Check for updates

Addressing Alzheimer's Disease (AD) Neuropathology Using Anti-microRNA (AM) Strategies

Vivian R. Jaber[1] · Yuhai Zhao[1,2] · Nathan M. Sharfman[1] · Wenhong Li[1,3] · Walter J. Lukiw[1,4,5]

Abstract
Disruptions in multiple neurobiological pathways and neuromolecular processes have been widely implicated in the etiopathology of Alzheimer's disease (AD), a complex, progressive, and ultimately lethal neurological disorder whose current incidence, both domestically and globally, is reaching epidemic proportions. While only a few percent of all AD cases appear to have a strong genetic or familial component, the major form of this disease, known as idiopathic or sporadic AD, displays a multi-factorial pathology and represents one of the most complex and perplexing neurological disorders known. More effective and innovative pharmacological strategies for the successful intervention and management of AD might be expected: (i) to arise from strategic-treatments that simultaneously address multiple interrelated AD targets that are directed at the initiation, development, and/or propagation of this disease and (ii) those that target the "neuropathological core" of the AD process at early or upstream stages of AD. This "Perspectives paper" will review current research involving microRNA (miRNA)-mediated, messenger RNA (mRNA)-targeted gene expression pathways in sporadic AD and address the potential implementation of evolving anti-microRNA (AM) strategies in the amelioration and clinical management of AD. This novel-therapeutic approach: (i) incorporates a system involving the restoration of multiple miRNA-regulated mRNA-targets via the use of selectively-stabilized AM species; and (ii) that via implementation of synthetic AMs, the abundance of only relatively small-families of miRNAs need be modulated or neutralized to re-establish neural-homeostasis in the AD-affected brain. In doing so, these strategic approaches will jointly and interactively address multiple AD-associated processes such as the disruption of synaptic communication, defects in amyloid peptide clearance and amyloidogenesis, tau pathology, deficits in neurotrophic support, alterations in the innate immune response, and the proliferation of neuroinflammatory signaling.

Keywords Alzheimer's disease · microRNA (miRNA) · messenger RNA (mRNA) · miRNA-mRNA linkage analysis · miRNA-7 · miRNA-9 · miRNA-34a · miRNA-125b · miRNA-146a · miRNA-155

Overview—AD Heterogeneity

Alzheimer's disease (AD) is a complex, progressive, and ultimately lethal neurodegenerative disorder whose primary risk factor is aging of the human CNS. The longitudinal course of AD progresses very differently amongst individuals and clearly evolves over different time scales commensurate with an individual's biomedical history, biophysical and hormonal signature, and highly interrelated patient characteristics including lifestyle, genetic constitution, and predisposition [1–4]. Epidemiological-, genetic-, epigenetic-, neuroimaging-, neuropathological-, biomarker-, lifestyle-, clinical-, etiopathological-, and ethnological-based evidence continue to suggest that AD is a particularly heterogeneous disorder [1–10]. Therapeutic interventions for AD combined with genome editing, RNA interference technologies, pharmacotherapeutics, and/or lifestyle intervention including dietary alterations (i) may provide the most promising approaches for modulating or repairing the primary molecular genetic deficits associated with such age-related and heterogeneous

✉ Walter J. Lukiw
wlukiw@lsuhsc.edu

1 LSU Neuroscience Center, Louisiana State University Health Sciences Center, New Orleans, LA 70112, USA

2 Department of Anatomy and Cell Biology, Louisiana State University Health Sciences Center, New Orleans, LA 70112, USA

3 Department of Pharmacology, School of Pharmacy, Jiangxi University of TCM, Nanchang 330004, Jiangxi, China

4 Department of Neurology, Louisiana State University Health Sciences Center, New Orleans, LA 70112, USA

5 Department of Ophthalmology, Louisiana State University Health Sciences Center, New Orleans, LA 70112, USA

neurodegenerative pathologies and (ii) may be the best example yet where a tailoring of "personalized or precision medicine" to each AD patient for disease amelioration may be successfully applied [7–10].

One attractive therapeutic strategy for AD intermediation would be an interventional therapy such as a pharmaceutical agent that (i) is non-toxic at physiologically realistic doses; (ii) has relatively high stability in vivo and is long acting and effective throughout the treatment regimen; (iii) has a modifiable structure that can increase or decrease intrinsic stability to shorten or prolong its pharmacological efficacy; (iv) is highly selective with minimal toxicity and limited off-target effects; (v) is well tolerated by the aged AD patient and their compromised physiological condition especially with respect to nephrotoxicity, hepatotoxicity, and neurotoxicity; (vi) has an intrinsic capacity to neutralize multiple disease targets; (vii) focuses on at least one molecular genetic component lying upstream from the branching of a critical pathological pathway relevant to the AD process; and (viii) can be effectively delivered into the CNS using viral-mediated transfer, exosomes or related packaging, and other advanced drug delivery techniques [8–10]. Currently, few drug choices for AD possess all of these qualities; however, all eight of these roles are fulfilled by microRNAs (miRNA) and anti-miRNAs (AMs). miRNAs and AMs are small 18–22-nucleotide (nt) highly soluble single-stranded non-coding (sncRNA) species whose stability can be greatly increased using 3′ adenylation, locked nucleic acids (LNAs, a bicyclic furanose unit locked in an RNA mimicking sugar conformation), or other RNA chemical modification techniques that significantly prolong miRNA half-life and stability both in vitro and in vivo. To put it simply, AM ribonucleotide sequences are complementary to respective miRNA sequences and the creation of a miRNA-AM hybrid reduces the abundance of that miRNA which can restore homeostasis to the system under study [11–17].

