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Omega-3 Essential Fatty Acids Modulate Initiation and Progression of Neurodegenerative Disease

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Abstract The significance of the selective enrichment in omega-3 essential fatty acids in photoreceptors and synaptic membranes of the nervous system has remained, until recently, incompletely understood. While studying mechanisms of cell survival in neural degeneration, we discovered a docosanoid synthesized from unesterified docosahexaenoic acid (DHA) by a 15-lipoxygenase (15-LOX), which we called neuroprotectin D1 (NPD1; 10*R*,17*S*-dihydroxydocosa-4*Z*,7*Z*,11*E*,13*E*,15*E*,19*Z* hexaenoic acid). This lipid mediator is a docosanoid because it is derived from the 22 carbon (22C) precursor DHA, unlike eicosanoids, which are derived from the 20 carbon (20C) arachidonic acid (AA) family member of essential fatty acids. We discovered that NPD1 is promptly made in response to oxidative stress, as a response to brain ischemia–reperfusion, and in the presence of neurotrophins. NPD1 is neuroprotective in experimental brain damage, in oxidative-stressed retinal pigment epithelial (RPE) cells, and in human brain cells exposed to amyloid-β (Aβ) peptides. We thus envision NPD1 as a protective sentinel, one of the very first defenses activated when cell homeostasis is threatened by imbalances in normal neural function. We provide here, in three sections, recent experimental examples that highlight the specificity and potency of NPD1 spanning beneficial bioactivity during initiation and early progression of neurodegeneration: (1) during retinal signal phototransduction, (2) during brain ischemia–reperfusion, and (3) in Alzheimer's disease (AD) and stressed human brain cell models of AD. From this experimental evidence, we conclude that DHA-derived NPD1 regulation targets upstream events of brain cell apoptosis, as well as neuroinflammatory signaling, promoting and maintaining cellular homeostasis, and restoring neural and retinal cell integrity.

Keywords Aging · Amyloid beta (Aβ) peptide · Beta-amyloid precursor protein (βAPP) · Cyclooxygenase-2 (COX-2) · Docosahexaenoic acid (DHA) · Hydroxynonenol (HNE) · Neuroprotectin D1 (NPD1) · Phospholipase A2 · Polyunsaturated fatty acid (PUFA)

Abbreviations

Aβ42	amyloid beta 42 amino acid peptide
AA	arachidonic acid
ADAM	a disintegrin and metalloprotease
AD	Alzheimer's disease
ALA	α-linolenic acid
ApoE4	apolipoprotein E4 allele
BACE	β-amyloid cleavage enzyme
βAPP	beta-amyloid precursor protein
COX-2	inducible cyclooxygenase-2
$cPLA_2$	cytosolic phospholipase A_2
DHA	docosahexaenoic acid
EPA	eicosapentanoic acid
HETE	hydroxyeicosatetraenoic acids
HNE	hydroxynonenal
LOX	lipoxygenase

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LPX	lipoxins
LTR	leukotrienes
NFT	neurofibrillary tangles
NPD1	neuroprotectin D1
NVU	neurovascular unit
PG	prostaglandin
PLA_2	phospholipase A_2
POS	photoreceptor outer segment
PUFA	polyunsaturated fatty acid
ROS	reactive oxygen species
RPE	retinal pigment epithelial
SP	senile plaque
sAPPα	soluble amyloid precursor protein alpha fragment
TNFAIP2	tumor necrosis factor alpha inducible protein-2 (B94)

Introduction

Dietary-essential omega-3 polyunsaturated fatty acids (PUFA) such as docosahexaenoic acid [DHA; *all-cis*-docosa-4,7,10,13,16,19-hexaenoic acid; 22:6(n–3); *cervonic* acid; docosa-hexaenoyl; chains of membrane phospholipids with 22C and 6 double bonds; MW 328.5] are vital for the normal, homeostatic operation of the central nervous system (CNS). Low DHA abundances are associated with cognitive deficits and impairments in visual function. Indeed, overwhelming evidence suggests that adequate dietary intake of PUFAs, and in particular life-long DHA bioavailability, provides considerable visual, neurovascular, cardiovascular, and neurological health benefits [1–13].

