachnoid space is commonly used to deliver miRNA-based drugs to the spinal cord or the cerebellar pool at the base of the brain [74]. In one study, miRNA-651 injected through a subdural catheter into rats three days after SCI inhibited the expression of leucine rich repeat and Ig domain containing 1 (LINGO-1), resulted in increased neuronal survival and enhanced axonal extension and myelin formation, and ultimately improved recovery of motor function in the hind limbs of SCI rats [75]. However, this invasive drug delivery method with relatively precise positioning may be difficult to apply in the clinical setting and may cause secondary injuries to patients.

(2) Intravenous injection. Due to the advantages of high dosing volume, ease of handling, low risk and the ability to reach almost all damaged tissues, they are currently probably the most suitable for clinical use in relative terms [76]. However, how to get miRNA-based drugs to bypass the blood brain barrier (BBB) or blood-spinal cord barrier (BSCB) is still a clinical challenge that needs to be addressed.

(3) Intranasal drug delivery. This delivery method has been shown to bypass the BBB or BSCB and allows access to the central nervous system in animal models [77]. It may also be a potentially non-invasive method of clinical drug delivery.

(4) Adeno-associated virus (AAV). This method allows the delivery of *in vitro* synthetic miRNA mimics or inhibitors into the target genome. Although this delivery method can be validated in both conceptual and animal models, there are still some issues with clinical application such as immunological responses and ethical aspects [78]. Most importantly, despite the conceptual validation of this approach, it is best performed prior to SCI to ensure that the vector material has sufficient time to function. Because viral-mediated miRNA knockdown or overexpression usually takes time to express, this approach may not be very useful in acute SCI injury, but could be considered for use during recovery from SCI [74].

(5) Exosomal delivery [79]. Has a strong biological barrier permeability to selectively penetrate tissue injury due to its natural ability to target donor cells based on its ability to deliver drugs and has nanomolecules of cell surface material [80]. This method of delivering miRNA to the site of spinal cord injury has great potential for application.

(6) The use of biological complexes such as chitosan [81]. As a natural complex, chitosan is the only positively charged edible fibre in nature, non-toxic, non-hazardous, readily degradable in humans, and has been experimentally corroborated as a miRNA delivery material in animal models of SCI [71]. From the chemical structure, chitosan is a cationic polyamine, which can bind to negatively charged miRNA through electrostatic interaction, thus encapsulating miRNA and making it less susceptible to destruction by RNA enzymes, effectively protecting miRNA [82]. At the same time, miRNAs are characterized by immediate degradation after action and can only play a regulatory role for a short period of time, whereas chitosan has good mucosal adhesion properties, which allows it to accomplish sustained release *in vivo* as well, and can effectively increase the action time of miRNAs [83]. It is worth mentioning the emerging biomaterial hydrogel, a physically entangled and/or chemically cross-linked polymer structure with a high water content, which mimics natural human tissue due to its similarity to the extracellular matrix [84]. Although most hydrogel-based reports have confirmed the delivery capacity of hydrogels, some studies have shown that certain hydrogels have intrinsic immunomodulatory properties that are well known to attenuate the inflammatory process of SCI [85]. However, few studies have been reported on miRNA-loaded hydrogels for SCI repair. Although the mode of miRNA administration in the clinic is still debatable, the mode of administration in animal models could certainly provide new avenues and inspiration for clinical drug delivery.

4.2.2 Administration Dose

The dose of a miRNA-based drug should have a significant impact on the predicted target gene, making it particularly important to assess the half-life of miRNA-based drugs, as it can determine whether multiple injections are required to ensure maximum drug benefit [86]. In practice, however, dosing ultimately depends on the method of delivery and the model of injection. Currently, miRNA dosing is mostly empirically determined in animal models and the dosing of miRNA-based drugs in the clinic remains open to debate.

4.2.3 Time Window of Administration

There are currently two main approaches to the time window for miRNA administration in animal SCI models: (1) pre-SCI administration and (2) post-SCI administration. The former is pre-protective for SCI models, but cannot be carried out in practical clinical applications. Although the

Table 1. Development and intended application of miRNA-based drugs and therapeutic limitations of clinical trial exposure.

Drug	Drug nature	Expected application	Therapeutic limitations of clinical trial exposure
Miravirsen	miRNA-122 inhibitor	HCV	Drug half-life, miRNA off-target effects and their side-effects
RG-101	miRNA-122 inhibitor	HCV	Drug half-life, miRNA off-target effects and their side-effects
RG-125/AZD4076	miRNA-103/107 inhibitor	Type 2 diabetes or nonalcoholic fatty liver disease	Discontinued
MRX34	miRNA-34 mimics	Primary liver cancer and small cell lung cancer or lymphoma Melanoma or multiple myeloma or renal cell carcinoma	Side effects of miRNA off-target effects
TargomiR	miRNA-16 mimics	Recurrent breast cancer	Drug half-life, miRNA off-target effects and their side-effects
MRG-106	miRNA-155 mimics	Mycosis fungoides skin or T cell lymphoma	Clinical trials may present problems that have arisen with other miRNA-based drugs

latter dosing time window is more in line with clinical applications, the dosing method and the dosing time window are often closely linked. Both the dosing method and the dosing time window need to be precisely optimised and tailored for each treatment application. This is because it may be relevant to the delivery vehicle, tissue exposure time, delivery route and target cell type [87].

