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MicroRNA in ischemic stroke etiology and pathology

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Rink C, Khanna S. MicroRNA in ischemic stroke etiology and pathology. Physiol Genomics 43: 521–528, 2011. First published September 14, 2010; doi:10.1152/physiolgenomics.00158.2010.—Small, noncoding, microRNAs (miRNAs) have emerged as key mediators of posttranscriptional gene silencing in both pathogenic and pathological aspects of ischemic stroke biology. In stroke etiology, miRNA have distinct expression patterns that modulate pathogenic processes including atherosclerosis (miR-21, miR-126), hyperlipidemia (miR-33, miR-125a-3p), hypertension (miR-155), and plaque rupture (miR-222, miR-210). Following focal cerebral ischemia, significant changes in the miRNA transcriptome, independent of an effect on expression of miRNA machinery, implicate miRNA in the pathological cascade of events that include blood brain barrier disruption (miR-15a) and caspase mediated cell death signaling (miR-497). Early activation of miR-200 family members improves neural cell survival via prolyl hydroxylase mRNA silencing and subsequent HIF-1α stabilization. Pro- (miR-125b) and anti-inflammatory (miR-26a, -34a, -145, and let-7b) miRNA may also be manipulated to positively influence stroke outcomes. Recent examples of successfully implemented miRNA-therapeutics direct the future of gene therapy and offer new therapeutic strategies by regulating large sets of genes in related pathways of the ischemic stroke cascade.

ischemia; brain; atherosclerosis; miRNA

THE DISCOVERY OF POSTTRANSCRIPTIONAL gene silencing by small, noncoding, microRNA (miRNA) has led to an explosion of new mechanistic hypotheses in human disease. To date, over 940 human miRNA sequences have been recorded in the miRNA database “miRBase” alone, each with the potential for hundreds of evolutionarily conserved mRNA targets (4). Altogether, miRNAs have been suggested to regulate as much as one-third of protein coding genes in humans (37) and have been implicated in diverse biological processes including embryonic development, cellular differentiation, apoptosis, metabolism, and oncogenesis (30).

The etiological origins of focal cerebral ischemia as well as the resulting pathology are mediated by a multifaceted cascade of molecular mechanisms that are in part regulated by posttranscriptional activity. Given the complexity of the vertebrate central nervous system, it is not surprising that a number of brain-enriched miRNAs have emerged as potential regulators of homeostatic function and, under pathological conditions of stroke, as mediators of neurodegeneration and inflammation (9). While cloning and expression profiling studies have provided direct evidence of their presence in brain tissue, fewer studies have gone as far as biologically validating the effects of miRNAs beyond predicted mRNA targets. Therein lies the greatest challenge to the nascent field of miRNA biology: to determine the biological significance of miRNA under normal and pathological tissue conditions. Here, we present an overview of the current knowledge of miRNA in focal cerebral ischemia and highlight miRNA that have been biologically validated to regulate miRNA targets, encoded protein, and subsequent biological functions.

miRNA BIOLOGY AND LIMITATIONS OF PREDICTIVE TARGET ALGORITHMS

miRNAs are generated from long precursor primary transcripts (pri-miRNA) that are cleaved into ∼60- to 70-nucleotide stem-loop intermediates, referred to as pre-miRNA. This processing is enacted by the Drosha RNA III endonuclease, which cuts both strands of the stem at sites near the base of the primary stem-loop (2, 35). Pre-miRNA is then exported from the nucleus to the cytosol, where it is processed by yet another RNase III endonuclease, Dicer, to form mature 22- to 25-nucleotide miRNA. Mature miRNAs enact mRNA translational repression or cleavage via incorporation into a multiprotein complex termed RISC (RNA-induced silencing complex) and subsequent complete or incomplete binding to the 3’-untranslated region (UTR) of their respective target mRNAs (75). Several noteworthy reviews are available to provide greater detail on miRNA biogenesis and putative mechanisms of posttranscriptional mRNA regulation (2, 17, 75).

MiRNA nomenclature has been facilitated by the adoption of a standard naming scheme that was applied since the first large-scale miRNA discovery (21). MiRNAs are assigned sequential numerical identifiers with homologous miRNAs...
across species abbreviated with three or four letter prefixes. Nomenclature guidelines only require that novel miRNA genes are experimentally verified by cloning or with evidence of expression and processing. Validation of biological targets is not a prerequisite of miRNA discovery, naming, and publication.

