Review Article

Role of microRNAs in stroke recovery

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Abstract

Ischemic stroke is an important cause of morbidity and mortality across the globe. It is associated with physical, social, and economic disability. Immediately after the stroke, there is a critical period of spontaneous recovery during which there is maximal return to near normal. Following stroke, there is a period of neurogenesis, angiogenesis, axonal growth, and synaptic plasticity. There have been several studies focusing on neuroprotection and enhancing recovery following stroke. However, translation of these into clinical practice has been disappointing. The role of microRNAs in treatment of cancer has been well documented, but studying their role in stroke recovery has been minimal. MicroRNAs serve as critical mediators of recovery following stroke. In this review article, we discuss the role of microRNAs in stroke recovery.

Keywords: Angiogenesis, axonal growth, microRNAs, neurogenesis, stroke, synaptic plasticity

Introduction

Stroke is one of the leading causes of neurological morbidity and mortality worldwide. Every year, >795,000 people in the United States have a stroke and 130,000 eventually die.[1] About 50% of stroke victims are living with hemiparesis, 35% are depressed, 26% are dependent for activities of daily living, and 19% have aphasia.[2]

With stroke being the fifth leading cause of mortality in the United States,[3] there is a considerable pressure on the health system to attempt measures for the prevention and early recognition of stroke. Globally, 90.5% of the disease burden of stroke was attributed to modifiable risk factors[4] implying the need for family physicians to focus efforts toward primordial prevention of modifiable risk factors. This need is very well understood, and steps have been taken for targeted interventions at controlling various modifiable risk factors in at-risk patients. However, there is very little understanding of the role of targeted molecular interventions that can be used in the tertiary prevention of stroke, which can result in the early or improved recovery in patients.

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Stroke recovery is an incredibly complex process. After a stroke, the most dramatic recovery occurs within the first 3 months.[5] The neural basis of spontaneous recovery without any rehabilitative interventions has intrigued researchers. There are three different processes responsible for spontaneous recovery-diaschisis reversal, kinematic changes, and cortical reorganization.[6] The plastic changes that occur following stroke injury are similar to the ones observed during brain development with learning. While stroke injury results in a devastating set of events, it also induces growth-related events in the perilesional area that enable the lesion area to repair and form new connections.[7] In this review article, we focus on the processes that occur following stroke and the role of microRNAs in stroke recovery. Understanding the changes in levels of different microRNAs may help us to prolong the recovery period after stroke injury and design targeted interventions for better recovery following stroke.

Neurogenesis

Contrary to the long-held belief, neurogenesis occurs in the adult mammalian brain including humans. Neurogenesis occurs primarily in two areas: subventricular zone and the subgranular zone of the hippocampus.[8] These neurogenic areas have

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populations of neural stem and progenitor cells, and changes within this niche can modulate the process of neurogenesis. Ischemic stroke induces neurogenesis that involves proliferation, differentiation, and migration of neural progenitor cells.[9] The proliferation phase of neural progenitor cells is tightly regulated by cell cycle kinetics. Studies in rodents indicate that stroke reduces the G1 phase of the subventricular zone (SVZ) neural progenitor cell cycle, resulting in early expansion of a neural progenitor pool in the SVZ.[10] Neural progenitor cells differentiate into neuroblasts that migrate to the injured area. Endogenous neurogenesis in response to stroke is limited and only a small population of newly generated neurons survives, whereas the vast majority of neuroblasts die in the ischemic boundary regions.

