

Review article

MicroRNA-31 regulating apoptosis by mediating the phosphatidylinositol-3 kinase/protein kinase B signaling pathway in treatment of spinal cord injury

Yali Wang^a, Yitong Yuan^a, Yuantao Gao^b, Xiao Li^a, Feng Tian^a, Fang Liu^a,
Ruochen Du^a, Pengfei Li^a, Fei Wang^a, Suming Xu^c, Xueqing Wu^{c,*},
Chunfang Wang^{a,*}

^a Laboratory Animal Center, Shanxi Medical University, Xinjian South Road 56#, Shanxi 030001, China

^b Nanchang University, Nanchang 330000, China

^c Center of Reproductive Medicine, Children's Hospital of Shanxi and Women Health Center of Shanxi, Shanxi 030013, China

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Abstract

Apoptosis is a highly conservative energy demand program for non-inflammatory cell death, which is extremely significant in normal physiology and disease. There are many techniques used for studying apoptosis. MicroRNA (miRNA) is closely related to cell apoptosis, and especially microRNA-31 (miR-31) is involved in apoptosis by regulating a large number of target genes and signaling pathways. In many neurological diseases, cell apoptosis or programmed cell death plays an important role in the reduction of cell number, including the reduction of neurons in spinal cord injuries. In recent years, the phosphoinositol 3-kinase/AKT (PI3K/AKT) signal pathway, as a signal pathway involved in a variety of cell functions, has been studied in spinal cord injury diseases. The PI3K/AKT pathway directly or indirectly affects whether apoptosis occurs in a cell, thereby affecting a significant intracellular event sequence. This paper reviewed the interactions of miR-31 target sites in the PI3K/AKT signaling pathway, and explored new ways to prevent and treat spinal cord injury by regulating the effect of miR-31 on apoptosis.

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Keywords: Apoptosis; MicroRNA; MicroRNA-31; PI3K/AKT signaling pathway; Spinal cord injury

1. Introduction

MicroRNAs (miRNAs) are non-coding regulatory RNA of 21–25 nucleotides generated by larger RNA precursors [1], usually inhibiting the translation and stability of messenger RNAs (mRNAs), controlling genes relevant to cell processes, and participating in regulation

of proliferation, differentiation, migration and apoptosis [2,3]. miRNAs down-regulate protein expression by binding to the complementary sequence of 3'-untranslation region (3'-UTR) in the target mRNA. About one-third protein-coding genes is regulated by miRNA and participated in the signal pathway. Each miRNA is likely to regulate multiple target genes, and each gene may also be regulated by multiple miRNAs [4,5]. Abundant miRNAs were found and showed high expression in mammalian central nervous system [6,7]. MicroRNA-31 (miR-31) is a highly conserved specific

* Corresponding authors.

E-mail addresses: xueqingwu416@126.com (X. Wu), wangchunfang@sxmu.edu.cn (C. Wang).

miRNA, which has a wide range of molecular targets and is specifically expressed in various tissues and organs. Mir-31 regulates different cell development processes by targeting genes involved in cell proliferation, apoptosis, cell differentiation and cell movement [5].

Apoptosis plays an important role in maintaining and balancing the normal growth and development of multicellular organisms. At the same time, it also has a significant influence on protecting the body against the external factors, maintaining homeostasis and maturity of embryo development. miRNAs exhibit complex functions in apoptosis, and changes in their expression may simultaneously stimulate and inhibit apoptosis. Abnormal regulation of apoptosis is intimately associated with the occurrence and development of diseases, such as neurodegenerative diseases, spinal cord injury (SCI), AIDS and cancer [8]. When studying the development of the nervous system, it was found that the number of neurons initially produced was higher than the neurons combined with mature brain circuits in most animals, suggesting that a large number of neurons eliminate through the special way of apoptosis. In addition, many miRNAs play key roles in neural development and may be important mediators for cell differentiation into specific tissues or organs [9]. Previous studies have shown that miRNAs may be connected with neurodegeneration which suggests that changes in miRNA expression result in secondary damage of central nervous system injury, including the spinal cord [10].

SCI is a central nervous system disease with high incidence, high mortality, high disability rate and high consumption. It is an acute disease with serious clinical harm, mainly leading to permanent loss of sensory and motor functions. It is difficult to carry out tissue repair and functional reconstruction for spinal cord due to the weak regeneration capacity of neurons after SCI and the rapid emergence of glial scar that mechanical obstructed the regeneration of axon [11–13]. Therefore, the treatment of SCI has been a difficult problem for the medical community. In the disease model, microRNA signal mediator is attractive as an upstream controller of secondary SCI progression, and is considered an important participant in pathophysiology of SCI [14,15], because miRNA can regulate the expression of specific functional genes. Based on the specific interaction between miRNAs and their target genes, RNA-based technologies are potential therapeutic strategies [16–18]. The Phosphoinositide 3-kinase/AKT (PI3K/AKT) signaling pathway is activated by various types of cell stimulation or toxins and regulates basic cell functions such as transcription, translation, apoptosis, growth and survival. The identification of Phosphoinositide 3-kinase-protein kinase B/AKT (PI3K PKB/AKT) pathway and activation of receptor tyrosine kinases (RTKs) started

in the early 1980s, through vigorously trying to characterization of insulin receptor signal [19]. These humble beginnings caused by insulin receptor substrate (IRS) to the appraisal of PI3K protein composition and mechanism of insulin receptor signal transduction, and then by 3'-phosphoinositide dependent protein kinase 1 (PDK1) mediated PKB/AKT activation. Growth factor binding to its RTK or g-protein-coupled receptor (GPCR) stimulates subtypes Ia and Ib PI3K. PI3K catalyzes the formation of phosphatidyl inositol-3,4,5-triphosphate (PIP3) on the cell membrane. PIP3 in turn acts as a second messenger to help activate AKT. Once activated, AKT can control key cellular processes by phosphorylation, which is involved in apoptosis, protein synthesis, metabolism and cell cycle substrates [20–22]. Great attention has been paid to the molecular mechanism and treatment of various diseases [23], and its role and influence also deserve attention in SCI.