microRNAs in Neurological Disease

microRNAs (miRNAs) represent a unique, evolutionary conserved class of sncRNAs which act in the post-transcriptional regulation and expression of genetic information in higher eukaryotes [18–29]. Of the ~2,650 human miRNAs so far characterized, only ~35-40 miRNAs are abundant in the human brain and retina, and these have a potential to regulate a transcriptome consisting of ~ 22,000–27,000 messenger RNAs (mRNAs; this is the total number of mRNAs in an average eukaryotic cell, but not all mRNAs are either generated or expressed in any one specific cell type). The major mode of action of miRNAs is to function as negative regulators of mRNA-mediated gene expression by recognizing and binding to complementary ribonucleotide sequences in the 3′-untranslated region (3′-UTR) of target mRNAs and causing

translational repression and/or mRNA target degradation [18–21]. Hence, small families of upregulated miRNAs might be expected to downregulate their target mRNAs and downregulate the expression of the genetic information that these mRNAs encode. This capability of small families of upregulated miRNAs to target and downregulate multiple mRNAs and combinatorial miRNA-mRNA-linked gene expression networks, which are altered in disease conditions, makes them amongst the most interesting and potentially useful candidates for effective therapeutic intervention using AM therapeutic strategies.

Interestingly, each miRNA can potentially target the mRNAs encoded by hundreds of genes simultaneously; most miRNAs display a tightly regulated expression pattern that is often tissue specific or even cell type specific, emphasizing the importance of miRNAs in defining the space, time, and developmental stage of specific gene expression patterns [24–29]. Importantly, (i) the abundance, complexity, speciation, and regulation of a cell's endogenous miRNA pool are critical events in the definition of cell identity and behavior, both under steady-state conditions, during development and aging and in health and disease, and (ii) other important components of the CNS including ceramides and sphingosine and their phosphates may modulate gene expression signaling as AD progresses—these compounds also are most likely involved in the regulation of gene expression in the CNS in other neurodegenerative disorders [28–31].

Upregulated NF-κB-Sensitive microRNAs in AD Versus Age- and Gender-Matched Controls

Our understanding of the regulation of the expression of human inflammatory–related microRNAs (miRNAs) in the CNS was advanced by experimentally inducing the pro-inflammatory transcription factor NF-κB (p50/p65) in human neuronal-glial (HNG) cell co-cultures in primary culture and by subsequently analyzing the resulting induced miRNAs using a high-density microRNA array approach (capable of quantifying all 2650 human miRNA species). Upregulated miRNA signals were further quantified and analyzed using LED-Northern micro-dot blots and RT-PCR [26–30]. NF-κB was induced using Aβ42 peptides, neurotoxic metal sulfates, TNF-α, and/or IL-1β; for example, these are known pro-inflammatory mediators observed to be upregulated in AD brain tissues [30–33]. Selective NF-κB-DNA binding and translocation inhibitors, including the chelator and anti-oxidant pyrrolidine dithiocarbamate (PDTC), the caffeic acid phenethyl ester (CAPE), and the polyphenolic resveratrol analog CAY10512 (trans-3,5,4′-trihydroxystilbene), reaffirmed the NF-κB sensitivity of a small family of upregulated miRNAs in HNG cells. The most highly induced miRNA promoters of interest were next sequenced and analyzed for NF-κB-DNA binding sites using

bioinformatics software, and verification of NF-κB-mediated regulation of these miRNAs was performed using pLightSwitch 3′-UTR luciferase reporter vector systems stably transfected into HNG cells in primary culture [21, 26, 27, 34]. For example, the inducible pathogen-associated miRNA-125b and miRNA-146a were found to have multiple NF-κB-DNA binding sites in their upstream promoters [26]. Verified mRNA targets for miRNA-125b and miRNA-146a, including (i) 15-lipoxygenase (15-LOX, a critical enzyme in neurotrophic support and the synthesis of neuroprotectin D1 (NPD1) from docosahexaenoic acid and other omega-3 fatty acids), (ii) synapsin (SYN-1 and SYN-2, synaptic phosphoproteins that act as key modulators of neurotransmitter release across the presynaptic membrane of CNS synapses; downregulated SYN-1 and SYN-2 proteins result in a deficit in synaptogenesis and disruption of normal synaptic signaling function), (iii) complement factor H (CFH; originally known as beta-1H globulin, a key soluble glycoprotein and regulator of complement activation in the innate immune response), and (iv) tetraspanin-7 and tetraspanin-12 (TSPAN-7 and TSPAN-12, a βAPP-associated transmembrane protein whose deficits are associated with amyloidogenesis), suggested complex and highly interactive roles for the NF-κB (p50/p65) complex, miRNA-125b and miRNA-146a in the regulation of multiple mRNAs, and hence their expression, in human CNS neural cells [20, 35]. These data further indicated that a small number of NF-κB-induced miRNAs have tremendous potential to contribute to or detract from the regulation of neurotrophic support, synaptogenesis, neuroinflammation, innate immune signaling, and amyloidogenesis in stressed HNG cells and in AD-affected tissues. Many of the pathologically upregulated miRNAs in the AD brain (including to date miRNA-7, miRNA-9, miRNA-34a, miRNA-125b, miRNA-146a, and miRNA-155; based on the analysis of ~ 90 female short postmortem interval (PMI) control and AD brains) and significantly downregulated mRNAs (resulting in critical deficits in gene expression, as is observed in AD) and their neuropathological consequences are shown in the schematic diagram in Fig. 1 and are further described in the legend to this figure [35, 38, 39, 42].