Potential mRNA targets for miRNAs of interest are commonly predicted on the basis of computational software (i.e., PicTar - http://pictar.mdc-berlin.de/, miRanda - http://www.microrna.org/microrna/home.do, and TargetScan - http://www.targetscan.org/), which incorporate distinct algorithms to forecast the probability of a functional miRNA binding site within a given mRNA sequence (32). Such algorithms are primarily focused on programming alignment to identify complementary elements in the 3’-UTR of the target mRNA with the miRNA seed sequence; a region consisting of nucleotides 2–7 of the mature miRNA when read in the 5’ to 3’ direction. Additional considerations of computational algorithms may include the stability of the RNA-RNA duplex, target site conservation across species, and the presence of multiple target sites in the same gene (3, 44).

While there have been numerous candidate miRNA targets identified through computational algorithms, relatively few have been biologically validated in the context of ischemic stroke (66). Limitations of computational algorithms have previously been highlighted and underscore the need for stringent biological validation to limit false-positive and false-negative reporting (3, 4, 32, 44, 57). False-positive rates for early computational algorithms of miRNA/mRNA interaction were estimated to be greater than 20% (47). Conversely, a false-negative rate of >25% was recently reported for a set of experimentally validated miRNA interactions which were not predicted in several of the most commonly used miRNA target prediction programs due to requirements for evolutionary conservation of the miRNA target site across different species (44, 66).

Recent works have proposed guidelines to experimentally validate that a given miRNA regulates a predicted mRNA target (3, 32, 44). Biological validation approaches include, but are not limited to, 1) demonstration of the miRNA/mRNA binding interaction, 2) coexpression of the miRNA/mRNA in spatial and cell-specific resolution, 3) appreciable miRNA-induced changes in the amount of protein encoded by the target mRNA, and 4) a change in biological function as a result of miRNA-mediated regulation of a target mRNA (32). Given the complexity of posttranscriptional regulation, it stands to reason that not all of these criteria will be met under experimental conditions. To strengthen the current review, however, only miRNA that meet at least one of the aforementioned biological validation criteria are discussed.

ISCHEMIC STROKE INCIDENCE AND ETIOLOGY

Worldwide, 15 million people suffer stroke every year (43). Of these, stroke claims the lives of 5 million while another 5 million are left permanently disabled (43). In the United States alone, stroke is the third leading cause of death and the leading cause of serious long-term disability with ~795,000 Americans afflicted by a new or recurring stroke event each year (40). Stroke is broadly defined by a cerebrovascular disruption of blood supply to brain tissue that is either ischemic or hemorrhagic in origin. Stroke pathology of ischemic origin accounts for 87% of all strokes subtypes presented clinically, while intracerebral and subarachnoid hemorrhagic constitute the remainder (10 and 3%, respectively) (40).

The etymology of the word ischemia is derived from the Greek words ischaimos, which means “to restrain” and haima meaning “blood” (61). In simple terms, ischemic stroke occurs when an artery is obstructed. The brain depends on continuous blood flow to deliver oxygen and nutrients (i.e., oxygen, glucose) and to remove carbon dioxide and cellular waste. When brain tissue is unable to maintain homeostasis and subsequent trafficking of essential lipids, proteins, nutrients, and waste, the terminal result is energetic failure (ATP depletion) and cell death. Ischemic stroke has several etiological origins. The most common is due to the narrowing of the arteries in the neck or head. This is most often caused by atherosclerosis, a chronic disease process directly influenced by diet (13, 68). High-fat diets leading to elevated low-density lipoprotein (LDL) cholesterol and triglyceride levels are significant risk factors for atherogenesis. If cerebrovascular arteries become too narrow, blood cells collect and form atheromatous plaques. These plaques can block the artery where they are formed (thrombosis) or can dislodge and become trapped in smaller or more distant arteries of the brain (embolism) (Fig. 1).

miRNA IN ISCHEMIC STROKE ETIOLOGY

Atherosclerosis

Apart from its physiological effects, the vulnerability of an atheromatous plaque is of the utmost importance for its pathogenic role in stroke. Vulnerable plaques are composed of a large lipid core, enriched with >40% of LDL-filled foam cells, and a thin fibrous cap depleted of smooth muscle cells (25). An additional hallmark is the abundant infiltration of inflammatory macrophages that contribute to the vulnerability of plaque rupture and intraplaque hemorrhage. A number of genetic risk factors for atherosclerosis and restenosis have been identified (26, 36, 73). Only recently have these risk factors been studied in the light of miRNA-mediated posttranscriptional regulation (23).