In the biosphere, DHA is primarily synthesized de novo in photosynthetic and heterotrophic microalgae, especially of the genus *Crypthecodinium* and *Schizochytrium*, where it becomes highly enriched as a major PUFA in these single cell marine heterotrophs. DHA is subsequently concentrated up the marine food chain where it reaches its highest concentrations, of up to 11.1% (w/w) in fish oil from salmon (Salmonidae). In addition to these marine sources, DHA may also be synthesized via an elongation and desaturation of the twenty carbon (C_{20}) eicosapentanoic acid [EPA; 20:5(n-3)], or elongation of the eighteen carbon (C_{18}) n-3 fatty acid, α-linolenic acid [ALA; 18:3(n-3)] which is particularly enriched in the photosynthesizing terrestrial plants chia (*Salvia hispanica*), flax (Linaceae), soybean (Glycinae) and walnut (Juglandaceae) [1–6].

DHA is the most abundant omega-3 PUFA in the mammalian brain and retina, and up to 60% of all fatty acids esterified in neuronal plasma membrane phospholipid consist of DHA [4–7]. Interestingly, mammalian endothelial cells lining the cerebrovasculature and astroglial support cells have a very limited capacity to synthesize DHA from ALA and other (n-3) precursor fatty acids, an attribute also lacking in neurons. Whether brain cell synthesis of DHA contributes significantly to total brain DHA is not clear. High concentrations of DHA in the vascular endothelium suggests that DHA is taken up from the diet, gastrointestinal and systemic circulation, via blood plasma DHA carriers including specific fatty acid binding lipoprotein transporters [4–6]. In humans during postnatal development, rapid accretion of DHA in the brain and retina takes place; dietary α-linolenic acid [ALA] is first taken up by the liver, where elongation and desaturation to DHA occur followed by transit through the blood stream to brain and retinal targets. These events coincide with photoreceptor development and synaptogenesis [4–7]. Cells of the brain and retina therefore have an accessible and expedient reservoir of esterified DHA, and through highly regulated phospholipase-mediated activities liberate membrane-esterified DHA to function in neuroprotective, anti-inflammatory, and cell-fate signaling roles [6–12]. In general, the beneficial neurophysiological actions of DHA in the brain and retina are thought to occur via several interrelated and probably highly integrated mechanisms, in part through DHA's direct support of plasma membrane structure, fluidity, and function, and in part through the generation of 22C docosanoids using DHA as the immediate precursor. Neuroprotectin D1 (NPD1) is the first identified DHA-derived oxygenated mediator that elicits potent neuroprotective effects at nanomolar concentrations in the brain and retina. This compound is formed through tandem phospholipase A_2 (PLA_2)-15-lipoxygenase (15-LOX) action on free DHA, via a 16,17S-DHA epoxide intermediate [6, 10–12]. The bioavailability of free, unesterified DHA is a highly regulated event, and free unbound DHA, normally undetectable under basal homeostatic conditions, increases during brain injury, cerebral ischemia, epileptic seizures, and in other neuropathological conditions. Changes in oxidative stress, modulated in part by bioavailable antioxidants, may further affect the kinetics and efficacy of both DHA and NPD1 actions [2–7]. For example, the overall bioavailability of DHA appears to be dependent, in part, upon the redox state of brain cells, as the co-supplementation of DHA with antioxidants such as the carotenoid lutein has been shown to significantly improve cognition and overall brain signaling functions in clinical studies of the aged [2, 14–16]. Interestingly, the neurotrophin pigment epithelium-derived factor (PEDF) and the brain-derived neurotrophic factor (BDNF), each stimulates NPD1 synthesis, which in turn, modifies the expression of Bcl-2 family members by activating anti-apoptotic proteins by decreasing pro-apoptotic proteins and by attenuating caspase-3 downstream effects during oxidative stress [11–13, 17].

This review is focused on the positive regulatory and neuroprotective actions of DHA and NPD1 (1) during retinal phototransduction, (2) DHA–NPD1-mediated neuroprotective events during homeostatic cerebrovascular functions and ischemia–reperfusion, and (3) in Alzheimer's disease (AD), the most prevalent neurodegenerative disorder of the aged in modern, industrialized societies.