2. Apoptosis and diseases

Cell apoptosis is common both in physiological state and pathological state in biology. Apoptosis plays a key role in ensuring the healthy survival of multicellular organisms. It is of great significance in tissue differentiation, organ development and maintenance of homeostasis of multicellular organisms. For example, the degradation of tadpole tail involves apoptosis [24]. Kerr firstly defined this phenomenon in 1972 and proposed the term “apoptosis” [25]. Apoptosis comes from the Greek word for “far away.” Apoptosis is significant to the normal development of multicellular organisms, as well as the maintenance of normal tissue and organ cell balance and growth balance, and even body aging.

Abnormal regulation of apoptosis is related to the occurrence and development of diseases, such as Alzheimer's disease, stroke, AIDS, cancer, and myocardial infarction [8,26]. A growing number of studies have shown that apoptosis occurs at multiple stages of nervous system development and damage repair, from neural epithelium to cells after migration and division, from neural tube formation to neuron matching in target area [27]. Apoptosis plays a major role in acute nerve injury [26]. A large number of studies have found that apoptosis is an important pathophysiological change of secondary SCI, and it is also an important mechanism for the progressive aggravation of secondary SCI and irreversible changes of neuronal cells [27]. Studies confirmed the widespread apoptosis after SCI [28]. Several studies [29,30] confirmed that the death of neurons and glial cells was secondary to apoptosis in both humans and animals after SCI. It is very complicated for the mechanism of apoptosis of nerve cells after SCI. And various stimulus signals can induce apoptosis through a variety

