Austin Journal of Pharmacology and Therapeutics

Austin Publishing Group

Open Access Full Text Article

Review Article

Peripheral Blood miRNAs as a Potential Biomarker for Ischemic Stroke

Jing-Jie Peng[1], Xiu-Ju Luo[2] and Jun Peng[3]*

[1]Grade 2013 and Class 1304, Xiangya School of Medicine, Central South University, China

[2]Department of Laboratory Medicine, Xiangya School of Medicine, Central South University, China

[3]Department of Pharmacology, School of Pharmaceutical Sciences, Central South University, China

***Corresponding author:** Jun Peng, Department of Pharmacology, School of Pharmaceutical Sciences, Central South University, Changsha 410078, China

Received: August 29, 2014; **Accepted**: September 26, 2014; **Published**: September 26, 2014

Abstract

Rapid and accurate diagnosis of ischemic stroke plays a crucial role in saving patients' life. Currently, the diagnosis of ischemic stroke relies on clinical assessment together with neuroimaging. A well-recognized biomarker for ischemic stroke is currently not available in clinical practice. MicroRNAs (miRNAs) are endogenous non-coding RNAs with small size (~22nucleotides) and single-strand. MiRNAs have recently been detected in serum or plasma of peripheral blood in a highly stable form. Until now, around 20 peripheral blood miRNAs were reported to be closely with ischemic stroke. In this mini-review, we introduced characteristics, sources and isolation of peripheral blood miRNAs and summarized recent available data on the correlation between peripheral blood miRNAs and ischemic stroke. Some miRNAs, such as miR-145, miR-21, miR-221, miR-210 and miR-107, were given special attention, since they may have potential prospect in diagnosis and prognosis for ischemic stroke.

Keywords: ischemic stroke, miRNAs, peripheral blood, biomarkers, diagnosis

Introduction

Ischemic stroke is one of a leading cause for death and disability worldwide. Rapid and accurate diagnosis of ischemic stroke plays a pivotal role in therapy and prognosis for the disease. Over the past two decades, clinicians have made steady progresses in the diagnosis, treatment and prognosis for ischemic stroke. In which the progress in biomarkers for ischemic stroke attracted a great deal of attention. Although a biomarker for ischemic stroke is currently not used in clinical practice, efforts to develop such a test are ongoing. A biomarker could be a molecule measured in blood, cerebrospinal fluid or tissue. Most of people tend to focus on peripheral blood based biomarkers, which are usually rapid, cost effective, specific and sensitive, as is the case for troponin in the assessment of myocardial infarction [1]. Up to date, a number of ischemic brain injury- related proteins, such as calcium binding protein B (S-100B) [2], Neuron-Specific Enolase (NSE) [3], Myelin Basic Protein (MBP) and Glial Fibrillary Acidic Protein (GFAP) [4,5], have been studied as possible biomarkers for the diagnosis of ischemic stroke. However, the brain injury-related proteins are not ideal biomarkers for ischemic stroke due to lack of specificity to ischemic stroke. Moreover, the Blood Brain Barrier (BBB) restricts release of these proteins into peripheral blood. Therefore, there is still a clinical need for novel biomarker, which is able to reliably rule-in or rule-out ischemic stroke immediately upon admission. MicroRNAs (miRNAs) seem to be a promising candidate of novel biomarker for early diagnosis of ischemic stroke. Altered miRNAs levels in peripheral blood have been detected in patients with ischemic stroke. This review will focus on a potential role of peripheral blood miRNAs in diagnosis of ischemic stroke as novel biomarkers.

Peripheral Blood miRNAs

MiRNAs are endogenous non-coding RNAs with small size (~22nucleotides) and single-strand, which regulate gene expression at the post-transcriptional level through binding to the 3` untranslated region (UTRs) of the target mRNAs [6]. Upon the complete binding of miRNAs to their target mRNAs, the degradation of target mRNA is initiated. However, if the binding is partial, the translation of target mRNA is repressed. By regulating protein expression of potential candidate targets, miRNAs are involved in multiple biological processes, such as proliferation, differentiation, migration, secretion, excitation, conduction, cell cycle, aging and apoptosis [7].

