

MicroRNAs as Potential Regulators of Docosahexaenoic Acid Benefits in Alzheimer's Disease

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Abstract

Alzheimer's disease (AD) is a highly prevalent neurodegenerative disease that imposes a prodigious burden on the society. Docosahexaenoic acids (DHA) are known to be beneficial in AD, in part through their anti-inflammatory properties. MicroRNAs (miRs) are important regulators of brain functions and this regulation becomes disrupted in AD.

Objectives: The purpose of this article is to propose the involvement of miRs in the anti-inflammatory effects of DHA on AD.

Methods: The literature surrounding this topic is extensively researched: miR involvement in the pathophysiology of AD, the mechanism of action of DHA, the effects of DHA on miRs and potential future therapeutic strategies for AD involving miRs.

Results: AD results in a disrupted miR network that relates to inflammation, but the altered miRs vary between studies. The effects of DHA on AD are generally positive but the mechanism remains enigmatic. Emerging studies demonstrate that one of the potential mechanisms of action of DHA is modulation of miRs. The miR mechanism offer possible future strategies against AD.

Discussion: Future AD studies investigating miRs needs to set experimental standards to enable valid comparisons. For DHA effects on AD, thorough considerations on the properties of the DHA and the population involved is necessary. Validation is required to verify miR involvement in the anti-inflammatory properties of DHA in the context of AD. The proposed miR-related strategies against AD remain to be substantiated.

Keywords: *Omega-3; Diet; Anti-inflammatory; Inflammation; Nutrigenetics; Food-derived microRNA; Exogenous microRNA*

1. Introduction

Sporadic Alzheimer's disease (AD) is the age-related neurodegeneration leading to memory impairments, sensory and locomotive dysfunctions, apathy, aggression and eventually leading to splanchnic and peripheral system failure due to diminished networks within the central nervous system (CNS) [1].

Currently, one in nine elderly above 65 years of age in the United States (US) has AD, excluding cases of preclinical AD [2]. For elderly above 85 years of age, this prevalence becomes as high as one in three. AD is proposed to be the third leading cause of death in the US [3]. It administers a heavy burden on the society which reaches approximately \$200 billion in the US every year [4]. With the forecast of AD affecting more than 100 million people worldwide by 2050, AD will create a significantly greater burden on the society [4].

The etiology of AD is multifactorial and several hypotheses have been proposed including aberrant amyloid precursor processing into neurotoxic amyloid beta ($A\beta$) metabolites, hyper-phosphorylation of the microtubule-stabilizing protein tau, apoptotic alterations of synaptic operations and mitochondrial dysfunction-mediated neurotoxicity [5,6].

There is also significant evidence that supports an inflammatory hypothesis for AD [7,8]. This inflammation is likely to be both the cause [9,10] and the consequence [11,12] of AD. Microglia are macrophages for the CNS and they are found to be excessively activated in AD [13]. Inflammation appears to be an important trigger of this phenomenon [13]. This activation leads to further microglial release of pro-inflammatory mediators which creates a vicious self-perpetuating inflammation cycle that exacerbates AD [13].

Currently approved pharmacotherapies of AD include the use of cholinesterase inhibitors (e.g. rivastigmine) and *N*-methyl-D-aspartate antagonist (e.g. memantine) to counteract neurotransmission impairments [14]. However, these pharmacotherapies only slow the progression but do not cure AD. Many other AD pharmacotherapies are being developed and investigated but most fail during clinical trials. Therefore, there remain major demands for any strategies that can prevent, retard, halt or cure AD.

Dietary modifications offer key strategies against AD as observed from beneficial dietary effects from vitamins, phytochemicals, Mediterranean diet and Souvenaid® [15-18]. In contrast, diet may also exacerbate AD, as witnessed for high-fat diets and excessive intake of food contaminated with metals such as cadmium, lead and arsenic [15,19].

Due to the underlying inflammation in AD, anti-inflammatory strategies have exhibited promising strategies against AD such as the use of non-steroidal anti-inflammatory drugs and anti-tumor necrosis factor alpha (TNFA) from human observational studies and animal trials [8]. Docosahexaenoic acid (DHA) is well-established for its anti-inflammatory properties, and it has been observed in the literature to improve AD [20]. However, its mechanism of action remains enigmatic. Recent evidence suggests potential involvements of the important post-transcriptional gene regulator, microRNAs (miRs) [21].