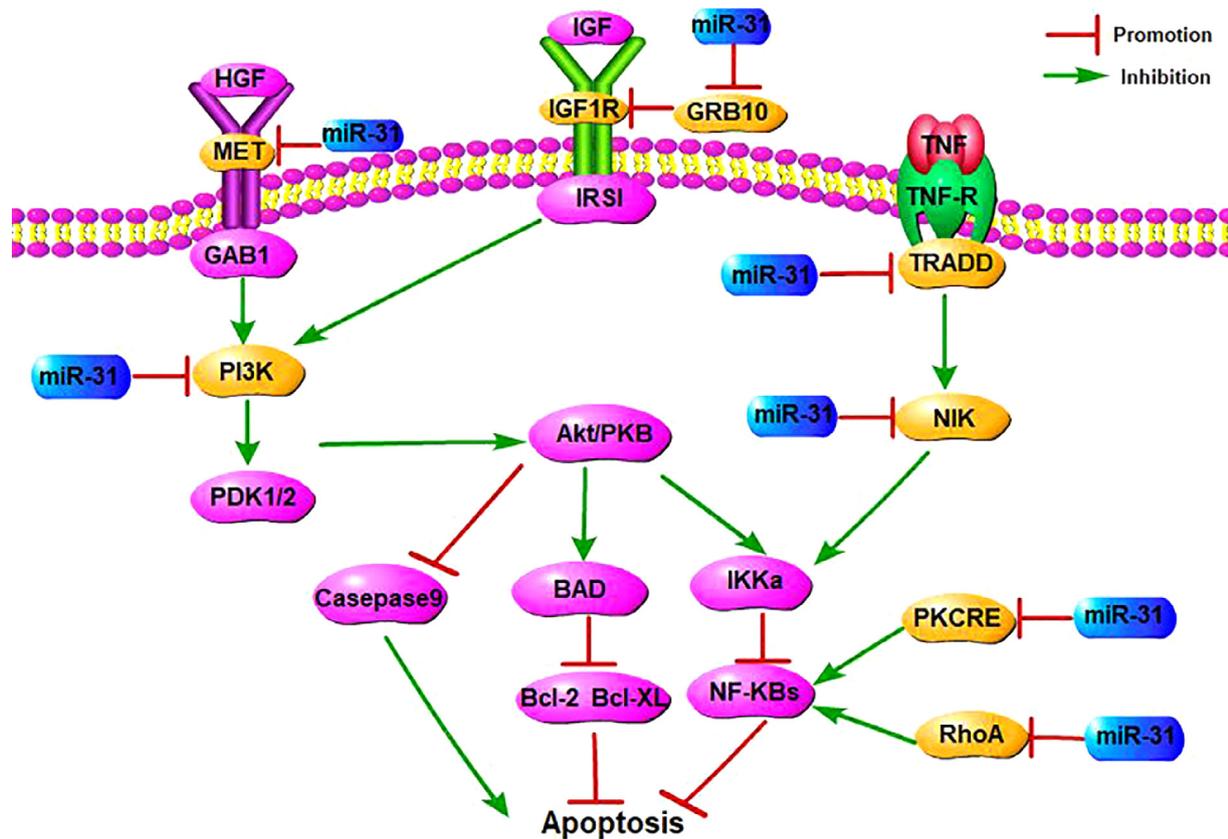


Fig. 1. The schematic diagram is the target gene of miR-31 regulating the apoptosis of PI3K/AKT signaling pathway and other pathways. The yellow molecule represents the target gene of miR-31. The blue color represents the miR-31 molecule. The red part represents the molecules in the pathway. PI3K: phosphatidylinositol 3-kinase; AKT/PKB: protein kinase B; TRADD: tumor necrosis factor receptor type 1-associated DEATH domain protein; MET: proto-oncogene tyrosine-protein kinase; GRB10: growth factor receptor-bound protein 10; RhoA: Ras homolog gene family, member A; NIK: mitogen-activated protein kinase kinase kinase 14; NF-κB: nuclear factor-k-gene binding; BCL-2: B cell lymphoma/leukemia-2; BAD: Bcl-2-antagonist of cell death; BCL-XL: B-cell lymphoma-extra large. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

of ways and involve a variety of pathological and physiological processes.

2.1. Expression of miRNA in the central nervous system

Numerous miRNAs were discovered and showed high expression in the central nervous system of mammals [31]. Similarly, in recent years, a large number of gene chip experiments on SCI, traumatic brain injury, stroke and other central nervous system diseases have shown that miRNAs have important regulatory effects on cell differentiation, growth, proliferation and apoptosis [32,33]. BAK used micromatrix, RT-PCR and in situ hybridization to find that there were 44 types of miRNAs in the brain and spinal cord, more than three times as many as other tissues, suggesting that many of the central nervous system of miRNAs may be associated with the special functions of these regions [6]. In the central nervous system, miRNA disorders are connected with neurodegenerative diseases, including Alzheimer's disease, Parkinson's disease and Huntington's disease, as well as traumatic injuries [34–37].

Studies have found that miRNAs play a crucial role in the morphology and function of the central nervous system [38,39]. Studies have shown that there are many miRNA enrichments in the brain of mice, including miR-9, miR-124a, miR-125b, miR-127, miR-128 and let-7 family members. Some miRNAs are also highly expressed in the long medulla, partially expressed in the cerebellum, such as miR-195, miR-497 and miR-30b [36]. And some miRNAs are clustered in the hypothalamus, such as miR-7 and miR-7b [40]. There are also many miRNAs highly expressed in the hippocampus and pituitary gland [41]. At the same time, miRNA is also abundantly expressed in the spinal cord, directly or indirectly affecting the development of the spinal cord [42].

3. miRNA and spinal cord injury

3.1. Pathogenesis of spinal cord injury

Study reported that the SCI was characterized by spine from mechanical damage in the early stages, leading to bundle of white matter, gray matter neurons,

major vascular and microvascular damage [43]. The second stage of subsequent injury is secondary injury, which starts a few minutes after the injury and lasts for several years, and is promoted by hypoxia, hemorrhage, glial scar formation and immune pathway changes [44]. Most treatments for SCI focus on the second phase of damage mitigation, aimed at preventing further damage and changing the patient's neurological processes [45–48]. Apoptosis is an important factor in secondary injury. Apoptosis seems to occur in at least two stages. In the initial stage, apoptosis is accompanied by necrosis. And in the late stage, it is mainly limited to white matter, affecting oligodendrocytes and microglia [49]. Due to prolonged pathological and physiological changes, spontaneous neuronal precursor nerve will be strongly suppressed, resulting in paralysis. The development of effective treatment methods is one of the focuses of SCI research. A lot of work has been done to improve the functional defects in patients with SCI including paralysis. There are treatments available to improve secondary injury by improving the SCI microenvironment and/or stimulating endogenous repair [50–53]. Although most studies have focused on the protection of the neuroprotective compounds against SCI secondary injury, the therapeutic effect is still controversial.

3.2. Changes of miRNA after spinal cord injury

In order to understand the relationship between miRNAs and SCI, the miRNAs expression changes were studied after SCI. With the advent of chip technology, most studies have shown that the level of miRNA changes in spinal cord tissues after SCI [54–57]. It has been reported that 269 miRNAs were found in normal spinal cord of rats, while 172 miRNAs remained unchanged. Among them, 97 miRNAs were significantly changed after SCI, and 30 miRNAs were up-regulated. The expression of 16 miRNAs was down-regulated. The expression of 14 miRNAs was up-regulated at 4 h after SCI, and then down-regulated at 1 d and 7 d after SCI [31]. Subsequently, it was reported that the microchip analysis of miRNAs was performed after SCI in rats. This study was different from the previous one. The microchip analysis was added at the 14th day after SCI. The results showed that four miRNAs were up-regulated at the damage site after SCI and 32 miRNAs were down-regulated. And the decline of these 32 miRNAs was higher than that at the 14th day after SCI [58]. Subsequently, miRNA expression profiling analysis on the spinal cord of rats was reported. It was concluded that a total of 343 miRNAs were changed after SCI, and it was found that SCI would cause the decrease of most miRNAs. Interestingly, the miRNAs expression profile showed a progressive change along with the damage of schedule after SCI. That is, with the progression of injury, the number of down-regulated miRNAs