Tissue-specific expression of miRNA is an importance determinant of biological activity. Indeed, one miRNA may be highly expressed in one tissue but have no or low expression in other tissues (33). Abundant miRNA expression has been reported in arteries where atherosclerotic plaques are known to accumulate. Of 180 miRNAs arrayed, 140 were found to be expressed in normal rat carotid arteries, with 49 of the 140 found to be highly expressed (29). It is well known that atherosclerosis manifests itself at certain predilection sites, such as side branches and curvatures in arteries. Studies over the past decade have provided evidence for a role of shear stress as a factor linking atherosclerotic plaque localization in such sites (7). Endothelial shear stress is a friction force, in plane with the cell body and induced by the movement of blood with respect to the endothelial layer. This force is transmitted to the cell nucleus, affecting the expression of several genes, including adhesion factors implicated in atheromatous plaque formation (7, 8). Recent work has described the induction of miR-21 in endothelial cells subjected to shear stress (82). Importantly, overexpression of miR-21 was associated with decreased phosphate and tensin homolog (PTEN) protein expression, and subsequent increasedendothelial nitric oxide synthase phosphorylation and nitric oxide production (82). Previ-
ous studies have demonstrated that PTEN reduces intimal hyperplasia in rat carotid artery and attenuates atherosclerotic lesion formation in high fat-fed rabbits (6, 53). miR-21 has also been shown to play a pivotal role in neo-intimal vascular smooth muscle cell (VSMC) proliferation following balloon injury in rodent carotid (29). While the molecular mechanism remains unclear, upregulation of miR-21 following angioplasty increases Bcl-2 expression, favoring VSMC survival and the subsequent proliferative phenotype.

New evidence also suggests miRNA play a distinct role in directing macrophage recruitment to atheromatous plaque. Vascular cell adhesion molecule-1 (VCAM1) is an immunoglobulin-like adhesion molecule expressed on activated endothelial cells (38). VCAM1 binds to αβ1-integrin, which is constitutively expressed on macrophages, mediating both rolling-type adhesion and firm adhesion. While not expressed under normal conditions, VCAM1 expression is rapidly induced by pro-atherosclerotic conditions in both mice and humans (52, 55). miR-126 has been biologically validated as a target of VCAM1. Decreased expression of miR-126 upregulates VCAM1 expression, which in turn enhances leukocyte adherence to the endothelium (22). miR-126 has also been reported to play essential roles in endothelial cells in maintenance of vascular integrity, angiogenesis, and wound repair (78). Indeed, targeted deletion of miR-126 causes leaky vessels, hemorrhage, and partial embryonic lethality due to a loss of vascular integrity and defects in endothelial cell proliferation, migration, and angiogenesis (78). A recent study revealed a distinct loss of endothelial cell specific miR-126 in patients with Type 2 diabetes (89). Considering the well-established link between Type 2 diabetes and an increased risk of atherosclerotic disease (i.e., stroke), miR-126 represents a strong candidate for microRNA mediated therapeutic intervention of atherosclerosis (27, 81).

Hyperlipidemia

Hyperlipidemia is widely recognized as a risk factor for decreased cerebral perfusion and stroke (50, 80). Studies during the late 1990s on the effects of 3-hydroxy-3-methyl-glutaryl-CoA reductase inhibitors (statins) in patients with coronary artery disease have shown significant stroke preventive effects, culminating in the Food and Drug Administration including stroke prevention as an indication for the use of both Simvastatin and Pravastatin (64, 83). Because plasma high-density lipoprotein (HDL) levels show a strong inverse correlation with atherosclerosis, there is strong interest in therapeutically targeting HDL and macrophage cholesterol efflux pathways. It has recently been shown that miR-33 regulates two such pathways: 1) HDL biogenesis in the liver and 2) cellular cholesterol efflux from macrophages, the first step in the reverse cholesterol transport pathway (60). In mouse and human cells, miR-33 inhibits the expression of the adenosine triphosphate-binding cassette transporter, ABCA1, thereby attenuating cholesterol efflux to apolipoprotein 1. Antagonism and overexpression of miR-33 in macrophages was shown to significantly alter cholesterol efflux, a critical first step in the reverse cholesterol transport pathway for the delivery of excess cholesterol.
cholesterol back to the liver (60). In mouse macrophages, miR-33 was biologically validated to inhibit ABCG1, reducing cholesterol efflux to nascent HDL.