2. MicroRNAs

MiRs were first identified in 1993 by the Ambros and Ruvkun laboratory from *Caenorhabditis elegans* experiments [22]. They were then identified to be conserved phylogenetically across the plant and animal kingdoms which then triggered a revolution in these newly classified non-coding RNAs.

For intergene- or exon-derived miRs, their canonical biogenesis pathway initiates with nuclear transcription of miR-coding genes into primary miR [23]. Cleavage via the microprocessor complex then occurs to form precursor miR (pre-miR), which

is transported into the cytoplasm via exportin5. Dicer cleavage leads to the formation of mature double stranded miR duplex, that is incorporated into the RNA-induced silencing complex (RISC), followed by the degradation of the passenger strand. This is slightly different for intron-derived miRs and other emerging non-canonical miR biogenesis pathways are being increasingly recognized [24].

The most well-known function of miRs are its gene-silencing effects on messenger RNA (mRNA) through Watson-Crick interactions between the miR 5' seed sequence nucleotides and the mRNA 3' untranslated region (3'UTR) [23]. This leads to either repression of protein translation or degradation of the mRNA transcript. Only partial complementarity is required for gene-silencing effects and therefore single miR can target multiple mRNAs and vice versa. The presence of "isomiRs" [23] and other miR functions further adds to the conundrum of miRs [25].

The regulation of miRs themselves are extremely complex and far from being understood. They can be regulated through changes in the miR biogenesis components, at the transcription level (transcriptional factors), the post-transcriptional level (pre-miR degradation and modification) as well as at the post-translational level (miR turnover and endogenous sponge activity) [24].

Some of these miRs have been determined to be specifically expressed or enriched in certain tissues. For the brain, a number of enriched miRs include *miR-128*, *miR-129*, *miR-133a*, *miR-138*, *miR-153*, *miR-181a*, *miR-181b*, *miR-218*, and *miR-219* [24]. Within neurons, there appears to be an enrichment of *miR-125b*, *miR-128*, *miR-32*, *miR-134* and *miR-139* in the synaptic and dendritic regions compared to the soma [24].

MiRs have been reviewed to play essential roles in the development and proper functioning of the brain [26]. At the molecular level, miRs are involved in the lineage determination, maturation, survival and neurotransmission [24]. A troika of miRs, *miR-134*, *miR-132* and *miR-138* were recently reviewed for their imperative functions in neurons including dendritogenesis, morphogenesis, neuron plasticity, synapse formation, dendritic spine size, cell migration and axon regeneration [27]. They also participate in the in the differentiation, activation and polarization of microglia [28]. Using dicer knockout experiments, miRs have shown to be crucial for memory formation within the brain [29].

This is further supported by recent research that *miR-34a* and *miR-132* as well as *miR-138* regulate memory in rats and humans, respectively [30,31]. These pivotal roles of miRs conveys their profound potential as paramount strategies against AD.

3. The Roles of MicroRNAs in Alzheimer's Disease Inflammation

MiRs are known to participate in human AD, and the studies conducted to date are summarized in **Error! Reference source not found.** The roles of miRs in AD have been reviewed previously [32]. The present review discusses newer studies conducted since the earlier review and reinforces the anti-inflammatory aspects as a proposal for novel strategies against AD. Since inflammation is implicated in AD and miRs are known to participate in inflammation [33], it is feasible to hypothesize some of the dysregulated miRs in AD are related to inflammation. From the clinical AD and miR studies conducted to date, several miRs were found to be differentially expressed in multiple studies. Some of these have shown to relate with inflammation and presents opportunities for counteracting the inflammation etiology underlying AD.

TABLE 1. Clinical studies on microRNA expression in Alzheimer's disease.