Downregulated messenger RNAs (mRNAs) Observed in the AD Brain

Multiple independent investigators have reported a generalized downregulation of gene expression in the AD brain neocortex and hippocampus to about 50–66% of what is found in healthy and age- and gender-matched controls [38, 43–45]. These deficiencies in genetic output include deficits in mRNA encoded genetic information necessary for homeostatic ubiquitination (UBE2A) and phagocytosis (TREM2); impairments in the end-stage clearance of amyloid from the cell

with ensuing amyloidogenesis (TSPAN-7, TSPAN-12); downregulation in neurotropic support including 15-lipoxygenase (15-LOX) and the vitamin D receptor (VDR); deficits in innate immune signaling (CFH), ectopic stimulation of NF-κB signaling, and neuroinflammation (IkBKG, IRAK-1; with a compensatory increase in IRAK-2 expression); and alterations in synaptogenesis, synaptic structure and organization, and the ability of synaptic vesicles to be "released" and "reabsorbed" at the synapse (TSPAN-7, TSPAN-12, SHANK3, SYN-1, and SYN-2). What is particularly interesting is the overlapping functions of groups of downregulated genes whose brain functions are highly interrelated, such as TSPAN-7, TSPAN-12, SHANK3, SYN-1, and SYN-2, in synaptic organization and signaling and in the coordinated downregulation in the expression of genes involved in inter-neuronal trans-synaptic communication and neurotrophic support, including SYN-1, SYN-2, 15-LOX, and VDR (Fig. 1).

Another highly illustrative example is the regulation of the superfamily of tetraspanin (TSPAN) proteins (including TSPAN-7 and TSPAN-12—whose expression levels are both significantly downregulated in AD by upregulated miRNA-7, miRNA-9, and miRNA-146a) [40, 46]. TSPAN proteins are normally engaged in organizing cellular interactions, membrane trafficking, compartmentalization, and the formation of complex protein networks known as "tetraspanin webs" with functions in neural activity-dependent synapse formation, function, and plasticity and are involved in both neurological and retinal degeneration [47–49]. Interestingly, the post-synaptic organizing and scaffolding protein SHANK3 (downregulated by increased miRNA-34a presence) and downregulated TSPAN proteins may jointly contribute to neurodegenerative aspects of neuropsychiatric illness [40, 41]. Deficits in such highly interrelated, and functionally inter-connected, gene networks may be exploited when the same overabundant miRNAs such as miRNA-9 and miRNA-125b target individual components of this complex system (Fig. 1). Hence, anti-miRNA strategies may be used to advantage to knock down miRNA-regulated networks of small families of genes to tailor the desired pharmacotherapeutic response and form a mechanistic basis for the personalization of treatments for progressive, age-related inflammatory neurodegenerative disorders such as AD.

anti-NF-κB Strategies

Firstly, because all of these six potentially pathogenic miRNAs are upregulated in abundance after the induction of the pro-inflammatory dimeric transcription factor NF-κB (p50/p65) complex, it may seem at first that inhibition of NF-κB could provide a novel and efficacious therapeutic strategy. There are currently about ~ 1000 NF-κB inhibitors (such as IκB phosphorylation, IκB activation, and NF-κB

Fig. 1 A highly schematized figure, taken from recent peer-reviewed publications in the upregulated-miRNA-in-AD field—indicating a miRNA-regulated expression network involving a small family of inducible NF-κB (p50-p65) controlled microRNAs (miRNAs, green ovals) that downregulate the expression of multiple messenger RNA (mRNA) targets (black rectangles) of relevance to the onset, development, and propagation of AD neuropathology [36, 37]. This graphic figure summarizes the effects of 6 upregulated miRNAs on the downregulation of 12 of their messenger RNA targets. All 6 miRNAs or 12 mRNAs are brain-enriched or brain-specific RNA transcripts [29, 38, 39]. White arrowheads indicate bioinformatics-derived evidence (based primarily on the free energy of association E_A, between the entire miRNA and the 3′-untranslated region (3′-UTR) of their target mRNA) and are generally free energies of association of less than − 21 kcal/mol (miRBase release 22.1; http://www.mirbase.org/; last accessed 25 April 2019; and TargetScan (TargetScanHuman release 7.2; http://www.targetscan.org/vert_72/; last accessed 25 April 2019; and/or other miRNA-mRNA bioinformatics algorithms); red arrowheads indicate both bioinformatics-derived and experimentally derived evidence and verification that has appeared in the recent peer-reviewed scientific literature; experimental verification is based mainly on miRNA-mRNA binding studies and the 3′-UTR luciferase reporter binding assay using pLightSwitch vectors transfected into human neuronal-glial (HNG) cell co-cultures; yellow dashed lines and yellow arrowheads indicate interrelated and/or coordinated gene expression; downregulated target mRNAs (and hence downregulated expression of multiple brain-enriched genes) appear to contribute, sometimes interactively, to impaired ubiquitination (UBE2A); deficits in phagocytosis (TREM2) and amyloidogenesis (TSPAN-7, TSPAN-12); deficits in innate immune signaling (CFH), stimulation of NF-κB signaling, and neuroinflammation (IkBKG, IRAK-1; with a compensatory increase in IRAK-2 expression); altered synaptogenesis, synaptic structure, and organization (SHANK3, SYN-1, and SYN-2); and deficits in neurotropic support (15-LOX, VDR) (see text for further details). Because miRNAs typically have multiple mRNA targets, only a few upregulated miRNAs can explain the observed downregulation of multiple mRNA targets; most of which reflect downregulated brain-enriched gene expression patterns that in part characterize AD neuropathology [18, 20, 22, 40, 41]. Neutralization of miRNA activities using anti-microRNA therapeutic strategies may be of immense benefit in the clinical management of AD and other neurodegenerative disorders with an inflammatory component. Other miRNA-mRNA-linked signaling circuits may be involved. This analysis was based on the temporal lobe neocortex and/or hippocampal CA1 regions of 90 female AD and age-matched control brain samples all having postmortem intervals (PMIs) of 2.5 h or less [38; unpublished observations]

