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REVIEW

Membrane Aging as the Real Culprit of Alzheimer's Disease: Modification of a Hypothesis

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Abstract Our previous studies proposed that Alzheimer's disease (AD) is a metabolic disorder and hypothesized that abnormal brain glucose metabolism inducing multiple pathophysiological cascades contributes to AD pathogenesis. Aging is one of the great significant risk factors for AD. Membrane aging is first prone to affect the function and structure of the brain by impairing glucose metabolism. We presume that risk factors of AD, including genetic factors (e.g., the apolipoprotein E ε4 allele and genetic mutations) and non-genetic factors (such as fat, diabetes, and cardiac failure) accelerate biomembrane aging and lead to the onset and development of the disease. In this review, we further modify our previous hypothesis to demonstrate "membrane aging" as an initial pathogenic factor that results in functional and structural alterations of membranes and, consequently, glucose hypometabolism and multiple pathophysiological cascades.

Keywords Alzheimer's disease · Membrane aging · Amyloid-β · Thiamine

Introduction

Alzheimer's disease (AD) is the most common form of dementia among the elderly and manifests as irreversible and progressive impairment of cognitive functions involving learning and memory, language and perceptual skills, and orientation and problem-solving functions. Late-onset AD diagnosed in people > 65 years of age accounts for $> 90\%$ of the total cases and early-onset AD that occurs in younger individuals only makes up $< 10\%$ [1]. Pathologically, AD is characterized by intracellular neurofibrillary tangles due to Tau hyperphosphorylation and extracellular amyloid plaques [2]. AD is predicted to affect 1 in 85 people globally by 2050 [3]. The number of AD patients in Mainland China is now > 5.7 million. Statistically, the incidence of AD is 6.3 per 1000 person-years. With the heavy economic burden for individuals, families, and society, AD is now of great concern [4]. However, there is still a lack of effective therapy and a convenient early-diagnostic approach for AD.

AD is an age-related neurodegenerative disease with explicit dysfunction of glucose metabolism. Furthermore, brain glucose hypometabolism appears decades prior to the overt clinical symptoms of AD and is closely correlated with the degree of cognitive impairment and progression of the disease [5, 6]. More specifically, Song *et al.* demonstrated that glucose deregulation is positively correlated with cognitive decline and could be an objective biomarker for pre-clinical dementia [7]. By assessing glucose metabolism in patients with early-onset and late-onset mild cognitive impairment (MCI) using 2-[(18)F] fluoro-2-deoxy-D-glucose positron emission tomography (FDG-PET), Kim and colleagues found dramatically reduced glucose metabolism in frontal regions of the brain in both groups [8]. Moreover, it has been concluded that the decreased rate of glucose metabolism is an objective indicator for dementia prior to the clinical onset of AD, and even for MCI [9].

Glucose metabolism includes two main processes – glucose transport and intracellular oxidative catabolism. Astrocytes are vital to glucose utilization by neurons. They first capture glucose from blood vessels, convert it

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into lactate, and secrete it into extra-neuronal space. Neurons then take up lactate as the substrate for energy metabolism [10]. Glucose transporter 1 (GLUT1) and GLUT3 are mainly responsible for glucose transport in the nervous system. GLUT1 is ubiquitously expressed in the mammalian brain [11]. In addition, ~44% GLUT1 is present on the abluminal membrane of the blood-brain barrier, while only 25% occurs on the luminal membrane. The luminal to abluminal ratio of GLUT1 characterizes the glucose uptake of the brain, and clearly a higher luminal level of transporter represents increased glucose metabolism [12]. GLUT3 is mainly localized in peripheral regions and in neurons. The insulin signaling pathway is also necessary for trans-membrane glucose transport. Extensive abnormalities in insulin and insulin-like growth factor I (IGF-I) and IGF-II signaling mechanisms have been demonstrated in the AD brain, suggesting that AD could be a third form of diabetes [13]. For instance, insulin resistance has been widely described in AD pathology. It is defined as insulin-sensitive organs or tissues becoming less sensitive to peripheral insulin, resulting in an increased blood glucose concentration [14]. There is a positive link between insulin resistance and cognitive impairment in AD [15]. By evaluating fasting insulin resistance, Morris *et al.* found dramatically higher insulin resistance in memory-impaired elderly than in normal controls; however, there was no significant cognitive recovery after infusing insulin [16]. In addition, Adzovic and colleagues suggested that insulin resistance is age-dependent, since insulin injection relieved the cognitive deficits in young but not in aged mice with chronic inflammation [17].

