TECHNICAL NOTE

MICRO RNA: A PROSPECTIVE DIAGNOSTIC BIOMARKER IN ALZHEIMER'S DISEASE

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ABSTRACT

Alzheimer's disease (AD) is regarded as the most prevalent neurodegenerative disease standing with cognitive decline, making individuals unable to carry out their normal activities unassisted. Based on epidemiological figure, it is stated that over 50 million people globally are affected by AD. None of the drugs till now have succeeded in reverting the AD progression. MicroRNAs (miRNAs) are small non-coding RNA molecules that comprised of ~22 nucleotides and endeavor their biological role by modulating intracellular protein expression at post translational level through translational degradation. Varying concentrations of miRNAs are found among peripheral and central nervous tissues of AD patients and control groups. This study will sum up the mechanism and potential role of miRNAs in the up and downregulation of genes involved in AD and may be considered as an ideal therapeutic agent for neurodegenerative disease.

Keywords: Alzheimer's disease, microRNAs, intracellular proteins, cognitive decline, non-coding RNA

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INTRODUCTION

Micro RNA (miRNA) constitutes a class of small and non-coding RNAs which are comprised of 17-25 nucleotides. Their function is to regulate mRNA through its degradation and to set up protein levels [1]. miRNA biogenesis process starts in the nucleus, where transcription results in the formation of primary RNA in the presence of RNA polymerases II and III [2,3]. As a consequence of this hairpin-like structure is formed with a loop at one end termed as pri-miRNA. Enzyme Drosha or DCGR8 residing in the nucleus act on pri-miRNA and forms a new hairpin-like structure containing 70 nucleotides called pre-miRNA. Exportin-5 protein then facilitates the transport of this pre-miRNA into the cytoplasm where it is acted upon by Ago2/Dicer complex and forms mature double stranded miRNA. Of them, one strand is the guide strand and will be incorporated into RNA-induced silencing complex (RISC) while another strand is called passenger strand that will be degraded. The mature miRNA or the guide strand will now combine with mRNA and inhibits its translation (Fig. 1) [4].

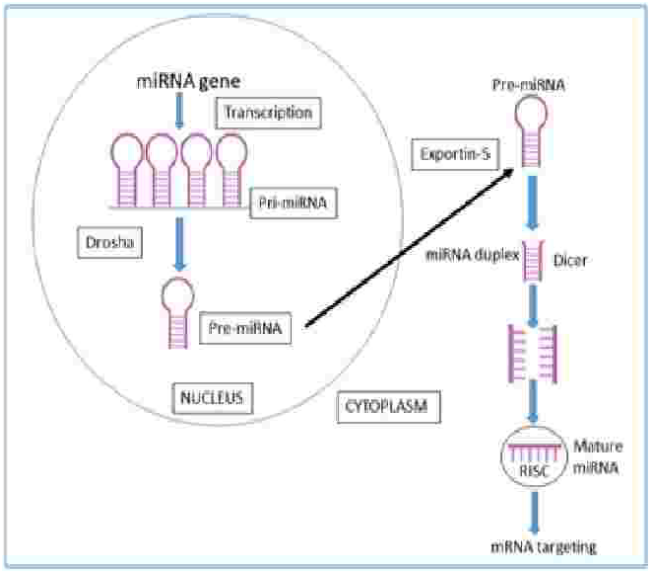

Figure I. Biogenesis of miRNA

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miRNA is also involved in intercellular signaling. Although majority of miRNA are present within the cell and a big fraction moves outside the cell and enter in body fluids [5,6]. These miRNAs are called circulating miRNAs and they are secreted in urine, blood, seminal fluid, saliva and breast milk. They are also discharged in other fluids by tissue damage, necrosis and apoptosis [7]. Other vital roles played by miRNAs include cell growth, organismal development, cell homeostasis, cell differentiation and physiological functioning [8]. Some miRNAs that are involved in apoptosis, likewise miR16 and miR15, contribute in the modulation of human anti-apoptotic BCL-2 gene [9]. Currently, efforts have been made in order to find out the origin and use of microRNAs in research practice in healthy and diseased individual. For instance, it has been postulated in few studies that some circulating miRNAs are implicated in angiogenesis, inflammation and cardiac muscle contractility [10, 11]. Other studies have shown the remarkable role of miRNAs in the induction of pluripotent stem cells. Nonetheless, most likely promising function of miRNA is to act as a biomarker. Evidence proposed that miRNA play crucial role as biomarkers in cancer by means of exome-mediated intercellular communication for the prognosis and diagnosis of Alzheimer's disease, epilepsy and other neurodegenerative disorders. miRNAs are also used in the diagnosis of cardiovascular disease and infectious disease such as sepsis [12-15].

