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Review Article

Ginseng gintonin, aging societies, and geriatric brain diseases

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ABSTRACT

Background: A dramatic increase in aging populations and low birth rates rapidly drive aging societies and increase aging-associated neurodegenerative diseases. However, functional food or medicinal formulations to prevent geriatric brain disorders are not readily available. *Panax ginseng* is a candidate, since ginseng has long-been consumed as a rejuvenating agent. However, the underlying molecular mechanisms and the components of ginseng that are responsible for brain rejuvenation and human longevity are unknown. Accumulating evidence shows that gintonin is a candidate for the anti-aging ingredient of ginseng, especially in brain senescence.
Methods: Gintonin, a glycolipoprotein complex, contains three lipid-derived G protein-coupled receptor ligands: lysophosphatidic acids (LPAs), lysophosphatidylinositols (LPIs), and linoleic acid (LA). LPA, LPI, and LA act on six LPA receptor subtypes, GPR55, and GPR40, respectively. These G protein-coupled receptors are distributed within the nervous and non-nervous systems of the human body.
Results: Gintonin-enriched fraction (GEF) exhibits anti-brain senescence and effects against disorders such as Alzheimer's disease (AD), Huntington's disease (HD), and Parkinson's disease (PD). Oral administration of gintonin in animal models of D-galactose-induced brain aging, AD, HD, and PD restored cognitive and motor functions. The underlying molecular mechanisms of gintonin-mediated anti-brain aging and anti-neurodegenerative diseases include neurogenesis, autophagy stimulation, anti-apoptosis, anti-oxidative stress, and anti-inflammatory activities. This review describes the characteristics of gintonin and GEF, and how gintonin exerts its effects on brain aging and brain associated-neurodegenerative diseases.
Conclusion: Finally, we describe how GEF can be applied to improve the quality of life of senior citizens in aging societies.

1. Introduction

Aging is an inevitable biological process and results in complex and physiological changes to the body.[1] Although there are many theories on aging, they fall into two generally accepted categories: programmed factors *vs.* damage-related factors.[2] The theory of programmed factors is that organisms have a biological timetable overseeing growth, development, aging, and death. During these periods, gene expression is altered to control maintenance, repair, and defense mechanisms. On the other hand, the theory of damage-related factors holds that internal, external, or environmental insults damage cells and organs, and accumulate in organisms.[2] Both theories agree that aging organisms are unable to maintain their normal functions, resulting in death. Despite the enormous molecular complexity of aging, there are several molecular mechanisms in which the aging process can considered in a generic sense, for example, disruption of hormonal axes, accumulated oxidative stress, chronic inflammation, increasing nucleic acid instability, and a reduction in metabolic efficiency. Among these common aging factors, inflammation and oxidative stress appear to be detrimental in all of the physiological aspects of aging.[3]

An aging society is a society whose median age rises due to rising life expectancy and/or declining birthrates.[4] The United Nations

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standards define an aging society as a country in which the share of population aged over 65 years exceeds 7% of the whole population.[5] Currently, both developed countries and many developing countries are rapidly becoming aging societies, with increasing life expectancies.[5] Aging societies usually accompany decreases in the economically active population.[6,7] Therefore, such countries make financial investments to boost birthrates.[6,7] In addition, senior citizens in aging societies show greater cognitive decline and higher occurrences of chronic diseases and age-related neurodegenerative diseases such as AD, PD and HD than younger populations, although some cases of these diseases are due to family heritage or genetic factors.[8–10] Thus, humans are now living longer but not always living healthier. Currently, although many countries around the world are rapidly become aging societies, there are no functional foods or medicines to delay brain aging and further prevent aged-related neurodegenerative diseases.[11] At a cellular level, brain aging is characterized by increasing inflammation, oxidative stress, increased genomic instability, altered metabolism and the destruction of protein homeostasis, and decline of autophagy activity, which causes the accumulation of cellular toxic peptides or proteins.[2] Recent evidence shows that ginseng gintonin, a herbal medicine, provides a promise in protecting the brain from aging and aging-related neurodegenerative diseases.[12]

This review provides a brief history of gintonin isolation from ginseng, chemical compositions of gintonin, and gintonin synthesis. We further focus on the underlying molecular mechanisms of gintonin-mediated effects against brain aging and neurodegenerative diseases, both *in vitro* and *in vivo*, and the possible applications of gintonin in an aging population that is accompanied by brain aging neurodegenerative disease.