Author	Clinical Samples	Up-regulated microRNA [1]	Down-regulated MicroRNA [1]
Wang et al. [85]	Cerebral cortex n=6		miR-103; miR-107; miR-23b
Hebert et al. [43]	Anterior temporal cortex n=5	miR-520h; miR-197; miR-511; miR-320; miR-516-3p;	let-7i; miR-22; miR-93; miR-26b; miR-9 ; miR-488; miR-363; miR-181c ; miR-106b; miR-101; miR-210; miR-15a ; miR-19b; miR-29b;
Sethi & Lukiw [40]	Temporal lobe neocortex n=6	miR-9 ; miR-125b; miR-146a	
Nunez-Iglesias et al. [44]	Parietal lobes n=5	miR-185; miR-382; miR-432; miR-486; miR-19790; miR-28648; miR-572; miR-18895; miR-35456; miR-320; miR-134; miR-45605; miR-10939; miR-10912; miR-617; miR-30184; miR-671; miR-188; miR-06383; miR-765; miR-575; miR-23974; miR-601;	miR-374; miR-582; miR-05109; miR-12504; miR-12497; miR-30e-5p; miR-376a; miR-44608; miR-181c ; miR-368; miR-95; miR-20546; miR-148b; miR-02532; miR-42448; miR-101; miR-20b; miR-08570; miR-29b; miR-15a ; miR-130a; miR-29c; miR-598; miR-494;
Shioya et al. [86]	Frontal lobes n=7		miR-29a
Geekiyanaige & Chan [45]	Frontal cortices n=7		miR-137; miR-181c ; miR-9 ; miR-29a; miR-29b-1; miR-15 ; miR-124
Long et al. [87]	Frontal cortex n=5		miR-153

Lau et al. [35]	Hippocampus n=41	let-7i-5p; let-7f-5p; miR-195-5p; miR-150-5p; miR-223-3p; miR-92b-3p; miR-362-3p; miR-23a-3p; miR-199a-3p; miR-199b-3p; miR-363-3p; miR-142-3p; miR-27a-3p ; miR-200a-3p; miR-455-5p;	miR-409-5p; miR-370; miR-769-5p; miR-132-3p ; miR-128; miR-138-5p; miR-129-5p; miR-433; miR-124-3p; miR-329; miR-425-5p; miR-127-3p; miR-487b; miR-129-2-3p; miR-487a; miR-543; miR-136-5p; miR-410; miR-495-3p; miR-219-2-3p;
Wong et al. [36]	Temporal cortex n=6		miR-132 ; miR-212
Bekris et al. [38]	Cerebellum n=21	miR-138; miR-208b; miR-181c ; miR-152; miR-126; miR-330-3p; miR-184; miR-191; miR-328; miR-342-3p; miR-370; miR-501; miR-331-3p; miR-139-5p; miR-149; miR-132 ; miR-98; miR-204;	
Bekris et al. [38]	Hippocampus n=21	miR-138; miR-208b; miR-181c ; miR-152; miR-126; miR-330-3p; miR-191; miR-328; miR-342-3p; miR-370; miR-501; miR-331-3p; miR-139-5p; miR-149; miR-132 ; miR-98; miR-204; miR-15a ; miR-346; miR-221	miR-184
Absalon et al. [34]	Temporal cortex n=6	miR-26a; miR-26b; let-7i; miR-125b; miR-134; miR-27a ; miR-27b ; miR-29c; miR-30a-5p	miR-132
Muller et al. [41]	Hippocampus n=5		miR-16; miR-107; miR-128a; miR-146a

The inflammation-related miRs that are discussed specifically in the review are bolded.

3.1 *miR-132*

MiR-132 has been consistently found to be down-regulated [34-36]. Further work in some of these studies discovered phosphatase tensin homolog (*PTEN*), forkhead box (*FOX*) O3a, *FOXO1a* and *p300* as its direct targets [35,36]. These genes participate in the inflammation-related phosphoinositide 3-kinase pathway (PI3K) [37].

Wong et al., demonstrated a -3.8 fold change in *miR-132* within their Braak VI stage AD patients [36]. However, *Lau et al.*, found a more diminished *miR-132* down-regulation of approximately -1.6 fold [35]. The robustness of the results from *Lau et al.* study is greater due to higher statistical significance ($p < 0.00001$) and verification of their nCounter miR data (Nanostrings) with locked-nucleic acids (Exiqon) quantitative real time polymerase chain reaction (qPCR) [35].

Disagreement was presented by *Bekris et al.*, where they found *miR-132* to be up-regulation in AD [38]. However, their study failed to divide samples into Braak stages and adopted inappropriate age-matched controls. Furthermore, their results did not persist following TaqMan qPCR (Applied Biosystems) validation.