increased, while the number of up-regulated miRNAs remained unchanged three days after SCI. Subsequently, they analyzed mRNAs expression profile and concluded that there was a negative correlation between miRNAs and mRNAs after SCI. The authors reevaluated a list of miRNA targets to confirm the influence observed miRNA expression changes on the transcriptome according to the miRanda algorithm and the mRNA expression data from De Biase et al. They first selected out the target mRNA of miRNA. Subsequently, the comparison between those miRNAs and target mRNA expression profiles showed that the down-regulated miRNA after SCI was parallel to the up-regulated target mRNA caused by equivalent damage. In addition, the correlation between low miRNA levels and high mRNA levels observed after SCI strongly supports miRNA regulation of mRNA levels [59,60]. Bioinformatics analysis showed that the target gene of miRNA changed after SCI. Using bioinformatics method, they analyzed potential targets of altered miRNA after SCI to understand the role of miRNA. Potential targets of these miRNAs include genes encoding components involved in many pathophysiological processes, such as inflammation, oxidation, and apoptosis after SCI. Several down-regulated miRNAs upstream of SCI regulate a variety of inflammatory and apoptotic genes, while other up-regulated miRNAs upstream of SCI regulate a variety of anti-inflammatory, antioxidant and anti-apoptotic genes [31].

Up-regulated miRNAs include miR-124, miR-129 and miR-1 after SCI, in which the expression changes of miR-129–2 and miR-146a are related to the severity of the initial injury, suggesting that these miRNAs can be used as biomarkers and therapeutic targets for SCI [58]. The expression patterns of these miRNAs are consistent with the emergence of SOX2, NESTIN and REST in time and space, indicating that these miRNAs reflect not only the emergence of the stem cell niche, but also the pre-neuronal phenotype of the surviving neurons after injury. Bioinformatics analysis of the target genes of these miRNAs showed that the regulatory abnormalities of these miRNAs were associated with the pathogenesis of secondary damage such as apoptosis and cell cycle abnormalities. Multiple miRNAs were varied after SCI, and participated in the secondary injury process of SCI. Bioinformatics analysis showed that some apoptosis-related genes such as caspase-3, calpain 1 and calpain 2, as well as apoptosis-inducing factors such as miR-235-3p, miR-137, miR-98 and miR-124 were potential targets of down-regulated miRNAs after SCI. In addition anti-apoptosis related genes including bcl2-1 and bcl2-2 were potential targets of up-regulated miRNAs after SCI, such as miR-145, miR-214, miR-133a, miR-133b, miR-674-5p and miR-15b. Meanwhile, some inflammatory mediators such as tumor necrosis factor- α (TNF- α), interleukin-1 β

(IL-1 β) and intercellular adhesion molecule 1 (ICAM1) mRNA are potential targets for down-regulating miR-181a and miR-411 after SCI. Conversely, some anti-inflammatory mRNAs such as annexin A1 and annexin A2 mRNAs were potential targets of miR-221 and miR-1 respectively, which were up-regulated after SCI. These processes are thought to play a major role in the pathogenesis of SCI. These findings suggest that the abnormal expression of miRNAs after SCI may contribute to the pathogenesis of secondary injury, therefore may be potential targets for therapeutic intervention [31,58].

3.3. The role of miRNAs in spinal cord injury

Numerous studies have shown that miRNAs can affect SCI in a variety of ways, including apoptosis, regeneration, inflammation and demyelination [34]. This paper mainly studies the clinical significance of miRNAs in SCI. Most of the existing research has been done on animals, especially mice. While some of the findings come from animal models, they may also provide proof and essential direction for further research. Multiple miRNAs participate in regulating the pathogenesis and development of SCI [61,62]. Bioinformatics analysis shows that the changes of miRNA expression affect

the key pathophysiological processes of SCI pathophysiology, and the pathological and physiological signs of SCI are cell death caused by apoptosis or other pathways. Unlike necrotic cell death associated with primary injury, apoptotic cell death is an excitatory or inhibitory event controlled by the gene acting on a number of regulatory factors, including some miRNAs [63]. Apoptosis can affect all types of cells in the spinal cord [34]. For the most part, SCI induces the expression of miRNAs, and miRNAs react to SCI by up-regulating or down-regulating the expression of apoptotic genes [35,64].

According to the current studies on miRNAs in SCI and part of neurodevelopment, the relationship was summarized between the expression of some miRNAs and pro-apoptosis (Table 1) as well as anti-apoptosis (Table 2). Mir-21 is one of the most obvious changes of miRNAs [64]. The regulatory function of miR-21 after SCI mainly has two aspects including apoptosis and astrocyte reaction [64–67]. The anti-apoptosis effect of miR-21 was firstly discovered in human and mouse glioblastoma studies, which inhibited apoptosis through downregulation of pro-apoptotic genes FASL, PTEN and PDCD4 [68], and was subsequently confirmed in studies of multiple diseases and different cell types. After

Table 1
Microarray analysis of the expression of miRNAs related to the pro-apoptosis of spinal cord injury.