DHA and NPD1 Neuroprotective Functions During the Process of Phototransduction

Photoreceptors renew membrane disks containing the phototransduction apparatus and DHA intermittently, via the shedding of their tips and subsequent phagocytosis by retinal pigment epithelial (RPE) cells [11, 12, 18–20]. This renewal is essential for vision, and it is thought that it engenders survival of the photoreceptors while maintaining RPE cell function. At the same time, new membrane disks are made at the base of the outer segments; their length remains constant, and phototransduction integrity remains remarkably unchanged throughout many decades of life. This process is maintained in spite of the fact that the photoreceptors are in an oxidative stress-prone environment involving high fluxes of visible light energy, high O_2 consumption, and high rates of PUFA flux [10, 11, 19]. We have demonstrated that phagocytosis of the photoreceptor outer segment (POS) markedly attenuates oxidative stress-induced apoptosis in cultured human RPE (ARPE-19) cells, and that this phenomenon does not seem to be a generalized outcome of phagocytosis because phagocytosed non-biological polystyrene microspheres do not elicit this specific form of protection [20]. Further, the free DHA pool size and NPD1 content were found to be increased during POS phagocytosis, but not during phagocytosis of polystyrene microsphere. Other retinal lipid mediators such as lipoxin A4, and 12(*S*)- and 15(*S*)-hydroxyeicosatetraenoic [12(*S*)- and 15(*S*)-HETE] acids under these conditions were not altered. Oxidative challenge to RPE cells undergoing POS phagocytosis further increased DHA and NPD1 content, and NPD1 was found within the RPE cells as well as in the culture medium, suggesting both autocrine and paracrine bioactivities. Using deuterium-labeled DHA, we also demonstrated that the bioavailability of free DHA increases during oxidative stress, with concomitant NPD1 synthesis and modulation in ARPE-19 cells [12, 20]. These data thereby suggest a distinct retinal mechanism of DHA–NPD1 signaling that promotes survival of photoreceptor and RPE cells by enhancing the synthesis of NPD1 during the phagocytosis of photoreceptor disks and promotes a specific refractoriness to oxidative stress-induced apoptosis in RPE cells. These mechanisms, in turn, underscore the critical roles of DHA and NPD1 in the maintenance of homeostatic retinal cell integrity and function [11, 12, 20–22]. Disruptions of the sentinel role of NPD1 in photoreceptor renewal may participate in age-related macular degeneration (AMD) and in other retinal degenerations leading to blindness [11, 12]. Recent clinical and epidemiological findings show an association of consuming a diet rich in DHA with a lower progression of AMD, in addition to the essential general support of critical synaptic signaling functions of dietary omega-3 fatty acids [23–26]. Importantly, however, these early findings should be interpreted with caution. One recent study of transgenic mice expressing mutant human ELOVL4 (a transgenic model of Stargardt macular dystrophy), when crossed with mice expressing the fat-1 protein (which can convert n-6 to n-3 PUFA) led the authors to conclude that DHA was not beneficial for the treatment of retinal degeneration in this animal model of human STGD3 dystrophic eye disease [27]. Hence, DHA might not, by itself, be able to overcome overwhelming genetic disturbances associated with transgenically driven degenerative retinal pathology [27, 28]. While clearly these in vivo and in vitro roles of DHA and NPD1 need to be reconciled, essential differences in immune, inflammatory biochemistry, physiology and genetic anomalies associated ELOVL4 transgenics in mouse and human retina may help resolve these discrepancies [28].

DHA–NPD1-mediated Neuroprotective Events During Brain Ischemia–Reperfusion

The beneficial health effects of dietary fish oils on cardiovascular and brain functions in rodents and primates have been known for at least 30 years [29–31]. Dietary DHA and its precursors were originally predicted to enhance CNS function by promoting plasma membrane flexibility and resistance to membrane peroxidization through up-regulation of the concentration of esterified DHA in membrane phospholipids [29]. The health benefits of substituting PUFA's for dietary saturated fatty acids typically result in lowered heart rate, relaxation of smooth cardiac muscle and decreased blood pressure, increased ventricular ejection, and reduced risk of developing malignant cardiac arrhythmias; these have important neurological implications as the brain is the most highly vascularized of all CNS tissues [30–32]. The potential benefits of DHA in modulating the pathogenic events surrounding the brain and retinal ischemia–reperfusion were further demonstrated when a protective effect from oxidative damage after post-ischemic oxidative stress in fetal rat brain and in retinal ischemic injury following dietary DHA supplementation was reported [32, 33].