The other process of glucose metabolism is intracellular oxidative catabolism, which consists of pathways in mitochondria and in cytoplasm. The mitochondria enclose the tricarboxylic acid (TCA) cycle and oxidative phosphorylation, while the pentose phosphate pathway (PPP) and glycolysis take place in the cytoplasm. The TCA cycle and glycolysis generate adenosine triphosphate (ATP) from ADP [18]. The PPP works as a metabolic pathway in parallel with glycolysis and produces nicotinamide adenine dinucleotide phosphate (NADPH) and pentose. NADPH is known to participate in the vital process of non-enzymatic anti-oxidation [19, 20]. Energy hypometabolism and oxidative stress are key, consistent, and the earliest abnormalities in AD and MCI. More specifically, increased levels of hippocampal oxidative stress and NADPH oxidase accompanied by amyloid plaque aggregation have been found in AD mice fed a high-fat diet [21]. By targeting the NADPH oxidase level, Cho *et al.* demonstrated that N-adamantyl-4-methylthiazol-2-amine (KHG26693) reduces the oxidative stress caused by amyloid-beta (Aβ) deposition [22]. The dysfunction of all processes in glucose metabolism, including abnormal glucose transport, mitochondrial disorder, and perturbed PPP, can be demonstrated in AD.

Three key enzymes in the Krebs cycle and PPP—the pyruvate dehydrogenase complex (PDHC), α-ketoglutarate dehydrogenase complex (KGDHC), and transketolase—play a vital role in glucose metabolism [23–25]. PDHC converts pyruvate into acetyl-CoA *via* the pyruvate decarboxylation process. In addition, acetyl-CoA may then be used in the citric acid cycle to perform cellular respiration, so that it links the glycolysis metabolic pathway to the citric acid cycle [26]. KGDHC is important in the TCA cycle as it is a rate-controlling step under physiological conditions [27]. In mammals, transketolase connects the PPP to glycolysis. It is a rate-limiting enzyme of the non-oxidative branch of the PPP, feeding excess sugar phosphates into the main carbohydrate metabolic pathways [28]. Thus, thiamine-dependent processes in brain glucose metabolism are critical to normal brain function.

There is an increased risk of thiamine deficiency (TD) among the aging population. Evidence indicates that TD is positively correlated with age rather than compound with other illnesses such as heart failure, angina, asthma and so on [29]. In mouse models of induced TD, Freeman *et al.* found that the activity of one of the thiamine-related enzymes—KGDHC—decreases in an age-dependent manner, further supporting the close relationship between TD and aging [30]. Studies have also shown that thiamine helps to protect hepatic cells from oxidative interference, which suggests that thiamine-related processes participate in the regulation of oxidative stress [31]. What is more, in a mouse model of cisplatin-induced oxidative stress, investigators found that pyrophosphate not only reduces lipid peroxidation but also reverses DNA damage [32].

Thus, abnormal brain glucose metabolism, especially thiamine-dependent processes, is critical to AD pathogenesis. The activities of the three key enzymes are significantly reduced in both brain and blood samples from AD patients [33]. Furthermore, as a common coenzyme of the three enzymes, thiamine diphosphate is significantly lower in AD patients than in controls with normal cognitive abilities and with other types of dementia [23, 34]. In addition, Pan *et al.* discovered that benfotiamine (a lipid-soluble thiamine derivative with better bioavailability) improved cognition in a small sample of AD patients [35]. However, the lack of direct genetic evidence suggests that brain glucose hypometabolism and thiamine deficiency may be secondary contributors to AD pathogenesis. The original pathogenic factor that results in abnormal brain glucose metabolism in AD should be further explored.

Membrane Aging and the AD Patient: Emphasis on Membrane Fluidity

As mentioned above, aging is the most significant risk factor for AD. It is evident that many elements such as DNA damage, genetic instability, and the accumulation of waste and free radicals lead to and/or accelerate the aging process [36–39]. From the macroscopic view, in terms of age-associated brain volume changes, neuroimaging studies have proposed that AD patients have specific morphological brain alterations with an augmented global aging shift. For instance, atrophy in brain regions such as the hippocampus, temporal poles, and entorhinal cortex is significant in AD and MCI patients [40]. From the microscopic view, aging is an irreversible process accompanied by the progressive accumulation of changes in cells and tissues. In senescence, age-associated physiological modifications of the membrane, such as alterations of the membrane potential, abnormal activity of enzymes and receptors, disturbance of membrane lipid composition, and changes of membrane fluidity, constitute the membrane aging process [41, 42]. Particularly, membrane fluidity is a hallmark of membrane aging. By studying the membrane of isolated CA1 pyramidal neurons, Oh *et al.* found calcium influx across the membrane and the consequent change of neuronal membrane potential were dramatically enhanced in elderly mice [43]. Meanwhile, several pieces of evidence have indicated that changes of membrane potential with aging are also present across mitochondrial membranes. For example, Leaver and colleagues found that membrane potential alteration in mitochondria *via* calcium flux is a direct pointer for caspase-independent cellular death [44]. On the other hand, with regard to changes of enzyme activity in membrane aging, Jang *et al.* demonstrated that the activities of enzymes such as gamma-glutamyltranspeptidase, disacchridase, and alkaline phosphatase on the mammalian membrane are considerably decreased in aging individuals [45]. Maslova found that the activities of Na, K-ATPase and acetylcholinesterase are significantly lower in "old" erythrocyte membrane than in young erythrocytes [46]. Calpain, a Ca^{2+}-dependent protease, has been shown to decrease in the erythrocyte membrane of individuals > 70 years of age [47]. Naeim and colleagues found an abnormal distribution of receptors in aging membrane. Compared with young persons, the elderly (> 85 years old) manifest significantly lower levels of concanavalin A and immunoglobulin (SmIg) receptors on erythrocyte membrane [48]. Similar findings have been reported by Christadoss and colleagues in the rat brain membranes (except cerebellum). They found a loss of binding in selective membrane-bound opioid receptor in the aged group, owing to the disruption of membrane phospholipids [49]. In addition, an altered Ca^{2+} flux also causes membrane lipid variation [50]. Changes in membrane phospholipids are thought to be a result of the membrane aging process. More specifically, Norris and colleagues summarized that the increase of docosahexaenoic acid and the decrease of arachidonic or adrenic acid across both mitochondrial and microsomal membranes are age-dependent [51]. Furthermore, Alvarez and colleagues found that the membrane composition of peritoneal neutrophils is impaired, with a remarkable decrease of phosphatidylinositols in old mice compared with young mice [52].