4.3 Some miRNA-Based Drugs are under Clinical Trial Development

Several miRNA-based drugs tested in animal models have entered human clinical trials, including Miravirsen, RG-101, RG-125/AZD4076 (which has been called off), MRX34, TagomiR, and MRG-106 (Table 1). Although theoretical and pre-clinical trials have demonstrated the potential of these miRNA-based drugs, their therapeutic limitations have also been exposed. Miravirsen is essentially a miRNA-122 inhibitor. Although short-term use of Miravirsen does not result in changes in the genetic material as well as the phenotype of Hepatitis C Virus (HCV), however a small proportion of HCV still proliferates slowly in the presence of Miravirsen. As the dose of Miravirsen is increased, mutations begin to occur in the 5'UTR region of HCV viral RNA, which may also lead to off-target effects of miRNA-based drug therapy [88]. RG-101 is also a miRNA-122 inhibitor by nature. In clinical trials with RG-101, doses of 2 mg/kg versus 4 mg/kg were shown to be safe for humans and to have a significant inhibitory effect on HCV replication. However, hepatitis C is prone to relapse at this dose, possibly due to unresolved half-life issues and miRNA off-target effects of the drug [89]. Subsequent studies used RG-101 in combination with Harvoni to determine whether treatment could be prolonged, but a relapse of hepatitis C and cases of jaundice were observed at 24 weeks and the clinical trial was eventually suspended by the US Food and Drug Administration (FDA) [90]. RG-125/AZD4076 is essentially a miRNA-103/107 inhibitor, which improves insulin sensitivity in type II diabetes and non-alcoholic fatty liver disease, but development of RG-125/AZD4076 has been halted as the clinical program was terminated in June 2017 [74]. MRX34 is essentially a miRNA-34 mimetic. It acts as a tumour suppressor through multiple pathways and is one of the most advanced miRNA-based drugs in the oncology field [91]. However, subsequent negative events (and even patient deaths), possibly related to the side effects of miRNA off-target effects, eventually led to the termination of the clinical programme [92]. TargomiR is essentially a mimic of miRNA-16 [93]. Although preliminary clinical trials corroborated the remission effect of TargomiR in patients with recurrent thoracic cancer, subsequent clinical trials revealed problems with drug dose selection and side effects arising from the off-target effect of miRNAs. Eventually, deaths were reported [94]. MRG-106 is essentially a miRNA-155 mimic. It is expected to be used in mycosis fungoides cutaneous T-cell lymphoma [95]. It does not appear to have been found in recent studies to produce evidence of serious adverse consequences [96]. But in-depth clinical trials may also face problems that have arisen with other miRNA-based drugs. The main problem currently facing miRNA-based drug development is the off-target effect. Because miRNAs can regulate one or more target genes, miRNAs may act on other target genes to produce unwanted side effects or may even activate pathways that counteract the protective effect [97]. Therefore, an urgent clinical task should be to improve the specificity of miRNAs for selected target genes and to develop methods to block off-target effects. Another common problem with miRNA-based therapies is their potential for rapid degradation by RNA enzymes. Repeated injections or chemical modifications of miRNA-based drugs may therefore be required to guarantee that they work for an effective circulation time [98]. Next is the mode of administration

of miRNA-based drugs and the window of administration. Different diseases may dictate different drug delivery methods due to the different sites of lesion involvement. In particular, the delivery of miRNAs following CNS injury is complicated by the need to bypass the BBB or BSCB. Although basic experiments have demonstrated several methods such as intrathecal and intranasal administration and these studies have proven the principle, there are considerable challenges. For example, intrathecal administration is likely to cause secondary harm to patients in the clinic and it is unclear whether intranasal administration is translatable to humans with different anatomical structures [74]. The dosing window may also have different time points in different diseases. For example, viral-mediated miRNA knockdown overexpression may often take time to express, so it is important to assess the precise timing of dosing. Although none of these drugs are relevant to SCI and the current clinical translation is still problematic, they may provide new avenues and guidance for the development of SCI-based miRNA drugs, which continue to demonstrate the great potential of miRNAs in the treatment of SCI.

5. Summary and Outlook

In summary, (1) miRNAs are likely to provide a new approach to SCI treatment. However, the animal models chosen for most of the current experiments demonstrating the recovery-promoting effects of miRNAs on SCI are non-primate and future studies will likely also be conducted in non-human primates. (2) Most of the RNAs present in organisms are non-coding RNAs [99], such as miRNA, circRNA, lncRNA, etc. As research progresses the interactions of these non-coding RNAs and the complex network of regulatory relationships are found to remain largely unknown. It supports the notion that ideal future miRNA-based therapies should focus on the regulatory network of non-coding RNAs. In 2018, a related study found that miRNA-7 and miRNA-671 cooperated to build a complex regulatory network to regulate brain function in mice [100]. However, similar studies have been rarely reported in recent years in SCI as well as other diseases. Future studies could therefore focus on the regulatory network of non-coding RNAs. (3) Although miRNAs have been shown to be useful for the alleviation of SCI in animal models, there are still many issues in clinical application. (i) How to effectively avoid the off-target effect of miRNAs and thus avoid the possible side effects of miRNAs acting on other target genes. (ii) How to choose the appropriate vector and delivery method, the appropriate dosing window, and the appropriate dose to ensure the maximum benefit of miRNA as a drug for SCI. (iii) How miRNA-based drugs can bypass the BBB or BSCB. Although much work remains to be done to develop miRNAs for application in clinical applications, the resolution of related issues will certainly enhance the development of this emerging field.

Author Contributions

ZZD wrote and revised the manuscript; YHC designed and critically revised the manuscript, and all authors read and approved the final manuscript.

Ethics Approval and Consent to Participate

Not applicable.

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Conflict of Interest

The authors declare no conflict of interest.

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