Ischemia is defined as an inadequate systemic blood supply to a local area due to blockage of the vasculature to that area; these events are among the most common causes

of debilitating heart attack and stroke in humans [33–35]. Reperfusion is defined as the restoration of blood flow to that formerly deprived area; it is the resurgence of nutritive support to formerly starved areas which underlie many key pathogenic manifestations [34]. The study of ischemia–reperfusion in experimental animals has greatly expanded our understanding of the molecular and physiological changes which accompany both heart attack and stroke [11, 32–36].

During ischemia–reperfusion, DHA appears to be initially released and used for the synthesis of stereospecific messengers such as NPD1 through oxygenation pathways, thus eliciting in situ neuroprotection via the local recruitment of anti-apoptotic signaling mechanisms [9–13, 34–37]. For example, immediate availability of anti-apoptotic members of the Bcl-2 family of proteins (such as Bcl-2, Bfl-1, and Bcl-xl) is positively modulated by NPD1, whereas pro-apoptotic Bcl-2 proteins (such as Bax and Bik) are negatively regulated, as are the arrival of leukocytes due to the breakdown of the neurovascular unit (NVU, 37). The NVU, consisting of a highly integrated complex of neurons, astrocytes, oligodendrocytes, microglia, and endothelial cells of the microvasculature also form the basis for the blood–brain barrier, whose function is, in part, to regulate lipid-mediator transport from the hepatic circulation into brain compartments [11, 35]. DHA also has important roles in pro-survival signaling cascades after ischemia–reperfusion injury. For example, DHA accelerates AKT translocation and activation and has binding affinity with an important PPAR-gamma family of ligand-activated nuclear receptors [11, 36]. Along with the PPAR-gamma receptors, phospholipase A_2 (PLA_2) and the inducible cyclooxygenase 2 (COX2), the two key enzymes in the metabolism of polyunsaturated fatty acids, play determinant roles in cytokine-induced inflammatory signaling suppressed by DHA and NPD1 [11, 36, 37].

Since the original reports of DHA's potential neuroprotective effects, treatment protocols have been optimized to maximize DHA's efficacy. For example, in Sprague–Dawley rats treated with DHA complexed to albumin after 2 h of middle cerebral artery occlusion (MCAO), improvement in neurobehavioral scores at 72 h significantly exceeded that of other treatment groups, and the extent of histological protection, such as an 86% reduction in cortical infarction size, was highly significant and tended to surpass the degree of cortical protection produced by native albumin at 1.25 g/kg. DHA-albumin, administered at 0.63 g/kg, but not native albumin, also significantly reduced subcortical infarction and markedly diminished brain swelling in MCAO. Importantly, lipidomic analysis of DHA-albumin-treated post-ischemic brains revealed a large accumulation of the neuroprotective DHA-derived metabolite NPD1 in the ipsilateral hemisphere [38].

DHA and NPD1 Contribute to Healthy Brain Aging and are Implicated in Alzheimer's Disease (AD)

Abundant epidemiological, molecular, neuropathological, and clinical data support the hypothesis that a complex interplay of aging, environmental, molecular, and genetic factors contribute to the etiogenesis of Alzheimer's disease (AD). Pathogenic processes which characterize AD neuropathology exhibit four consistent features: (a) they are progressive with aging; (b) they act in a chronic, cooperative, and integrative fashion; (c) they are highly specific to the neurons of the association neocortex, hippocampus which constitute the evolutionarily recent limbic system of the human brain; and (d) once initiated, the pathogenic effects of these processes exhibit positive feedback, perpetuating until brain cell defenses are exhausted, culminating in the irreversible degeneration and death of neurons. Mitochondrial dysfunction and focused oxidative damage, including peroxidation of membrane lipids and PUFAs by ROS and heightened pro-inflammatory gene expression, appear to be among the earliest events in AD affected tissues [39–45]. The ~110 kDa integral type-1 transmembrane glycoprotein beta-amyloid precursor protein (βAPP) holoprotein, central to the 'amyloid cascade hypothesis' of AD, and the generation of neurotoxic Aβ peptides 37 to 43 amino acids in length (Aβ37–Aβ43) from βAPP via the tandem actions of the 'beta–gamma-secretase systems' appear to be intimately involved with these oxidative stress, pro-inflammatory signaling, and pro-apoptotic events [44, 45].

