Brief Review

The Involvement of miRNA in Carotid-Related Stroke

Pierre Maitrias, Valérie Metzinger-Le Meuth, Joseph Nader, Thierry Reix, Thierry Caus, Laurent Metzinger

Abstract—Cardiovascular disease is the leading cause of morbidity and mortality in developed countries. Stroke is associated with a marked disability burden and has a major economic impact; this is especially true for carotid artery stroke. Major advances in primary and secondary prevention during the last few decades have helped to tackle this public health problem. However, better knowledge of the physiopathology of stroke and its underlying genetic mechanisms is needed to improve diagnosis and therapy. miRNAs are an important, recently identified class of post-transcriptional regulators of gene expression and are known to be involved in cerebrovascular disease. These endogenous, small, non-coding RNAs may have applications as non-invasive biomarkers and therapeutic tools in practice. Here, we review the involvement of several miRNAs in cell-based and whole-animal models of stroke, with a focus on human miRNA profiling studies of carotid artery stroke. Lastly, we describe the miRNAs’ potential role as a biomarker of stroke.

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Key Words: biomarkers ▪ cerebrovascular disorders ▪ microRNAs ▪ secondary prevention ▪ stroke

Stroke is one of the main causes of death and disability worldwide. Carotid artery atherosclerosis is the underlying cause of >20% of strokes.1 Turbulent flow makes the carotid bifurcation particularly prone to the development of atherosclerotic plaques.2 A carotid artery plaque can trigger a stroke in 2 ways. First, high-grade stenosis may lead to hypoperfusion. Second, weaknesses, such as an intraplaque hemorrhage, ulceration, and inflammation of the carotid plaque, can lead to embolism—even in the absence of a high-grade lesion.3 The current clinical guidelines recommend surgical endarterectomy for (1) symptomatic patients with a transient ischemic attack or ischemic stroke caused by >60% stenosis of the carotid artery and (2) asymptomatic patients with >70% stenosis of the carotid artery.4,5 Duplex ultrasound and computed tomographic angiography are the diagnostic methods of choice for plaque-related stenosis. The degree of carotid stenosis can then be determined according to the NASCET (North American Symptomatic Carotid Endarterectomy Trial) criteria,6 and diagnostic techniques also provide information on the plaque’s composition. Optimal medical treatment to limit disease progression associates control of atherosclerosis risk factors (including smoking, hypertension, diabetes mellitus, and dyslipidemia) with antiplatelet agents, statins, and angiotensin-converting enzyme inhibitors. At present, there are no biomarkers to predict plaque rupture and embolic stroke and to target high-risk subgroups of patients for whom preventive surgery would be formally indicated. However, recent research results suggest that miRNAs may be valuable in this respect.

MiRNAs are small, non-coding, regulatory RNAs composed of 18 to 22 nucleotides. They regulate target gene expression at the post-transcriptional level by either inhibiting translation or causing degradation of the corresponding messenger RNA.7 Many studies have shown that miRNAs have well-documented roles in a variety of biological processes and diseases.8,9 Hence, the involvement of miRNAs in the progression and rupture of carotid artery plaques and in carotid artery stroke warrant investigation. Here, we review current knowledge about how miRNAs are involved in carotid artery stroke in general and in the rupture of unstable carotid artery plaques in particular.

Cellular miRNAs Contribute to Atherosclerosis

Atherosclerosis is a complex process of arterial wall inflammation and remodeling. The starting point for atheroma formation is endothelial dysfunction. Hemodynamic disturbances, hypercholesterolemia, and inflammation are the main causes of endothelial dysfunction.6,10 Etiologic factors also include cigarette toxins, homocysteine, and a wide spectrum of infectious agents. Chronic endothelial injury leads to endothelial dysfunction and increased permeability.11 The injury also induces oxidation and accumulation of low-density lipoprotein in the subendothelial space of the intima, together with the expression of adhesion molecules (eg, VCAM-1 [vascular cell adhesion molecule 1],...
ICAM-1 [intercellular adhesion molecule], and P selectin] and chemokines (eg, monocyte chemoattractant peptide) that participate in platelet aggregation and lymphocyte/monocyte adhesion and infiltration—thus, initiating the inflammatory process.12,13 Because monocytes are attracted to the endothelium and migrate to the subendothelial space, they mature into macrophages, take up oxidized low-density lipoprotein, and transform into foam cells that form the lipid-rich core of the atherosclerotic plaque.14,15 This inflammatory mediator cascade promotes a phenotype change of vascular smooth muscle cells (VSMCs) from the contractile phenotype to the active synthetic phenotype. Synthetic VSMCs can proliferate and migrate from the media to the intima, where they produce excessive amounts of extracellular matrix (eg, collagen, elastin, and proteoglycans); this transforms the lesion into a fibrous plaque.16

