A New Discovery of MicroRNA-455-3p in Alzheimer’s Disease

Subodh Kumar and P. Hemachandra Reddy

Department of Internal Medicine, Texas Tech University Health Sciences Center, Lubbock, TX, USA
Department of Pharmacology and Neuroscience, Texas Tech University Health Sciences Center, Lubbock, TX, USA
Department of Neurology, Texas Tech University Health Sciences Center, Lubbock, TX, USA
Departments of Speech, Language and Hearing Sciences, Texas Tech University Health Sciences Center, Lubbock, TX, USA
Garrison Institute on Aging, South West Campus, Texas Tech University Health Sciences Center, Lubbock, TX, USA

Accepted 24 July 2019

Abstract. MicroRNA-455-3p (miR-455-3p) is identify as a member of broadly conserved miRNA family expressed in most of the phylum and species. In humans, miR-455 is present on the human chromosome 9 at locus 9q32 and encoded by the human COL27A1 gene (collagen type XXVII alpha 1 chain). The role of miR-455 has been implicated in various human diseases such as cartilage development, adipogenesis, preeclampsia, and cancers, e.g., colon cancer, prostate cancer, hepatocellular carcinoma, renal cancer, oral squamous cancer, skin cancer, and non-small cell lung cancer. Recently, our laboratory discovered the biomarker and therapeutic relevance of miR-455-3p in Alzheimer’s disease (AD). Our global microarray analysis of serum samples from AD patients, mild cognitive individuals (MCI), and healthy subjects unveiled the high level of miR-455-3p in AD patients relative to MCI and healthy controls. Further, validation analysis using different kinds of AD samples such as serum, postmortem brains, AD fibroblasts, AD B-lymphocytes, AD cell lines, AD mouse models, and AD cerebrospinal fluid confirmed the biomarker potential of miR-455-3p. The mechanistic link of miR-455-3p in AD was determined via modulation of amyloid-β protein precursor (AβPP) and amyloid-β (Aβ) levels. Luciferase reporter assay confirmed AβPP as validated target of miR-455-3p. Our study on mouse neuroblastoma cells revealed the protective role of miR-455-3p against Aβ-induced toxicities. We also noticed that miR-455-3p enhances cell survival and lifespan extension. High level of miR-455-3p reduces Aβ toxicity, enhances mitochondrial biogenesis and synaptic activity, and maintains healthy mitochondrial dynamics. Based on these evidences, we cautiously conclude that miR-455-3p is a promising peripheral biomarker and therapeutic candidate for AD.

Keywords: Alzheimer’s disease, amyloid-β, biomarker, microRNA-455-3p

INTRODUCTION

Alzheimer’s disease (AD) is a progressive brain disease in elderly individuals that affect over 50 million people worldwide, including more than 5.5 million Americans [1]. AD is currently ranked as the sixth leading cause of death in the United States, but recent estimates indicate that the AD may rank third,
just behind heart disease and cancer, as a cause of death for older people [2]. AD is associated with the loss of memory, mental decline, difficulty in thinking, confusion, and changes in the personality and behavior [3–6]. Currently, there are no definite treatments and drugs, which can delay and/or prevent disease progression. In the last several decades, tremendous progress has been made to understand the molecular basis of both early-onset familial and late-onset sporadic AD. Early-onset “familial AD” caused by the genetic mutations and 2) late-onset “sporadic” AD involves aging and other lifestyle factors. Genetic mutations in amyloid precursor protein (APP), presenilin 1 (PS1), and presenilin 2 (PS2) cause a small proportion of familial AD [7]. Genetic polymorphisms in the Apolipoprotein E (APOE) gene with the E4 genotype, sortilin related receptor 1, clusterin, complement component receptor 1, CD2AP, EPHA1, and MS4A4/MS4A6E are responsible for the late-onset AD [7].

In addition to the genetic aspects, lifestyle factors such as diet, exposure to toxic environments, oxidative mitochondrial DNA damage and epigenetics transformation are major elements responsible for late-onset of sporadic AD [7, 8]. Maloney and Lahiri (2016) pointed out that AD and other idiopathic dementias are associated with epigenetic transformations, and these transformations connect the environment and human genes to the pathogenesis [8]. This epigenetic evidence suggests that AD is not a sudden and sharp delineated state, but rather it is a result of gradual change in the crucial cellular pathways, that leads the healthy state to the dysfunctional state, i.e., neurodegeneration [8]. For example, a cumulative lifetime lead (Pb) exposure in adult humans and an animal model was associated with accelerated declines in cognition [9]. Meta-analysis of 100 publicly available microarray datasets unveiled that different patients react to AD progression by variable single transcript alterations that lead to similar changes in functional gene groups indicate changes in epigenetic state and microRNA (miRNA) profiles. Moreover, early-onset decline in alternative splicing, protein folding, and transport transcripts occur simultaneously with decreases in synaptic transmission; however, at later stages, these changes progressed into enhanced oxidative stress and inflammation [10].