translocation inhibitors) available to neurological researchers and clinicians including a variety of natural and synthetic antioxidants, peptides, small RNA/DNA, microbial and viral proteins, antibodies, and engineered dominant-negative or constitutively active polypeptides that have direct anti-NF-κB activities [50–52; unpublished]. Interestingly, the therapeutic, preventative, and beneficial anti-inflammatory effects of many natural products such as allicin, curcumin, ellagic acid, resveratrol, and sulforaphane may, at least in part, be due to their ability to inhibit NF-κB signaling [29, 30, 35, 51, 53–56].

There are however important limitations to the implementation of anti-NF-κB therapeutic strategies due to (i) the hepatotoxicity (liver toxicity), nephrotoxicity (renal toxicity), and neurotoxicity (toxicity in the CNS) of many anti-NF-κB
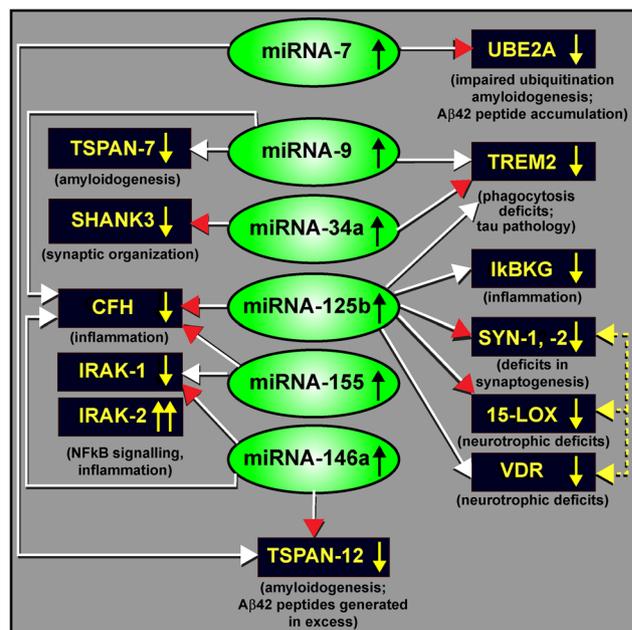
compounds, especially in aged humans [50, 51, 57], (ii) difficulties in drug delivery and the selective targeting of NF-κB signaling pathways within CNS compartments [51, 54], and (iii) significant off-target effects for NF-κB inhibitors, many of which were recognized at least 13 years ago [50, 51, 57]. Related to these limitations, NF-κB is often a critical and pluripotent transcription factor that orchestrates many diverse physiological and pathological processes and responses, especially those involved in cell proliferation, inflammation, immunity, growth, apoptosis, and necrosis, the expression of pathogenic viral genes, and cancer development and progression—thereby, unwanted off-target effects may be of genuine concern when strategically implementing anti-NF-κB compounds therapeutically [29, 30, 51, 52, 54–57]. The singular importance and significance of NF-κB in the generation of potentially pathogenic miRNA in cancer and neurodegenerative disease have been presented in a series of well-illustrated peer-reviewed papers from multiple laboratories [27–31, 37, 40, 54–59].

Anti-microRNA (AM) Strategies

There are multiple useful intrinsic properties, advantages, and benefits in both the molecular structure and function of anti-miRNAs (AMs) which impart to them a significant potential for their successful therapeutic application as AM-based therapies in the human CNS. These include the following: (i) both miRNAs and AMs are non-toxic at physiologically realistic doses in human brain cells in primary culture; (ii) because AMs (and miRNAs) are typically less than ~ 1/375th the size of the smallest known single-stranded RNA virus (poliovirus, 7500 nt), miRNAs (at 18–22 nt) are

probably too small to elicit a robust immune response in the host; (iii) the regulation of their stabilities, and hence functional longevity, in the cell is extremely versatile; although many miRNAs and AMs are short lived in the brain and retina, their structure can be chemically and/or physically modified to significantly increase their stability in vivo and can be made to be long acting and effective throughout the treatment regimen; miRNAs in the brain and retina can also be relatively unstable entities and this may be useful in limiting the efficacy of the AM itself; (iv) just like antibody-protein recognition, miRNAs are highly selective with minimal toxicity and negligible off-target effects; (v) AMs can be designed so that their complementary anti-miRNA ribonucleotide sequence is either fully or only partly complementary to modulate the full AM effect; (vi) miRNAs are well tolerated by the aged AD patient and their often compromised physiological condition; (vii) miRNAs have an intrinsic capacity to neutralize multiple disease targets; (viii) depending on complementarity between the AM and the miRNA, the abundance and actions of a particular miRNA can be "throttled down" or completely negated; (ix) a strategic approach of an AM targeting a specific miRNA could define a pharmacological treatment that focuses on at least one critical molecular genetic component lying upstream from the branching of a critical pathological pathway relevant to the AD process; (x) an extremely high selectivity—based on complementary AM-miRNA pairing that is one of the most specific and selective links in human neurobiology; (xi) delivery of AMs via exosomes and viral vectors; (xii) especially in the brain and retina, many sncRNA and AM stabilities can be kept relatively short, indicating that the therapeutic effect of miRNAs or sncRNAs can be tailored to be self-limiting; and (xiii) if necessary, anti-NF-κB and AM strategies may be combined to achieve the desired pharmacotherapeutic effect.