	miRNA	Targets gene(s)	Biological Role of Target Genes	Refs
Up-regulation	Let-7a	RAS, Myc	Pro-apoptosis	[66]
	miR-10b	BIM	Pro-apoptosis	[134,135]
	miR-16	BCL-2	Pro-apoptosis	[66]
	miR-21	FasL, PTEN, PDCD4, TPM1	Pro-apoptosis	[65,67,136,137]
	miR-96	FOXO1, FADD caspase-3	Pro-apoptosis	[138,139]
	miR-145	c-Myc, caspase-3	Pro-apoptosis	[140–142]
	miR-146a	FAS	Pro-apoptosis	[143]
	miR-133b	caspase-9, RhoA	Pro-apoptosis	[144,145]
Down-regulation	miR-98, miR-124, miR-30b-3p, miR-137, miR-235-3p	caspase-3, calpain2 AIF	Pro-apoptosis	[33]
	miR-125b	BMF, ERBB2,3	Pro-apoptosis	[146,147]

Table 2
Microarray analysis of the expression of miRNAs related to the anti-apoptosis of spinal cord injury.

	miRNA	Targets gene(s)	Biological Role of Target Genes	Refs
Up-regulation	miR-1		Anti-apoptosis	[148,149]
	miR-7	EGFR-regulated AKT signaling pathway	Anti-apoptosis	[150]
	miR-15b	BCL-2	Anti-apoptosis	[31,151,152]
	miR-17		Anti-apoptosis	[31]
	miR-20a, miR-133a	BCL2-1, BCL-2	Anti-apoptosis	[31]
	miR-103, miR-107	CDK5R1	Anti-apoptosis	[31,153]
	miR-133b	BCL-W, MCL-1	Anti-apoptosis	[31,154]
	miR-138	BCL-2	Anti-apoptosis	[155]
	miR-145	c-Myc	Anti-apoptosis	[31]
	miR-206, miR-672, miR-674-5p	BCL2-1, BCL-2	Anti-apoptosis	[33]
	Down-regulation	miR-34	SIRT1, BCL-2	Anti-apoptosis
miR-29b		MCL-1	Anti-apoptosis	[158–160]

Table 3
Sequences of miR-31 in different species.

Species	miRNA Name	Sequence
Human	hsa-miR-31	AGGCAAGAUGCUGGCAUAGCU
Mouse	mmu-miR-31	AGGCAAGAUGCUGGCAUAGCUG
Rat	rno-miR-31	AGGCAAGAUGCUGGCAUAGCUG
Cow	bta-miR-31	AGGCAAGAUGCUGGCAUAGCU
Horse	eca-miR-31	AGGCAAGAUGCUGGCAUAGCU
Pig	ssc-miR-31	AGGCAAGAUGCUGGCAUAGCUG
Zebrafish	dre-miR-31	GGCAAGAUGUUGGCAUAGCUG

SCI in rats, inhibition of miR-21 expression led to up-regulation of FASL and PTEN expression, increased apoptosis, enlarged lesion area, and hindered hindlimb motor function. In summary, miR-21 plays an important role in the pathophysiological development after SCI [65]. So far, PDCD4 has been widely confirmed to be a target gene of miR-21. However, inhibition of miR-21 expression after SCI does not affect the expression level of PDCD4. The researchers believe that although a single miRNA can regulate hundreds of genes, and this regulation has tissue or cell specificity [65]. In the process of astrocyte proliferative hypertrophy, miR-21 plays a regulatory role, and inhibition of miR-21 expression can promote apoptosis. In general, low-expression miRNAs usually act on pro-apoptotic genes such as the caspase family. While over-expressed miRNAs act on anti-apoptotic genes such as Bcl2, which contributes to the treatment of SCI. A large number of miRNAs regulated apoptosis through target genes after SCI. And miR-31, as a miRNA upregulation after spinal cord injury [69], also plays an important role in spinal cord injury. miR-31 is a highly conserved, specific miRNA with a wide range of molecular targets that are specifically expressed in various tissues and organs. Meanwhile, the sequence of hsa-miR-31 can be obtained by searching the miRBase database, which is highly conserved among different species (Table 3). Therefore, miR-31 may also regulate apoptosis by regulating target genes in spinal cord injury, which will serve as a therapeutic target for spinal cord injury in the future.

4. miR-31 and apoptosis

4.1. PI3K and AKT

Phosphoinositide 3-kinase (PI3K), discovered in 1985, is a previously unknown Phosphoinositide kinase [70,71]. PI3K is the intracellular phosphoinositide kinase composed of a p110 catalyzed subunit and a p85 regulated subunit. It has lipid kinase activity and protein kinase activity and can be activated by receptor tyrosine kinase or G2 protein coupled receptor. PIP2 and PIP3, the products of PI3K, are significant second messengers in the cell, which act on the PI3K/AKT

pathway and play an important role in cell proliferation, apoptosis and metabolism [72,73]. It was found that mammalian PI3K signaling family was classified into three types (I, II, III) according to its structure and substrate specificity [74,75]. In the following studies, a class of isomers has been studied extensively. The type IA, which consists of PI3K beta, PI3K intellect and PI3K receptor isoforms, is usually activated by hormones, cytokines, spacing cells and growth factors through the tyrosine kinase receptor. And PI3K cabinet (the only member of category IB) is activated by G protein coupled receptor (GPCR) [76–78].