Several cellular, morphological, and pathological examinations revealed that AD is mainly associated with synaptic damage, loss of synapses, loss of synaptic proteins, proliferation of reactive astrocytes, activated microglia, defects and alterations in cholinergic neurons, defective autophagy/mitophagy, age-dependent imbalance in hormones, and structural and functional changes in mitochondria [11–21]. In addition to the major AD hallmarks, intracellular neurofibrillary tangles (NFTs) and extracellular amyloid-β (Aβ) plaques, miRNA changes are also involved in AD [11–22]. Among these changes, synaptic protein damage and the loss of synapse activity and mitochondrial oxidative dysfunction are widely recognized as early events in the pathogenesis and progression of AD. In terms of AD biomarkers, genetic testing of APOE, APP, PSEN1, and PSEN2 genes and cerebrospinal fluid (CSF) detection of Aβ (40 and 42) and tau/p-tau proteins are used for AD detection. However, early-detectable, non-invasive peripheral biomarkers are urgently needed for AD. In recent years, several published reports suggested that microRNAs play a large role in AD from biomarkers and therapeutic perspectives [22–24].

MICRONRNAS

MiRNAs are small single stranded, non-coding RNA molecules, endogenously present in humans, plants, fungi, bacteria, and viruses [25, 26]. These are ubiquitous, stable, and very abundant, evolutionary conserved molecules involved in regulation of multiple genes in the cells. MiRNAs are involved in almost all cellular process such as cell cycle control, apoptosis, developmental processes, stem cell differentiation, hematopoiesis, hypoxia, cardiac and skeletal muscle development, neurogenesis, insulin secretion, cholesterol metabolism, aging, immune responses, and viral replication [25–32]. As per the latest release of miRBase (v22.1), a total of 38,589 hairpin precursors and 48,860 mature sequences of miRNAs have been identified from 271 organisms (http://www.mirbase.org) [33]. In the human genome, a total of 1,917 annotated hairpin precursors and 2,654 mature miRNAs sequences have been reported so far [33]. MiRNAs regulate gene expression by interacting at the untranslated regions of mRNAs in a sequence-specific manner and lead to abortive transcription, mRNA degradation, translation suppression, or improper protein formation [25, 26, 34]. Depending on the miRNAs’ origins and expression patterns, some miRNAs are highly tissue-specific and localized at certain cellular niches, while some families of miRNA are expressed throughout the human body [25, 26, 35]. The synthesis of
miRNA(s) starts in the nucleus; however, mature miRNA formation occurs in the cytoplasm. Mature miRNAs interact with several cytoplasmic proteins such as AGO2 to form RISC complex to execute gene modulation at mRNA and protein levels of host cells [35–38]. In several circumstances, miRNAs (so called circulating miRNAs) encapsulated within exosomes, microvesicles, and apoptotic bodies are released from the cells to extracellular spaces where they participate in governing intracellular communication [36–38]. Research into miRNAs as potential biomarkers and therapeutics have focused on cancer, aging, and neurological conditions [24–26, 39–41]. In the brains of persons with neurodegenerative diseases, such as AD, miRNA expression is deregulated in extracellular fluid, such as CSF, blood, plasma, and serum of AD patients [22].

MICRORNAS AND ALZHEIMER’S DISEASE

Several miRNAs have been implicated in AD as potential biomarkers and/or therapeutic targets. We have reviewed the biomarker potential of miRNAs in AD previously [22]. Besides, several miRNAs are identified as therapeutic candidates, e.g., miR-101 downregulates AβPP levels in human cell cultures and could be a therapeutic target for AD [42]. Another miRNA, miR-339-5p, downregulates the protein expression of β-site amyloid precursor protein-cleaving enzyme1 (BACE1) in human primary brain cultures and is reduced in brain tissue specimens of AD subjects [43]. Hence, regulation of BACE1 by miR-339-5p makes it an important drug target for AD. Kong et al. (2014) investigated the miRNA expression profile in a Drosophila AD model [44]. Using μParaflo™ miRNA microarray assay, 17 miRNAs consistently dysregulated in adult-onset AD Drosophila: eight were upregulated (miR-8, miR-13b, miR-277, miR-279, miR-981, miR-995, miR-998, miR-1017) and nine were downregulated (let-7, miR-1, miR-9a, miR-184, miR-193, miR-263b, miR-276a, miR-285, miR-289). KEGG pathway analysis showed the involvement of these miRNAs in several important cellular pathways [44].

MICRORNA-455 FAMILY

MicroRNA-455 (miR-455) is a class of highly conserved miRNAs expressed in most of the species from Chordata to Mammalia [41]. MiR-455 precursor sequence is transcribed by human COL27A1 gene (collagen type XXVII alpha 1 chain) at position 114, 209, 434,114, 209, 529 [41]. In humans, miR-455 is expressed in two forms: miR-455-5p and miR-455-3p. MiR-455 has been implicated in very limited human diseases, in particular cancer and chondrogenesis [45–47]. The role of miR-455 has been identified in human non-small cell lung cancer [47], oral squamous cancer cells [48], colorectal cancer [49], hepatocellular carcinoma [50], breast cancer [51], pancreatic cancer [52], renal cancer [53], skin cancer [54], and prostate cancer [55]. In the case of neurodegenerative diseases, information about the role of miR-455-3p is in a very primitive stage. For the past four years, the Reddy laboratory has been investigating miRNAs in aging, age-related neurodegenerative disease such as AD, and stroke. For the first time, the Reddy laboratory debuted the role of miR-455-3p in AD. Using global microarray analysis in AD serum samples, they reported higher expression of miR-455-3p in patients with AD and other AD sources [40]. Further, they validated the level of miR-455-3p in different kinds of AD samples and assessed its biomarker capabilities [41]. Their findings on different AD sources concluded that miR-455-3p could be a potential biomarker for AD. Next, they studied the molecular mechanisms that are associated with miR-455-3p in AD; what is the role of miR-455-3p in AD progression and pathogenesis and what could be the reason for high level of miR-455-3p found in AD [56]. MiR-455-3p target prediction analysis showed AβPP as a putative target and luciferase reporter assay confirmed the interaction of miR-455-3p at 3'UTR in the APP gene. They discovered the protective role of miR-455-3p against toxic effects of Aβ on mitochondrial biogenesis, mitochondrial dynamics, synaptic activity, and cell survival.