Membrane fluidity is the viscosity of the lipid bilayer of the cell membrane, and is considered to be a core factor of membrane aging. It is influenced by lipid packing, which is impacted by many factors. For instance, variations of thermal energy [53], membrane phospholipid composition, lateral pressure [54], membrane voltage [55], release and binding of neurotransmitters [56], ion flux [57], signal transfer, and enzyme activity [58] are all elements that participate in changes of mammalian membrane fluidity. Consequently, the rotation and distribution of proteins and other molecules within the membrane are affected and their functions are impaired due to membrane aging [54].

It is well known that the mitochondrion is involved in aging and senescence, especially in AD. A double membrane-bound organelle and the main site of production of chemical energy as ATP *via* the respiratory chain, the mitochondrion is the major source of reactive oxygen species (ROS) [59]. The brain is the dominant consumer of ATP in the human body. Thus, the relationship between brain and mitochondrial function has been universally studied. As glucose is a dominant substrate of energy metabolism, and due to limited glycolytic capacity, neurons are enormously dependent on mitochondrial energy. Mitochondria are mostly present in neuronal synapses owing to the maximal demand for ATP production and energy utilization [60]. Therefore, mitochondrial dysfunction has been implicated in several neurodegenerative disorders such as Parkinson disease (PD) and AD, permanently or temporarily [61]. Mitochondrial dysfunction has also been found in other diseases, including mitochondrial disorders [62], type II diabetes, and heart failure [63]. It is not surprising that the heart is preferentially affected by mitochondrial disorders due to its high ATP consumption. Compromised oxidative phosphorylation and defective function of the electron transport chain are key to the majority of cardiac diseases associated with mitochondrial dysfunction [64]. Speculative mechanisms have been discussed in order to identify the relationship between mitochondrial dysfunction, insulin resistance, and type II diabetes. It has been indicated that in skeletal muscle, heart, and other tissues, destruction of mitochondrial

function alters the Ca^{2+} concentration and the activation of CaMKII, followed by a reduction of glucose transport isoform 4 (GLUT4) translocation, leading to insulin resistance [65]. Along with this, peptides released by mitochondria under cellular stress have parallel effects [66]. Under most circumstances, such insulin action is a crucial contributor to the pathogenesis of type II diabetes and is a critical factor of glucose metabolism [67].

On the other hand, mitochondrial function is closely linked to the structural integrity of the membrane, which depends principally upon the adequate interactions of proteins and lipids within the membrane [68]. Changes of mitochondrial membrane composition are critical indicators of the aging process. By using *Nothobranchius rachovii* as a model for analyzing mitochondrial membrane phospholipids, Lucas-Sanchez *et al.* found age-associated changes in lipid composition, including a decrease of cardiolipin and fatty acids, and an increase of sphingomyelin [69].

With regard to the aging process, the change of biomembrane fluidity may be interpreted as the major age-related structural change underlying the deterioration of brain function. Membrane fluidity is a main contributor to membrane aging. Evidence suggests that biomembrane fluidity is altered in ageing animals and AD patients. Eckmann and colleagues revealed that increased mitochondrial permeability and increasing oxidative phosphorylation are caused by a significant variation in plasma and mitochondrial membrane mobility. This might be an underlying biogenetic factor for neurodegenerative diseases such as AD and Huntington disease [70]. Similarly, Scheuer and colleagues found a slight reduction in the membrane fluidity of AD parietal cortex. Such an alteration specifically correlated with the histopathological modifications of AD [56]. Van Rensvurg *et al.* found that membrane fluidity increases in platelets and decreases in erythrocytes in blood samples from AD patients [71]. Li *et al.* focused on frontal cortical and hippocampal synaptosomes, and found significantly elevated membrane fluidity in aged mice as compared with a young group [72]. For other cell types, Hashimoto and colleagues detected remarkably lower membrane mobility of aortic endothelial cells in aged rats. This change was accompanied by an irreversible increase of the cholesterol component and lipid peroxide [73].