Concluding Comments

The utilization and implementation of anti-miRNA and related anti-sncRNA therapeutic treatment strategies in human neurological diseases such as AD are still in their infancy, given that the actual discovery of altered miRNA abundance, speciation, and complexity in AD and miRNA inducibility by oxidative stress was only scant 12 years ago [58–60]. The first in vivo experiments using AM strategies in human brain cells in primary culture in vitro occurred only about 6 years ago [42]. Here, we cite five relevant examples on the design and utilization of AM strategies and the potential usefulness of AM therapeutic approaches:

(i) The efficient design of specific AMs can help to improve their performance and potency towards the target miRNA by increasing, for instance, nuclease resistance and target affinity; the design of AM compounds including locked nucleic acid (LNA) oligonucleotides that have recently proved their worth in silencing miRNAs is progressing and is being optimized in vivo [61]

(ii) Expression of the integral membrane triggering receptor expressed in microglial cells (TREM2) and post-synaptic organizing protein SHANK3 has been shown to be down-regulated due to a significantly upregulated miRNA-34a in AD and in stressed HNG cells in primary culture, and a specifically stabilized AM-34a was shown to both knock down miRNA-34a expression and restore normal TREM2 and SHANK3 expression and homeostasis in vivo [20, 41, 42]

(iii) The pro-inflammatory miRNA-146a has been shown to be significantly up-regulated in both sporadic AD neocortex and in stressed HNG cells in primary-co-culture [26–28]. In preliminary experiments, anti-miRNA-146a (AM-146a)-based treatment strategies using viral vector delivery (AAV) systems have been shown to improve cognitive performance in the aging 5xFAD murine model of AD [26–28; unpublished].

(iv) Very recently, an AM for miRNA-1 has been successfully used in the experimental treatment of middle cerebral artery occlusion (MCAO) in a rodent model—resulting in an improvement in neurological deficits and reduced infarction volume, brain edema, and blood-brain barrier (BBB) permeability, indicating that AMs are able to protect the brain and provide a promising therapeutic approach after stroke and MCAO [63]

(v) There is also recent evidence that miRNA-155 upregulated after traumatic brain injury (TBI) can be reduced to homeostatic levels in the regulation of post-traumatic neuroinflammatory responses and neurological recovery using an AM-155 strategy, indicating that miRNA-155 may be a therapeutic target for post-TBI-related neuroinflammatory signaling [36, 64, 65]

Lastly, the successful implementation of AM-based clinical management AD patients may require a highly "*individualistic*" treatment strategy and clinical regimen based on multiple factors including highly interactive aspects of a particular patient's gender, epidemiology, multi-factorial pathological factors such as familial-genetic and medical history, disease-duration, disease-course, interceding illness, and the timing of the strategic miRNA-based intervention itself. For example, it has been recently shown that specific timeframes during the endocrine aging process, such as during the menopause-transition in female AD patients, may provide an optimal-window of opportunity for therapeutic-intervention to further prevent or delay progression of AD [64, 65]. Such "*personalized AM-based therapeutic approaches*" would more effectively address the spectrum of specific miRNA,

mRNA, and gene expression deficits in individual AD-patients, including combinatorial and/or tailored multiple AM-therapeutic approaches that will take both clinician-focus and dedication, and probably individual customization, to maximize the probability of success in the clinical management of the disease course.

Authors' Contributions VRJ, YZ, NMS, and WJL performed all microRNA and messenger RNA analysis, miRNA-mRNA verification and linking, and preliminary AM experiments, organized and tabulated all the data, and performed statistical analysis from age-, gender-, and PMI-matched control and AD brains, and collaborated interactively in the synthesis of the material presented in this Frontiers "Perspective"

article. WJL wrote the paper. YZ and WJL have published in excess of 50 peer-reviewed manuscripts into this research area. All authors agree on the content of this publication.

Compliance with Ethical Standards

Ethics Statement All acquisition, handling, experimental, and analytical procedures involving postmortem human brain tissues were carried out in an ethical manner in strict accordance with the ethics review board policies at brain and tissue donor institutions and at the Louisiana State University (LSU) Health Sciences Center. Informed consent from next of kin was obtained at brain and tissue donor institutions for all tissue samples prior to autopsy and donation; coded postmortem brain tissue samples (containing no personal identifying information of the donors) were obtained from the 18 brain and tissue banks listed in the "Acknowledgments" section above. The ethical use of postmortem human brain tissues and their analyses were also carried out in strict accordance with the Institutional Biosafety Committee and the Institutional Review Board Committee (IBC/IRBC) ethical guidelines IBC#18059 and IRBC#6774 at the LSU Health Sciences Center, New Orleans, LA 70112, USA.

Conflict of Interest The authors declare that they have no conflict of interest.