In the current review, we summarized the complete picture of miR-455-3p in human disease and cellular processes. Our main emphasis was the newly discovered roles of miR-455-3p in AD and its estimated diagnostic and therapeutic capabilities in AD. This is the first review on miR-455, which compiled the biological importance of miR-455 in human health and diseases.

MICRORNA-455 AND HUMAN DISEASES

Initially, research investigating the role of miR-455 was in chondrogenesis and cartilage development in past decade. Over this period, studies reported
the implication of miR-455-3p in several human cancers, adipogenesis, preeclampsia, amyotrophic lateral sclerosis, and AD (Fig. 1). Here, we summarized the role of miR-455 in human health and diseases.

Osteoarthritis and chondrogenesis

Osteoarthritis (OA) is a degenerative joint disease, characterized by the degradation of articular cartilage, thickening of subchondral bone, and formation of osteophytes [57]. It is the most common disabling joint disease where chondrocytes are the only cells in cartilage that are responsible for the synthesis and turnover of extracellular matrix and crucial for tissue function. In 2012, Swingler et al. reported that miR-455 resides within an intron of COL27A1 that encodes a cartilage collagen. The comparison of human OA cartilage with cartilage obtained from patients with femoral neck fractures showed the increased expression of miR-140-5p and miR-455-3p in OA cartilage [57]. The expression of miR-455-3p was regulated by transforming growth factor β (TGFβ) ligands, and miRNA regulated TGFβ signaling. The other important targets of miR-455-3p were ACVR2B, SMAD2, and CHRD1 that may mediate its functional impact on TGFβ signaling [57]. The diminished TGFβ signaling during the aging process and in OA chondrocytes contribute to the cartilage destruction and exacerbates OA pathogenesis [57]. Zhang et al. reported the high levels of miR-455-3p in human adipose-derived stem cells (hADSCs). In this study, the miRNA expression was examined in hADSC before and after chondrogenic induction using miRNA microarray essay [58].

Recent studies demonstrated the potential role of miR-455-3p as an activator of early chondrogenic differentiation. Zhang et al. (2015) reported that miR-455-3p functions are an activator for early chondrogenic differentiation, most likely by inhibiting the expression of Runx-related transcription factor 2 (Runx2) [45]. Runx family genes are a set of multi-function transcriptional factors that regulate the expression of several genes involved in cellular differentiation, including chondrogenesis. Particularly, Runx2 is a key transcription factor associated with osteoblast and chondrocyte differentiation and endochondral ossification [45]. In 2016, Chen et al. characterized the important role of miR-455-3p in regulation of histone acetylation regulated by class I histone deacetylases (HDACs) during cartilage development and degeneration [46]. HDACs plays a pivotal role in regulation of matrix-specific gene transcription and cartilage development. MiR-455-3p levels were higher in proliferating and pre-hypertrophic chondrocytes, while HDAC2 and HDAC8 were primarily expressed in hypertrophic chondrocytes. In human SW1353 chondrocyte-like cells, miR-455-3p suppressed the activity of 3′-untranslated regions of HDAC2/8 and inhibited the expression of HDAC2/8, and promoted histone H3 acetylation at the collagen 2 promoter [46].

Adipogenesis

Other important role of miR-455 has been investigated in the regulation of brown fat adipogenesis. Brown adipocytes are specialized for thermogenic energy expenditure in human. It dissipates excess fuel energy as heat. Brown adipose tissue counteracts obesity by enhancing the development and activity of brown adipocytes [59]. Zhang et al. (2015) unveiled that miR-455 was induced by a bone morphogenetic protein 7 and cold exposure and promote brown adipogenesis of committed preadipocytes and non-committed progenitor cells by inducing the expression of PGC1a and mitochondria biogenesis [59]. This study showed that miR-455 targets several potential adipogenic regulator genes such as Necdin, Runx1t1, and hypoxia-inducible factor 1α (HIF1α) [59]. Necdin and Runx1t1, and hypoxia-inducible factor 1α inhibitor (HIF1α). Necdin and Runx1t1 are the important adipogenic suppressors that are involved in adipocyte differentiation program, whereas HIF1α is a class of hydroxylase that mainly modifies the AMP-activated kinase α1 subunit (AMPKα1) by
hydroxylation. Since, HIF1α can directly hydroxylate AMPKα1 and thereby inhibit its activity. Here, miR-455 plays an important role; it suppresses Neddin and Runx1t1 and initiates the adipogenic program. On the other hand, suppression of HIF1α gene activates AMPKα1 activity, which in turn acts as a metabolic trigger and induces the brown adipogenic program [59].