It has been found that stroke upregulates the expression of members of miR 17-92 cluster, miR-18a, miR-19a, miR-19b, and miR-92a, in neural progenitor cells or SVZ of adult mice. Elevated miR-18a and miR-19a downregulate the expression of phosphatase and tensin homolog (PTEN) and promote cell proliferation. The expression of miR 17-92 cluster is upregulated by Shh signaling pathway through myc oncogene. Therefore, administration of exogenous Shh protein increases the expression of miR 17-92 cluster and hence increases neurogenesis.[11]

miR-124 decreases neurogenesis by targeting SOX9 or Notch ligand jagged1 in nonischemic and ischemic brain, respectively. In the ischemic brain, activation of Notch signaling pathway promotes neurogenesis, whereas abolishing Notch pathway suppresses neurogenesis. Administration of exogenous miR-124a inhibits the NOTCH signaling pathway and decreases neurogenesis.[12]

Post-stroke Angiogenesis

Post-stroke penumbra is a site of active angiogenesis and the number of new vessels in ischemic penumbral regions correlates with longer survival in ischemic stroke patients.[13] A balance between angiogenic (vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF), metalloproteinases MMP-2/9) and angiostatic factors (thrombospondin-1, thrombospondin-2 (TSP-2)) marks the angiogenic activity. Postischemic angiogenesis has multiple roles:

1. Facilitates the macrophage infiltration and removes the necrotic tissue[14]
2. The growth factors promote the survival of endothelial, glial, and neuronal cell types in the penumbra
3. Formation of neurovascular niche in which neural stem cells are generated and migrated.[15]

miR-210 is involved in the regulation of angiogenesis in response to ischemic injury to the brain. Upregulation of miR-210 can activate the Notch signaling pathway, which may contribute to angiogenesis after stroke.[16,17]

It has been shown that overexpression of miR-15a can suppress poststroke angiogenesis by inhibition of proangiogenic factors (VEGF, FGF). Thus, decreasing the levels of miR-15a can increase poststroke angiogenesis.[18]

Inflammation

Following ischemia, damaged neurons release proinflammatory cytokines such as Interleukin (IL)-1β, IL-6, and tumor necrosis factor (TNF)-α, as well as other potential cytotoxic molecules including nitric oxide (NO), reactive oxygen species (ROS), and prostanoids. These lead to the activation of microglia and expression of cellular adhesion molecules (ICAM, selectins, etc.) on the endothelial cells and migrating inflammatory cells. Activated microglia and inflammatory cells secrete additional cytokines and reactive oxygen species and activation of matrix metalloproteinases leading to disruption of blood–brain barrier (BBB) causing edema and cerebral damage.

miR-424 has been thought to play a therapeutic role in stroke by suppressing microglia activation through depression of factors required for G1-S transition, including CDC25A, CCND1, and CDK6.[19] miR-181c has been known to suppress the levels of TNF-alpha after ischemia and therefore prevents neuronal death.[20]

The inflammatory process due to stroke increases astrogliosis (astrocyte proliferation) and scar formation. miR-125b increases astrogliosis by decreasing the expression of CDKN2A, a negative regulator of cell growth. Interferon β is an anti-inflammatory cytokine and could prevent the neuron from ischemic injury for it could decrease the infarct volume by 30%. Some miRNAs such as miR-26a, miR-34a, miR-145, and let-7b increase the expression of interferon (IFN)-beta and are neuroprotective.[21]

Excitotoxicity

After an ischemic insult, the major excitatory neurotransmitter glutamate accumulates in the extracellular space as a result of depletion of neuronal oxygen and energy reserves, ion pump failure as well as a failure of reuptake mechanisms by glutamate transporter-1 (GLT-1) and GLAST glutamate transporters. The excess of glutamate leads to prolonged stimulation of Alpha-Amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) and N-methyl-D-aspartate receptor (NMDA) inotropic receptor (glutamate receptors) that causes a generalized ionic imbalance in the neurons, especially the increase of intracellular calcium. Massive calcium influx activates catabolic processes mediated by proteases, lipases, and nucleases causing cell death.

miR-223 protects against the excitotoxic injury by regulating the expression of glutamate receptor subunits Glur2 (a subunit of AMPA receptor) and nr2b (a subunit of NMDA receptor). Overexpression of miR-223 lowers the levels of Glur2 and nr2b and prevents the excessive Ca^{+2} influx. Therefore, miR-223 is neuroprotective.[22]