PI3K is a lipid kinase that phosphorylates the D3 position of the myosidic ring of phosphoinositol, thus producing intracellular signaling molecules such as Thr308 and pAkt phosphorylation of Ser473 [79]. Silymaric acid/threonine acid kinase Akt, was also known as egg white kinase PKB. It is an important downstream target in the PI3K signal transduction pathway. Three subtypes of AKT were found in mammals, including AKT1 (PKB), AKT2 (PKB) and AKT3 (PKB). AKT can be divided into N terminal (PH domain), central catalytic domain and C terminal (similar to PKC regulatory region). The PH domain can be combined with the lipid second messenger PIP3 to enable AKT to be transposed to the plasma membrane and activated. When cells are stimulated by extracellular signals, PIP3 generated by PI3K activation binds to the PH domain at the end of AKT N, enabling AKT to be transposed to the plasma membrane and activated. The latter catalyzes the phosphorylation of Ser124 and Thr450 by itself, while PIP3-dependent Kinase, PDK1 and PDK2, respectively catalyze the phosphorylation of Thr308 and Ser473 of AKT, further activating AKT, giving rise to a cascade reaction [79].

4.2. Mechanism of PI3K/AKT regulates apoptosis

After decades of research, the PI3K/AKT pathway is still worth studying due to its multiple functions. PI3K/AKT signaling pathway plays a core role in key processes of body growth and cells (such as glucose homeostasis, lipid metabolism, protein synthesis, cell proliferation and survival) through mediating growth factor signals, and it also plays an important role in apoptosis. AKT is activated under the regulation of PI3K and regulates cell apoptosis through various pathways. It is mainly regulated by inhibiting FKHR, NF-KB, YAP and promoting the activity of CREB, Mdm2 and other transcription factors [80]. Apoptosis is inhibited by directly inhibiting phosphorylation of Bad. Bad is the first directly confirmed target gene for regulation of apoptosis and survival downstream of the PI3K/AKT signaling pathway. Bad belongs to the BCL-2 family of proteins and is a BCL-2 homology domain 3-related protein that directly forms a

pro-apoptotic complex with the anti-apoptotic factor BCL-2 or BCL-XL, activating pro-apoptotic members such as BAX or BAK, together promote cell apoptosis [81]. Activated AKT directly phosphorylates Ser136 of Bad, which binds to 14-3-3 protein, promotes the expression of BCL-2/BCL-XL and inhibits apoptosis [82]. And it can exert myocardial protective effect by inhibiting Bad and reducing apoptosis [83]. Inhibition of caspase-9 phosphorylation and inhibition of gsk-3 activity, which is also a way to inhibit apoptosis, ultimately inhibits the release of apoptosis-related factors such as cytochrome C and AIF, inhibits apoptosis, and simultaneously regulates apoptosis.

PI3K/Akt signaling pathway plays an important role in the regulation of apoptosis. Studies have shown that it also plays an important role in the inhibition of apoptosis in the ischemia–reperfusion injury of heart, kidney, liver and other organs [84–87]. At the same time, PI3K/Akt pathway, as a pro-survival signaling pathway, is activated to play a protective role on nerve cells [88,89]. It is involved in the survival, differentiation and apoptosis of glial cells and neurons [90,91], and mainly regulates and manages cell survival by regulating the apoptosis of the nervous system. Activation of PI3K/Akt signaling pathway can promote endothelial cell survival, reduce nerve damage, reduce inflammatory cell death, and block neuron damage. Then, some studies explored the role of PI3K/Akt signaling pathway in apoptosis after secondary spinal cord injury and its possible mechanism, indicating that the involvement of PI3K/Akt signaling pathway in spinal cord injury may be related to apoptosis [92]. In conclusion, the downstream molecules of the PI3K/Akt signaling pathway are diverse and complete, which is a key factor in inhibiting apoptosis and promoting cell survival. Therefore, the development of PI3K/Akt signaling pathway has broad prospects as a new therapeutic target.

4.3. Expression of apoptosis genes

Under physiological conditions, PI3K is normally activated by extracellular signals, and a variety of stimuli, including growth factors, cytokines, and hormones. Growth factors such as hepatocyte growth factor (HGF), insulin-like growth factor (IGF) [93,94], bind to the n-terminal extracellular region of the corresponding transmembrane receptor tyrosine kinase (RTKs), leading to autophosphorylation of the RTKs cytoplasmic region and tyrosine residues in the connecting molecules. Then PI3K is recruited into the RTKs by interacting with the phosphorylated tyr residues on the RTK complex members via the p85 SH2 domain, resulting in allosteric activation of PI3K. Activated PI3K phosphorylates phosphatidylinositol 4,5-diphosphate [PtdIns(4,5)P₂] to form phosphatidylinositol 3,4,5-triphosphate in the cell membrane [PtdIns(3,4,5)P₃],

the activation signal is transmitted to the downstream molecule. Phosphorylated active AKT is subsequently transferred from the cell membrane to other cell compartments, phosphorylating multiple downstream substrates to achieve AKT function [95], and activated AKT phosphorylates a large number of substrates that are almost controlled Physiological and pathological aspects of cell function, including cell survival, growth, metabolism, tumorigenesis, and metastasis, specifically regulating apoptosis. Studies have shown that GRB10 (growth factor receptor-bound protein 10) participates in the IRS-1/IRS-2-PI3K/Akt signaling pathway by disrupting IRS-1/IRS-2 [96]. A met-dependent mechanism that affects apoptosis resistance is the activation of the PI3K/AKT signaling pathway [10]. Experiments have shown that MET and GRB10 are respectively target genes of miR-31 [96,97]. Thompson MA demonstrated that PIK3C2A and miR-31 had a targeted effect, and PIK3C2A was the oncogene that inhibits apoptosis [98].