A recent study by Pahlavani et al. (2018) investigated the effect of eicosapentaenoic acid (EPA) on miRNAs expression in brown adipose tissue metabolism [60]. The miRNA sequencing analysis revealed that miR-455-3p level was higher in C57BL/6J (B6) mice fed with high-fat-EPA diet compared to high-fat diet. Ingenuity pathway analysis demonstrated that miR-455 downregulated Tgfβr3 level and its downstream member, Smad2/3 [60]. This study identified a link between miR-455 and its TGF-β/Smad3 targets. These findings unveiled the important role of miR-455-3p in adipogenesis and obesity.

**Preeclampsia**

Preeclampsia is a severe multisystemic pregnancy-related disorder, which causes maternal and fetal mortality in 2–5% cases worldwide [61]. Lalevée et al. (2014) identified the role of miR-455-3p in preeclampsia [62]. Study demonstrated that miR-455 family members, miR-455-3p and miR-455-5p, were both significantly downregulated in the placenta of preeclampsia patients, whereas the levels of other placenta-specific human miRNAs remain unchanged. Further, miR-455-3p target prediction and validation analysis revealed a potential link of miR-455-3p to hypoxia signaling. The high expression levels of miR-455-3p during trophoblast differentiation suggested that miR-455-3p represses the hypoxia response that might otherwise prevent cytotrophoblasts from syncytiotrophoblast differentiation [62]. This report revealed that deregulated expression of miR-455 responsible for aberrant hypoxia signaling in preeclampsia patients. Further, due to the circulatory nature of miR-455 in the blood, it could help in development of noninvasive prenatal tests for early diagnosis of preeclampsia [62].

**MICRORNA-455 AND HUMAN CANCERS**

Both members of the miR-455 family, miR-455-5p and miR-455-3p, participate in human cancers such as hepatocellular carcinoma, melanoma, prostate cancer, colorectal cancer, breast cancer, squamous cell carcinoma, pancreatic cancer, non-small cell lung cancer, and renal carcinoma (Fig. 2). However, for each cancer very few reports are available regarding miR-455-3p. Here, we summarized several important findings of miR-455-3p in each cancer and explain the molecular mechanism of miR-455 in cancer.

**Hepatocellular carcinoma**

Hepatocellular carcinoma (HCC) has become the second most common cause of cancer-related deaths worldwide [63]. Qin et al. (2016) found significant downregulation of miR-455 expression levels in both HCC tissues and cell lines. Low levels of miR-455 were significantly associated with the poor prognostic features: multiple tumor nodes, high Edmondson-Steiner grading, advanced tumor-node-metastasis stage, and venous infiltration of HCC patients. Qin et al. (2016) revealed that gain- and loss-of-function of miR-455 significantly suppressed migration and invasion abilities of HCC cells in vitro [50]. The level of miR-455 was inversely correlated with runt-related transcription factor 2 (Runx2) expression in HCC samples. Moreover, Runx2 was a direct downstream target of miR-455, and miR-455 inversely regulated Runx2 expression in HCC cells. Evidently, alteration in Runx2 expression suppressed the effect of miR-455 on HCC cell migration and invasion. Hence, miR-455 could be a potential prog-
nostic indicator and valuable therapeutic strategy for HCC [50].

Melanoma

Melanoma is the most aggressive type of skin cancer. Adenosine-to-inosine (A-to-I) RNA editing of miRNAs, as well as other post-transcriptional modifications, increases protein diversity from a limited set of genes; this process can promote tumor growth and progression [64]. One A-to-I edited miRNA is miR-455-5p, which has two A-to-I RNA-editing sites. The biological function of edited miR-455-5p is different from that of the unedited form, as it recognizes a different set of genes. Indeed, wild-type miR-455-5p promotes melanoma metastasis through inhibition of the tumor suppressor gene CPEB1. Moreover, wild-type miR-455 enhances melanoma growth and metastasis in vivo, whereas the edited form inhibits these features. These results demonstrate a previously unrecognized role for RNA editing in melanoma progression [54].

Prostate cancer

Prostate cancer (PCa) is the most commonly diagnosed cancer among men and is the third leading cause of cancer-related deaths in the United States [65]. Arai et al. (2019) identified the anti-tumor function of both passenger and guide strands of the miR-455-duplex-miR-455-5p and miR-455-3p in PCa cells by RNA sequencing analysis [55]. The Cancer Genome Atlas expression analysis of a large patient cohort found eight miR-455-5p/3p target genes, PIR, LRP8, IGFBP3, DMBX1, CCDC64, TUBB1, KIF21B, and NFAM1, that were significantly associated with poor prognosis of PCa patients. Of them, PIR (pirin) was a potential target and directly regulated by miR-455-5p. The high expression of PIR was detected in hormone-sensitive prostate cancer surgical specimens and castration-resistant prostate cancer autopsy specimens. Loss-of-function analysis of PIR gene using siRNA or inhibitor showed the inhibition of cancer cell migration and invasion. Interestingly, the miR-455-5p/PIR axis complex contributed to PCa aggressiveness [55].