3.2 *miR-146a*

MiR-146a has been described extensively as an inflammation-related miR [39]. It was identified to be up-regulated in earlier studies [40], but observed to be down-regulated in a more recent study [41]. It regulates nuclear factor kappa B (NFkB), which is a vital pro-inflammatory transcription factor [42].

The disparity between these two AD studies may arise from the difference in the tissues used, where *Muller et al.*, [41] profiled only the hippocampus but *Sethi & Lukiw*, [40] profiled the whole temporal lobe neocortex. In addition to that, it was shown by *Muller et al.*, [41] that there was actually an up-regulation of *miR-146a* at Braak III, followed by a decrease in Braak VI.

Since *Sethi & Lukiw*, [40] did not provide Braak staging for their AD patients, *Muller et al.*, contributes to more valuable *miR-146a* changes that allows its surveillance across different Braak stages within AD [41].

3.3 *miR-15a*

The up-regulation of *miR-15a* in AD was determined [38], but contrasting results were identified in earlier studies [43-45]. *MiR-15a* is validated to target inflammation-related genes including peroxisome proliferated-activated receptor (*PPAR*)-delta46 and coactivator-associated arginine methyltransferase 1 [47].

Studies that found *miR-15a* down-regulation revealed a -1.5 magnitude with statistical significance $p < 0.01$, based on microarray (LC Sciences & Ambion) data [43,44]. Comparatively, the methodology used by *Bekris et al.*, was more robust in that they had three phases of miR screening using TaqMan qPCR (Applied Biosystems) and normalization to three housekeeping genes [38]. Although, valid comparison was further complicated by the use of different brain sections in these studies as well as the absence of considerations for Braak staging.

3.4 *miR-181c*

Down-regulation of *miR-181c* was detected in three AD studies [43-45]. It was shown to be up-regulated in the preliminary screening by *Bekris et al.*, but their statistics were not significant enough to proceed to the third stage validation of this study [38]. *MiR-181c* has been reviewed in terms of its involvement in inflammation in aspects of their regulatory roles on immunosenescence, T cells activity and mitochondrial encoded cytochrome c oxidase 1 [48].

Within the AD studies that found *miR-181c* down-regulation, the resulting magnitudes were discrepant [43-45]. This is likely attributable to the use of different brain tissues as well as RNA extraction methodology. In terms of the miR profiling, two of these studies adopted a microarray (LC Sciences & Ambion) approach but did not proceed with conventional qPCR validation. (*Geekiyana & Chan*) practiced a comparatively more robust methodology with quantification using singleplex miScript qPCR (Qiagen) and their AD cohort being more defined to specifically profile samples at Braak V [45]. They found a -2.5 fold change within the frontal neocortex.

3.5 *miR-27*

Up-Regulation of *miR-27* was recognized in two of the AD miR studies with similar fold changes around 1.7 [34,35]. It was ascertained in other literature to target the anti-inflammatory genes. *IL-10* and *PPARG* expression [49,50].

Since the Braak stages in AD patients investigated by *Absalon et al.*, [34] were only at Braak III, and *Lau et al.*, [35] at Braak VI, this may lead to the perception that *miR-27* change occurs at an early stage of AD. Nonetheless, several differences between these two studies prohibit this inference to be made. Variations exist for their brain sections profiled, sample size and miR profiling methods.

While *Lau et al.*, [35] had a much greater sample size of 41 and extra miR purification steps, the confirmation of *miR-27* up-regulation found in their nCounter miR assay (Nanostrings) failed to persist with miRCURY locked nucleic acid qPCR (Exiqon) validation.

3.6 *miR-9*

Two studies demonstrated *miR-9* down-regulation [43,45]. It has been revealed to activate microglia through NF κ B signaling [51] and targeting *PPARD* [52].

Geekiyana & Chan, [45] found higher down-regulation of -3.3 compared to -1.3 found by *Hebert et al.*, [43]. As mentioned previously, the methodology presented by *Geekiyana & Chan*, [45] has higher credibility. Albeit, differences may likewise derive in that the temporal cortex was profiled by *Hebert et al.*, [43] in contrast to the frontal neocortex used by *Geekiyana & Chan*, [45].

No gold standard currently exists for miR work [53], and therefore the comparison between AD miR studies are difficult. This can be further complicated by the discrepancies in the use of brain sections, Braak stages, ethnicity, genotypes [54], gender and polymorphisms in miR target sites [32,54]. Nevertheless, these provide valuable grounding for future research.