Oxidative Stress

Central nervous system (CNS) is more prone to oxidative damage because of the high metabolic activity and oxygen consumption that leads to increased ROS production. Also, the high lipid content of the brain reacts with ROS to generate peroxyl radicals. After a stroke, the primary mechanism of free radical generation is decreased redox potential of mitochondria. There is increased superoxide production by nicotinamide adenine dinucleotide phosphate (NADPH) oxidase following neuronal NMDAR receptor activation. During a stroke, there is increased production of NO due to the activation of neuronal and inducible forms of nitric oxide synthase (NOS). Another mechanism for the production of free radicals is the activation of Ca^{+2} dependent enzymes, Phospholipase 2 and cyclooxygenase. Free radical accumulation is fatal as they cause damage to different cellular components, including oxidation of lipids, proteins, and DNA as well as initiating cascade reactions that lead to mitochondrial dysfunction, caspase activation, and finally, neuron death.

miR-424 has been known to suppress oxidative stress and thus is neuroprotective. miR-424 treatment reduces H_2O_2 induced injury, increases MnSOD activity, and cell viability. The level of miR-145 is increased after stroke. Prevention of miR-145 expression by antagomir administration in lateral ventricles of rats led to an increased protein expression of its downstream target superoxide dismutase-2 in the postischemic brain and reduced the damage of the infarct.[23]

Apoptosis

During a stroke, due to the depletion of oxygen and glucose in neurons, there occurs cell death. Necrosis and apoptosis are the primary mechanisms of cell death. Necrosis occurs in the core of the infarct, whereas apoptosis occurs in the penumbra. Apoptosis can occur by several pathways; the mitochondrial pathway (intrinsic pathway) and death receptor pathway (extrinsic pathway). The mitochondrial pathway can proceed through either caspase-dependent or caspase-independent mechanisms. The death receptor pathway involves the activation of fas-associated protein with death domain (FADD) by signal receptors, i.e. TNFR and FAS, followed by activation of caspase-8 and the subsequent caspase cascade leading to apoptosis.

A neurotrophin receptor p75 (NTR) has been implicated in causing apoptosis after stroke. miR- 592 is a key regulator of p75 (NTR) expression. miR-592 levels are inversely correlated with the levels of p75 (NTR). After ischemia, there is an increase in the level of p75 (NTR) with a corresponding fall in level of miR-592. Similarly, overexpression of miR-592 decreases the p75 (NTR)-mediated apoptosis and is a potential therapeutic target.[24] Overexpression of miR-21 has also proven to be neuroprotective. This is because of the decreased expression of Fas ligand that is an inducer of cell death. miR-15a has been shown to reduce the levels of Bcl-2, an anti-apoptotic protein by inhibiting its translation. The expression of miR-15a is under the control of peroxisome proliferator-activated receptor (PPAR)-delta and PPAR-delta inhibits the pro-apoptotic miR-15a. Thereby, it decreases caspase-3 activity and provides neuroprotection.[25]

Inhibition of miR-181a has been shown to reduce neuron loss after cerebral ischemia. miR-181a antagomir administration post stroke prevents apoptosis by increasing the levels of BCL-2 and X-linked inhibitor of Apoptosis.[26] Overexpression of miR-497 amplified the ischemic injury via inhibition of Bcl-2 while antagomir-497, its specific inhibitor, could reduce the infarct volume, decrease the mortality, and improve the neurological function in experimental stroke.[27] Increased expression of miR- 134 is associated with downregulation of HSPA12B (Heat Shock 70kd protein 12B) and thus enhanced apoptosis and cell death. Conversely, inhibition of miR-134 will be neuroprotective.