Colon or colorectal cancer

Colorectal cancer (CRC) is the third most common type of cancer and the fourth most common cause of cancer-related deaths worldwide [66]. Zheng et al. (2016) demonstrated the role of miR-455-3p in cell proliferation and apoptosis in HCT116 human colon cancer cells [49]. Overexpression of miR-455-3p significantly inhibited the cell proliferation of HCT116 cells and increased apoptosis. Proliferation of HCT116 cells was restricted by the regulation of cell cycle regulator p27 kinase inhibition protein (KIP) 1 expression. The overexpression of miR-455-3p significantly elevated the protein levels of p27 KIP1, Bax, pro-caspase-3, and active caspase-3, and markedly downregulated the levels of B-cell lymphoma-2 [49]. MiRNA-455-3p functions as an anti-oncogene in HCT116 cells by inhibiting cell proliferation and inducing apoptosis.

Another report investigated the role of miR-455-3p in CRC through the modulation of histone deacetylase 2 (HDAC2), class I of HDACs. Overexpression of HDAC2 proved to confer oncogenic potential to human lung cancer cells by deregulating apoptosis-associated proteins. HDAC2 is involved in prognosis and therapy of CRC [67]. HDAC2, a class I of HDACs, is a crucial factor in health and disease [68]. Overexpression of HDAC2 induces oncogenic potential in human lung cancer and laryngeal squamous cell carcinoma, and suppression of HDAC2 could induce cell apoptosis and inhibit cell proliferation [69, 70]. Recently, Mao et al. (2017) unveiled the molecular associations between HDAC2 and miR-455-3p in order to search for therapeutic candidates for CRC. The study showed that miR-455 potentially suppressed the oncogenic function of HDAC2 and effectively inhibited cell proliferation while inducing cell apoptosis in CRC cells [67].

Triple-negative breast cancer

Triple-negative breast cancer (TNBC) is define by its lack of estrogen-receptor and progesterone-receptor expression, along with the absence of human epidermal growth factor receptor 2 overexpression or gene amplification [51, 71]. TNBC accounts for 15–20% of all breast cancers, and the majority are basal-like [72]. Recent findings suggested that miR-455-3p may be involved in TNBC development and progression, and regulation of key tumor associated genes. Li et al. (2017) found that miR-455-3p expression was upregulated in TNBC cell lines and TNBC tissues as well [51]. In vitro analysis showed that miR-455-3p enhanced cell proliferative, invasive, and migrational abilities in TNBC cell lines by binding to 3’ UTR of targeting etoposide-induced 2.4 (E124)
gene. As the p53-target gene, EI24 is involved in the suppression of cell growth, induction of apoptosis, and the activation of autophagy. EI24 expression was dramatically downregulated, while miR-455-3p was upregulated in TNBC cell and tissues [51]. Interactions of miR-455-3p/EI24 axis provides a potential novel therapeutic target for prevention and treatment of TNBC in the future.

*Esophageal squamous cell carcinoma*

Esophageal cancer is the eighth most common cancer and causes the sixth highest cancer-related mortality worldwide [73]. Liu et al. (2017) examined the effects of miR-455-3p on chemoresistance and tumorigenesis in esophageal squamous cell carcinoma (ESCC) using *in vivo* and *in vitro* models [74]. This study showed a positive role for miR-455-3p in the activation of Wnt/β-catenin and transforming growth factor-β (TGF-β)/Smad pathways in ESCC. Suppression of miR-455-3p using antagonir dramatically chemosensitized ESCC cells and reduced the subpopulations of CD90 + and CD271 + T-ICs via deactivation of multiple stemness-associated pathways, including Wnt/β-catenin and TGF-β signaling [74]. These observations confirmed the essential role of miR-455-3p in ESCC chemoresistance and tumorigenesis.

*Pancreatic cancer*

Pancreatic ductal adenocarcinoma is one of the most lethal malignancies and accounts for >200,000 deaths annually worldwide [75]. Drug resistance is a major cause of treatment failure in pancreatic cancer. Zhan et al. (2018) unveiled the involvement of miR-455-3p in chemoresistance in pancreatic cancer [52]. MiR-455-3p expression analysis revealed a significantly decreased level of miR-455-3p in pancreatic cancer tissues and cell lines. The low level or inhibition of miR-455-3p increased the cell proliferation and gemcitabine resistance of pancreatic cancer. On the other hand, induced expression of miR-455-3p had the opposite effects on cell proliferation and gemcitabine resistance of pancreatic cancer. On the other hand, induced expression of miR-455-3p had the opposite effects on cell proliferation and drug resistance. MiR-455-3p targets the TAZ gene, which is associated with drug resistance of pancreatic cancer and contributes to cell proliferation and drug resistance in pancreatic cancer cells [52]. TAZ is a transcriptional co-activator with PDZ-binding motif, also known as WW domain-containing transcriptional regulator 1 (WWTR1), and is a key downstream component of the Hippo pathway. Evidences suggest that TAZ promotes chemotherapy resistance in various cancers [76]. These findings unveiled the important role of miR-455-3p in drug resistance.

*Non-small cell lung cancer*

Non-small cell lung cancer (NSCLC) is the most common malignancy worldwide and is characterize by high recurrence, metastasis, and mortality rates [77]. MiRNAs have been reported to play significant roles in the initiation and progression of NSCLC. Likewise, as in other cancers, miR-455-3p could function as a tumor suppressor in NSCLC. The levels of miR-455-3p was prominently downregulated in NSCLC tissues and cell lines [47]. The correlation analysis between miR-455-3p level and clinico-pathological features of NSCLC tissues revealed poorly differentiated cancer and advanced tumor stage. The gain and loss of function analysis of miR-455-3p revealed the inhibition of cell proliferation and migration *in vitro*. MiRNAs target prediction analysis, and luciferase reporter assay revealed that miR-455-3p directly targets and suppresses HOXB5 in NSCLC. Furthermore, knockdown of the HOXB5 gene attenuated the effects of miR-455-3p downregulation on cell proliferation and migration. This study suggested that downregulation of miR-455-3p in NSCLC was correlated with the poor prognosis of NSCLC, and its tumor suppressor functions by directly targeting HOXB5 in NSCLC [47].