MicroRNAs have also been implicated in the regulation of LDL uptake by atheroma-associated macrophages. Macrophages that have infiltrated the LDL-enriched plaque play important roles in the formation of atherosclerotic lesions by taking up oxidized LDL, which leads to their conversion into foam cells (56, 67). Once formed, foam cells secrete proinflammatory cytokines such as interleukin (IL)-1β, IL-6, and tumor necrosis factor-α (58). Cholesterol-loaded foam cells eventually undergo secondary necrosis to form the lipid core of mature atherosclerotic plaques. When exposed by plaque disruption, this core triggers acute thrombotic events leading to stroke. miR-125a-5p has been shown to be significantly upregulated in macrophages following oxidized LDL exposure (5). A biologically validated target of miR-125a-5p is oxysterol binding protein (OSBP)-related protein 9, characterized by a COOH-terminal OSBP domain that binds cholesterol, ergosterol, and phospholipids (5). Inhibition of miRNA-125a-5p by a complimentary binding miRNA “antagomir” significantly increased lipid uptake of macrophages and enhanced expression of the oxidized LDL receptor-1 (LOX-1). Indeed, LOX-1 contributes to plaque instability and development of acute coronary syndromes as demonstrated by LOX-1 deletion reducing proinflammatory signaling and attenuation of atherogenesis (48).

Hypertension

Elevated blood pressure, or hypertension, has long been recognized as a significant risk factor for stroke. There is increasing evidence that an angiotensin II type 1 receptor (AT1R) polymorphism and increased vascular oxidative stress contributes to sympathetic hypertension and vascular disease (10, 49). miRNAs have been directly implicated in mechanisms by which the AT1R polymorphism contributes to increased AT1R activity and associated hypertension (65). Prior to this discovery, and because the polymorphism occurs in the 3’-UTR of the human AT1R gene, the biological significance of the mutation has been questionable. Recently, however, it has been observed that the +1166 A/C polymorphism occurs in a cis-regulatory site that is recognized by miR-155 (45). When the +1166 C allele is present, base pairing complementarity is interrupted and the ability of miR-155 to interact with the cis-regulatory site is decreased. As a result, miR-155 no longer attenuates translation as efficiently. Importantly, mature miR-155 is abundantly expressed in the endothelial and vascular smooth muscle. Downregulation of miR-155 expression in human primary vascular smooth muscle cells induced endogenous human AT1R expression and angiotensin II-induced ERK1/2 activation. Compared with that of Wistar-Kyoto rats, miR-155 expression was decreased in aorta of adult spontaneously hypertensive rats and is negatively correlated with blood pressure, suggesting that miR-155 is possibly involved in the development and pathologic progression of hypertension (86). miR-155 expression was reported to be significantly lower in the aorta of 16-wk-old spontaneously hypertensive rats than in age-matched Wistar-Kyoto rats. Furthermore, miR-155 expression in the aorta is also negatively correlated with blood pressure and age.

Plaque Rupture

Rupture of arterial plaque and subsequent embolic occlusion is one of the leading causes of stroke. Angiogenesis plays a key role in atherothrombotic plaque vulnerability to rupture and is thought to be responsible for the majority of coronary and carotid artery symptomatic events (23, 54). In atherosclerotic intima, newly formed blood vessels are distributed irregularly but ubiquitously across zones of atheromatous plaque disruption. In most cases, these vessels are immature and leaky, permitting infiltration of inflammatory cells as well as an influx of blood and related factors (51, 71). A recent study identified signal transducer and activator of transcription 5α (STAT5a) as a target for miR-222 (11). Importantly, the upregulation of STAT5a due to IL-3/basic fibroblast growth factor-induced downregulation of miR-222 was shown to increase endothelial cell proliferation and migration and, therefore, intraplaque neovascularization during atherosclerosis (71). miR-222 has also been biologically validated to modulate c-Kit (59), a tyrosine kinase cytokine receptor expressed in mature endothelial cells and believed to play a role in microtubule formation and angiogenesis (46). In addition to miR-222, inhibition of miR-210 has also been shown to attenuate endothelial cell migration and angiogenesis (19). Specifically, decreased miR-210 expression inhibits hypoxia induced vessel formation and expression of ephrin-A3 (18). Ephrin-A3 modulation by miR-210 has been demonstrated to possess significant functional effects, including the prevention of tubulogenesis in endothelial cells.