Factors Influencing Physical Properties of Membranes

It is well established that the cell membrane participates in a variety of cellular functions, including cytoskeletal anchoring, cellular transport, interaction or communication with other cells, and metabolic activities, which are implicated in some neurological disorders. For example, dysfunction of axonal transport is considered to be an early pathogenic feature of AD, amyotrophic lateral sclerosis, and PD [74].

In order to investigate neurotransmitter uptake in cellular senescence, Ando and colleagues used mouse neuronal synaptosomes, and found reduced enzyme activity as well as a less negative membrane potential in senescence, which may be caused by disorganization of the membrane lipid environment [75]. Regarding AD, neurotransmission and synaptic plasticity have been found to be decreased in an Aβ-induced AD mouse model [76]. Putting these all together, membrane aging, as one property of cellular senescence, will lead to changes in membrane neurotransmission, and may also be an inducer of AD pathogenesis.

The Effect of Amyloid-β on Membrane Fluidity

Extracellular senile plaques (SPs) and intracellular neurofibrillary tangles are classical pathological characteristics of AD [77]. SPs are composed of Aβ peptides surrounded by degenerating neuronal components and activated glial cells. Moreover, Aβ is derived from the sequential cleavage of amyloid precursor protein (APP), an integral membrane protein with a short cytoplasmic C terminus, a bigger extracellular glycosylated N terminus, and a membrane-spanning domain. APP695 as the most abundant isoform in the brain, is mainly located on neuronal membranes [78]. APP processing can be briefly described as two main pathways, the amyloidogenic and the non-amyloidogenic pathways. In the amyloidogenic pathway, APP is successively cleaved by β- and γ-secretases to generate Aβ [79, 80]. More specifically, β-secretase (BACE-1) cleavage produces the membrane-bound fragment CTFβ and the soluble β-secreted APP (sAPPβ). Interestingly, CTFβ seems to be cleaved in the middle of the membrane domain by γ-secretase [81], demonstrating that the generation of the diversity of Aβ isoforms (Aβ38/40/42) is associated with membrane properties. Alternatively, in the non-amyloidogenic pathway, APP is cleaved by α-secretases and further initiates neuroprotective and neurotrophic soluble APP (sAPPα) between amino-acids 16 and 17 within the Aβ domain, which has been suggested as a potential therapeutic target for AD [82, 83]. Since APP, α-, β-, and γ- secretases are all proteins located in the membrane, APP processing may be affected by the membrane properties through biophysical means such as molecular order and membrane fluidity [84]. To be more precise, the cleavage of APP by β-secretase occurs in lipid rafts of highly-ordered membrane microdomains rich in sphingolipids, saturated phospholipids, and cholesterol

[85, 86], whereas the cleavage by α-secretase commonly occurs in non-raft domains [87]. Recent research has demonstrated that the activity of γ-secretases can also be modulated by membrane thickness [88].

Several types of Aβ peptide can be generated in the membrane through the cleavage of APP. Furthermore, Aβ40 is considered to be the major species, while Aβ42 is regarded as the most toxic and fibrillogenic element in senile plaques [89]. Aβ can reinsert into a membrane after its release, forming ion-conducting pores or enhance accelerated aggregation on a membrane surface and initiate nonspecific structures, which finally result in the deformation and thinning of the membrane. The environmentally sensitive probe laurdan has been used by Parasassi and colleagues, who, using fluorescence microscopy, discovered that the insertion of Aβ peptide into artificial membrane bilayers modifies membrane lipid packing and more water molecules are partitioned into the membrane core [90]. In order to investigate the behavior of Aβ in the cell membrane, zwitterionic and anionic lipid bilayers were prepared artificially, and researchers found that the β-sheet tetramer is more stable than Aβ monomers. Moreover, the membrane permeabilization is mostly determined by membrane-bound Aβ oligomers rather than monomers [91].

Aβ has been demonstrated to promote the amyloidogenic processing of APP as well as reducing membrane fluidity [84, 92]. After Aβ treatment, synaptosomes exhibit decreased membrane fluidity [72]. Kremmer and colleagues demonstrated that the structure and function of the membrane can be affected by the aggregation state and pH of Aβ. By using the DPH (1, 6-diphenyl-1, 3, 5-hexatriene) florescent probe, they found that unaggregated Aβ peptides and neutral pH have no effect on membrane fluidity. In contrast, aggregated Aβ at pH 6 or 7 decreases membrane fluidity in a time- and dose-dependent manner [93]. It is evident that Aβ reduces membrane variability in a concentration-dependent manner, and this is caused by the effect of Aβ on Ca^{2+} signaling as well as neurotoxicity in the brain [92, 94].

Other studies have shown contradictory results. Chochina and colleagues found that Aβ accumulation increases the annular and bulk fluidity of synaptic plasma membrane (SPM) in cerebral cortex and hippocampus using the fluorescent probe pyrene and energy transfer, whereas Aβ has no effect on fluidity of the SPM in the cerebellum. The contradiction of the effect of Aβ on membrane fluidity may be due to the different types and lifetimes of fluorescent probes and their locations in the membrane environment. It might also be due to the different ages of the organism, the diversity of sources and preparation of the tissues, or whether Aβ is soluble or aggregated [95].