*Renal cell carcinoma*

Renal cell carcinoma (RCC) is the most common kidney-associated neoplasm. RCC constitutes 2–3% of human cancers, and the proportion is increasing worldwide, more than 350,000 people [78]. Yamada et al. (2018) investigated the role of miR-455 family members in RCC [53]. The miR-455-5p (the passenger strand) and miR-455-3p (the guide strand) based on miRNA expression signatures were downregulated in RCC tissues and their low levels were significantly associated with poor prognosis of RCC. The ectopic expression of miR-455-5p and miR-455-3p significantly inhibited the proliferation, migration, and invasive abilities of RCC cell lines [53]. The oncogenic targets of miR-455-5p and miR-455-3p were identified by a combination of genome-wide gene expression and in silico miRNA database analyses. The spindle and kinetochore-associated proteins, SKA1 and SKA3, were the potential target and
directly regulated by miR-455-5p and miR-455-3p, respectively. The overexpression of SKA3 is very frequent in RCC clinical specimens. Therefore, the miR-455-3p/SKA3 axis complex contributed to pathogenesis and aggressiveness of RCC [53].

MICRORNA-455 AND NEURODEGENERATIVE DISEASES

Amyotrophic lateral sclerosis

Amyotrophic lateral sclerosis (ALS), the most common adult-onset neurodegenerative disorder, is characterized by the progressive loss of motor neurons [79]. Freischmidt et al. (2014) identified several miRNAs that were significantly downregulated in serum of patients with familial ALS and presymptomatic ALS mutation carriers compared with healthy control subjects using Affymetrix TM miRNA3.0 arrays [80]. Among them, miR-455-3p levels were found to be downregulated by 0.19-fold ($p = 0.00315$) in familial ALS serum samples compared to healthy controls [80].

MicroRNA-455 and Alzheimer’s disease

Several potential miRNAs have been identified in AD based on their biological relevance in AD diagnosis, progression, and therapeutics. We have reviewed the biomarker characteristics of several potential miRNA candidates in AD [22]. However, we still do not have any universally accepted miRNAs that can be used as a biomarker in clinical settings.

MicroRNA-455-3p and Alzheimer’s disease

For the first time, our laboratory investigated the role of miR-455-3p in AD [40, 41]. The primary aim of our research was to discover peripheral circulating miRNAs as biomarkers for AD. Upon completion of global microarray analysis of serum samples, we identified several miRNAs that were deregulated in the serum samples of AD patients, MCI individuals, and healthy control subjects. In microarray data analysis, miR-455-3p was the top candidate, which showed the highest fold variation among AD, MCI, and controls with significant $p$-values. Based on the primary screening results of serum samples, we focused on miR-455-3p and validated its levels in different kinds of AD samples, and unveiled its biomarker potential.

BIOMARKER POTENTIAL OF MIR-455-3P IN ALZHEIMER’S DISEASE

Due to the lack of a non-invasive, early detectable, peripheral biomarker for AD, the purpose of our study was to identify miRNAs as early detectable peripheral biomarkers in AD. To achieve our objective, we assessed circulating miRNAs in serum samples from AD patients and MCI subjects relative to healthy controls. We used the Affymetrix GeneChip miRNA Array, v.4.0 platform to analyze the miRNAs expression patterns in disease versus healthy state. We analyzed the data using Transcriptome Analysis Console software v.3 using the following criteria: bi-weight average (log2) intensity, a fold change of less than 2 or more than 2, and an ANOVA/false discovery rate (FDR), $p < 0.05$. Comparison of AD versus MCI versus healthy controls showed a total of 68 miRNAs (32 mature and 36 precursors) that were deregulated among three groups of serum samples. Our focused was on the miRNAs, whose expressions are either gradually increased or decreased with disease progression (healthy controls $\rightarrow$ MCI $\rightarrow$ AD). Of the 32 mature miRNAs that were identified, four were gradually upregulated: hsa-miR-455-3p, hsa-miR-3613-3p, hsa-miR-4674, and hsa-miR-4668-5p, while one, hsa-mir-6722, was downregulated in AD and MCI subjects compared with controls. The top candidate was miR-455-3p, which showed a log2 intensity of 2.53-fold increase in the controls, a 4.9-fold increase in MCI subjects and a 6.03-fold increase in AD patients [40].

Further, we validated the levels of these five miRNAs on serum samples and postmortem brains from
AD patients and healthy controls. Careful validation analysis verified only miR-455-3p, which showed a similar expression trend in other AD samples as obtained from microarray data. Further, we elaborated our validation analysis on AD postmortem brains, AD fibroblasts, AD B-lymphocytes, AD cell lines, and APP transgenic mice [40, 41]. In all biospecimens, the levels of miR-455-3p expression was higher in AD case compared to the samples from healthy controls. Further, receiver operating characteristics curve analysis of miR-455-3p in serum, postmortem brains, fibroblasts, and B-lymphocytes unveiled a significant area under curve value of miR-455-3p in AD relative to controls. MiR-455-3p validation analysis on different AD sources together with ROC curve analysis confirm that it could be a potential peripheral biomarker for AD and capable of discriminating persons with and without AD [40, 41].