miRNA IN ISCHEMIC STROKE PATHOLOGY

Poststroke miRNA Transcriptome

Cerebral ischemia triggers a cascade of pathological events that ultimately cause irreversible neuronal injury in stroke-affected brain tissue within minutes of stroke onset (14). The pathophysiological order of events includes excitotoxicity within minutes, a robust inflammatory response within hours, and programmed cell death (apoptosis) within hours and days of stroke onset. Preclinical studies in rodents have demonstrated that focal brain ischemia produced by the intraluminal thread model of middle cerebral artery occlusion (MCAO) induces a profound temporal change in the cerebral miRNA transcriptome (12, 28, 39). Table 1 collates differentially expressed miRNA common to preclinical studies in early (<24 h) and late (24–72 h) phases associated with reperfusion injury following stroke. To date, none of these miRNA targets have been adequately validated in the context of stroke. Additionally, observed changes in the miRNA transcriptome in all three preclinical studies were at the tissue level. Newly developed laser capture microdissection techniques permit cell-specific resolution of miRNA transcription, which represents a powerful tool to strengthen biological validation of differentially expressed candidates (63, 79).

Of particular note, however, is that changes in the miRNA transcriptome following focal cerebral ischemia were found to occur independently of an effect on miRNA synthesis machinery. Specifically, focal ischemia produced no significant effect in the miRNA expression of the miRNA processing RNases Drosha and Dicer, Drosha cofactor Pasha, or the pre-miRNA transporter exportin-5 up to 24 h postreperfusion (12). Further-
Table 1. Temporal changes in the microRNA transcriptome following ischemic stroke

<table>
<thead>
<tr>
<th>miRNA</th>
<th>Early Phase ≤24 h</th>
<th>Late Phase 24–72 h</th>
<th>Authors</th>
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<td>Upregulated at one or both phases</td>
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<tr>
<td>17</td>
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<td>up (D)</td>
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<td>up</td>
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<td>up</td>
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<td>292-5p</td>
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<tr>
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<td>324-5p</td>
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<td>422b</td>
<td>up (J)</td>
<td>up (D)</td>
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<tr>
<td>223</td>
<td>NC</td>
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<td>Mixed at both phases</td>
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<td>down (D), up (J)</td>
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<td>down (D)</td>
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<tr>
<td>539</td>
<td>NC</td>
<td>down</td>
<td>D, J</td>
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</table>

Table collates microRNA common to at least 2 of 3 preclinical studies to perform transcriptome-wide analysis following focal cerebral ischemia in rat. Final column designates studies in agreement: D(12), J(28), L(39). NC, no change. Note: specific conditions of experimental MCAO differ across studies and likely contribute to variability in transcriptome changes. For example, D and J employed transient ischemia while L employed permanent ischemia. Refer to original manuscripts for detailed descriptions of experimental stroke procedures.

more, changes in the miRNA transcriptome of stroke-affected cortical brain tissue have been observed as early as 3 h postreperfusion (12), at a time when posttranscriptional regulation by miRNA would influence acute phase molecular mechanisms associated with oxidative stress, inflammation, and apoptosis. Taken together, these important clues suggest that miRNA play a pivotal role in regulating the complex cascade of molecular signaling associated with stroke pathology and neuron cell death.

To date, only two miRNA have undergone extensive investigation to biologically validate a physiological effect following acute ischemic stroke: miR-15a and miR-497 (87, 88). miR-15a has recently been shown to contribute to the pathogenesis of ischemic vascular injury through direct inhibition of the proapoptotic gene bcl-2 (87). Gain or loss of miR-15a significantly reduced or increased oxygen-glucose deprivation-induced cerebral vascular endothelial cell death, respectively.