References

1. Latta CH, Brothers HM, Wilcock DM (2015) Neuroinflammation in Alzheimer's disease; a source of heterogeneity and target for personalized therapy. Neuroscience 302:103–111. https://doi.org/10.1016/j.neuroscience.2014.09.061

2. Lane CA, Hardy J, Schott JM (2018) Alzheimer's disease. Eur J Neurol 25:59–70. https://doi.org/10.1111/ene.13439

3. Rogaev EI (2018) Different pathways to neurodegeneration. Biochemistry (Mosc) 83:1007–1008. https://doi.org/10.1134/S0006297918090018

4. Melis RJF, Haaksma ML, Muniz-Terrera G (2019) Understanding and predicting the longitudinal course of dementia. Curr Opin Psychiatry 32:123–129. https://doi.org/10.1097/YCO.0000000000000482

5. Dong A, Toledo JB, Honnorat N, Doshi J, Varol E, Sotiras A, Wolk D, Trojanowski JQ et al (2017) Heterogeneity of neuroanatomical patterns in prodromal Alzheimer's disease: links to cognition, progression and biomarkers. Brain 140:735–747. https://doi.org/10.1093/brain/aww319

6. Blennow K, Zetterberg H (2018) Biomarkers for Alzheimer's disease: current status and prospects for the future. J Intern Med 284:643–663. https://doi.org/10.1111/joim.12816

7. Bhagwat N, Pipitone J, Voineskos AN, Chakravarty MM, Alzheimer's disease neuroimaging initiative (2019) An artificial neural network model for clinical score prediction in Alzheimer

disease using structural neuroimaging measures. J Psychiatry Neurosci 44:1–15

8. Berkowitz CL, Mosconi L, Scheyer O, Rahman A, Hristov H, Isaacson RS (2018) Precision medicine for Alzheimer's disease prevention. Healthcare (Basel) 6(3). https://doi.org/10.3390/healthcare6030082

9. Hodes JF, Oakley CI, O'Keefe JH, Lu P, Galvin JE, Saif N, Bellara S, Rahman A et al (2019) Alzheimer's "prevention" vs. "risk reduction": transcending semantics for clinical practice. Front Neurol 9:179. https://doi.org/10.3389/fneur.2018.01179

10. Isaacson RS, Ganzer CA, Hristov H, Hackett K, Caesar E, Cohen R, Kachko R, Meléndez-Cabrero J et al (2018) The clinical practice of risk reduction for Alzheimer's disease: a precision medicine approach. Alzheimers Dement 14:1663–1673. https://doi.org/10.1016/j.jalz.2018.08.004

11. Kauppinen S, Vester B, Wengel J (2006) Locked nucleic acid: high-affinity targeting of complementary RNA for RNomics. Handbook of Exp Pharmacol 173:405–422

12. Katoh T, Sakaguchi Y, Miyauchi K, Suzuki T, Kashiwabara S, Baba T, Suzuki T (2009) Selective stabilization of mammalian microRNAs by 3′ adenylation mediated by the cytoplasmic poly(A) polymerase GLD-2. Genes Dev 23:433–438. https://doi.org/10.1101/gad.1761509

13. Sethi P, Lukiw WJ (2009) Micro-RNA abundance and stability in human brain: specific alterations in Alzheimer's disease temporal lobe neocortex. Neurosci Lett 459:100–104. https://doi.org/10.1016/j.neulet.2009.04.052

14. Rüegger S, Großhans H (2012) MicroRNA turnover: when, how, and why. Trends Biochem Sci 37:436–446. https://doi.org/10.1016/j.tibs.2012.07.002

15. Pogue AI, Hill JM, Lukiw WJ (2014) MicroRNA (miRNA): sequence and stability, viroid-like properties, and disease association in the CNS. Brain Res 1584:73–79. https://doi.org/10.1016/j.brainres.2014.03.042

16. Zhao Y, Alexandrov PN, Lukiw WJ (2016) Anti-microRNAs as novel therapeutic agents in the clinical management of Alzheimer's disease. Front Neurosci 10:59. https://doi.org/10.3389/fnins.2016.00059

17. Exiqon-Qiagen, Germantown MD, USA; http://www.exiqon.com/lna-technology; last accessed 12 March 2019).

18. Guo H, Ingolia NT, Weissman JS, Bartel DP (2010) Mammalian microRNAs predominantly act to decrease target mRNA levels. Nature 466:835–840. https://doi.org/10.1038/nature09267

19. Towler BP, Jones CI, Newbury SF (2015) Mechanisms of regulation of mature miRNAs. Biochem Soc Trans 43:1208–1214. https://doi.org/10.1042/BST20150157

20. Zhao Y, Jaber VR, LeBeauf A, Sharfman NM, Lukiw WJ (2019) microRNA-34a (miRNA-34a) mediated down-regulation of the post-synaptic cytoskeletal element SHANK3 in sporadic Alzheimer's disease (AD). Front Neurol 10:28. https://doi.org/10.3389/fneur.2019.00028

21. Bhattacharjee S, Zhao Y, Dua P, Rogaev EI, Lukiw WJ (2016) microRNA-34a-mediated down-regulation of the microglial-enriched triggering receptor and phagocytosis-sensor TREM2 in age-related macular degeneration. PLoS One 11(3):e0150211. https://doi.org/10.1371/journal.pone.0150211

22. Bartel DP (2018) Metazoan microRNAs. Cell. 173:20–51. https://doi.org/10.1016/j.cell.2018.03.006

23. Iwakawa HO, Tomari Y (2015) The functions of microRNAs: mRNA decay and translational repression. Trends Cell Biol 25:651–665. https://doi.org/10.1016/j.tcb.2015.07.011

24. Jonas S, Izaurralde E (2015) Towards a molecular understanding of microRNA-mediated gene silencing. Nat Rev Genet 16:421–433. https://doi.org/10.1038/nrg3965