Of particular interest is the self-aggregating, amyloidogenic, pro-inflammatory Aβ42 peptide that progressively accumulates during the course of AD, and a soluble form of a 612 amino acid protein known as sAPPα, which is neuritogenic and neurotrophic, promoting neuronal survival and whose formation precludes the generation of more neurotoxic Aβ peptide species [44–48]. Aβ oligomers have been shown to significantly increase JNK and tau phosphorylation in cultured hippocampal neurons while DHA was shown to inhibit JNK activation. The alternate neurotoxic and pathogenic or neuroprotective roles of the Aβ42 peptide and the neurotrophic sAPPα protein in neurodegeneration have been extensively addressed in a series of recent comprehensive reviews [2, 46–53].

One fundamental feature of the 'amyloid cascade hypothesis' of AD is the progressive generation of neurotoxic Aβ42 peptides derived from the tandem beta–gamma-secretase cleavage of βAPP into ragged, pathogenic Aβ peptide fragments. The unusual gamma-secretase cleavage site within the hydrophobic transmembrane domain of βAPP suggests that pathological events, which are membrane disruptive or which alter or disorganize lipid bilayer structure or fluidity, contribute to Aβ peptide generation.

The progressive accumulation, condensation, and aggregation of fibrillar Aβ42 peptides into neuritic senile plaques further support ROS generation, oxidative stress, pro-inflammatory and pro-apoptotic gene expression and signaling, resulting in neuronal dysfunction and an irreversible loss of brain cell homeostasis [44–48]. The neurotrophic effects of DHA on membrane molecular biology in aging are well-known, and DHA may repress lipid raft-mediated mechanisms involving Aβ42 peptide generation while favoring the mechanism of generation of the more neurotrophic sAPPα [47, 48, 53]. Beta-secretase and/or gamma-secretase activities that down-regulate neurotoxic Aβ42 peptide production and subsequent ROS generation appear to be affected by the lipid raft composition and microenvironment of these membrane integral and peripheral enzyme systems (Fig. 1). The effects of NPD1 on the biophysics and kinetics of the membrane-imbedded secretase-mediated cleavage mechanisms of βAPP are just beginning to be fully understood, and knowledge of how NPD1 impacts specific secretase activities in the lipid raft environment will be essential in the future design of more effective and selective Aβ42 peptide lowering strategies in the clinical management of AD [54, 47, 53].

We now know that both DHA and NPD1 abundance, and their neuroprotective roles, are significantly reduced in the hippocampal CA1 and association neocortical areas from AD patients when compared to age-matched controls. DHA and NPD1 changes are not significant when regions such as the thalamus that typically exhibit no significant AD-specific neuropathology are compared between AD and controls [9, 12, 54]. AD affected brain tissues are further associated with pathological markers for excessive oxidative stress including prominent markers of lipid peroxidation, such as 4-hydroxy-2-trans-nonenal (HNE), whose levels positively correlate with AD severity [51, 52]. Notably, and because of its extensive desaturation, DHA is also a primary lipid peroxidation target during oxidative brain cell injury and in AD [51, 52]. During periods of up-regulated oxidative stress, DHA is readily oxidized non-enzymatically into the prostaglandin-like F4-, D4-, E4-,