Of particular interest, miR-15a itself was found to be transcriptionally regulated by peroxisome proliferator-activated receptor δ (PPARδ). Intracerebrovascular infusion of a specific PPARδ agonist significantly reduced ischemia-induced miR-15a expression, increased bcl-2 protein levels, and attenuated caspase-3 activity, leading to decreased blood brain barrier (BBB) disruption and reduced cerebral infarction in mice after transient focal cerebral ischemia (87). Another bcl-2 targeting miR, miR-497 was found to be induced in mouse brain transient MCAO. miR-497 was demonstrated to directly hybridize to the predicted 3'-UTR target sites of bcl-2 and inhibit translation. Demonstrating the physiological significance of this effect, in vivo repression of miR-497 using antagonirs was found to effectively lower miR-497 levels, reduce MCAO-induced infarct, and improve neurological deficits with a corresponding increase in bcl-2 protein (87).

Neural Cell Survival

Acute focal cerebral ischemia causes energetic failure in hypermetabolic neural cells, resulting in loss of transmembrane potential, Ca^2+ influx, glutamate release, subsequent excitotoxicity, and cell death. One strategy known to improve poststroke neural cell survival is to induce a sublethal threshold of ischemic insult to brain tissue prior to stroke, an approach termed ischemic preconditioning (IPC) (70, 74, 90). While the therapeutic relevance and clinical significance of IPC are questionable, this approach has contributed to the identification of a number of neuroprotective mechanisms in preclinical models of acute ischemic stroke (15). To date, the only miRNA directly implicated in poststroke neural cell survival have been identified through IPC screening (34). Members of the miR-200 family (miR-200a, miR-200b, and miR-429) were all found to be significantly upregulated 3 h after IPC in rodents. Transfection of these miRNA in Neuro-2a cells increased neural cell survival when subjected to oxygen glucose deprivation. Predicted miRNA binding targets of the miR-200 family include the untranslated 3' region of prolyl hydroxylase 2 (PHD2). PHD2 is known to hydroxylate hypoxia inducible factor 1α (HIF-1α) marking it for proteosomal degradation under normoxic conditions. HIF-1α is a well-established transcription factor that is rapidly induced by hypoxia and accounts for transcriptional regulation of both prosurvival and prodeath genes including Bcl homology 3-only protein. The overall physiological effect of HIF-1α activation following stroke is believed to be toward prosurvival, however, as neuron-specific inactivation of HIF-1α has been demonstrated to increase brain injury following stroke (1). Indeed, miR-200 family overexpression was shown to increase HIF-1α expression, suggesting effective silencing of PHD2 target (34). Outcomes advocate strategies to enhance early upregulation of miR-200 family members to promote poststroke neural cell survival.

Stroke-mediated Inflammation

The cascade of molecular events following focal brain ischemia transforms the cerebrovascular endothelium from a quiescent to a proinflammatory state. Cytokine induction of cell adhesion molecules on the vascular endothelium promote BBB disruption and leukocyte recruitment (69). This exacerbates the proinflammatory state, with an increase in reactive oxygen species, cerebral edema, and release of cytotoxic enzymes. While no miRNA have been biologically validated in stroke-induced cytokine signaling, several have been implicated in...
other models of proinflammatory brain injury and serve as starting points for investigation. IL-6 is a proinflammatory cytokine known to induce proinflammatory astrocytic scarring (astrogliosis) following stroke (76). After stroke, astrogliosis is particularly localized in regions of neural cell death. Astrogliotic phenotype and miR-125b levels were found to be increased in IL-6 stressed normal human astrocytes (42). Antagomir-125b attenuated glial cell proliferation and increased mRNA and protein expression of putative mRNA target cyclin-dependent kinase inhibitor 2A (CDKN2A), a negative regulator of cell growth. CDKN2A expression is known to be downregulated in chronic neurodegenerative diseases associated with astrogliosis, such as Alzheimer’s disease, suggesting a role in reactive astrocyte proliferation (62). Taken together, these findings support IL-6 mediated inflammation and miR-125b upregulation as a potential mediator of poststroke astrogliosis.