It has been shown that high shear stress significantly affects the gene expression of endothelial cells and that miRNAs are even involved in the flow regulation of atherosclerosis.17 Mir-10a has been shown to be implicated in KLF-2 (Krüppel-like factor 2)–dependent mechanisms that regulate lesions of endothelial cells in vascular niches.18 Moreover, miRNAs are involved in the regulation of gene expression in above-mentioned cells and mediate cell-to-cell communication. The miRNAs’ main roles in the atherosclerotic process are summarized in the Figure.

Rupture-prone, vulnerable plaques are typically associated with the presence of (1) a high inflammatory cell count, (2) a large necrotic core count, and (3) a thin fibrous cap.19 Several studies have shown that miRNAs have several roles in the processes underlying atherosclerotic plaque rupture. Vulnerable plaques show evidence of VSMC death and, thus, low numbers of VSMCs in the fibrous cap—suggesting that treatment approaches aimed at stabilizing existing plaques should target VSMCs. Upregulated miR-21 expression has been shown to inhibit reactive oxygen species–induced VSMC apoptosis and death.20 In mice, miR-126–treated arteries displayed higher intimal smooth muscle cell counts, a higher collagen content, and fewer apoptotic cells (relative to controls), which is consistent with greater plaque stability.21 Promoting a contractile VSMC phenotype may increase the integrity of the fibrous cap. Indeed, transdifferentiation of VSMC from the normal, quiescent, and contractile, phenotype to a synthetic, proliferative phenotype is associated with atherosclerosis. We and others have shown that several miRNAs, including miR-126, are involved in this process.22 In this context, overexpression of miR-145 in SMCs in ApoE−/− mice reduced the plaque volume and increased features of plaque stability (such as a higher collagen content and a greater fibrous cap surface area) in a manner consistent with the promotion of a quiescent smooth muscle cell phenotype.23 Therefore, upregulation of miR-143/145 may not only decrease VSMC proliferation in recently formed atherosclerotic plaques but also stabilizes the fibrous cap in advanced plaques.

MMPs (macrophage-derived matrix metalloproteinases) have a crucial role in fibrous cap thinning and plaque destabilization. It has notably been shown that miR-24 downregulation enhances macrophage apoptosis and MMP-14 proteolytic activity and, thus, promotes atherosclerotic plaque progression and the characteristics associated with plaque instability.24 It has also been shown that miR-29 represses collagen synthesis.25 Macrophage and VSMC apoptosis contribute significantly to the formation and expansion of the plaque’s necrotic core. In this context, miR-155 has been implicated in the induction of macrophage apoptosis in response to specific stimuli.26 In the necrotic core, cholesterol crystals have a direct breaching effect on the fibrous cap but also may trigger an inflammatory cascade via activation of the NLRP3 (NOD [nucleotide oligomerization domain]-like receptor family, pyrin domain containing 3) inflammasome, which makes the atherosclerotic plaque even more unstable. miR-223 reportedly downregulates the NLRP3 inflammasome and thereby prevents the associated inflammatory response.27 It has also been shown that miR-30c reduced lipid synthesis and miR-30c mimics could be used to lower plasma cholesterol and atherosclerosis without causing steatosis usually induced by decrease in lipoprotein production.28 Advanced plaques are marked by an expansive remodeling process and increased angiogenesis, which leads to invasion of the intima by neovessels—a process that is closely linked to plaque growth, instability, and rupture. Several studies have demonstrated that miRNAs have a functional role in vascular remodeling29 and can alter lesion characteristics through either proangiogenic or antiangiogenic effects.30

Target genes and biological effects of the main miRNAs involved in atherosclerosis are summed up in the Table.