MiRNAs have recently been detected in serum or plasma of peripheral blood, which are referred to peripheral blood or circulating miRNAs. Despite intense research, the origin of peripheral blood miRNAs remains poorly understood. Numerous studies have reported that miRNAs are actively secreted in microvesicles or exosomes from different cell types, which are a possible source of peripheral blood miRNAs [8]. Up to date, over 100 miRNAs were identified in exosomes from mast cells and sphingomyelinase 2 (nSMase2), a rate-limiting enzyme for ceramide biosynthesis, controls the secretion of exosomes to the extracellular matrix [8,9]. Besides the microvesicles or exosomes, microparticles and lipoprotein complexes (such as high-density lipoprotein complexes) are other potential sources for circulating miRNAs. Microparticles are bigger than exosomes and actively control cell communication process. In addition to its basic role as a delivery vehicle for excess cellular cholesterol, High-Density Lipoprotein (HDL) is able to transport endogenous miRNAs and deliver them to recipient cells with functional targeting capabilities[8, 9]. Interestingly, the human HDL-miRNA profile from normal subjects is significantly different from the familial hypercholesterolemia subjects. So far, the human genome is estimated to encode up to 1000 miRNAs and more than 100 miRNAs in serum were identified from healthy subjects. The detection of peripheral blood miRNAs may have extra meaning beyond the cell function and can act as potential biomarkers for diseases.

Citation: Jing-Jie Peng, Xiu-Ju Luo and Peng J. Peripheral Blood miRNAs as a Potential Biomarker for Ischemic Stroke. Austin J Pharmacol Ther. 2014; 2 (9).3

Isolation of Peripheral Blood miRNAs

Different from miRNAs isolated from tissues or cells, peripheral blood miRNAs are highly stable in boiling water, in solution with very high or low pH [10]. Plasma miRNAs levels remain stable when they are subjected to prolonged room temperature incubation or freeze-thawed multiple times. It has been shown that endogenous peripheral blood miRNAs exist in a form that is resistant to degradation by plasma RNase. Differently, synthetic miRNAs (such as cel-miR-238, cel-miR-54 and cel-miR-39 from caenorhabditis elegans) are found to be degraded within minutes when they are added into human plasma [10]. If RNase is inactivated before the addition, the exogenous miRNAs could escape from the degradation. There are multiple factors that account for the stability of circulating miRNAs. First of all, the plasma miRNAs can form the protein-miRNA complexes with some special proteins (such as nucleolar RNA-binding protein, nucleophosmin 1), which may protect the miRNAs from the degradation. Secondly, plasma miRNAs are normally included in microvesicles or exosomes, which are different from the exogenously added miRNAs [11,12].

Although plasma miRNAs are stable, reproducible isolation of cell-free miRNAs with high purity is still a technical challenge due to multiple reasons [11,12]. Firstly, plasma or serum contains high concentration of proteins, which may potentially interfere with plasma miRNAs isolation and detection. Thus, how to avoid protein contamination and hemolysis is a big challenge to plasma miRNAs preparation. Secondly, the quantity of total RNA in plasma or serum is very low. The yield of total RNA from plasma or serum is very limited by regular methods. Finally, control miRNAs (such as U6 RNA, cel-miR-39) are present in extremely low concentrations in serum or plasma. Thus, how to choose a reliable control miRNAs is another big challenge for plasma miRNAs isolation. Fortunately, numerous commercial kits are developed to isolate plasma miRNAs, which may greatly promote the research on peripheral blood miRNAs [11,12].

Alterations in Peripheral Blood miRNAs in Ischemic Stroke

Rapid and correct diagnosis is crucial to treatment and prognosis of ischemic stroke. Recently, numerous studies have shown that plasma levels of many miRNAs are significantly changed in patients with ischemic stroke or in animal model of cerebral ischemia. Secreted by brain cells and accumulated in blood, miRNAs are expected to reflect brain injury in response to ischemia. Thus, the peripheral blood miRNAs, particularly the brain-specific or-enriched miRNAs may provide unique biomarkers for diagnostic and therapeutic interventions of ischemia stroke.

miR-145

MiR-145 has been extensively studied and its role in modulating the oscillating state of smooth muscle cells has been elucidated. Compiling evidence has demonstrated that miR-145 may work as a modulator of smooth muscle cell phenotype [13]. Because ischemic stroke is a vascular disease, its relationship with miR-145 has been attracted attention. By using real-time quantitative PCR, Gan et al have examined the expression profile of circulatory miR-145 in healthy control subjects and ischemic stroke patients [14]. They have found that plasma level of miR-145 in peripheral blood was significantly higher in ischemic stroke patients than in control subjects. The authors concluded that circulating miR-145 had potential as a biomarker for ischemic stroke [14]. However, in a similar study, the serum miR-145 level in healthy controls and ischemic stroke patients were reported to be very low and over 50% of the subjects did not detect miR-145 expression in the serum [15]. These authors suggest that miR-145 may not be an ideal marker to predict stroke.