Interferon-beta (IFN-β) is a regulatory cytokine with anti-inflammatory properties that has been approved for the treatment of multiple sclerosis (MS), an inflammatory, demyelinating disease of the central nervous system. In rodents subjected to autoimmune encephalomyelitis, a preclinical model of human MS, IFN-β therapy strongly inhibited extravasation of proinflammatory blood-derived monocytes into the central nervous system by preventing upregulation of vascular cell adhesion molecule 1 (20). Several miRNAs targeting IFN-β have been biologically validated to silence IFN-β in monocytes-derived macrophages isolated from human subjects, including miR-26a, -34a, -145, and let-7b (85). Importantly, IFN-β therapy has already shown promise in stroke. In a preclinical model of focal cerebral ischemia, systemic IFN-β delivery attenuated infiltration of neutrophils and monocytes to brain tissue and reduced stroke-induced lesion volume by 70% compared with controls (77). A phase I clinical trial to test the dosing safety of IFN-β1a in patients with acute ischemic stroke was completed in 2008 (ClinicalTrials.gov Identifier: NCT00097318). Whether miRNA-26a, 34a, -145, and let-7b could be augmented as a therapeutic strategy to improve IFN-β anti-inflammatory signaling and poststroke outcome remains to be seen.

miRNA THERAPEUTICS FOR PREVENTION AND TREATMENT OF ISCHEMIC STROKE

Examples of therapeutic opportunities for miRNA regulation already abound. Antagomirs, although incapable of silencing miRNA in the central nervous system when injected systemically, efficiently target miRNAs when injected locally in the mouse cortex (31). Furthermore, antagomir treatment has been shown to be effective in abolishing tumor growth in vivo, specifically in therapy-resistant neuroblastoma. The potential for antagomir therapy has also been proven in the context of acute ischemic stroke by the aforementioned regulation of miR-97 (88). In contrast to antagomir-directed miRNA silencing, miRNA overexpression strategies also exist in the form corrective synthetic miRNA delivery. miR-34a targets gene products that promote cell cycle progression and counteract apoptosis (24). In an oncogenic environment and in many forms of human cancer, homeostatic miR-34a expression is decreased (41, 72). Systemic delivery of miR-34a in a lipid-based delivery vehicle has been shown to block lung tumor growth in vivo (84).

Barriers to successful therapeutic intervention of miRNA activity include tissue and cell-specific delivery in vivo, degradation avoidance, and target specificity. Chemically modified miRNAs have shown significant promise in preclinical studies to overcome such hurdles. The locked nucleic acid-modified oligonucleotide antagomir (LNA-antimiR) construct contains a methylene bridge that connects the ’2’,’O-oxygen and the ’4’,’C atom of the ribose ring. This conformation increases the thermal stability of duplexes and confers resistance to exo- and endonuclease activity for improved in vivo stability. miR-122 is a liver expressed miRNA implicated in cholesterol and lipid metabolism that has been successfully targeted in vivo using a LNA-antimiR. Systemic administration of a phosphate-buffered solution formulated LNA-antimiR for miR-122 demonstrated potent antagonism of liver-expressed miR-122 in nonhuman primates (16). Biological validation of the LNA-antimiR action revealed cytoplasmic uptake in hepatocytes, formation of stable heteroduplexes between the LNA-antimiR and miR-122, and subsequent depletion of mature miR-122 with appreciable physiologic effect of lowering plasma cholesterol. Chemical modification and delivery systems for miRNA to efficiently cross the BBB and target brain tissue are the focus of ongoing investigation.

In a setting where one miRNA regulates more than one hundred gene targets, and one gene can be regulated by a number of miRNA, a critical barrier is to improve the knowledge of regulatory loops that govern miRNA-mRNA interaction and functional outcome (65). Robust biological validation of miRNA targets is moving us ever closer to better understanding the complex molecular mechanisms associated with pathological disorders such as ischemic stroke. An improved understanding of miRNA biology in disease will also direct new therapeutic strategies to modulate discordant gene expression toward a favorable outcome. Given the complexity of pathophysiologically molecular signaling in the context of ischemic stroke, it comes as no surprise that targeting single genes for therapeutic intervention have failed in the clinic. Targeting noncoding genes such as miRNAs, which have the capacity to regulate large sets of evolutionary conserved coding genes, represents the future of gene therapy.

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DISCLOSURES

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microRNA IN STROKE