On the other hand, changes in membrane fluidity and membrane potential can influence the cooperative structure of Aβ, the means of Aβ-membrane binding, and membrane permeability [96]. For instance, when Aβ40 is exposed to small amounts of sodium dodecyl sulfate, which simulates the negatively-charged membrane environment, it is then converted into β-sheets [97]. Decreases in membrane fluidity can also hinder the function of ion channel proteins and cell surface receptors. For example, a reduction in membrane fluidity hampers the interaction of G protein and cholecystokinin (CCK) receptors in rat cortical bilayers [98]. CCK is a neuroendocrine peptide involved in many biological functions, including cell growth and proliferation. In addition, such reductions have been found to be accompanied by a decrease of Na^+/K^+–ATPase activity [99].

It has been demonstrated that specific Aβ–membrane interactions induce changes in the physical properties of membrane and may have deleterious consequences for cellular functioning. Such consequences may further lead to cellular toxicity and thus become diagnostic biomarkers of AD. Since APP, PS1 and PS2 are all trans-membrane proteins, mutations may promote membrane aging, especially changes of membrane fluidity.

The Composition and Biophysics of the Membrane are Influenced by Oxidative Stress

Increasingly, evidence has shown that the physical properties of membranes are most likely transformed in AD due to the specific effects induced not only by Aβ but also through downstream cellular signaling, including oxidative stress and Aβ-induced oxidant pathways.

ROS are formed as a kind of natural byproduct of normal oxidative metabolism and participate in cell signaling and homeostasis [100]. More specifically, they respond to a variety of pathological and physiological elements, and play a vital role in the pathogenesis of AD. Cells may generate ROS *via* two pathways – normal metabolism and signaling processes. Profound deleterious effects include the direct oxidation of molecules (e.g. lipid, protein, and DNA), indirect alteration of cellular functions and structures, and provoking cell death through the stimulation of over-production of ROS within the cell.

Direct interactions between ROS and the cell membrane lead to changes in its biophysical properties, such as changes of membrane molecules and fluidity. Zhu and colleagues demonstrated that homeostasis of the astrocyte membrane becomes more molecularly-ordered (or gel-like) by using H_2O_2, which has a joint effect of direct oxidation and indirect modifications mediated by the MAPK pathway [101]. Additional evidence has shown that the oxidant menadione also changes the molecular order and fluidity of

the plasma membrane, making it more gel-like [102]. Furthermore, both NADPH oxidase and phospholipase A2 (PLA2) regulate such alterations, except for the direct oxidation by ROS.

Membrane Components Impact Membrane Fluidity by Modulating Aβ–Membrane Interactions

Cholesterol is a sterol and an essential structural component of membranes. It is required to maintain both membrane structure and fluidity. Many biophysical parameters of lipid bilayers such as oxygen diffusion, membrane fluidity, membrane thickness, bilayer polarity, water permeability, and thermomechanical properties, are affected by the percentage and distribution of cholesterol [103–105]. Igbavboa and colleagues found higher levels of free cholesterol inside SPMs in the cytofacial bilayer leaflet than in the exofacial leaflet. Moreover, substantial cholesterol, which is located in the exofacial leaflet, mostly condenses in lipid rafts. This asymmetric distribution of cholesterol changes dramatically with aging [106]. It is known that the level of membrane cholesterol can be modulated by specific inhibitors such as statins. Three different statins, simvastatin, lovastatin, and atorvastatin, can translocate brain cholesterol from the cytofacial leaflet to the exofacial leaflet of the plasma membrane when non-transgenic mice are given these statins [107].

Many studies support the notion that cholesterol enrichment reduces membrane fluidity. After exposure to cholesterol, the expression of sAPPα is inhibited and cell mobility reduced, whereas lovastatin administration accelerates α-secretase processing and thus increases the membrane fluidity of astrocytes [108]. Similar results have been found in cultured glial cells and primary neurons [109, 110]. It is worth noting that high levels of cholesterol that reduce membrane fluidity may decrease sAPPα secretion by impeding the interaction of the substrate with its proteases [111]. Endogenous membrane β-secretase activity has also been investigated in human SH-SY5Y neuroblastoma cells and human platelets exposed to different cholesterol levels. The results showed that the membrane cholesterol level is positively correlated with the activity of β-secretase [112]. After cholesterol depletion, a reversible effect on membrane fluidity has been demonstrated. More precisely, a lower cholesterol concentration reduces APP internalization and finally leads to an increase in membrane fluidity and sAPPα generation [113]. What is more, Barrett *et al*. established that a domain in APP called C99 can be directly cut by the γ-secretase of Aβ oligomer generation. This domain has also proved to be a specific site that binds with cholesterol, which better explains the effect of cholesterol on amyloidogenesis [114].