It is equally important to validate the inflammation-related mRNAs targeted by the differentially expressed miRs in future AD studies to facilitate development of anti-inflammatory strategies against AD.

4. The Effects of Docosahexaenoic Acids on Alzheimer's Disease

Docosahexaenoic acids (DHA) are well known for its anti-inflammatory properties [55]. It is a long chain omega-3 polyunsaturated fatty acid (n3) made up of 22 carbons and 6 *cis* double bonds in a homoallylic arrangement [56]. The brain is made up of 60% lipids and 15% of these are DHA, which implicates its essential roles within the brain. Since n3 cannot be synthesized *de novo*, they must be obtained from external sources such as seafood or in the form of alpha linolenic acid (ALA) within certain plant foods [55].

ALA undergoes elongase and desaturase-mediated metabolism into eicosapentaenoic acid (EPA) and then into DHA [55]. They can then be carried across the endothelial cells lining the blood brain barrier via MFSD2A [57] and then esterified into phospholipids at the stereospecific number-2 position [55]. Its liberation from neuronal membranes can be made via phospholipase to mediate intracellular anti-inflammatory actions by inhibiting activity of NFkB. Current hypotheses of these actions are proposed to involve the docosanoid pathway via lipoxygenase conversion into resolvins, protectins and maresins [58] or the activation of G-coupled protein receptor 120 and PPARG [55]. DHA is also important for neuronal membrane fluidity, long term memory, neurotransmission and synaptic plasticity [55].

A systematic review concluded from meta-analysis of 18 observational studies, that n3 was beneficial for AD [59]. Furthermore, higher levels of direct n3 biomarkers in the elderly were associated with superior brain white matter [60], less executive decline [61] and generally positive brain characteristics [20]. In addition, a meta-analysis of animal trials with direct n3 supplementation revealed improvements of AD-related pathophysiologies including reduced A β , diminished neuronal loss and improved cognitive function [62]. In contrast to these, in a recent meta-analysis of 34 human clinical trials, n3 did not benefit cognition or AD in the elderly [63].

The inconsistency of these results may emanate from differences in the n3 consumed in terms of the food source, food processing and form of supplementation. These disparities can all alter the chemistry of n3 such as its isomerism, homoallylic arrangement, oxidation and stereospecific numbering [56]. Oxidized n3 can lead to development of various diseases through damage to physiological systems and 62% of marketed n3 supplements have been found to be significantly oxidized [64]. As described earlier, the population used within the study is also paramount and supplementation at later stages of AD appeared to be less effective [55]. These are key considerations for newer n3 clinical trials, but decades of evidence do suggest DHA to be beneficial for the CNS. Through elucidation of the underlying miR mechanism, anti-inflammatory strategies of DHA against AD can be optimized.

5. The Effects of Docosahexaenoic Acids on MicroRNAs

MiR studies investigating dietary modifications in the CNS are extremely exiguous and none of these are directly relevant to AD. With regards to the relationship between DHA and miR, only eight published studies exist and these findings are summarized in TABLE 1. The effects of DHA on miR have been reviewed partially in 2012, but many more miR studies of

DHA have been generated since then [65]. Most of these were addressed in other physiological systems with only two conducted within the CNS. These studies in other physiological systems still provide relevant insights to potential miR mechanisms in DHA anti-inflammatory effects on AD. For example, one study illustrated an up-regulation of *miR-107* and their further validation revealed the amyloid processing enzyme, beta-secretase 1, as a *miR-107* target [66].

TABLE 1. Docosahexaenoic acid effects on microRNA expression.

Author	Model	Up-regulated microRNA	Down-regulated MicroRNA
Davidson et al. [66]	Sprague-Dawley rats colon (induced colon tumour by azoxymethane)	Let-7d; miR-15b; miR-107; miR-191; miR-324-5p	
Farago et al. [67]	GBM2 Glioma cells	miR-143	miR-30c; miR-145
Farago et al. [67]	GBM5 Glioma cells	miR-20b	miR-22; miR-30c; miR-143; miR-145
Farago et al. [67]	U373 Glioma cells	miR-145	miR-22
Shah et al. [88]	Sprague-Dawley rats colon (induced colon tumour by azoxymethane)		miR-19b; miR-27b; miR-497; miR-93; miR-18a; miR-203; miR-26b
Mandal et al. [89]	Mice breast cancer tumour		miR-21
Mandal et al. [89]	Breast cancer cells (MCF-10A, MDA-MB-231, MCF-7)		miR-21

