The Application Progress of MicroRNA in Cerebral Ischemia-reperfusion Injury

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Abstract. Ischemia-reperfusion injury (I/R) is caused by the blood flow obstruction and is further aggravated by restoration of the blood supply suddenly. The brain is the most sensitive organ in the human body; ischemia can cause irreversible damage to brain cells after a few minutes. Therefore, exploring the prevention and treatment of cerebral ischemia-reperfusion injury are of great significance. In the current studies, scholars have found that microRNAs are closely related to the mechanism of cerebral ischemia-reperfusion injury, and gene therapy based on microRNAs is expected to be a new method for it.

Introduction

MicroRNAs (miRNA) are non-coding RNA with 22 nucleotides encoded by endogenous genes, approximately 1/3 of human genes are regulated by miRNAs. They are post-transcriptional regulators that specifically recognize the 3’ non-transcribed region of the target mRNA, lead to inhibiting the translation or degradation of the respective mRNAs. They have been implicated for regulating varieties cellular processes and diseases such as apoptosis, differentiation, proliferation, ischemic diseases, neurodegenerative diseases and tumor. miRNA-based gene therapy has become a new type of treatment due to the development of genetic engineering treatment. Whatsmore, biomarker-specific miRNAs have been detected, owing to the application of detection methods. Recent years, studies found that miRNAs were expressed by RNA molecules and had key roles in the pathophysiological processes involved in I/R. In this review, we focus on the role of miRNAs as potential diagnostic biomarkers and promising therapeutic agents in the development of cerebral ischemia-reperfusion injury.

Biosynthesis and Functions of miRNAs

In 1993, the Ambros group first discovered miRNA-lin-4 in the nematode, it interacted with a specific region of the 3’ UTR of the lin-4 gene mRNA in an incompletely complementary manner, ultimately inhibited lin-4 protein synthesis and regulated nematode development[1]. Pre-miRNA is formed with the help of RNA polymerase II, ribonuclease III Drosha and output protein 5 (Exportin-5). The pre-miRNA is cleaved by ribonuclease Dicer and formed a double strand miRNA with a length of about 22 bases in the cytoplasm, and then, the miRNA duplex binds to the Argonaute protein assisted by the Hsc70/Hsp90 chaperone in an ATP-dependent manner, and initiates the unzipping process through the N
domain of the Ago protein, leaving one strand to form a functional miRNA-induced silencing complex (miRISC)[2]. The RISC degrades the target mRNA if the miRNAs are perfectly matched to the targeting mRNA sequence, then regulates targeting mRNA at transcriptional levels. If the two are not fully matched, RISC closes the mRNA and inhibits protein translation, which is the most popular combination type of miRNAs. The third type of action has two modes at the same time: when the target gene complements the combination, it directly targets the cutting mRNA, when the target gene is not completely integrated, it plays a regulatory role in gene translation.

**miRNAs and Cerebral Ischemia-reperfusion Injury**

**The Mechanism of I/R**

The pathophysiological mechanism of I/R is a complex process involving mitochondrial damage, free radical accumulation, calcium overload, inflammatory response, apoptosis and many other aspects. Mitochondrial damage is a top priority, the radical cascade reaction runs through the mechanisms, and eventually the calcium overload causes the death of neurons. The above processes lead to a vicious cycle, and eventually cell necrosis[3].

**Expression of miRNAs During I/R**

miRNAs (such as miRNA-15a, -15b, -29b, -29c, -124, -145, -181c, -let-7b) have been found to expressed in brain differentially after I/R and have changed the reactions of I/R, as well as the expression of cell survival and apoptosis key elements, indicated that miRNAs may be a potential therapeutic target for I/R.

**miRNAs Regulate the Production of ROS During I/R**

Mitochondria contains a large number of nuclear genome encoded proteins, which not only produces ROS, but also be the main target of ROS. Recent studies have shown that miRNAs were associated with mitochondria, regulated the production of ROS[4]. In MACO mice, the study found that miRNA-424 could induce the upregulation of MnSOD, EcSOD and Nrf2 expression after I/R. MnSOD and EcSOD have antioxidative activity to reduce ROS. Nrf2 is a redox-sensitive transcription factor that activates the expression of SOD. The experiment demonstrated that miRNA-424 positively regulated the antioxidant system in neurons and reduced the brain damage caused by I/R[5].

