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Progress on the Role of MicroRNAs and GSK3 β in the Regulation of Inflammatory Signal Pathway in Ischemic Stroke

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Abstract: *The inflammatory response is the core content of pathophysiology after cerebral ischemia reperfusion injury (cerebral ischemia reperfusion injury, IRI), involving the inflammatory cells activated by different pathways and immunity response. More and more studies have shown that MicroRNAs (miRNAs) play an important role in the regulation of inflammation after IRI, while glycogen synthase kinase-3 β (GSK-3β), as one of the most important kinases, is also involved in the regulation of inflammatory response, autophagy, apoptosis and other pathological processes of IRI. This article reviews the related studies on the inflammatory response pathway regulated by miRNA and GSK-3 β in IRI, in order to provide a new strategy for the prevention and treatment of IRI.*

Keywords: *ischemic stroke, miRNAs, GSK-3β, neuro-inflammation*

Cerebral ischemia reperfusion injury (IRI) refers to the injury of brain cells caused by cerebral ischemia. After the recovery of blood reperfusion, the ischemic injury is further aggravated. It is closely related to excessive production of reactive oxygen species (ROS), inflammation, glutamate excitotoxicity and apoptosis during ischemia[1-3]. However, due to the time window of thrombolysis and the complexity of pathophysiological process of ischemic stroke, an effective treatment has not been found at present [4]. The "cascade amplification" of early inflammatory reaction and secondary oxidative stress reaction further aggravates apoptosis. Therefore, how to effectively control the early inflammatory response to reduce secondary nerve cells injured has been one of the hotspots of IRI neuro-protection research.

MiRNAs are one small non-coding RNAs with a length of 18~25bp. After binding to its target mRNA, it can directly regulate gene expression at the transcriptional level.It has been found that targeting specific miRNAs could prevent neuronal injury in both in vitro and in vivo experiments, suggesting that miRNAs may be one potential target to cerebral ischemic reperfusion injury[5-6].Glycogensynthasekinase3 (GSK-3 β) is a serine / threonine kinase that exists widely in cells, which is involved in inflammation, oxidative stress, autophagy and apoptosis after IRI. It has been found that a large amount of GSK-3β can further aggravate the injury of brain neurons after IRI[7-8]. However, at present, there is no neuroprotective drugs for GSK-3 β, and some of the schemes targeting GSK-3β for the treatment of ischemic stroke are still in the experimental stage, and the potential side effects of GSK-3 β inhibitors in animal experiments are not clear too[9]. Therefore, it may be of certain significance to explore whether miRNAs could specifically regulate the expression of GSK-3β to reduce neurons injured after IRI. In this article we reviews the mechanism of inflammatory response pathway regulated both the miRNAs and GSK-3β in order to provide a new strategy for the prevention and treatment for IRI.

1. MiRNAs participate in the inflammatory response pathway after IRI

Inflammatory responses are the core content of pathophysiology after ischemic stroke, and its process involves immune and inflammatory cells. There are different ways and sources of activated inflammatory factors, such as pro-inflammatory factors IL-1 β, IL-6 and TNF- α. Many miRNAs has been proved to

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regulate the inflammatory response after cerebral ischemia, and the main mechanism of regulating inflammation is to regulate the expression of cytokines in target cells.

1.1. The signal pathway of TLRs

The Toll-like receptors (TLRs) are one of a family of pathogen-related molecular pattern receptors that recognize and bind to the conserved sequence of pathogenic microorganisms, and its also the most important trigger of inflammatory response in IRI [10]. Among them, there are many studies on the inflammatory response of TLR4 and TLR3 in IRI. For example, the MyD88 could downstream TLR4 signal transduction pathway, and activate the NF-κB, which in turn promotes the expression and release of inflammatory factors IL-1 β, IL-6 and TNF- α[11]. Xu[12] has found that miR-1906 could specifically inhibit the expression of TLR4 and reduce the brain cells injury caused by inflammatory response to IRI. MiR-155 could up-regulate the expression of TLR4 and MyD88 to promote the expression of TNF- α and IL-1 β, resulting in the aggravation of cerebral ischemic injury in IRI. However, down-regulation of miR-155 expression can delay the progression of IRI. Yang[13] also proved that miR-155 could regulate the inflammatory response of TLR4/NF- κB pathway during cerebral ischemia-reperfusion injury. Chen[14] found that overexpression of miR-497 can inhibit TLR4-MyD88-NF- κB signal pathway and reduce inflammatory response in acute IRI.In addition, it was also found that[15], the TLR3 combined with ligands to recruit TRIF (TIR-domain-containing adaptor protein inducing, INF- β) containing TIR (Toll/1L-1 receptor homologous region, TIR) after IRI. The IRF3 could be activated and phosphorylated, forming IRF3/IRF3 homodimer, and forming IRF3/IRF7 heterodimer with IRF7, which enters the nucleus and causes specific genes such as TNF-α, IL-6 protein expression, to mediate the inflammatory injury of cerebral ischemia-reperfusion[16-17].