2. Ginseng, gintonin, and gintonin-enriched fraction (GEF)

2.1. Ginseng

Panax ginseng is one of the most widely consumed herbal nutritional products in the world. Ginseng has been used to promote rejuvenation and longevity; against stress, weakness and fatigue; and for both mental and physical states.[13] Although the root of *Panax ginseng* is used in medicinal food recipes, it is also added to confectionary, food products, and drinks.[14–16] Recent studies have shown that medicinal formulations of the extract are consumed for their medicinal purposes in several countries. However, relatively little is known about the underlying molecular mechanisms and which components of ginseng are responsible for its efficacy. The active components, ginsenosides (also called ginseng saponins), which are a kind of plant glycoside, were first found in ginseng.[17] and gintonin was recently isolated from a crude ginseng total saponin fraction.[18] Since ginsenosides have been well characterized in previous reports,[17] we will focus on gintonin.

2.2. Gintonin: a non-saponin bioactive component of ginseng

Before technical methods to purify individual ginsenosides from ginseng root were developed, a simple butanol fraction of ginseng, which is also known as a crude ginseng total saponin fraction, was usually used for *in vitro* and *in vivo* studies.[19] As a dried powder, the color of crude ginseng total saponin fraction ranges from weak to thick brown depending on the content of other ginseng components besides ginsenosides, since the color of pure ginsenosides is white.[20] Thus, the percentage of pure ginsenosides is low in crude ginseng total saponin fractions, although active components that are brown in crude ginseng total saponin fraction have been unidentified. In early studies before novel components were isolated from crude ginseng total saponin fraction, they were shown to work in a different manner from pure ginsenosides, in terms of elicit of $[Ca^{2+}]_i$ transients and Ca^{2+}-activated Cl^- channel regulation in *Xenopus* oocytes, since they activate Ca^{2+}-activated chloride channels through unknown membrane protein signaling pathways through phospholipase C (PLC)-IP_3-transient cytosolic Ca^{2+} release.[21] However, individual pure ginsenoside are unable to do this, indicating the possibility that crude ginseng total saponin fraction contains unknown active ingredients besides ginsenosides.[21–24] Interestingly, the degree of Ca^{2+}-activated Cl^- channel activation by crude ginseng total saponin fraction is dependent on its color, since thick brown crude ginseng total saponin fraction is more active in activation of Ca^{2+}-activated Cl^- channels than that of a weak brown color.

Later, Pyo et al.[25] isolated the active ingredient that induced Ca^{2+}-activated Cl^- channel activation from crude ginseng total saponin fraction through multiple steps using organic solvents (*i.e.*, methanol and butanol), fractionation, and diethylaminoethyl (DEAE) sepharose anion exchange chromatography. The active ingredient was named gintonin: a $[Ca^{2+}]_i$ transient induction agent of ginseng in mammalian cells. However, individual pure ginsenosides did not activate Ca^{2+}-activated Cl^- channels at all.[25] Chemical characteristics of gintonin include carbohydrates, lipids, and ginseng proteins complex. The yield of gintonin is about 0.2% in 4-year-old white and 6-year-old red ginseng, although the amount gintonin is still higher than individual ginsenosides of ginseng.[25] Other ginseng species, such as American ginseng, also contain an amount of gintonin comparable with *Panax ginseng*.[25] It appears that gintonin can be further divided into six different subtypes through size-exclusion gel filtration. The native molecular weight of gintonin is about 67 kDa, but its apparent molecular weight is about 13 kDa according to SDS-PAGE analysis, indicating that gintonin might be a kind of pentamer.[25] In qualitative assays, gintonin consists of ginseng proteins with rich hydrophobic amino acids, and carbohydrates rich with glucose $\gg$ glucosamine, and lipid components of linoleic $\gg$ palmitic > oleic = stearic acids.[25]

Hwang et al.[26] for the first time identified that the active ingredient of gintonin is lysophosphatidic acids (LPAs). which are known as endogenous ligands of plasma membrane G protein-coupled LPA receptors (Fig. 1). Before their identification in animal systems, LPAs were first known as serum factors in trace amounts, since serum factors induce mitogenic effects in cultured fibroblasts and retraction in neuronal cells.[27–29] Interestingly, the fatty acid composition of LPAs in gintonin is mostly linoleic acid ($C_{18:2}$), whereas the fatty acid components of LPAs in animals and other plants are oleic acid ($C_{18:1}$), palmitic acid ($C_{16:0}$), or stearyl acid ($C_{18:0}$).[30] In addition, LPAs, which are hydrophobic lipid components of gintonin, tightly bind to ginseng protein components, since LPAs are partially separated from ginseng protein components only after long-term methanol extraction of gintonin, as LPAs are tightly bound to serum albumin.[30] Proteomic analysis of protein components of gintonin shows that the main protein components of gintonin are ginseng latex-like protein 151 and ginseng major protein.[31,32]