The "prototype" ganglioside, GM1 (monosialotetrahexosylganglioside), is a member of the ganglio series of gangliosides, which contain one sialic acid residue. GM1 has important physiological properties and impacts neuronal plasticity and repair mechanisms [115]. It is negatively-charged in the outer leaflet of the membrane bilayer in most cells, especially in neurons. GM1 not only maintains the proper functional state of neurons but also participates in protein aggregation and amyloid cytotoxicity [116, 117]. In addition, Matsuzaki *et al*. concluded that Aβ interacts with GM1 in the neuronal membrane in the brains of AD patients, and further leads to the formation of GM1−Aβ complexes, which can act as seeds for Aβ aggregation [118]. It has been suggested that alterations of ganglioside composition can modify cell behavior and a drop in the cholesterol concentration leads to the augmented expression of GM1 [119]. This finding could help to interpret the increased vulnerability to amyloid aggregation in cells with depleted cholesterol [120].

Two major components of the cell membrane, phosphatidylserine (PS) and phosphatidylcholine (PC) can be obtained from a variety of readily-available sources such as meat, milk, egg-yolk, and soybeans. In particular, PS plays a key role in cell-cycle signaling, specifically as related to apoptosis [121]. Fewer reports have focused on the relationship between PS or PC and Aβ-membrane interactions. Simakova and colleagues proposed that the concentration of PS in neuronal membranes in AD brains contributes directly to the neurotoxicity of Aβ as well as Aβ-membrane binding [122]. In terms of PC, studies have revealed that compactly-packed PC in membranes provides a better environment for non-electrostatic Aβ binding and self-aggregation [123].

The Effect of Fatty-Acids on Membrane Fluidity

Fatty-acids are usually derived from triglycerides or phospholipids and are divided into saturated or unsaturated types. Fatty-acids with carbon–carbon double-bonds are unsaturated, whereas those without double-bonds are saturated. Fatty-acids are indispensable constituents of the cell membrane and play a vital role in brain development and function [124]. Polyunsaturated fatty acids (PUFAs) are abundant in the brain and have multiple functions. For instance, PUFAs participate in boosting synaptogenesis and neurogenesis, stimulating gene expression and neuronal activity, inducing antinociception, and preventing apoptosis and neuroinflammation [125]. By administering docosahexaenoic acid (DHA; a long-chain ω-3 PUFA) to C57/BL6 mice and mouse primary cortical neurons, Tan and colleagues found that endogenous DHA is neuroprotective since it acts against the Aβ oligomer-induced neuronal damage both *in vitro* and *in vivo* [126].

Similar results have been demonstrated by administering other types of fatty-acid, such as eicosapentaenoic acid (EPA) [127].

Fatty-acids directly integrate into cell membranes to alter cellular function by affecting their physical properties. The ability of fatty-acids to change membrane functions rely both on the trans/cis ratio of the unsaturated fatty-acids and the PUFA level [128, 129]. More specifically, studies have shown that membrane fluidity remains unchanged when the membrane PUFA level is between 80% and 90%. In contrast, membrane fluidity changes remarkably when the PUFA level is $< 80\%$, and this is accompanied by alterations of cellular composition and cell growth [130]. Studies have also found that not all types of unsaturated fatty-acid, but only those with 4 or more double-bonds, such as arachidonic acid, DHA, and EPA, are capable of promoting neuronal membrane fluidity and increasing the secretion of sAPPα [129]. By using a fluorescence probe, this study found that an increase of cytoplasmic membrane fluidity corresponds with an increase of the trans/cis ratio of the unsaturated fatty-acid [128]. Nevertheless, the incorporation of saturated acid in the membrane leads to a decrease of membrane fluidity [131].

The Effect of Phospholipases A2 on Membrane Fluidity

Phospholipases A2 (PLA2s) are enzymes characterized by the ability to specifically hydrolyze the sn-2 ester bond of phospholipids to generate arachidonic acid, and are universally found in mammalian tissues. PLA2s consist of several protein families with common enzymatic activities. The two most notable families are secreted and cytosolic A2 phospholipases. Other families include Ca^{2+}-independent PLA2 and lipoprotein-associated PLA2s (Lp-PLA2). Lp-PLA2 is also known as platelet activating factor acetylhydrolase [132]. These enzymes are responsible for the maintenance of phospholipid homeostasis in the cell membrane. Changes of membrane-associated PLA2 activity have been found in several neurological disorders, such as cerebral ischemia, multiple sclerosis, PD, and in particular, AD [133]. The activity of sPLA2 is significantly higher in the cerebrospinal fluid of patients with AD and multiple sclerosis, and may be regarded as a biomarker of increased permeability of the blood-cerebrospinal fluid barrier [134].