Baselga- Escudero et al. [90]	Dyslipidemic rats liver		miR-33a; miR-122
Gil- Zamorano et al. [91]	Caco-2 cells	let-7e; let-7f; miR-1283; miR-330; miR-374b; miR-658; miR-1; miR-221-3p; miR-181a-5p; miR-141-3p; miR-143-3p; miR-191-5p; miR-29b-3p; miR-192; miR-30c	miR-30a
Siddesha et al. [92]	Primary mouse cardiac fibroblasts (treated with Ang II)		miR-21
Antal et al. [21]	Glioma cells U87 MG (treated with radiation)	miR-146; miR-181a	

5.1 Farago et al., 2011 study [67]

In this CNS study, the highly fatal malignant glioma was investigated [67]. Their study explored the effects of multiple PUFAs, including DHA based on previous literature evidence that these can combat gliomas. Their aim was to elucidate the miR mechanism of DHA action on gliomas.

For this, they treated glioblastoma cells (U373, GBM2, GBM5) with 50 & 100 μ M DHA for 24 hrs and then extracted for their miR (Roche). The miRs were profiled using a megaplex TaqMan qPCR (Applied Biosystems) followed by further validation with singleplex TaqMan qPCR (Applied Biosystems). They also quantified levels of selected apoptotic mRNA targets that were predicted for the differentially expressed miR using qPCR.

The only miR that was found to change in all three cell lines was *miR-145*, but the direction of change was discrepant for U373. This suggests possible specificity of DHA action on different cell lines. This notion is further supported by disparities in the magnitude of *miR-145* change between GBM2 of -1.5 fold with GBM5 of -4.7 fold. Therefore, it would be more appropriate to discuss these cell lines separately.

In GBM2, other miRs that were altered include down-regulation of *miR-30c* and up-regulation of *miR-143*. Down-regulation of *miR-22*, *miR-30c* and *miR-143* were discovered for GBM5 as well as up-regulation of *miR-20b*. In U373, aside from the *miR-145* up-regulation, *miR-22* was found to be down-regulated.

In terms of the mRNA targets, correlation was made to their complementary miR. Successful inverse relationships were found for *miR-20b* with tumor protein p53 inducible nuclear protein 1 (*TP3INP1*), *miR-22* with sirtuin 1 (*SIRT1*), *miR-30c* with integrin, beta3 (*ITGB3*), *miR-143* with v-Ki-ras2 oncogene (*KRAS*) and prostaglandin-endoperoxide synthase 2 (*COX2*) as well as *miR-145* with insulin receptor substrate 1 (*IRS1*). These miR associations with apoptotic genes suggest DHA involvement in apoptosis, which is closely related with inflammation [68].

5.2 Antal et al., 2014 Study [21]

This CNS study was conducted by the same research group and they again investigated glioma but this time they focused on DHA enhancement of radiotherapy against glioma cells [21]. The mechanism of this radiotherapy enhancement by DHA remains enigmatic and therefore the researchers intended to determine the miR mechanism to optimize radiotherapy against glioma.

They subjected U87 glioblastoma cells under 10Gy cobalt irradiation and then treated with 25 μ M DHA for 48 hrs. RNA was extracted from cells (Bioneer) and selected miR expression were quantified using singleplex TaqMan qPCR (Applied Biosystems). Candidate mRNAs were similarly quantified using qPCR.

The combination of DHA with radiation did not alter any of the miRs that were measured (*miR-34a*; *miR-96*; *miR-146*; *miR-181a*; *miR-148a*; *miR-148b* and *miR-152*), but DHA alone up-regulated *miR-146a* and *miR-181a*. With DHA treatment alone, it was sufficient to pose significant negative effects on U87 cells. In the case of mRNAs, they found up-regulation of oxidative stress related genes including anti-inflammatory heme oxygenase (decycling) 1 (*HMOX1*) and pro-apoptotic NAD(P)H dehydrogenase, quinone 1 (*NQO1*).