These target genes of miR-31 interact on the PI3K/AKT pathway and related pathways (Fig. 1), among which the main one is the nuclear factor- κ -gene binding (NF- κ B) signal pathway. There is evidence to support a crosstalk between PI3K/AKT signaling pathway and NF- κ B [99–101]. NF- κ B is not a molecule within the core PI3K/AKT pathway, but it is downstream of AKT. NF- κ B pathway contains several important molecules such as NF- κ B, I κ B, IKK, etc. Under normal circumstances, NF- κ B in the cytoplasm is combined with its inhibiting factor I- κ B and loses its transcriptional activity. AKT activates IKK by phosphorylation (I- κ B kinase), which leads to the phosphorylation and degradation of I- κ B and separation from NF- κ B, and the released NF- κ B is transferred to the nucleus and induces the expression of the target gene. AKT IKK is essential for mediated I- κ B degradation and NF- κ B activation and is a key regulator of NF- κ B dependent gene transcription [102–104]. Tumor necrosis factor (TNF), the most studied activator, binds to the receptor and recruits a protein called TNF receptor death domain (TRADD). Subsequently, TRADD acted on TNF receptor-related factor 2 (TRAF-2) and absorbed NF- κ B induced kinase (NIK) [105–107].

Among them, TRADD is an upstream activator of NF- κ B [108], and TNFR-1 related death domain (TRADD) recruits RIP and tumor necrosis factor (TNFs) receptor-related factors, leading to the activation of NF- κ B and inhibiting TNF- α -induced apoptosis. Rajbhandari R proved that TRADD was the target gene of miR-31. Similarly, Yamagishi M indicated that MAP3K14 was the target gene of miR-31 using the luciferase-3'UTR reporter gene assay. MAP3K14, also known as NIK, plays an important role in non-classical NF- κ B signal transduction through IKK α phosphorylation [109]. Two genes in NF- κ B signal transduction including NF- κ B induced kinase

(MAP3K14) and RhoA GTPase are published effective targets of miR-31, which have been verified by bioinformatics analysis, siRNA knock, western blot and other methods [109,110]. At the same time, these direct targets are activators of NF- κ B signal. Korner C1 and others proved the protein kinase C epsilon (PKC by PRKCE gene encoding epsilon) as miR-31 new target directly and showed that down-regulation of PKC ϵ leads to impaired NF- κ B signaling. In terms of mechanism, we attributed this sensitization to the down-regulation of anti-apoptotic factor BCL2 by PRKCE, and there was a negative correlation between miR-31 and BCL2 expression [111].

Therefore, miR-31 may mediate apoptosis through this pathway to promote the repair of the body, which may become a potential therapeutic target for future diseases.

5. Treatment of spinal cord injury

5.1. Current research on the treatment of spinal cord injury

There have been a number of treatments for SCI in recent years [112–114], some of which are aimed at promoting neurological recovery by inhibiting apoptosis. Currently, stem cell transplantation has become a research hotspot in the treatment of SCI. The transplantation of adipose stem cells to treat SCI can promote the repairment of SCI by secreting various cytokines, regulating inflammatory responses, improving hypoxia, promoting angiogenesis, inhibiting apoptosis or directly differentiating and replacing damaged cells [115,116]. The therapeutic mechanism of umbilical cord mesenchymal stem cells (UC-MSCs) is mainly the result of the comprehensive effect of multiple factors, that is, the neurotrophic effect, anti-inflammatory effect, anti-apoptosis effect and promoting angiogenesis of UC-MSCs [45,117,118]. Bone marrow-derived mesenchymal stem cells (BMSCs) were transplanted, thereby glial scar, inflammatory response and apoptosis in the damaged area were significantly reduced and secondary injury was improved [117].

Some of the medications are also used to treat SCI by inhibiting apoptosis. Riluzole can inhibit the death and apoptosis of ischemic neurons in the brain and spinal cord, so as to alleviate the damage of nerve function after ischemic brain and spinal cord and improve the prognosis of patients. Wu used TUNEL assay to detect apoptosis levels in injured spinal cord. The apoptotic cell count showed that Riluzole had anti-apoptotic effect [119]. Erythropoietin (EPO) which administered after SCI 24 h, can weaken the inflammatory response and cell apoptosis by maintaining the integrity of microvessels and tissues, it also can play a broad neuroprotective role and improve the kinematic function of SCI model

animals [120,121]. The role of interleukin-10 (IL-10) in SCI is also a research hotspot. As it inhibits the generation of inflammatory factors such as TNF- α , IL-6 and IL-1, it can up-regulate the expression of anti-apoptotic genes and increase the amount of neurotrophic factors in the damaged area, which plays an important role in the functional recovery and anti-inflammation [122].

Studies have shown that calpain inhibitors (such as calpeptin) can improve the large kinematics of SCI model animals by reducing apoptosis and increasing residual tissue. Das took primary cultured motor neurons as model cells and added glutamic acid to build in vitro excitatory toxic cell model. After calpeptin treatment, apoptosis was reduced and its function was maintained, further demonstrating the neuroprotective effect of calpeptin [123]. Studies showed that minocycline hydrochloride could inhibit the release of cytochrome C caused by SCI, thereby reducing apoptosis, which was conducive to the restoration of neurological function in SCI rats [124].