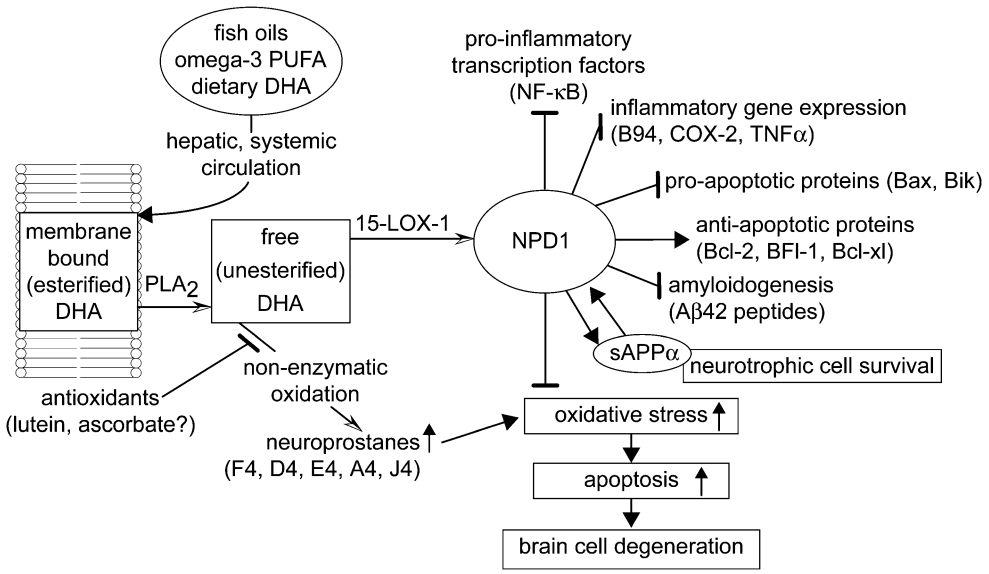

Fig. 1 Fish oils, omega-3 PUFAs, dietary DHA, and neuroprotectin D1 (NPD1) signaling circuits in health and disease. DHA obtained through the diet is transported via gastrointestinal, hepatic, and systemic circulations where it preferentially esterifies into neuronal, synaptic, and retinal membranes. From here, membrane-esterified DHA may be liberated from membrane-bound stores via a highly regulated membrane-associated phospholipase A_2 (PLA_2) to generate free (unesterified) DHA. Subsequent lipoxygenase (15-LOX) or 15-LOX-like activities generate the 10*R*,17*S-dihydroxy-docosa-4Z,7Z,11E,13E,15E,19Z* hexaenoic acid docosatriene known as NPD1. As is described more fully in the text, NPD1 has been shown to convey multiple neuroprotective benefits including inhibition of pro-inflammatory transcription factors such as NF-κB, coupled to inhibition of inflammatory gene expression, repression of the expression of pro-apoptotic proteins Bax and Bik, up-regulation of the anti-apoptotic proteins Bcl-2, Bfl-1(A1) and Bcl-xl, a suppression of amyloidogenesis via inhibition of the generation of Aβ42 peptides, and an up-regulation of sAPPα and neurotrophic cell survival. Reactive oxygen species (ROS) are a consistent feature of degenerating brain and retinal tissue, suggesting a role for oxidation-related decrease in protein function, mis-regulation of the cellular redox-balance, oxidative stress and neurodegeneration leading ultimately to cell death. During oxidative stress, non-enzymatic oxidation of free DHA results in the formation of a family of prostaglandin-like neuroprostanes, a class of peroxidized lipids that further support oxidative stress, neuronal dysfunction and cellular apoptosis. That non-enzymatic reactions may be quenched by specific antioxidants and free radical scavengers such as lutein and curcumin supports the idea that the redox state of brain cells has bearing on either the neurotrophic or oxidative-neurotoxic pathways for DHA and NPD1 conversion [2, 20, 58, 64]. The potential effects of other common dietary antioxidants such as ascorbate (vitamin C) on DHA and NPD1 bioactivity may be beneficial, and but further cellular, animal and clinical studies are urgently required