PLA2s take part in the inflammatory response, cell membrane remodeling, and fluidity. It is evident that PLA2 activity is significantly reduced in AD, especially the Ca^{2+}-dependent cytosolic PLA2 (cPLA2). The levels of Ca^{2+}-independent intracellular PLA2 (iPLA2) and phospholipid metabolism are also reduced in the hippocampus and frontal cortex of Wistar rats. Similarly, after the administration of MAFP (an inhibitor of cPLA2 and iPLA2), membrane fluidity parameters decrease, as assessed using three different fluorescence probes: DPH, TMA-DPH, and pyrene [58]. Further, the inhibition of PLA2 activity hampers neurite outgrowth and results in the loss of membrane mobility in cultured cortical and hippocampal neurons [135]. In order to explore the relationship between PLA2 and APP cleavage, Yang and colleagues exposed SH-SY5Y cells to type III secreted PLA2 and found an increased level of sAPPα secretion and a decreased level of Aβ (1-42) intracellularly, along with increased membrane fluidity [136]. Taken together, these results support the idea that PLA2 activity enhances the non-amyloidogenic cleavage of APP and increases membrane fluidity.

Others

When the N-methyl-D-aspartate receptor (NMDAR) is activated, it allows positively-charged ions to flow into cells through the cell membrane [137]. NMDAR density is significantly decreased in the frontal cortex but not in the parietal cortex of brain samples of postmortem AD patients. Meanwhile, membrane fluidity is unchanged in the frontal cortex, but slightly reduced in the parietal cortex, which means the neuronal membrane fluidity depends on the density of NMDARs [56].

It has recently been confirmed that there are substantial gender-related differences in the clinical features of AD. Platelet membrane Na^+/K^+–ATPase activity and fluidity have been studied by dividing subjects according to gender. Male AD patients show a significantly higher DPH fluorescence anisotropy than controls, indicating higher fluidity in males [57]. Studies on gender differences could lead to further therapeutic targets in AD.

Other factors such as SiO_2 nanoparticles, intracellular Ca^{2+} concentration, and Zn^{2+} concentration are all elements that are known to disturb membrane fluidity by altering the pathophysiological conditions of cells either *in vivo* or *in vitro* [138–140].

New Hypothesis: Membrane Aging as a Primary Contributor to AD Pathogenesis

Most of the former hypotheses of AD pathogenesis are Aβ- and tau-related. Although the studies of familial AD provide solid genetic evidence associated with abnormal Aβ metabolism in the disease, sporadic AD still lacks evidence for genetic mutation directly related to abnormal Aβ metabolism [141]. In addition, most of the fundamental studies and clinical trials involving such assumptions have been frustrated [142–144]. AD is a typical age-dependent

disease and has a long incubation period over decades from the development of pathophysiological alterations to overt clinical symptoms. Given such a complicated disease, can a simple linear model clarify it?

Cerebral glucose hypometabolism is an invariant characteristic of AD and is significantly correlated with the degree of cognitive decline and the progression of the disease. Further, brain Aβ deposition does not correlate with brain glucose hypometabolism in AD. Brain glucose hypometabolism also cannot be explained by the reduction of neurons and/or synapses because the hypometabolism appears prior to the onset of clinical symptoms over decades. Thus, the dysfunction of brain glucose metabolism contributes to AD pathogenesis [33, 145]. However, what causes cerebral glucose hypometabolism in AD?

Aging is a vital risk factor of AD. Membrane aging is an age-related manifestation which consists of modifications of membrane potential, abnormal activity of enzymes and receptors, disturbance of membrane lipid composition, and in particular, changes of membrane fluidity; such changes foster the process of membrane aging and *vice versa*. Besides, normal functions of membrane, such as cytoskeleton anchoring, cellular transport, intercellular interactions, or neurotransmission, could all be disrupted by abnormal membrane fluidity. Furthermore, mitochondrial dysfunction as well as glucose hypometabolism could be induced by the reformation of membrane structure.

Chronic elements such as Aβ aggregation, phospholipids disorders, oxidative stress, dysfunctions of membrane receptors and ion channels, and alterations in the concentrations of specific molecules, are all factors contributing to membrane modification. In addition, they are also of great importance to the process of AD and pathological changes in senescence.

Membrane changes have been discovered in other age-related systemic diseases. For instance, in coronary artery disease, Namazi and colleagues found that peroxidation of the red blood cell membrane as well as reduced Na^+/K^+-ATPase activity are significant in patients compared with normal controls [146]. In type 2 diabetes, the lipophilic index of membrane fluidity has been assessed in a large population and followed up for 7 years; the positive result demonstrated that decreased membrane fluidity might be a risk factor for glucose metabolic disorders [147]. In terms of brain injury, when using a closely-coupled repeated blast exposure mouse model, it is found that the destruction of membrane integrity increases membrane permeability, which contributes to polytrauma of the brain [148]. With regard to epidemiological factors, it has been demonstrated that AD can be classified into two main types – early-onset (EOAD) and late-onset (LOAD). Most EOAD cases have a autosomal dominant mutation, including the PS1, PS2, or APP genes [149–151]. Down Syndrome (DS), which derives from trisomy of chromosome 21, is associated with EOAD [152]. DS adults with diverse levels of Aβ peptides appear to have an extraordinary risk of AD [153]. Bueno and colleagues found a decrease in the membrane phospholipids of erythrocytes in DS adolescents compared with controls [154]. Such an important finding reminds us that membrane aging could take part in the pathophysiological changes of DS, thus better explaining the Aβ deposits in DS patients many years and even decades before the onset of AD.