Neurodegenerative diseases are intricate and intensifying diseases that breakthrough and in due course cause death. Researchers have come across that miRNAs have crucial role in the growth and plasticity of the nervous system and also have role in the progression of neurodegenerative disorders. Deposition of Aβ is hallmark of Alzheimer's disease (AD), comprised of insoluble plaques and protein inclusions such as Aβ42 and Aβ40 in case of AD. Several studies showed that Aâ serve as potential biomarker employed in diagnostic procedure for AD [16]. In the meantime, tau is considered as the major protein contributing in the stabilization of microtubules in neuronal axons [17]. It has been exemplifying that involvement of miRNAs could modulate gene expression upon binding to mRNA, either to block its translation or paramount its degradation. Furthermore, in recapitulation about the stability of miRNAs in body fluids and also its regulation capability, researchers have hypothesized that miRNAs conceivably could serve as AD biomarkers. Excessive expression and maladjustment of miRNAs could cause different neurodegenerative diseases [18].

METHODOLOGY

Criteria for sample collection: miRNAs are the propitious candidate clinical biomarker as they are most stable molecules and present in plethora of bio-fluids. Bio-fluids storage is important when accounting for biomarkers, although, it is reported that miRNAs remain stable at room temperature for short term storage whereas for long term storage at -20 °C and -80 °C. Additionally, freeze-thaw procedure does not affect quality of miRNAs. Conversely, variations in concentration of miRNAs reported in blood samples due to hemolysis and change in miRNA profiles due to platelet activation, this effect might be considering due to extent of gender, race and donor age [19]. As mentioned earlier that micro RNAs can be quantified from different circulations within the body such as blood, serum and plasma. The first step of miRNA analysis is the sample collection. The type of tube used for sampling affects the quantity of miRNA collected, because RNAs degrade rapidly in untreated tubes, also due to the adsorption of RNA to plastic. Anticoagulant tubes are usually employed to collect the sample such as EDTA, sodium citrate, heparin sodium fluoride/ potassium oxalate. After the sample collection, miRNAs are isolated from other constituents present in fluids [20].

Extraction: Extraction methods depends silica columns or phase separation extraction that adsorb RNAs specifically. Such purification strategies are crucial for last step analysis and proper enzymatic reactions. In phenol-chloroform extraction method phenol and chloroform associates with chaotropic agent so that protein can be broken down, specifically

RNA binding proteins and RNases. The single step procedure separates DNA, RNA and proteins and is accordant with purification of small RNA sequences. Later isopropanol or ethanol is used to precipitate total RNA molecules. Although, it's difficult to recover low concentration RNAs pellet because it cannot be seen properly thus not easy to handle [20, 21].

Quantification: For the quantification of RNA, spectroscopy is considered as most preferred analytical tool with sensitivity in ng/µL. Although, it is difficult to distinguish between DNA and RNA through this method but alternatively nucleic acids can be labeled with fluorescent dyes to show specificity among single or double-stranded DNA, RNA, or even miRNA. Electrophoresis is another technique that can be used to deduce nucleic acids on the basis of size. Other quantification techniques include the amplification based method which involves the use of reverse transcription PCR and accounted as a gold standard method for the quantification of RNAs as shown in fig. 2 [20, 21].