25. Pu M, Chen J, Tao Z, Miao L, Qi X, Wang Y, Ren J (2019) Regulatory network of miRNA on its target: coordination between transcriptional and post-transcriptional regulation of gene expression. Cell Mol Life Sci 76:441–451. https://doi.org/10.1007/s00018-018-2940-7

26. Lukiw WJ, Zhao Y, Cui JG (2008) An NF-kB-sensitive miRNA-146a-mediated inflammatory circuit in AD and in stressed human brain cells. J Biol Chem 283:31315–31322. https://doi.org/10.1074/jbc.M805371200

27. Pogue AI, Li YY, Cui JG, Zhao Y, Kruck TP, Percy ME, Tarr MA, Lukiw WJ (2009) Characterization of an NF-kB-regulated, miRNA-146a-mediated down-regulation of complement factor H (CFH) in metal-sulfate-stressed human brain cells. J Inorg Biochem 103:1591–1595. https://doi.org/10.1016/j.jinorgbio.2009.05.012

28. Cui JG, Li YY, Zhao Y, Bhattacharjee S, Lukiw WJ (2010) Differential regulation of IRAK-1 and IRAK-2 by microRNA-146a and NF-kappaB in stressed human astroglial cells and in Alzheimer's disease. J Biol Chem 285:38951–38960. https://doi.org/10.1074/jbc.M110.178848

29. Lukiw WJ (2012) NF-κB-regulated, pro-inflammatory miRNAs in Alzheimer's disease. Alzheimers Res Ther 4:47. https://doi.org/10.1186/alzrt150

30. Xiong W, Dong S, Yuan J, Li J, Liu J, Xu X (2012) miRNA-146a promotes proliferation and migration of rat vascular smooth muscle cells in vitro in a NF-κB-dependent manner. Nan Fang Yi Ke Da Xue Xue Bao 32:270–273

31. Czubowicz K, Jęśko H, Wencel P, Lukiw WJ, Strosznajder RP (2019) The role of ceramide and sphingosine-1-phosphate in Alzheimer's disease and other neurodegenerative disorders. Mol Neurobiol. https://doi.org/10.1007/s12035-018-1448-3

32. Ng A, Tam WW, Zhang MW, Ho CS, Husain SF, McIntyre RS, Ho RC (2018) IL-1β, IL-6, TNF- α and CRP in elderly patients with depression or Alzheimer's disease: systematic review and meta-analysis. Sci Rep 8:12050. https://doi.org/10.1038/s41598-018-30487-6

33. Shen XN, Niu LD, Wang YJ, Cao XP, Liu Q, Tan L, Zhang C, Yu JT (2019) Inflammatory markers in Alzheimer's disease and mild cognitive impairment: a meta-analysis and systematic review of 170 studies. J Neurol Neurosurg Psychiatry 90:590–598. https://doi.org/10.1136/jnnp-2018-319148

34. LightSwitch Assay, Switchgear Genomics (an Active Motif Company), Menlo Park CA USA; https://switchgeargenomics.com/resources/science-technology; last accessed 14 March 2019.

35. Lukiw WJ, Alexandrov PN (2012) Regulation of complement factor H (CFH) by multiple miRNAs in Alzheimer's disease (AD) brain. Mol Neurobiol 46:11–19

36. Lukiw WJ (2012) NF-κB-regulated micro RNAs (miRNAs) in primary human brain cells. Exp Neurol 235:484–490. https://doi.org/10.1016/j.expneurol.2011.11.022

37. Mosconi L, Rahman A, Diaz I, Wu X, Scheyer O, Hristov HW, Vallabhajosula S, Isaacson RS et al (2018) Increased Alzheimer's risk during the menopause transition: a 3-year longitudinal brain imaging study. PLoS One 13:e0207885. https://doi.org/10.1371/journal.pone.0207885

38. Colangelo V, Schurr J, Ball MJ, Pelaez RP, Bazan NG, Lukiw WJ (2002) Gene expression profiling of 12633 genes in Alzheimer hippocampal CA1: transcription and neurotrophic factor down-regulation and up-regulation of apoptotic and pro-inflammatory signaling. J Neurosci Res 70:462–473

39. Burmistrova OA, Goltsov AY, Abramova LI, Kaleda VG, Orlova VA, Rogaev EI (2007) MicroRNA in schizophrenia: genetic and expression analysis of miR-130b (22q11). Biochemistry (Mosc) 72:578–582

40. Jaber V, Zhao Y, Lukiw WJ (2017) Alterations in micro RNA-messenger RNA (miRNA-mRNA) coupled signaling networks in sporadic Alzheimer's disease (AD) hippocampal CA1. J Alzheimers Dis Parkinsonism 7(2):312. https://doi.org/10.4172/2161-0460.1000312

41. Alexandrov PN, Zhao Y, Jaber V, Cong L, Lukiw WJ (2017) Deficits in the proline-rich synapse-associated Shank3 protein in multiple neuropsychiatric disorders. Front Neurol 8:670. https://doi.org/10.3389/fneur.2017.00670

42. Zhao Y, Bhattacharjee S, Jones BM, Dua P, Alexandrov PN, Hill JM, Lukiw WJ (2013) Regulation of TREM2 expression by an NF-κB-sensitive miRNA-34a. Neuroreport 24:318–323. https://doi.org/10.1097/WNR.0b013e32835fb6b0