miR-21 and miR-221

Like miR-145, miR-21 and miR-221 have also been implicated in the cardiovascular system. In a recent study, the serum levels of these two miRNAs in patients with ischemic stroke or in patients with atherosclerosis were compared [15]. The results showed that stroke patients and atherosclerosis subjects had significantly higher miR-21 and lower miR-221 serum levels than healthy controls. There was a 6.2-fold increase for stroke risk when miR-21 levels increased by $\log_{10}2(-\Delta Ct) = 1$, while a 10.4-fold increase was observed as miR-221 decreased by $\log_{10}2(-\Delta Ct) = 1$ [15]. The authors concluded that miR-21 and miR-221 were novel biomarkers for atherosclerosis and stroke.

miR-210

miR-210 is a pleiotropic hypoxia-miRNA and plays multiple roles in brain ischemia. Recently, the correlation of blood miR-210 with clinical findings in acute ischemic stroke patients was evaluated [16]. miR-210 was measured within 3, 7 and 14 days after stroke by a quantitative PCR technique. Stroke severity and clinical outcome were evaluated by NIHSS and modified Rankin Score. Compared to healthy controls, blood miRNA-210 was significantly decreased in stroke patients, especially at 7 and 14 days of stroke onset. miR-210 level in stroke patients with good outcome was significantly higher than that in patients with poor outcome. The authors also examined both serum and brain miR-210 in ischemic mice [16]. The results showed a positive correlation between blood and brain miR-210 in ischemic mice. Based on the results, the author demonstrated that blood miR-210 might be a novel sensitive biomarker for clinical diagnosis and prognosis in acute cerebral ischemia.

miR-107

To date, thousands of miRNAs have been identified and many of them are specifically expressed or highly enriched in brain, the so called brain-specific or enriched miRNAs [17]. In a recent study, we have measured the expressions of 9 brain-specific or enriched miRNAs (miR-9, miR-107, miR-124, miR-128b, miR-134, miR-153, miR-219, miR-329 and miR-381) in brain tissues and plasma in a cerebral ischemia/reperfusion (I/R) injury rat model [18]. Interestingly, among the 9 screened miRNAs, only miR-107 level was elevated in both brain tissue and plasma. Further study has found that plasma levels of miR-107 were also dramatically elevated in patients with acute ischemic stroke concomitant with an increase in plasma levels of glutamate. There was a positive correlation between miR-107 and glutamate level [18]. Thus, the plasma level of miR-107 may serve as a novel biomarker for monitoring excitotoxicity in the ischemic stroke patients.

So far, more and more peripheral blood miRNAs were reported to be closely correlated with ischemic stroke [19-21]. For example, miR-422a, miR-488, miR-627, miR-125b-2* and miR-27a* were

consistently altered in acute stroke. Differential expression of these 5 miRNAs was also present in rat stroke models [19]. Whereas circulating miR-30a and miR-126 levels were significantly down-regulated in ischemic stroke patients until 24 weeks. In theory, brain specific or enriched-miRNAs should have the advantages (such as more specific or sensitive) over those unspecific ones in diagnosis of ischemic stroke. However, more studies are needed before drawing a firm conclusion.

Conclusion

Currently, the diagnosis of ischemic stroke relies on clinical assessment in combination with neuroimaging, such as CT (computed tomography) or MRI (magnetic resonance imaging). The application of CT or MRI is generally limited to specific scenarios where time and/or imaging resources are limited. For example, in a pre-hospital setting or facilities where acute neuroimaging is not available. In such situations, a blood test that rapidly identifies ischemic stroke could speed patient transfer to centers and physicians are able to perform evaluation for thrombolysis. In some cases, the diagnosis of ischemic stroke remains unclear in spite of clinical evaluation and imaging. At this moment, a blood test may strengthen physicians' confidence in diagnosis of stroke. Unfortunately, a well-recognized biomarker for ischemic stroke is currently not available in clinical practice. Peripheral blood miRNAs are resistant to endogenous ribonuclease activity and can be present in human plasma or serum in a remarkably stable form. It is believed that peripheral blood miRNAs provide a novel class of minimal invasive biomarkers for ischemic stroke diagnosis. Until now, around 20 miRNAs have been reported as potential biomarkers for ischemic stroke. However, most of findings were based on relatively small samples, which led to many divergences between different reports. Therefore, it is necessary to conduct independent and large cohort studies to identify those peripheral blood miRNAs with real value in ischemic stroke diagnosis.

Acknowledgments

This work was supported by National Nature Science Foundation of China (grants 81373409), Hunan Provincial Natural Science Foundation of China (13JJ2008) and Doctoral Fund of Ministry of Education of China (20120162110056).