1.2. The signal pathway of JAK2/STAT3

The JAK/STAT signaling pathway is closely related to inflammatory response and plays an important role in the pathogenesis of IRI. Among them, JAK2 protein was mainly expressed in the cytoplasm of cerebral neurons and a few glial cells, while STAT3 protein was widely distributed in the whole cerebral nervous system. The expression of phosphorylated JAK2 and STAT increased significantly after cerebral ischemia-reperfusion injury[18]. Studies have shown that phosphorylated STAT3 can release pro-inflammatory mediators and promote the inflammatory response to aggravate the injury of brain, while inhibited the activation of STAT3 could reduce the inflammatory response in IRI[19]. Tian[20] has confirmed that miR-216a directly targeted 3'UTR binding to JAK2 by luciferase reporter gene assay. Overexpression of miR-216a can inhibit the level of JAK2 protein in cerebral ischemic area of IS model. It also colud reduce the production of inflammatory mediators and inflammatory cytokines throw negatively regulating the JAK2/STAT3 signal pathway, which reduced the volume of cerebral infarction area and improve neurological impairment.

1.3. The signal pathway of Notch

The Notch signaling pathway is consists of four Notch receptors (Notch1 / 2 / 3 / 4), five Notch ligands (Delta-like1 / 3 / 4, Jagged1, and Jagged2), and effector molecules (CS and Hes). In the central nervous system, the microglia express molecules related to Notch pathway. In the model of cerebral ischemia-reperfusion injury[21], the Notch signal pathway is activated, and Notch1 activates microglia through its ligand Jagged1, which promotes the secretion of proinflammatory cytokines and the infiltration of inflammatory cells. Cao[22] has found that blocking Notch1 pathway could reduce the expression of proinflammatory cytokines, such as IL-1 β and IL-6, to reduce the inflammatory response. Shi[23] confirmed that Notch1 is the target gene of miR-137. In the model of cerebral ischemia and hypoxia injury, miR-137 targets negative regulation of Notch1 and inhibits Notch signal pathway, thus protecting neurons from cell injury caused by cerebral ischemia and hypoxia.

2. The GSK-3β mediates inflammatory response to cerebral ischemia-reperfusion injury.

The neuro-inflammation plays an important role in the pathological changes after cerebral ischemia and can be mediated by ROS produced by oxidative stress. GSK-3 β participates in the process of oxidative stress after cerebral ischemia and causes the inflammatory response of nerve cells. ROS and inflammatory factors induce the apoptosis of nerve cells, resulting in brain injury after cerebral ischemia[24]. The GSK-3 β can directly act on NF-κB signal pathway and activate its expression from

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many aspects as a pro-inflammatory role[25]. Some studies have found that GSK-3 β can phosphorylate p65Ser-468 and promote its transcriptional activity[26]. And other studies have found that[27]， the P100 binds to NF- κ B dimer in the nucleus and inhibits its activity. GSK-3 β mediates its ubiquitin degradation by phosphorylating p100Ser707 in the nucleus, which in turn promotes the activation of NF- κ B signal.In addition, GSK-3 β can activate IKK complex or mediate the binding of NF- κ B to target genes by phosphorylating multiple serine sites at the N-terminal of NEMO subunit of IKK complex[28-29]. Therefore, the neuroprotective effect can be produced by inhibiting oxidative stress and neuroinflammation by reducing the expression of GSK-3 β in the brain after ischemia. Vasoactive polypeptide Apelin13 attenuates brain injury by inhibiting the activity of GSK-3 β, increasing the expression of Nrf2 and reducing the expression of oxidative stress products and inflammatory factors in the brain of rats with cerebral ischemia-reperfusion[30]. The remote limb ischemic post conditioning(RIPOC) has been proved to be an effective postprocessing method to reduce reperfusion injury in experiments. RIPOC can inhibit the increase the expression of GSK-3β level in rats, reduce oxidative injury and nerve inflammation, and attenuate cognitive impairment in rats with cerebral nerve injury [31]. In the neonatal mouse model of hypoxia-ischemia, GSK-3 β was significantly activated, and its specific inhibitor SB216763 could reduce the expression of GSK-3 β, increase the antioxidant capacity of nerve cells, reduce inflammation and brain injury [32]。

3. Summary

To sum up, the study on the pathological mechanism of cerebral ischemia-reperfusion injury is the subject of neuroprotection research, and a variety of miRNAs participate in the inflammatory response of IS and play an important regulatory role, which has become the focus and focus of research in recent years. However, it has been found that GSK-3 β participates in oxidative stress, inflammation and autophagy of IRI neurons, induces neuronal apoptosis and aggravates cerebral ischemia-reperfusion injury. For example, targeted regulation through miRNA can inhibit inflammatory response in IRI, reduce oxidative stress secondary to stroke, and avoid large area apoptosis. It has important clinical significance for the prevention, treatment and prognosis evaluation of IRI.

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Declaration of interest

The authors declare that they have no conflict of interest.

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