A4- and J4-neuroprostanes that in turn trigger reactive oxygen species (ROS) evolution at the cytoplasmic or extracellular interface of the plasma membrane [52, 53, 55, 56]. Thus, these pathogenic cycles of oxidative stress and progressive lipid peroxidation are further perpetuated [51–53, 56]. Synthesis of F4-neuroprostane-containing amino-phospholipids may further adversely affect neuronal function as a result of alterations they induce in the biophysical properties of neuronal plasma membranes and membrane lipid raft domains [53, 56]. The abundance and speciation of neuroprostanes and HNE thereby reflect the general state of lipid peroxidation and abundance of ROS in stressed brain cells, and may be useful biomarkers for the extent of brain cell oxidation, degeneration, and neuronal dysfunction, as well as for the therapeutic efficacy DNA, NPD1, anti-oxidative drugs, and their neuroprotective actions. The nature of the switch from neuroprotective to membrane disruptive and oxidative roles for DHA, such as the generation of NPD1 versus neuroprostanes, is under extensive research study, as the signaling axis along the PLA_2-15-LOX axis and related enzyme pathways may be profitably exploited to modulate NPD1 generation, bioactivity, and the ensuing neuroprotective effects on brain cell survival [2, 57] (Fig. 1). The potential paracrine interactions of pro-inflammatory and pathogenic signaling among the neurons, astroglia, endothelial cells, and the extracellular space of the NVU are only beginning to become understood [58]; aforementioned, DHA and NPD1 autocrine and paracrine signaling appears to be a defining feature of RPE cells during photoreceptor outer segment-mediated phagocytosis [20].

Our laboratory has further explored the significance of NPD1 in cellular models of AD that involve human primary brain cells and that when stressed with pro-inflammatory cytokines such as interleukin-1β (IL-1β) or Aβ42 peptides can recapitulate, to a high degree, pro-inflammatory gene expression patterns and pro-apoptotic aspects characteristic of AD neuropathology. For example, primary co-cultures of human neurons and astrocytes when challenged with Aβ42 peptides or transiently transfected to overexpress APPsw (using a plasmid containing APP695sw, bearing the double Swedish mutation K595N-M596L) using the FuGENE 6 transfection protocol (Roche Applied Sciences, Mannheim, Germany) show that NPD1 down-regulates the amyloidogenic processing of βAPP and switches off pro-inflammatory gene expression of tumor necrosis factor alpha (TNF-α), COX-2, and a TNF-α inducible pro-inflammatory element (TNFAIP2) known as B94, thereby promoting long-term neural cell survival [9–12, 59–61; manuscript in preparation]. Unpublished data from our lab suggest that NPD1 is up-regulating the beneficial α-secretase (a disintegrin and metalloprotease, ADAM10) and down-regulating a β-secretase (β-amyloid cleavage enzyme, BACE1) to shift the processing of the membrane-spanning βAPP into more neurotrophic, non-amyloidogenic pathways. Interestingly, the TNF-α, COX-2, and B94 genes all contain NF-κB as a prominent regulatory transcription factor in their immediate upstream promoters, and up-regulation of NF-κB-DNA binding is a consistent feature of brain regions exhibiting AD inflammatory neuropathology [13, 62, 63]. Hence, NPD1 not only targets the upstream regulatory events of apoptosis at multiple checkpoints, but also simultaneously directs the down-regulation of neuro-inflammatory gene signaling. This, in turn, rescues and promotes the homeostatic maintenance of brain cell integrity.

Conclusion

Important insights into the neurobiology of DHA and NPD1 both in the aging brain and in neurodegeneration have been obtained. In summary, the beneficial contributions of DHA and NPD1 to brain and retinal health appear to occur via several interdependent mechanisms that include (a) the enhancement of membrane functional integrity including superior plasma and synaptic membrane flexibility, lipid bilayer fluidity, and a 'beneficial' configuration of membrane lipid raft order and viscosity; (b) the recruitment and up-regulation of anti-apoptotic members of the Bcl-2 gene family such as Bcl-xl and Bfl-1 (A1); (c) the repression of the expression of pro-apoptotic signaling including the apoptotic drivers Bax and Bik; (d) the down-regulation of the activation of inflammatory signaling mediators such as the prostaglandin synthesizing arachidonic acid cascade enzyme COX-2; and (e) the modulation of kinase-mediated Bcl-2 gene family phosphorylation, such as directed inhibition of the JNK survival signaling cascade (Fig. 1). How specific cellular and signaling components of the NVU contribute to these neuroprotective signaling mechanisms are currently not totally clear; however, each of the major cell types of the human NVU—neurons, astrocytes, oligodendrocytes, microglia, and endothelial cells of the brain's microvasculature—can now be propagated in primary culture so their individual contribution to DHA–NPD1 signaling, integration, and cross-talk can be further explored.

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