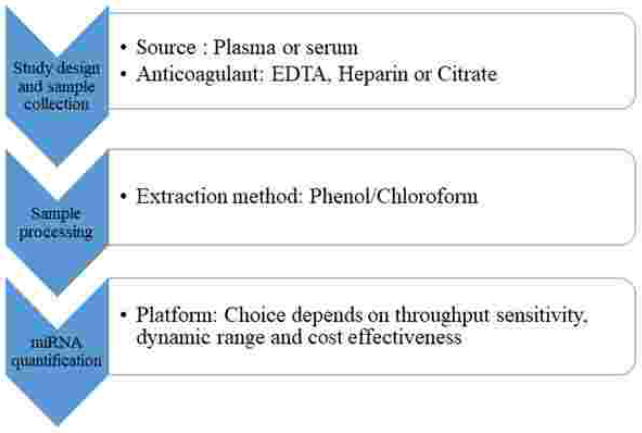

Figure II: Flow chart summarizing step for the detection of miRNAs

DISCUSSION

miRNA, Aβ and BACE1: Based on amyloid theory, AD pathogenesis is due the abnormal deposition of Aâ. BACE1 enzyme process amyloid protein precursor (APP) and forms Aβ. Aβ peptides comprised of 37 to 43 amino acids of those Aâ42 are considered the most noxious. When the equilibrium between the Aβ formation and clearance is broken then Aβ gets accumulated outside the cell leading to neuronal degeneration. Aβ formation is markedly influence by the expression of APP and BACE. miRNA-20a, miRNA17 and miRNA101 are considered as the negative regulators of APP and unsteady level of these miRNAs produces a profound effect in the pathogenesis of AD [22,23]. It is also demonstrated that some of the miRNAs such as miR-9, miR-128, and miR-1256 expressed abundantly in AD patient's brains when compared with brains tissues of control adult group [24]. It is also seen that the expression of miRNA107 down regulated as the AD progresses. Beta-secretase 1 (BACE1) control the formation of Aβ-amyloid, miR-328 and miR-298 regulate BACE1 expression eventually affection Aβ-amyloid production. miR-328 and the miR-298 can have recognized the BACE1 locations thus controlling the enzyme expression [25, 26].

miRNAs and Aβ clearance: Deposits of Aβ form when imbalance occurs between the formation and clearance of amyloid peptides. Different miRNAs are involved in the clearance of Aβ. Function of endosomal lysosome is to degrade deposited proteins and serve as protective entity in central nervous system. It is postulated that upregulation of miRNA128 impedes Aβ clearance by targeting lysosomal enzymes present in monocytes of AD patients. Improvised degradation is observed when miRNA128 is inhibited. MiRNA34a in upregulated from also inhibits Aβ clearance through the suppression of TREM2 expression [27]. Different biomarkers implicated in the upregulation and downregulation of APP, BACE-1 and Aβ clearance are illustrated in fig: 3.

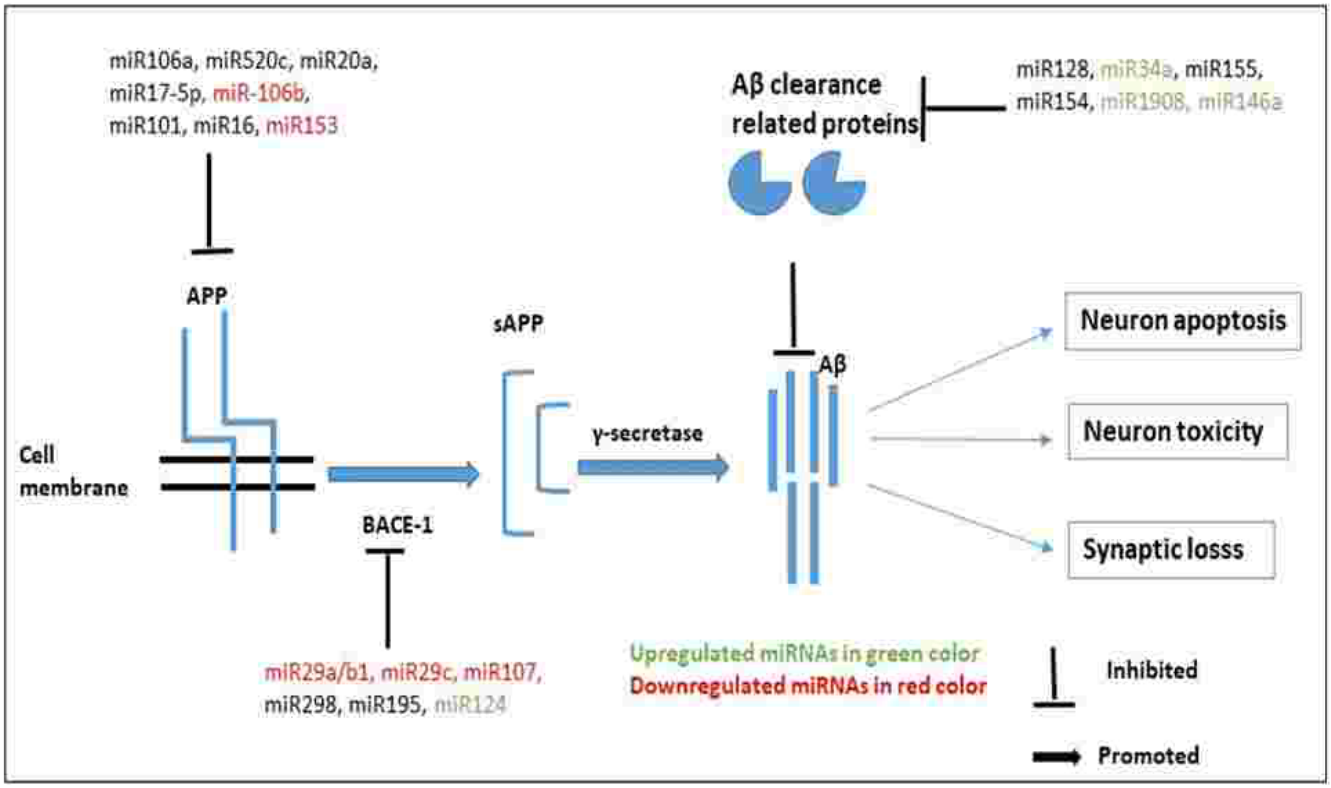

Figure III: A schematic presentation of Aβ postulation in the pathogenesis of AD and association of miRNAs on each step