THERAPEUTIC POTENTIAL OF MIR-455-3P IN ALZHEIMER’S DISEASE

The results obtained from primary findings further led us to conclude a possible role of miR-455-3p in AD progression. Our study showed an upregulation of miR-455-3p expression levels in cells treated with Aβ [40]. These findings led us to hypothesize that Aβ toxicity might be responsible for the high levels of miR-455-3p found in the brain/neurons of patients with AD. Based on these findings, we also hypothesize that the higher expression levels of miR-455-3p in persons with AD (and in the brains of APP mice) might be a compensatory response to Aβ toxicity.

Next, we investigated the link of miR-455-3p with AβPP and Aβ toxicity. In-silico analysis of miR-455-3p identified several molecular targets of miR-455-3p. Important ones are: APP, NGF, NRXN1, ATXN1, USP25, PDRG1, SMAD4, UBQLN1, SMAD2, TP73, VAMP2, and HSPBAP1. Among them, APP was the top predicted target, which has never been validated in relation to miR-455-3p [41]. Luciferase reporter assay confirmed the binding of miR-455-3p at 3’UTR of APP and significant suppression of APP gene at mRNA and protein levels [56]. Abnormal AβPP processing and Aβ level reduces mitochondrial biogenesis, alters mitochondrial dynamics, and reduces synaptic activity [82]. We studied the roles of miR-455-3p in AβPP processing, Aβ levels, mitochondrial biogenesis, mitochondrial dynamics, and synaptic activity in AD progression. Our laboratory was the first to investigate the relevance of miR-455-3p in AD progression and pathogenesis [56].

MicroRNA-455-3p and AβPP processing

Abnormal AβPP processing and lack of excess Aβ clearance in the brain is a major pathogenic hallmark of AD progression. Overexpression of miR-455-3p construct (pRP[Exp]-U6 > hsa-miR-455-3p-CAG-EGFP) reduces the expression of mutant APP cDNA (pCAX-APP Swe/Ind) in mouse neuroblasta cells. The levels of full-length mutant APP, c-terminal fragments of APP (C99 and C83), and the levels of Aβ (40) and (42) were significantly decreased in cells by miR-455-3p. Co-localization studies of miR-455-3p construct and mutant APP cDNA showed significant reduction of Aβ level by miR-455-3p overexpression [56]. High levels of miR-455-3p further reduce the toxic effects of Aβ on mitochondrial biogenesis, mitochondrial dynamics, synaptic activities, cell viability, and apoptosis.

MicroRNA-455-3p, mitochondrial biogenesis, and mitochondrial dynamics

The high level of mutant AβPP and Aβ reduces the mitochondrial biogenesis proteins and alters mitochondrial dynamics events in hippocampal neurons [82]. The key biogenesis genes (PGC1α, NRF1, NRF2, and TFAM) were significantly reduced by mutant APP cDNA over production. Our study indicated that the high level of miR-455-3p was able to suppress the mutant APP cDNA, abnormal AβPP processing, and Aβ levels. Further, mutant APP cells (cells transfected with mutant APP cDNA) that expressed miR-455-3p construct showed increased mRNA and protein levels of mitochondrial biogenesis genes. Additionally, the fission proteins DRP1 and FIS1 were decreased while the fusion proteins (OPA1, Mfn1, and Mfn2) were significantly increased by miR-455-3p. In brief, miR-455-3p rescued the mitochondrial function from toxic effects of Aβ. Transmission electron microscopy analysis of mitochondria morphology showed a decrease in the number of mitochondria and an increase in the average size of mitochondrial length in mutant APP cells transfected with miR-455-3p [56].
MicroRNA-455-3p, neuron proliferation, and synaptic activity

The roles of miR-455-3p in cell survival and cell proliferation have been identified previously [45, 48]. In our studies, we sought to determine the protective and deleterious roles of miR-455-3p in AD. We found that miR-455-3p overexpressed cells showed improved cell proliferation with extended dendrites. MiR-455-3p targets several apoptotic pathways genes (e.g., BAX, BMF, TGFBR3L, and HIF1) as shown by bioinformatics analysis (http://targetscan.org). High level of mutant APP induces apoptotic cell death, while high level of miR-455-3p suppress Aβ levels and reduces apoptotic cell death [56]. MiR-455-3p also rescued cells from toxic effects of Aβ on synaptic proteins synaptophysin and PSD95. MiR-455-3p increased the levels of synaptic proteins and improved synaptic activity. Based on these observations, we cautiously conclude that miR-455-3p regulates AβPP processing and is protective against mutant AβPP-derived mitochondrial and synaptic abnormalities in AD.