5.2. miRNAs for the treatment of spinal cord injury

Previous studies showed that miRNA had different expressions after SCI and was involved in different physiological and pathological processes, such as apoptosis, inflammation and regeneration. Therefore, misaligned miRNA is considered as a potential target for therapeutic interventions after SCI [31,125]. Studies have demonstrated that some miRNAs have anti-apoptotic capacity, such as miR-17-92 cluster (miR-17 and -20), miR-146a and miR-138 [108,109]. Among them, miR-34a may protect SCI by inhibiting anti-apoptotic proteins BCL-2 and XIAP [110]. Nevertheless, the expression of miR-34a was notably downregulated in SCI, suggesting that miR-34a can protect cells from death due to the high level of anti-apoptotic protein expression. After SCI, the expression of miR-384-5p was continuously down-regulated. Studies have shown that the expression of cleaved caspase-3 was up-regulated by miR-384-5p and down-regulated by miR-384-5p inhibitors, suggesting that miR-384-5p plays an important role in regulating the signaling pathway, such as apoptotic pathways [97]. Regardless of the pathway identified, down-regulation of miR-384-5p exerts a protective influence on SCI.

Mir-21 and miR-146a, among the misregulated miRNAs after SCI, promote neural function by reducing apoptosis and the hypertrophic response of astrocytes to injury [126]. The study found that the expression level of miR-21 in mouse tissue of SCI was significantly higher than that in normal spinal cord tissue [108]. Through bioinformatics retrieval prediction, its target genes may include programmed cell death factor 4 (programmed cell death, PDCD4). Studies have shown that

PDCD4 is a kind of related gene of apoptosis, and reducing its expression can inhibit the occurrence of apoptosis, thus having a protective effect on cells [98,111,127]. It was verified by experiments that miR-21, which is highly expressed in mouse SCI, directly acts on PDCD4, thereby inhibiting the expression of PDCD4 at the mRNA and protein levels, suggesting that miR-21 may have an important protective effect during SCI. This is basically consistent with the results of studies on the protective effect of miR-21 by inhibiting the expression of PDCD4 in various damage models [128,129]. In addition, several studies have shown that miRNA can also regulate the endogenous antioxidant system after SCI [7,130], and regulate the redifferentiation by targeting superoxide dismutase (SOD), namely antioxidant enzyme defense system [131]. Therefore, miRNAs are considered as biomarkers and therapeutic targets in the pathologic process of SCI [132].

Studies have shown that miR-31 regulates multiple target genes, and some target genes regulate apoptosis through the PI3K/AKT pathway. Because the previous literature also showed that miR-31 was up-regulated after SCI, we hypothesized that it could up-regulate miR-31 or inhibit miR-31 to up-regulate its target gene and ultimately play an anti-apoptotic role. It can reduce neuronal apoptosis, thereby treating SCI, and contributing to human medicine, is also expected to become a hot spot in the future, and a major step in the treatment of SCI. At present, miR-31 has available intervention drugs, such as miR-31 mimic microspheres drugs [133], and it is mature in animal models, which provides a substantial foundation and direction for miR-31 intervention in the clinical setting. Therefore, with the continuous development and improvement of technology as well as the efforts of researchers, miR-31 may provide a new opportunity for the treatment and rehabilitation of SCI through the ethical review and the early development stage, namely the preclinical research stage.

6. Conclusion and perspective

miRNAs are involved in a series of pathological and physiological processes. There is abundant evidence that miRNAs play an important regulatory role in many neurobiological processes (such as neurogenesis, neural differentiation, growth, proliferation and apoptosis) and play a key role in maintaining internal balance after SCI. Because which miRNAs are small molecules that are easy to transmit, and usually have tissue-specific expression characteristics, they can simultaneously fine-tune the expression of multiple genes, thus have great therapeutic potential for SCI gene therapy. Basic research on miRNA has increased rapidly in the past 20 years, and the research results strongly confirm the potential of miRNA for clinical treatment of SCI. A

large number of studies have found that miRNA may be a potential marker of nerve cell death after SCI, and may be an effective target for intervention of SCI [37,47,55,59,130]. It also provides new ideas, new methods and new technologies for the recovery and reconstruction of neurological function in patients with SCI.

At present, much energy has been invested in the research field of PI3K/AKT pathway. Many studies have revealed the regulatory effect of PI3K/AKT pathway in apoptosis, and miR-31 is likely to regulate apoptosis through this pathway, thereby inhibiting the reduction of neurons and minimizing the secondary SCI as much as possible, bringing great changes to the functional recovery of SCI. But so far, we know very little about the molecular mechanisms of neuronal regeneration and the pathogenesis of SCI, and the complete regulatory network involving genetic and epigenetic factors such as miRNAs is largely unclear. Efforts should also be made to study the therapeutic potential of miRNAs and develop effective methods for the treatment of SCI. Apoptosis is an important research topic in the pathogenesis of SCI. It is a new way for treatment of SCI to make use of the law of cell apoptosis and effectively regulate cell apoptosis. How miR-31 regulates SCI through the PI3K/AKT pathway may be the direction of future research. In the future, more attention should be paid on exploring the gene targets and signaling pathways of SCI-related miRNAs, minimizing secondary SCI. The specific miRNA related to SCI will bring significant changes the recovery process of SCI in the future. It is expected that breakthrough will occur in the treatment of SCI.

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