**Animal Models**

Middle cerebral artery occlusion (MCAo) has often been used to simulate stroke in animal models. The occlusion is usually maintained for 60 to 120 minutes and is followed by varying periods of reperfusion and sampling. MCAo can variously be achieved by mechanical obstruction, electrocoagulation, or embolic techniques.

In 2008, Jeyaseelan et al30 were the first to describe the involvement of miRNA regulation in the pathogenesis of brain ischemia. Using Sprague Dawley rats having undergone MCAo and reperfused for either 24 or 48 hours; they screened for 236 miRNAs in both blood and brain. A comparison with the corresponding DNA microarray data revealed that target messenger RNA expression was correlated with miRNA regulation. Laminin-1 and integrin-1 were confirmed as targets of miR-124; VSNL1 (visinin-like 1 protein—a neuronal calcium sensor) was targeted by miR-124 and miR-290; AQP4 (aquaporin 4) was targeted by miR-30a-3p and miR-383; and
MMP-9 was targeted by miR-132 and miR-66. In a second study, Jeyaseelan group focused on miRNAs expression in brain tissue in later phases of stroke (48–168 hours after ischemic stroke). Relative to the acute phase, the authors found higher levels of miR-21, miR-142-3p, miR-142-5p, and miR-146a and lower levels of miR-196a/b/c, miR-224, and miR-324-3p. Levels of miR-206, miR-290, and miR-291a-5p were positively correlated with the infarct volume.51

Dharap et al52 used a similar model of transient MCAo in spontaneously hypertensive rats. Animals were euthanized at 5 different time points (3, 6, 12, 24, and 72 hours after the end of MCAo). In brain tissue, 49 miRNAs were deregulated (with 24 upregulated and 25 downregulated) at one or more of the time points. The expression of miR-140, miR-145, miR-260, and miR-292-5p tended to increase over time, whereas the expression of miR-376-5p and miR-153 tended to decrease. Moreover, inhibition of miR-145 led to the upregulation of superoxide dismutase; this might protect neurons from cell death.

Gubern et al53 also showed that the expression of 32 miRNAs changed during the acute and late phases of stroke (7 and 14 days postocclusion). They focused on miR-247 and its targets (Acs14, Bnip31, and Phyhip) and showed that this miRNA is of importance in the regulation of neuronal cell death.

Selvamani et al54 studied the impact of age and sex on miRNA expression in young adult and middle-aged female and male rats having undergone MCAo. Circulating miRNAs...
Table. Target Genes and Biological Effects of the Main miRNAs Involved in Atherosclerosis