**miRNAs Regulate Apoptosis after I/R**

Ischemia and hypoxia itself, oxygen free radicals, calcium overload, mitochondrial damage can induce apoptosis. For the past few years, it has been found that miRNAs such as miRNA-15a, miRNA-29b, miRNA-124, miRNA-145, miRNA-let-7f were associated with various apoptotic genes and played different roles in promoting apoptosis[6]. Peroxisome proliferator-activated receptors (PPARs) belong to the nuclear receptor family of ligand-activated transcription factors, which exert an anti-apoptotic effect through bcl-2 protein and induce endothelial cell proliferation. Yin and his team confirmed that miRNA-15a was regulated by PPARδ, overexpressing of PPARδ could increase the level of bcl-2 protein and inhibit the expression of miRNA-15a[7]. MiRNA -124 targets iASPP, an inhibitor of p53 family apoptosis stimulating protein, inhibits its expression and promotes apoptosis mediated by p53[8]. MiRNA-let-7a inhibits the activation of MAPK signaling pathway by regulating MKP1 expression and exerts anti-apoptosis effection[9].
miRNAs Regulate Nerve Regeneration after I/R

Matrix metalloproteinase 9 (MMP9) is one member of the MMP protein family, whose main function is to degrade and restore the dynamic balance of extracellular matrix. MMP9 degrades the membrane components around the capillaries, results in the destruction of the blood-brain barrier and the formation of new blood vessels during the later stage of I/R. Deng et al. have found that miRNA-21 was a key factor in increasing level of MMP-9 protein mediated by the ERK cascade after ischemia. Therefore, anti-miRNA-21 could be used as a target for promoting angiogenesis[10].

Gene Therapy Based on miRNAs

miRNAs have been recognized as a key component of gene expression regulatory substrates. LNA-antimiRTM-122 (SPC3649) was the world’s first microRNA drug tested in humans. Harry and his team used Miravirsen (Locked nucleic acid modified DNA thiophosphate antisense oligonucleotides isolated from mature miR-122) to carry out experiments in patients with long-term infection of HCV, he found that Miravirsen could significantly inhibit the blood signs caused by HCV[11]. Hou and his team used adenovirus mediated miRNA-22 vector in rat and cell models, they found that miRNA-22 has a remarkable neuroprotective effect through targeting genes and inhibiting neuronal apoptosis[12].

Currently, miRNAs delivery system include chemically synthesized single-stranded miRNA inhibitors, liposomes, nanomaterials, and miRNAs viral vector delivery. miRNAs inhibitors or analogue synthesized by chemical substances can be transfected into cells simply by encapsulation with transfection reagents. Liposomes, such as cationic liposomes, can protect the miRNAs from the degradation of nuclease. LPS of mirna-29b (lp-mir-29b) can effectively deliver mirna-29b to non-small cell lung cancer (NSCLC)[13]. However, it also causes a strong immune response. Nanoparticles with diameter of 50~70nm transmission system, such as polyethylene glycol (PEG) cationic lipid coated layer and polyethyleneimine (PEI) -miRNA compound, they can significantly reduce the immunogenicity of miRNA preparations, enhance the stability of miRNAs, and even achieve the effect of sustained release and controlled release[14]. Nevertheless, the circulating time of nanoparticles is shorter, which needs repeated administration. Viral vectors are the most efficient way to transfec miRNAs, recent studies have successfully used AAV9 - mediated miRNA analogue to treat heart disease animal models[15]. However, virus vectors can disrupt the host’s normal genes and activate harmful genes, such as cancer genes. In recent years, the nasal drug delivery has been used to target the brain, which can bypass the blood brain barrier system to absorb molecules. Whereas, the intracranial method is not a simple way, and accurately target the specific areas of the brain without damaging the entire brain still be a big challenging.

Prospect

Studies have found that miRNAs were key regulators of gene expression in the past 20 years. As mentioned above, miRNAs are related to the mechanisms of I/R, which has opened new fields of research for not only biomarkers, but also therapeutics of ischemic diseases. And iPSC (inducible pluripotent stem cells) derived BMEC (brain microvascular endothelial cells) have been designed to transfer miRNAs over the years, overcome the difficulty of crossing the blood brain barrier[16], locked nucleic acid (LNA) and chemically modified
miRNAs vectors overcome the stability of miRNAs in vivo, make it possible for miRNAs based gene therapy to cure I/R. Yet, miRNAs treatment is far from safe and effective clinical application. In a large number of clinical trials, miRNAs modulating drugs have missed target due to the multi-target characteristics; chemically modified miRNAs are also susceptible to accumulation in organs such as the liver; In addition, there is a problem of saturated drug concentration in miRNAs therapeutics, which should be further studied in clinical trials. At the same time, due to the high risk and complexity of central nervous system diseases, the experimental study of miRNAs is still limited to animal experiments. Nonetheless, it is believed that we shall get much more evidence for the miRNAs gene therapy since more and more miRNA drug intervention experiments have been tried in clinical trials.

References