43. Ginsberg SD, Alldred MJ, Che S (2012) Gene expression levels assessed by CA1 pyramidal neuron and regional hippocampal dissections in Alzheimer's disease. Neurobiol Dis 45:99–107. https://doi.org/10.1016/j.nbd.2011.07.013

44. Zhao Y, Bhattacharjee S, Jones BM, Hill J, Dua P, Lukiw WJ (2014) Regulation of neurotropic signaling by the inducible, NF-kB-sensitive miRNA-125b in Alzheimer's disease (AD) and in primary human neuronal-glial (HNG) cells. Mol Neurobiol 50:97–106. https://doi.org/10.1007/s12035-013-8595-3

45. Zhao Y, Bhattacharjee S, Dua P, Alexandrov PN, Lukiw WJ (2015) microRNA-based biomarkers and the diagnosis of Alzheimer's disease. Front Neurol 6:162. https://doi.org/10.3389/fneur.2015.00162

46. Seipold L, Saftig P (2016) The emerging role of tetraspanins in the proteolytic processing of the amyloid precursor protein. Front Mol Neurosci 9:149. https://doi.org/10.3389/fnmol.2016.00149

47. Termini CM, Gillette JM (2017) Tetraspanins function as regulators of cellular signaling. Front Cell Dev Biol 5:34

48. Saint-Pol J, Eschenbrenner E, Dornier E, Boucheix C, Charrin S, Rubinstein E (2017) Regulation of the trafficking and the function of the metalloprotease ADAM10 by tetraspanins. Biochem Soc Trans 45:937–944. https://doi.org/10.1042/BST20160296

49. Murru L, Moretto E, Martano G, Passafaro M (2018) Tetraspanins shape the synapse. Mol Cell Neurosci 91:76–81. https://doi.org/10.1016/j.mcn.2018.04.001

50. Gilmore TD, Herscovitch M (2006) Inhibitors of NF-kB signaling: 785 and counting. Oncogene. 25:6887–6899

51. Kaur U, Banerjee P, Bir A, Sinha M, Biswas A, Chakrabarti S (2015) Reactive oxygen species, redox signaling and neuro-inflammation in AD: the NF-κB connection. Curr Top Med Chem 15:446–457

52. Yu L, Li L, Medeiros LJ, Young KH (2017) NF-κB signaling pathway and its potential as a target for therapy in lymphoid neoplasms. Blood Rev 31:77–92. https://doi.org/10.1016/j.blre.2016.10.001

53. Khuda-Bukhsh AR, Das S, Saha SK (2014) Molecular approaches toward targeted cancer prevention with some food plants and their products: inflammatory and other signal pathways. Nutr Cancer 66:194–205. https://doi.org/10.1080/01635581.2014.864420

54. Wu J, Ding J, Yang J, Guo X, Zheng Y (2018) MicroRNA roles in the nuclear factor kappa B signaling pathway in cancer. Front Immunol 9:546. https://doi.org/10.3389/fimmu.2018.00546

55. Che J, Stark LA (2018) Crosstalk between NF-κB and nucleoli in the regulation of cellular homeostasis. Cells. 7(10):E157. https://doi.org/10.3390/cells7100157

56. Mendiola AS, Cardona AE (2018) The IL-1β phenomena in neuro-inflammatory diseases. J Neural Transm (Vienna) 125:781–795. https://doi.org/10.1007/s00702-017-1732-9

57. Hong JT (2017) NF-kB as a mediator of brain inflammation in AD. CNS Neurol Disord Drug Targets 18:3–10. https://doi.org/10.2174/1871527316666170807130011

58. Lukiw WJ (2007) Micro-RNA speciation in fetal, adult and Alzheimer's disease hippocampus. Neuroreport. 18:297–300

59. Lukiw WJ, Pogue AI (2007) Induction of specific micro RNA (miRNA) species by ROS-generating metal sulfates in primary human brain cells. J Inorg Biochem 101:1265–1269

60. Schipper HM, Maes OC, Chertkow HM, Wang E (2007) MicroRNA expression in Alzheimer blood mononuclear cells. Gene Regul Syst Biol 1:263–274

61. Lima JF, Cerqueira L, Figueiredo C, Oliveira C, Azevedo NF (2018) Anti-miRNA oligonucleotides: a comprehensive guide for design. RNA Biol 15:338–352. https://doi.org/10.1080/15476286.2018.1445959

62. Zhao Y, Jaber V, Lukiw WJ (2017) Secretory products of the human GI tract and their potential impact on Alzheimer's disease (AD): detection of lipopolysaccharide (LPS) in AD hippocampus. Front Cell Infect Microbiol 7:318. https://doi.org/10.3389/fcimb.2017.00318

63. Talebi A, Rahnema M, Bigdeli MR (2019) Effect of intravenous injection of antagomiR-1 on brain ischemia. Mol Biol Rep 46:1149–1155. https://doi.org/10.1007/s11033-018-04580-y

64. Li YY, Alexandrov PN, Pogue AI, Zhao Y, Bhattacharjee S, Lukiw WJ (2012) miRNA-155 upregulation and complement factor H deficits in Down's syndrome. Neuroreport 23:168–173. https://doi.org/10.1097/WNR.0b013e32834f4eb4

65. Henry RJ, Doran SJ, Barrett JP, Meadows VE, Sabirzhanov B, Stoica BA, Loane DJ, Faden AI (2019) Inhibition of miRNA-155 limits neuro-inflammation and improves functional recovery after experimental traumatic brain injury in mice. Neurotherapeutics 16:216–230. https://doi.org/10.1007/s13311-018-0665-9