<table>
<thead>
<tr>
<th>miRNA</th>
<th>Main Targets</th>
<th>Biological Effects</th>
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<tbody>
<tr>
<td>let-7f</td>
<td>TIMP-1</td>
<td>MMP expression, macrophage infiltration, plaque formation (Rous et al, 1999; Circulation)</td>
</tr>
<tr>
<td>let-7g</td>
<td>TGF-β and SIRT1</td>
<td>Suppressor of endothelial inflammatory activation (Liao et al, 2014; J Am Coll Cardiol)</td>
</tr>
<tr>
<td>17-92</td>
<td>ABCA1, LDLR, and SEMA6A</td>
<td>Cholesterol efflux; promoting of angiogenesis by regulation of ECs repulsion (Suarez et al, 2008; Proc Natl Acad Sci USA)</td>
</tr>
<tr>
<td>21</td>
<td>MMP-9, Bcl-2, and FABP7</td>
<td>Macrophage infiltration, inflammatory response, proliferation of SMCs, and senescence (Fan et al, 2014; Exp Mol Pathol)</td>
</tr>
<tr>
<td>29a</td>
<td>LPL</td>
<td>Lipid uptake and inflammatory cytokine secretion (Li et al, 2009; J Biol Chem)</td>
</tr>
<tr>
<td>29b</td>
<td>DNTMP3b</td>
<td>Regulation of oxLDL-mediated human aortic SMCs (Chen et al, 2011; FASEB)</td>
</tr>
<tr>
<td>92a</td>
<td>Integrin 5</td>
<td>oxLDL-induced NFκB activation and monocyte adhesion (Yurdagul et al, 2014; Arterioscler Thromb Vasc Biol)</td>
</tr>
<tr>
<td>99a</td>
<td>TGF-β</td>
<td>Inflammation, chemotaxis, fibrosis, proliferation, and apoptosis (Turcatel et al, 2012; Plos One)</td>
</tr>
<tr>
<td>100</td>
<td>mTOR</td>
<td>Antiangiogenic function (Grundmann et al, 2011; Circulation)</td>
</tr>
<tr>
<td>125a-5p</td>
<td>LOX-1, CD68, SRB1, and ORP9</td>
<td>Lipid uptake, cytokine expression, and oxLDL internalization (Huang et al, 2010; J Invest Med and Chen et al, 2009; Cardiovasc Res)</td>
</tr>
<tr>
<td>125b</td>
<td>IRF4</td>
<td>Inflammatory expression of macrophages (Goettsh et al, 2011; Am J Pathol)</td>
</tr>
<tr>
<td>126</td>
<td>TNF-α, VCAM1, and VEGF</td>
<td>Endothelial dysfunction and vascular remodeling (Santovito et al, 2012; Nutr Metab Cardiovasc Dis and Mandadori et al, 2015; Biomed Res Int)</td>
</tr>
<tr>
<td>127</td>
<td>CD40 and BCL6</td>
<td>Vascular inflammation and oxidative stress (Santovito et al, 2012; Nutr Metab Cardiovasc Dis)</td>
</tr>
<tr>
<td>133a</td>
<td>MMP-9 and type I collagen</td>
<td>VSMC proliferation and collagen synthesis (Castoldi et al, 2012; J Cell Physiol)</td>
</tr>
<tr>
<td>143</td>
<td>COX-2</td>
<td>Plaque formation (Burleigh et al, 2002; Circulation)</td>
</tr>
<tr>
<td>145</td>
<td>KLF4, myocardin, and Elk-1</td>
<td>Promote differentiation and repress proliferation of SMCs (Loren et al, 2012; Circulation)</td>
</tr>
<tr>
<td>146a/b</td>
<td>TLR4, IKAR1, and TRAF6</td>
<td>Lipid accumulation and inflammatory response (Guo et al, 2010; Immunol Cell Biol)</td>
</tr>
<tr>
<td>155</td>
<td>AT1R and Ets-1</td>
<td>Protective role in development of endothelial inflammation (Huang et al, 2010; J Invest Med and Chen et al, 2009; Cardiovasc Res)</td>
</tr>
<tr>
<td>221/222</td>
<td>c-kit, p27, and p57</td>
<td>Proliferation of SMCs and contractile gene transcription (Liu et al, 2012; J Mol Cell Cardiol)</td>
</tr>
<tr>
<td>223</td>
<td>Tissue factor</td>
<td>Plaque formation and thrombosis (Li et al, 2014; Atherosclerosis)</td>
</tr>
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ABCA1 indicates ATP-binding cassette 1; AT1R, angiotensin-2 type 1 receptors; BCL6, B-cell lymphoma 6 protein; c-kit, tyrosine-protein kinase kit; CD, cluster of differentiation 68; COX-2, cyclo-oxygenase-2; EC, endothelial cell; FABP7, fatty acid binding protein 7; IKAR1, interleukin-1 receptor-associated kinase 1; IRF4, interferon regulatory factor 4; KLF4, Krüppel-like factor 4; LDLR, low-density lipoprotein receptor; LOX-1, lectin-like oxidized low-density lipoprotein (LDL) receptor-1; LPL, lipoprotein lipase; MMP-9, matrix metalloproteinase 9; mTOR, mammalian target of rapamycin; ORP9, oxysterol binding protein-related protein 9; SEMA6A, semaphorin-6A; SIRT1, sirtuin 1; SMC, smooth muscle cell; SRB1, scavenger receptor class B member 1; TGF-β, tumoral growth factor β; TIMP-1, tissue inhibitor of β, tumoral growth factor β; VEGF, vascular endothelial growth factor.

were analyzed in blood samples 2 and 5 days postocclusion, and brain miRNAs were analyzed 5 days postocclusion. Two days after occlusion, 21 circulating miRNAs were differentially regulated, and most of the variance was because of age. In each case, a main effect was attributed to age, indicating a significant difference in miRNA expression between adult and middle-aged animals, irrespective of sex. In contrast, 5 days after occlusion, 78 circulating miRNAs were differentially regulated, and most of the variance was because of sex. Five miRNAs (miR-15a, miR-19b, miR-32, miR-136, and miR-199a-3p) were found to be highly expressed, exclusively in adult females compared with middle-aged females, adult males, and middle-aged males.