References

1. Eggers KM, James S, Venge P, Lindahl B. Prognostic implications of changes in cardiac troponin I levels in patients with non-ST elevation acute coronary syndrome. Biomarkers. 2013; 18: 668-672.
2. Scarcello E, Morrone F, Piro P, Tarsitano S, Intrieri F, Vaccarella S, et al. Protein S-100B as biochemical marker of brain ischemic damage after treatment of carotid stenosis. Ann Vasc Surg. 2011; 25: 975-978.
3. Oertel M, Schumacher U, McArthur DL, Kästner S, Böker DK. S-100B and NSE: markers of initial impact of subarachnoid haemorrhage and their relation to vasospasm and outcome. J Clin Neurosci. 2006; 13: 834-840.
4. Becker KJ, Kindrick DL, Lester MP, Shea C, Ye ZC. Sensitization to brain antigens after stroke is augmented by lipopolysaccharide. J Cereb Blood Flow Metab. 2005; 25: 1634-1644.
5. Mayer CA, Brunkhorst R, Niessner M, Pfeilschifter W, Steinmetz H, Foerch C. Blood levels of glial fibrillary acidic protein (GFAP) in patients with neurological diseases. PLoS One. 2013; 8: e62101.
6. De Rosa S, Curcio A, Indolfi C. Emerging role of microRNAs in cardiovascular diseases. Circ J. 2014; 78: 567-575.
7. Ghelani HS, Rachchh MA, Gokani RH. MicroRNAs as newer therapeutic targets: A big hope from a tiny player. J Pharmacol Pharmacother. 2012; 3: 217-227.
8. Wang K, Zhang S, Weber J, Baxter D, Galas DJ. Export of microRNAs and microRNA-protective protein by mammalian cells. Nucleic Acids Res. 2010; 38: 7248-7259.
9. Tsui NB, Ng EK, Lo YM. Stability of endogenous and added RNA in blood specimens, serum, and plasma. Clin Chem. 2002; 48: 1647-1653.
10. Turchinovich A, Weiz L, Langheinz A, Burwinkel B. Characterization of extracellular circulating microRNA. Nucleic Acids Res. 2011; 39: 7223-7233.
11. Sayed AS, Xia K, Yang TL, Peng J. Circulating microRNAs: a potential role in diagnosis and prognosis of acute myocardial infarction. Dis Markers. 2013; 35: 561-566.
12. Sayed AS, Xia K, Salma U, Yang T4, Peng J. Diagnosis, prognosis and therapeutic role of circulating miRNAs in cardiovascular diseases. Heart Lung Circ. 2014; 23: 503-510.
13. Cheng Y, Liu X, Yang J, Lin Y, Xu DZ, Lu Q, et al. MicroRNA-145, a novel smooth muscle cell phenotypic marker and modulator, controls vascular neointimal lesion formation. Circ Res. 2009; 105: 158-166.
14. Gan CS, Wang CW, Tan KS. Circulatory microRNA-145 expression is increased in cerebral ischemia. Genet Mol Res. 2012; 11: 147-152.
15. Tsai PC, Liao YC, Wang YS, Lin HF, Lin RT, Juo SH. Serum microRNA-21 and microRNA-221 as potential biomarkers for cerebrovascular disease. J Vasc Res. 2013; 50: 346-354.
16. Ouyang YB, Stary CM, Yang GY, Giffard R. microRNAs: innovative targets for cerebral ischemia and stroke. Curr Drug Targets. 2013; 14: 90-101.
17. Roshan R, Shridhar S, Sarangdhar MA, Banik A, Chawla M, Garg M, et al. Brain-specific knockdown of miR-29 results in neuronal cell death and ataxia in mice. RNA. 2014; 20: 1287-1297.
18. Yang ZB, Zhang Z, Li TB, Lou Z, Li SY, Yang H, et al. Up-regulation of brain-enriched miR-107 promotes excitatory neurotoxicity through down-regulation of glutamate transporter-1 expression following ischaemic stroke. Clin Sci (Lond). 2014; 127: 679-689.
19. Sepramaniam S, Tan JR, Tan KS, DeSilva DA, Tavintharan S, Woon FP, et al. Circulating microRNAs as biomarkers of acute stroke. Int J Mol Sci. 2014; 15: 1418-1432.
20. Long G, Wang F, Li H, Yin Z, Sandip C, Lou Y, et al. Circulating miR-30a, miR-126 and let-7b as biomarker for ischemic stroke in humans. BMC Neurol. 2013; 13: 178.
21. Li WY, Jin J, Chen J, Guo Y, Tang J, Tan S. Circulating microRNAs as potential non-invasive biomarkers for the early detection of hypertension-related stroke. J Hum Hypertens. 2014; 28: 288-291.

Austin J Pharmacol Ther - Volume 2 Issue 9 - 2014
ISSN: 2373-6208 | www.austinpublishinggroup.com

Citation: Jing-Jie Peng, Xiu-Ju Luo and Peng J. Peripheral Blood miRNAs as a Potential Biomarker for Ischemic Stroke. Austin J Pharmacol Ther. 2014; 2 (9).3