Putting this all together, elements that impair membrane fluidity can be potential causal factors of AD. This clarifies why AD is aging-dependent and is correlated with so many pathogenic clues. We further assume that genetic risk factors of AD, including the apolipoprotein E ε4 allele, APP/PS1/PS2 genetic mutations, and non-genetic risk factors of AD (e.g. Aβ aggregation, oxidative stress, changes in phospholipid composition, and inflammation), contribute to the onset and development of the disease. We summarize the new hypothesis in Fig. 1.

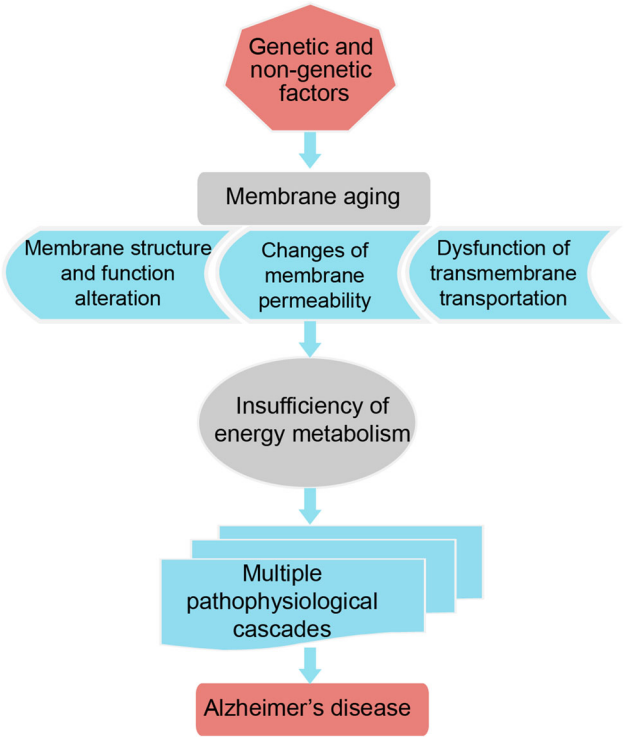

Fig.1 The diagram of relationship between membrane aging and AD. Due to the cause of genetic and non-genetic factors, membrane aging results in the alteration of membrane structure and function, leading to the changes of membrane permeability and the impairment of transmembrane transportation. The maintenance of normal biological functions in cells needs abundant energy metabolism and across-membrane transportation of a great quantity of substrates and key molecules for energy metabolism. Thus, the insufficiency of energy metabolism will cause multiple pathophysiological changes such as glucose hypometabolism, thiamine deficiency, inflammatory responses, etc. and finally lead to AD.

It is well known that AD progresses for decades before patients present relevant clinical manifestations. Thus, it is reasonable to use preventative therapies at the earlier stages of the disease based on its etiological factors. Here, we assume that membrane aging may be the original contributor to AD. Several measurements can be taken based on such pathological changes. As noted above, DHA, a major fatty-acid in brain phospholipids and the retina, has neuroprotective effects and may further participate in stabilizing normal membrane functions and reversing the membrane aging processing. Therefore, it may become a potential etiology-based therapy for AD. In addition, an increasing body of evidence indicates that there is a positive correlation between DHA concentration and cognition. For example, from the Framingham study, individuals with lower plasma DHA concentrations are more likely to show cognitive decline during 9 years of follow-up [155]. As specific inhibitors of cholesterol, statins play a crucial role in balancing brain phospholipids and may further take part in relieving the membrane aging process. There is good evidence demonstrating that statins prevent cognitive impairment due to their action of reducing the level of cholesterol [156].

Conclusions

In this review, by the "membrane aging" hypothesis, we try to give a better explanation for the correlation between aging-dependent AD and so many and diverse risk factors, including genetic and non-genetic factors. Membrane aging results in a change of membrane permeability and the impairment of transmembrane transport. The maintenance of normal biological functions in cells needs abundant energy metabolism and the trans-membrane transport of a multitude of substrates and key molecules for energy metabolism. Probably, membrane aging first attacks the energy metabolism of cells. The brain has the highest energy consumption, predominantly depending upon glucose catabolism. Thus, the brain is particularly vulnerable to energy insufficiency due to membrane aging. In addition, lipid components in the plasma membrane of neurons are more abundant than in other types of cells. Neurons are prone to impairment under lipid-unfriendly conditions, including oxidative stress, which are increasingly significant with age. Theoretically, all factors that directly or indirectly promote "membrane aging" are risk factors for AD. This can reasonably explain why so many genetic factors, other diseases, and an unhealthy life-style are correlated with AD.

The new understanding of AD pathogenesis based on membrane aging will provide insight into diagnostic and therapeutic targets for the disease. The alterations of critical processes and molecules in membrane permeability and energy metabolism may not only be markers for AD diagnosis but also targets for intervention. Etiology-based therapies such as DHA and statins as well as genetic intervention may contribute to alleviating age-related membrane dysfunction and further stabilizing membrane fluidity, and might become a new breakthrough for treating the disease.

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