miR-155 has been shown to be instrumental in macrophage differentiation and consequently promotes atherosclerosis because macrophages modulate vascular inflammation and lipid deposits, become macrophage-derived foam cells and, therefore, constitute the main players in the atherosclerotic process. Jahantigh et al used a murine model of partial carotid ligation in mice that were both deficient for miR-155 (miR-155ΔΔ) and ApoEΔΔ. Interestingly, miR-155ΔΔ mice presented a significant reduction in plaque size and macrophage number per plaque. Another effect of miR-155 inhibition is that it improved functional recovery after mouse experimental stroke by altering the expression of major cytokines and thereby influencing the inflammatory process and tissue repair. The sum of this research would lead to the tentative conclusion that miR-155 acts as an inflammatory miRNA promoting atherosclerosis. On the contrary, other authors have reached the opposite conclusion, by showing that miR-155 knockout mice had increased plaque development and decreased plaque stability, concomitant with altered white blood cell counts (decreased monocytes and increased granulocytes and helper T cells).

miR-181b has been shown to be atheroprotective in several studies. Serum miR-181b levels were decreased in acute...
stroke patients with atherosclerotic plaque.29 This team also used a murine model of ApoE−/−, in which miR-181b is overexpressed. This overexpression leads to a decrease of the artery disease burden and atherosclerotic plaque vulnerability. The molecular mechanism involved was modulation of macrophage polarization by downregulation of transcription factor Notch1 by miR-181b.30 In a complementary study in the same ApoE−/− model, Sun et al31 showed that mir-181b targets and inhibits nuclear factor-xB in the intima, leading to a decrease of vascular inflammation and atherosclerosis. In a recent study, Deng et al32 showed that electroacupuncture therapy in stroke enhanced miR-181b expression in the penumbra part of the brain, in turn leading to neurobehavioral function rehabilitation through miR-181b direct targeting of paired immunoglobulin-like receptor B. Nonetheless, miR-181b has recently been shown to be proatherogenic. Indeed, Di Gregoli et al33 interestingly found that miR-181b was overexpressed in symptomatic human atherosclerotic plaques and abdominal aortic aneurysms and correlated with decreased expression of miR-181b targets, tissue inhibitor of MMP-3, and elastin. Further studies are, thus, required to precise the complex role of miR-181b in the regulation of atherosclerotic process.62

Overall, these results show that various miRNAs in rat brain and blood are deregulated after an ischemic event—confirming the complexity of stroke’s effects on the organism. It must be stressed that MCAo models only highlight miRNAs that are altered after stroke and would, therefore, not serve as reliable markers for predicting a primary event.

**Human Studies**

Unlike miRNA profiling studies in animals (where the availability of brain tissue allows a much deeper focus on the molecular biology of stroke), only bodily fluids and endarterectomy tissue samples are available for miRNA analysis in humans with stroke. Fortunately, miRNAs in blood are highly stable.63 In diseases such as cancer, the clear correlation between plasma miRNA levels and tissue miRNA levels64 highlights a potential role for these small, noncoding RNAs as noninvasive biomarkers.65 Circulating miRNAs are stable because they are protected from degradation either by their inclusion in different types of vesicles (such as exosomes) or by their association with RNA-binding proteins.66

In 2009, Tan et al67 reported on the first study of circulating miRNAs in 136 patients with stroke. Of a total of 836 miRNAs, 157 were deregulated, and let-7f, miR-15b, miR-126, miR-142-3p, miR-186, miR-519e, miR-768-5p, and miR-1259 were found to be significantly downregulated. Plasma levels of miR-106b-5p and miR-4306 were higher after stroke, and plasma levels of miR-320d and miR-320e were lower. Li et al68 identified 115 differentially expressed miRNAs in patients with ischemic stroke; miR-32-3p, miR-105-5p, miR-532-5p, and miR-1246 were identified as potential biomarkers of stroke. Jickling et al69 determined miRNA profiles in peripheral blood mononuclear cells from patients with stroke, and the results suggested a link between immunity, inflammation, and stroke. Intracellular levels of let-7i, miR-19a, miR-122, miR-148a, miR-320d, and miR-4429 were abnormally low and those of miR-363 and miR-487b were abnormally high—suggesting that these miRNAs were involved in the regulation of leukocyte adhesion, extravasation, and thrombus formation. Sorensen et al70 published interesting data on potential miRNA biomarkers of acute stroke by studying not only blood but also cerebrospinal fluid. miR-151a-3p and miR-140-5p were found to be upregulated in the blood, and miR-18b-5p was found to be downregulated. Interestingly, miR-523-3p was detected in over half the patients with stroke but in none of the controls.