Genes related to endoplasmic reticulum (ER) stress were also altered encompassing the pro-survival G protein-coupled receptor 78 (*GPR78*) and pro-apoptotic DNA-damage-inducible transcript 3 (*DDIT3*). Early growth response protein 1 (*EGR1*) is an early-response gene in radiotherapy that coordinates cell differentiation and growth. It was up-regulated with DHA treatment. The Notch signaling pathway was likewise altered through up-regulation of *NOTCH1*. Despite validation of direct *miR-146a* and *miR-181* targets were not performed in this study, the up-regulation of multiple genes indicates probable involvement of these miRs in these pathways. Many of these pathways including oxidative stress, ER stress and Notch signaling are known to relate with inflammation [69].

Portions of the DHA-regulated miRs similarly correspond to miRs that were altered in AD (**Error! Reference source not found.**). Some of these have also been discussed earlier to engage in inflammation including *miR-15*, *miR-27*, *miR-181* and *miR-146*. Aside from these inflammation-related miRs already discussed, some of the other DHA-regulated miRs are likewise known to associate with inflammation. These includes RECK, lipid metabolism, oncology and stress pathways [70-71]. Through this elucidation of DHA anti-inflammatory mechanisms, they offer valuable insight as strategy against AD.

The two studies described demonstrate the potential for DHA to affect miR expression within CNS. While they do provide beneficial preliminary intuition, the cell specificity of DHA effects [72] signifies the importance of establishing their effects on AD-related neuronal and neuroglial miR expression.

The objective to investigate their underlying anti-inflammatory mechanism in AD requires thorough considerations. For *in vitro* studies, the considerations for AD-relevant cell lines or primary cells are necessary. As described above, the parameters of the DHA supplemented is equally important. Furthermore, the miR methodology demands the adoption of robust procedures that are comparable to high quality studies. Animal models and human studies will also be useful. As addressed above, regards need to be paid for the characterization of AD patients, brain section that is profiled and sample size.

6. How MicroRNAs Contribute to Future Strategies against Alzheimer's Disease

By understanding miRs that are responsible for anti-inflammatory effects of DHA on AD, improvements can be made to existing tactics as well as development of novel strategies. The first miR therapy is presently being tested under clinical trial to explore *miR-34* replacement as an anti-tumor therapy [73].

Exogenous miRs offer possible solution where exogenous miR transfer from dietary origin was first documented in 2012 that discovered the presence of rice-derived miRs in human serum [74]. It is feasible for miRs to survive gastrointestinal digestion due to their well-appreciated stability [75]. This property is ascribed to their selective cellular export into various transport mechanisms [75,76]. The extracellular miRs can then be taken up by recipient cells to mediate function distally [75,77,78].

Newer studies further support food-mediated miR transfer into human, porcine and murine biological fluids from plants and milk [79-81]. By contrast, there are similarly studies that refute this notion as shown in bees, mice, macaques and human [82,83]. The presence of miRs from other species have been recently reported to possibly arise from undesirable contamination or artefacts of sequencing methodologies [84]. Based on these arguments, dietary transfer of miRs remains to be concluded. Additional considerations needs to be made whether the amount of exogenous miR transferred translate into biological significance. Nevertheless, these demonstrate the feasibility of using exogenous miRs as strategies against AD.

The concept of exogenous miR supplementation for AD requires knowledge of miR mechanisms of how DHA antagonizes AD inflammation. The miRs that are responsible can be supplemented to create novel food products or nutraceuticals. Genetic engineering can likewise be adopted to enhance levels of these miRs within foods. In an alternative perspective, the efficiency of miR-modulation by DHA in AD can be enhanced through understanding which aspects of the DHA molecule (e.g. unsaturation, stereospecificity, allylism) are responsible for its anti-inflammatory effects. This can lead to fabrication of optimized DHA and derivatives to maximize their miR effects to antagonize AD inflammation.

AD is afflicted with a pathological state of inflammation and this can be counteracted through anti-inflammatory effects of DHA. They underlying mechanism likely involves miRs and through its elucidation, novel strategies can be developed to combat AD. AD is highly prevalent, affecting 1 in 3 elderlies above 85 years of age. It is the third leading cause of death in United States and attributes to a financial burden of \$200 billion annually. Any prevention, retardation, termination or reversal strategies against AD will reduce the significant and rapidly growing societal burden attributed by AD.

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