Association between miRNAs and phosphorylated tau-protein: In AD axonal degeneration results in the massive release of tau from neurons. Thereafter, dysfunctional tau accumulates to form neurofibrillary tangles in axoplasm [17]. Neurofibrillary tangles are mainly comprised of hyper phosphorylated tau which results in the imbalance between phosphorylated and de-phosphorylated tau through the regulation and expression activities of corresponding kinases and phosphatases [28]. Consequently, hyper phosphorylation of tau such p-tau181, p-tau217, and p-tau231 in cerebrospinal fluid and axoplasm contemplate as biomarkers of AD. On the other hand, microglia and chronic inflammation are also considered as AD biomarkers [17]. It has been hypothesized that miRNA132 and miRNA125b expression is remarkably upregulated in brain tissues of AD patients while the miRNA124 and miRNA425-5p expression is downregulated when compared with control group. miRNA 132 and miRNA125b increase tau protein phosphorylation and cause neuronal apoptosis. On contrary miRNA124-3p impedes abnormal phosphorylation of tau protein [29, 30].

Diagnostic potential of miRNAs: Strikingly, pathological role of different miRNAs regulating AD genes is highly interactive and overlapped, and the cumulative functions are much influential than the single one. Another remarkable feature is that some miRNAs rich brain areas are continuously changing and are short-lived, thus they may encounter degradation in oxidative condition in an AD brain. Therefore, miRNAs that are downregulated may be considered as the residual of degenerative side of the disease. On contrary, up-regulated miRNAs with their down-regulated mRNAs are considered appropriate to study post-mortem tissues. The National Institute of Aging and Alzheimer's Association suggested that "Alzheimer's Disease" is an accumulated neuropathological condition and it cannot be defined by a vivo or postmortem

examination or by clinical manifestations. Therefore, there is a quest to look for the effective biomarker. Commonly employed biomarkers include A42 or A42/A40, phosphorylated and total tau from cerebrospinal fluid. Although none of these procedures can individually diagnose Alzheimer's disease (AD), they are often identified too late for effective intervention. Studies have proved that miRNA involvement in AD pathogenesis and its alterations found in plasma, serum and CSF are regarded as surprising postulant for AD biomarkers [31].

LIMITATIONS IN USING miRNAs

miRNAs can be obtained from circulating plasma or serum and it has been demonstrated that miRNAs profiles of venous and arterial blood are very similar. In disease detection, it is challenging to use venous miRNAs in some cases. Therefore, it is stated that care must be taken while choosing blood sampling method for miRNAs as biomarkers. miRNAs are the small and highly conserved molecules, presents high homology and challenges in the detection process. Overcoming these challenges depends on technological progress for their detection. The variety of sensitive and specific techniques are available for the detection of miRNAs such as in situ hybridization, northern blot examination, miRNA microarray, real-time PCR and next generation sequencing and many have already been employed as diagnostic biomarker, thus advocating their applications in both personalized and clinical medicine. Some researches related to circulating miRNAs failed to produce highly validated biomarkers due to the lack of considerations about some factors including age, sex and previous treatment [19]. Additionally, problem in reproducibility of data presents the lack of steadiness. It is crucial to lessen experimental variations while processing the miRNA detection so that accurate expression of miRNA in a tissue, cell type or fluid can be analyzed. Poor diagnostic reproducibility and specificity of miRNA, has introduced the limitation for the usage of miRNA as diagnostic biomarker. There is immediate need to standardize the miRNA detection methods to produce useful hypothesis. In order to minimize limitations, it is crucial to account for different aspects including sample size, so that variants can be detected with only minor differences. Further prospective studies are important so that results of observational study can be confirmed along with validation process [32].

CONCLUSION

Despite significant efforts, time, and financial resources invested globally in treating AD, the results have been largely unsatisfactory. At Aβ level it is not possible to induce anti-AD effects through traditional drugs. miRNAs can be taken as therapeutic tools with substantial research ability in the milestone of AD. This review article highlighted the role of miRNAs in the progression of AD and provided the possibility for the treatment of AD.

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Author's Contribution:

SUH: Concept & design, manuscript writing, final approval of manuscript and responsible for accuracy and integrity of research

AHA: Concept & design, final approval of manuscript and responsible for accuracy and integrity of research

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