A few human studies have focused on the miRNAs’ involvement in carotid artery atherosclerosis.

The tampere vascular study71 compared miRNA expression profiles in human atherosclerotic plaques (removed during aortic, carotid, and femoral surgical procedures) and nonatherosclerotic left internal thoracic arteries. Of >800 miRNAs analyzed, 10 showed statistically significant differences in expression levels in atherosclerotic plaques versus controls. Expression levels of miR-21, miR-34a, miR-146a, miR-146b-5p, and miR-210 were significantly higher in atherosclerotic arteries than in control arteries. In the atherosclerotic plaques, 187 predicted targets of these 5 miRNAs were found to be downregulated. Most of these genes were involved in signal transduction, the regulation of transcription, or vesicular transport.71

Cipollone et al72 revealed that expression levels of miR-100, miR-127, miR-133a, miR-133b, and miR-145 were significantly higher in 2 cohorts of symptomatic carotid plaques than in asymptomatic plaques removed during endarterectomy. In vitro experiments on endothelial cells transfected with miR-145 and miR-133a confirmed the importance of these miRNAs in the modulation of stroke-related proteins. Supplementation with miR-133a led to the downregulation of MMP-9 protein levels. Similarly, plasminogen activator inhibitor-1 protein levels were downregulated by both miR-133a and miR-145.

In the agreement with Cipollone results, we recently showed that miR-100, miR-125a, miR-127, miR-133a, miR-145, and miR-221 were significantly upregulated in symptomatic plaques versus asymptomatic plaques. In the symptomatic group, miR-125a expression was significantly and inversely correlated with the circulating level of low-density lipoprotein cholesterol.73 In a similar study, miR-21 and miR-143 were found to be significantly upregulated in patients with asymptomatic plaques.74

Santovito et al75 have shown that miR-145 expression was higher in carotid plaques from hypertensive patients, who are prone to develop stroke if their hypertension is not properly treated. This finding suggests that high carotid plaque expression of miR-145 is a risk factor for plaque instability. Bloods levels of miR-145 are significantly higher in patients with ischemic stroke than in control subjects, which suggest that this miRNA is a potential biomarker. Furthermore, it was recently shown that plasma miR-92a levels are higher in hypertensive patients (regardless of the carotid intima-media thickness) than in healthy controls. miR-92a levels were positively associated with carotid–femoral pulse wave velocity and ambulatory blood pressure monitoring parameters. These findings suggest that miR-92a may be involved in the pathophysiology
of hypertension and atherosclerosis in humans and might be a predictor of atherosclerosis in hypertensive patients.76

Circulating levels of miR-21 and miR-221 were shown to be higher in patients with stroke and carotid atherosclerosis than in healthy controls. In a population of patients with type 2 diabetes mellitus, urine levels of miR-29b were shown to be significantly correlated with carotid intima-media thickness. All 3 miRNAs (miR-21, miR-221, and miR-29b) might be potential biomarkers of carotid atherosclerosis.77,78

**Potential Biomarkers of Stroke**

Profiling studies in animal models and humans have shown that many different miRNAs are associated with stroke. Levels of circulating miRNAs and tissue miRNAs are altered during and after stroke and may be differentially deregulated. We must keep in mind that the miRNAs altered after stroke cannot be used as reliable predictive markers of stroke, but some miRNAs have been studied in detail and may constitute diagnostic and prognostic biomarkers of stroke.