MOLECULAR MECHANISMS OF MICRONA-455-3P IN ALZHEIMER'S DISEASE

The newly discovered biomarker and therapeutic role of miR-455-3p in AD leaves some questions to be addressed: Why is the level of miR-455-3p elevated in AD, and if elevated, is it suppressing endogenous AβPP and Aβ or not? Recent findings from our laboratory reported that mutant APP cDNA caused abnormal AβPP processing and high Aβ in hippocampal neurons [82]. The excess Aβ accumulation in neurons leads to the reduction in mitochondrial biogenesis, mitochondria fusion, and synaptic activity and increases mitochondrial fission activity [82]. On the other hand, Aβ toxicity in the brain also induces the level of miR-455-3p in human and mouse neuroblastoma cells treated with Aβ40 peptide [40]. These observations indicate that upregulation of miR-455-3p could be a compensatory response to the increased levels of Aβ in AD cells. Here, we propose that induced level of endogenous miR-455-3p in response to Aβ may not be enough to counter suppress AβPP and Aβ. However, overexpression of miR-455-3p using the miR-455-3p construct is potentially able to control abnormal AβPP processing and reduce Aβ levels [56]. Moreover, high levels of miR-455-3p rescue the cells from the toxic effects of Aβ on mitochondrial biogenesis, mitochondrial dynamics, and synaptic activity (Fig. 3).

Mouse models of microRNA-455-3p in AD and other neurodegenerative diseases

To determine the protective and/or deleterious roles of miR-455-3p in both peripheral and central nervous systems, it is important to create overexpressed (transgenic) and/or depleted (knockout) mouse models for miR-455-3p and characterize these mouse models systematically in different organs, such as kidney, heart, liver, lung, pancreas, brown adipose tissue, and brain in the body. Currently, our laboratory is undertaking this task and actively making both transgenic and knockout mouse models for miR-455-3p.

It is important to create both transgenic and knockout mouse models in genetic backgrounds of several rodent models for neurodegenerative diseases. And, it is equally important to cross these transgenic and knockout lines of miR-455-3p mice with different mutant APP mice (APP - Tg2576, APP/PS1, 5XFAD, and others) and study abnormal AβPP processing, C-terminal fragments such as C83 & C99, Aβ levels, mitochondrial biogenesis, mitochondrial dynamics, and synaptic activities in double mutant mice (Tg miR-455-3p × APP Tg mice; KO miR-455-3p × APP Tg mice) relative to wild type and APP Tg mice alone. Therefore, further investigations of cell and animal models of aging, other human diseases, including neurodegenerative diseases, cancer, cardiovascular, and kidney diseases are urgently needed.

MiR-455-3p exosomes as therapeutic targets in Alzheimer’s disease

Exosomes are biological nanocarriers that play a large role in basic physiological events in the body [83, 84]. Exosomes exert their effects by targeting their cargos, such as DNAs, messenger RNAs, miRNAs, and proteins to host cells that led to changes in the cellular and molecular behaviors of recipient cells [85]. One of the important aspects of exosomes is to protect cells and organs against mutant proteins such as Aβ and phosphorylated tau. As discussed above, miR-455-3p is a strong candidate to treat patients with AD. Therefore, preparation and delivery of exosomes enriched with miR-455-3p to AD cells, AD mice, and even AD patients are important. Therefore, further
research on miR-455-3p exosomes are important and urgently needed in AD.

CONCLUSION AND FUTURE DIRECTIONS

The purpose of this review was to understand the role of miR-455-3p in human health and diseases. Past studies identified miR-455 as mainly being involved in chondrogenesis and several human cancers. miR-455 is expressed during chondrogenesis and in adult articular cartilage, where it can regulate TGFβ signaling, suppressing the Smad2/3 pathway. For the first time, the Reddy laboratory investigated the role of miR-455-3p as a biomarker in AD. Consistent upregulation of miR-455-3p in AD serum, AD postmortem brains, AD fibroblasts, AD B-lymphocytes, AD mouse models, and AD cell lines strengthen our hypothesis that miR-455-3p is a potential biomarker and provoke us to extend our research to the translation level. Further, interaction of miR-455-3p with key AD genes raises the hope for its possible therapeutic application as well. However, certain issues need to be clear and rectified before establishment of miR-455-3p as AD biomarkers. Expression of circulatory miR-455-3p needs to be checked in the biofluids (serum/plasma) of certain cancer patients and in other non-AD dementia such as Lewy body dementia, Huntington’s and Parkinson’s diseases. In addition, it is very important to determine the changes/fluctuations in the levels of miR-455-3p with AD progression, and how it correlates with disease severity. Furthermore, a protective role of miR-455-3p against abnormal AβPP processing, Aβ levels, and Aβ-induced toxicities unveiled its therapeutic importance in AD. Importantly, induction of mitochondrial biogenesis, synaptic activity, and regulation of healthy mitochondrial dynamics by miR-455-3p made it a valuable molecule to study in more detail.

Lastly, based on all these findings, we hypothesize that the overexpression of miR-455-3p in a mouse model will reduce Aβ pathology, enhance mitochondrial biogenesis, mitochondrial function, and synaptic activity, and ameliorate cognitive decline in AD. We also hypothesize that depleted level of endogenous miR-455-3p in a mouse model will increase Aβ, reduce mitochondrial biogenesis, mitochondrial function, and synaptic activity, and enhance cognitive decline in AD.

ACKNOWLEDGMENTS

The research presented in this article was supported by NIH grants AG042178, AG047812, and
NS105473; the CH Foundation; and Alzheimer’s Association through a SAGA grant (to PHR).
Authors’ disclosures available online (https://www.j-alz.com/manuscript-disclosures/19-0583r1).

REFERENCES