BBB Disruption

Disruption of BBB following stroke causes cerebral edema and brain damage because of increased accumulation of proteins and fluids into the cerebral parenchymal extracellular space. Poststroke BBB disruption is associated with poor functional outcome in patients receiving thrombolytic therapy.[28] Metalloproteinases, especially MMP-2 and MMP-9, are implicated in BBB disruption as they cause degradation of extracellular matrix and tight junctions around the BBB.

miR-320 is found to be a contributor to cerebral edema because it downregulates the expression of aquaporins 1 and 4, which play an essential role in water reabsorption. Thus, after a stroke, there is an increased level of miR-320.[29] It has been found that inhibition of miR-155 prevents the cytokine-induced increase in BBB permeability and BBB dysfunction.

Axon Sprouting and Growth

Surrounding the infarct where there is an area of apoptotic cell death, there is a formation of glial scar that prevents axonal regrowth by the formation of gap junctions that constitutes the physical barrier and also the secretion of growth inhibiting molecules that chemically prevent the axonal extensions. The growth inhibiting molecules include chondroitin sulfate proteoglycans (aggrecan, phosphocan, versican, and neurocan), myelin-associated glycoprotein, ephrin B2, slit proteins, and semaphorin IIIa ligand and its receptor neurophilin 1. However, adjacent to the glial scar, there is peri-infarct cortex that is characterized by expression of growth promoting factors, such as CAP23, GAP43, MARCKS, and small proline-rich protein-1 that are permissive to axonal sprouting.[30,31] The immediate 2- to 3-week poststroke period is the window for axonal sprouting.[32] Understanding the epigenetic mechanisms regulating the establishment of permissive and inhibitory environments for axon growth will help to further illuminate the means by which network remodeling occurs after a stroke, and possible ways to facilitate it.

miR-9 is downregulated in ischemic white matter. MiR-9 is expressed in the axons of primary cortical neurons in the developing brain where it represses microtubule-associated protein 1b (Map1b) translation. Inhibition of miR-9 by RNA interference resulted in significantly increased axon length but reduced branching patterns, effects that were dependent on the regulation of Map1b translation.[33,34]

miR-17-92 cluster expression is upregulated after stroke. miR-19a, one of the members of miR 17-92, is known to downregulate the protein level of PTEN and activate the phosphorylated mammalian target of rapamycin (mTOR) pathway. mTOR activity is known to be required for local protein synthesis in axonal development and regeneration. Thus, miR-17-92 promotes axon growth.[35]

Synaptic Plasticity

miR-134 is a negative regulator of synaptic spine volume. Schratt *et al.* showed that miR-134 suppresses Limk1 expression that is essential for spine development. Gao *et al.* showed that miR-134 downregulated the expression of cAMP response element binding protein (CREB) and brain-derived neurotrophic factor (BDNF), thereby impairing synaptic plasticity. Importantly, both the studies show that inhibition of miR-134 increased the levels of Limk1 and CREB, respectively, and improves synaptic plasticity.[36] miR-138 is also known to impair synaptic plasticity. miR-138 interacts with acyl protein thioesterase 1 and causes palmitoylation of alpha subunits of G proteins and increases rho activity thereby decreasing the size of dendritic spines.[37]

The immediate early gene Arc is an important regulator of synaptic plasticity.[38,39] Arc expression is decreased in the ischemic core but significantly increased in the peri-infarct cortex soon after a stroke. It has been shown that Arc expression is regulated by multiple miRNAs. Ectopic expression of miR-34a, miR-193a, or miR-326 has been found to downregulate the endogenous Arc protein expression in response to BDNF treatment. However, treatment with cell penetrating, peptide nucleic acid inhibitors of miR-326 enhanced Arc mRNA expression and synaptic plasticity.

Conclusion

We herein described various processes that occur following stroke and discussed the relevance of microRNAs in affecting these processes. Clinical epigenetics is an advancing field. Understanding the role of microRNAs in the pathophysiology of stroke and its recovery would help design interventions to prolong the period of recovery following stroke and also hasten the process of recovery.

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Conflicts of interest

There are no conflicts of interest.

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