miR-124 is involved in the regulation of neuronal differentiation and adult neurogenesis and is often considered to be a brain-specific miRNA. In 2009, Laterza et al79 showed that the brain injury induced by MCAo led to elevated levels of plasma miR-124 levels. Wang et al80 confirmed that miR-124 was preferentially expressed in the nervous system. An elevation of circulating miR-124 levels was found in the profiling study by Jeyaseelan et al,50 who reported that VSNL1 was targeted by miR-124 in an animal model of stroke. Elevated circulating miR-124 levels have been reported in 2 independent studies81,82 of animal models; this upregulation was significant for at least 48 hours after stroke induction—making miR-124 as a potential biomarker of stroke. In these animal studies, miR-124 levels were not correlated with the stroke outcome or the infarct size. In human studies, the results are contradictory; both upregulation and downregulation of miR-124 have been reported after acute stroke. Leung et al83 showed high circulating miR-124 levels in the first 6 hours poststroke. Levels of miR-124 were also higher in patients with hemorrhagic stroke than in patients with ischemic stroke. In contrast, Liu et al84 found that miR-124 levels were lower after stroke and were negatively correlated with infarct volume, CRP (C-reactive protein) levels, and MMP-9 levels. Hence, large multicenter studies are required to determine the potential value of miR-124 as a biomarker.

The let-7 family (a group of 7 miRNAs denoted as let-7a to let-7i) is one of the most abundant in the brain and has an important role in neurogenesis and differentiation.85 Profiling studies in animals have shown that the expression of let-7 family members is downregulated after stroke.50,53,54 Specific blockade of let-7f (using antagomir) led to increased neuroprotection in the rat model via modulation of the IGF-1 (insulin-like growth factor) signaling pathway.86 A large population-based study found that let-7 levels were low after stroke and only returned to normal values after 24 weeks. Interestingly, levels of let-7 were lowest in patients with an atherosclerosis-related stroke.87 A cutoff value of 1.675 for let-7 at 24h yielded a sensitivity of 92% and a specificity of 84%. The serum expression of let-7c was recently evaluated in another atherosclerotic disease—the coronary artery disease. Faccini et al88 especially showed that the combination of let-7c, miR-145, and miR-155 managed to deliver a specific signature for diagnosing coronary artery disease.

Brain miR-21 expression reportedly increases in animal models of stroke and in patients with small artery stroke.50,89 miR-21 is known to be involved in ischemia and hypoxia-related processes and has an antiapoptotic effect.90 In the MCAo animal model, the expression of miR-21 is significantly elevated in the ischemic boundary zone. However, upregulation of miR-21 did not occur in cortical neurons exposed to oxygen-glucose deprivation in culture. Introduction of miR-21 into the oxygen-glucose deprivation–exposed cells decreased apoptosis and increased survival via the downregulation of Fas-ligand.90 A larger study of circulating levels of miR-21, miR-145, and miR-221 was performed in 233 patients (167 patients with ischemic stroke and 66 stroke-naive patients with carotid atherosclerosis) and 157 healthy controls.77 The 2 patient groups had significantly higher miR-21 levels and significantly lower miR-221 serum levels than healthy controls. In this study, miR-145 was not detected in over half of the patients and, therefore, did not provide useful information. However, miR-145 was shown to be overexpressed after ischemic stroke in the profiling study by Sepramaniam et al90 and in a smaller study by Gan et al91; this suggests that an increase in miR-145 levels is predictive of a better stroke outcome—perhaps because of miR-145’s targeting of KLF4 and 5 and its effect on re-endothelialization.

miR-210 has been described as a hypoxia-induced, anti-apoptotic miRNA.92 Eken et al93 recently showed that miR-210 was substantially downregulated in the fibrous cap of unstable carotid atherosclerotic plaques and has an antiapoptotic role in smooth muscle cell-enriched fibrous cap. The overexpression of miR-210 in normoxic human endothelial cells stimulates the formation of capillary-like structures and increases cell migration and differentiation.94 miR-210 expression is reportedly elevated in the ischemic brain, where it protects against ischemia-induced injury via a variety of mechanisms.95 In the setting of cerebral ischemia in the mouse, use of a lentiviral vector to increase miR-210 levels stimulated angiogenesis and neurogenesis in the subventricular zone and protected the animals against ischemia-induced injury.96 In this context, miR-210 represents a potential therapeutic target in stroke. In patients with stroke, a continuous poststroke decrease in peripheral blood leukocyte levels of miR-210 has been reported; patients with higher miR-210 expression levels had a better prognosis than patients with the lowest expression levels.97 Zeng et al98 determined levels of miR-210 and other potential biomarkers (including various cytokines and hemostasis markers) in peripheral blood mononuclear cells; a combination of miR-210, fibrin degradation product, and interleukin 6 had higher sensitivity and specificity for stroke-outcome prediction than the individual markers alone.

In a study of the diagnostic value of atherosclerosis-related, circulating miRNAs in acute stroke, levels of miR-126 were found to be positively correlated with cerebral atherosclerosis. Levels of miR-17 are elevated after acute stroke and are also predictive of early stroke recurrence.100 Another study
on ischemic stroke, induced by distal MCAo in mice, showed a significant decrease of miR-126 expression levels in serum and heart. miR-126 is known to be a vascular remodeling miRNA, targeting genes such as vascular cell adhesion molecule 1 and monocyte chemoattractant peptide. The decrease of miRNA logically induced an increase of the expression of these proteins in the heart compared with nonstroke mice. Because stroke directly induces cardiac dysfunction, the decrease in miR-126 expression may contribute to cardiac dysfunction after ischemic stroke in mice. Furthermore, miR-126 is overexpressed in the cerebral microvasculature of mice with chronic kidney disease. It has recently been shown that miR-126 overexpression exerts a protective role in intracerebral hemorrhage by modulating angiogenesis and apoptosis. Finally, a study analyzing miR-126 expression in intracerebral hemorrhage by modulating angiogenesis and a novel biomarker for ischemic stroke diagnosis.

Perspectives and Conclusions

Much progress has been made with regard to understanding the miRNAs’ roles in the pathophysiology of carotid artery stroke. miRNAs have potential value as diagnostic and prognostic markers and as therapeutic targets. Well-defined protocols for miRNA detection, extraction, and isolation are now available—even though a variety of internal or external controls (eg, exogenous cel-miR-39, U6, or ubiquitous miRNAs) have been used for quantification. To normalize results, standardized controls should now be adopted. Multicenter studies of large number of patients are needed to confirm the miRNAs’ potential value as biomarkers.

In addition to the miRNAs’ potential as biomarkers, gene therapy designed to modulate the expression of a given miRNA might be especially useful. Depending on the miRNA in question and the target tissue, 2 contrasting strategies can be considered: premiRNAs and antisense RNAs could be, respectively, used to increase or decrease miRNA levels. Antisense oligonucleotides can be synthesized and modified chemically and then delivered systemically via IV injection. miRNA sponges (a succession of complementary binding sites for a given miRNA of interest) also constitute a means of silencing miRNAs. However, these delivery methods lack specificity because the chemically modified RNA is distributed throughout the body and may well lead to side effects. Naturally occurring nanomaterials already present in the human body (notably microvesicles) could also be used for delivery. Indeed, microvesicles have a crucial role in the protection and long-distance transport of endogenous miRNAs and have already been used to deliver synthetic, therapeutic miRNAs (such as miR-21). Viruses designed for gene therapy (such as lentiviruses or adeno-associated viruses) can also be used to deliver either premiRNAs (to increase miRNA levels) or antisense miRNAs (to decrease miRNA levels). Developments in nanotechnology are also promising; for example, gold nanoparticles with a mean±SD diameter of 13±1 nm have been functionalized with a monolayer of alkylthiol-modified, double-stranded RNA molecules. Mirkin group has demonstrated that these gold nanoparticles can enter cells (without the aid of cationic cocarriers), traffic through the endocytic pathway into late endosomes, reside there for ≤24 hours after transfection, and ultimately mimic the in situ function of endogenous miRNAs.

The miRNAs’ potential in stroke therapy should be further investigated, with a focus not only on the effects of injected agomiRs or antagomiRs but also on potential side effects and uptake/elimination mechanisms.

Disclosures

None.

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miRNAs are potential noninvasive biomarkers able to identify high-risk groups of embolic stroke among patients with carotid artery stenosis. This review summarizes the various miRNAs involved in stroke.

miRNAs represent new pharmacological targets for plaque stabilization in atherosclerosis.

### Highlights

- **miRNA expression is deregulated in human carotid plaques**, leading to plaque growth, instability and rupture.
- **miRNAs are potential noninvasive biomarkers able to identify high-risk groups of embolic stroke among patients with carotid artery stenosis.**

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Pierre Maitrias, Valérie Metzinger-Le Meuth, Joseph Nader, Thierry Reix, Thierry Caus and Laurent Metzinger

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