Gintonin binds and activates six subtypes of LPA receptor in a differential manner to elicit $[Ca^{2+}]_i$ transients in mammalian cells.[26] Thus, gintonin exhibits different affinities for six LPA receptor subtypes for Ca^{2+} mobilization, in increasing order of affinity from LPA2, LPA5, LPA1, LPA3, LPA4, to LPA6, but does not induce changes to intracellular cAMP concentration.[26] Gintonin has also been isolated from ginseng leaf and stem, in addition to ginseng root.[33] Interestingly, although ginseng leaf contains a lot of gintonin, it was not easy to remove lead-derived chlorophyll from gintonin without using hexane.[33] Gintonin has also been isolated using only ethanol instead of methanol and butanol.[34] *In vitro* studies using neuronal and non-neuronal cells show that gintonin, but not pure ginsenosides, induces $[Ca^{2+}]_i$ transients via LPA receptor activation and acts as an exogenous LPA receptor ligand, which is

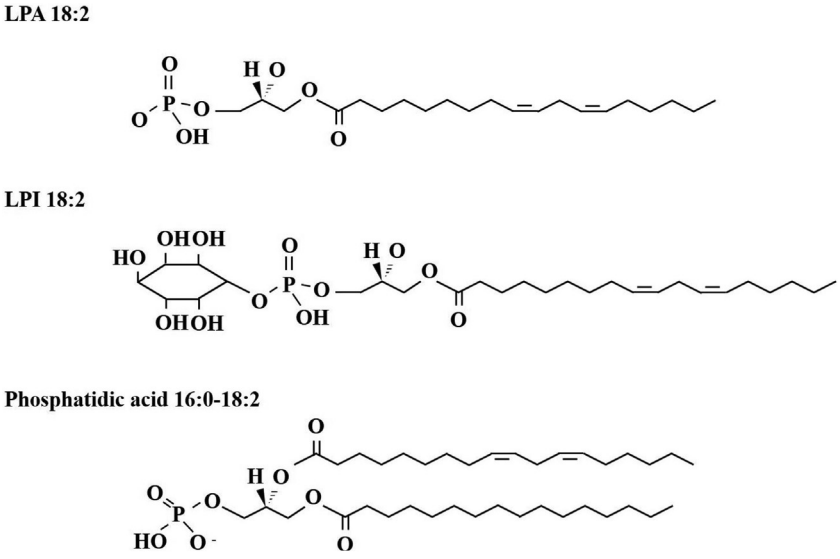

Fig. 1. The chemical structures of four main bioactive components of gintonin. Gintonin was prepared from *Panax ginseng* root. LPAs was proved as the main functional ingredients of gintonin. Gintonin LPAs act as exogenous ligand for G protein-coupled LPA receptors. Recently, gintonin LPI and linoleic acid was also proved as a ligand of GPR40 and GPR55.[49]

key to gintonin's *in vitro* and *in vivo* biological effects, as described below.

2.3. Gintonin-enriched fraction (GEF)

Instead of using multiple steps for gintonin preparation using organic chemicals such as methanol and butanol, a brief and cost-effective method was developed to obtain a large amount of gintonin using only fermented ethanol and water, after much trial and error.[35] After extraction of ginseng with ethanol, the extract is fractioned with water to obtain water-soluble and water-insoluble fractions. The water-insoluble precipitate, rather than the water-soluble supernatant, still induced a large $[Ca^{2+}]_i$ transient in neuronal cells, as does gintonin.[35] The water-insoluble precipitate fraction was designated as the gintonin-enriched fraction (GEF). The yield of GEF was approximately 1.3%, which is 6-fold higher than that obtained previously. GEF utilizes the same signal transduction pathway as gintonin does, and induces $[Ca^{2+}]_i$ transients in cultured mouse cortical astrocytes. Most of the water-soluble supernatant fractionation does not induce $[Ca^{2+}]_i$ transients and mainly contains ginsenosides and carbohydrates.[35] The apparent molecular weight of GEF is about 13 kDa by SDS-PAGE, which is equivalent to that obtained by the previous gintonin preparation method.[35] Interestingly, the main difference between gintonin and GEF is that more ginseng proteins exist in GEF preparations, since GEF staining by Coomassie brilliant blue after SDS-PAGE is stronger than that for gintonin.[35] Because GEF can be prepared through water precipitation of ginseng ethanol extract and is easily reproducible with high yields, it could be used for preclinical animal studies and commercially utilized for the development of gintonin-derived medicinal food and natural medicine for application in humans.[36]

3. Characteristics of the lipid components of gintonin and GEF

Gas chromatography–mass spectrometry for fatty acid analysis and liquid chromatography–tandem mass spectrometry for GEF phospholipids analysis has revealed that gintonin contains many kinds of bioactive lipids.[37] Further quantifying analysis for fatty acids, lysophospholipids (LPLs), and phospholipids (PLs) revealed that GEF contains about 7.5% linoleic ($C_{18:2}$), 2.8% palmitic ($C_{16:0}$), and 1.5% oleic acids ($C_{18:1}$). GEF contains about 0.2% LPA $C_{18:2}$, 0.06% LPA $C_{16:0}$, and 0.02% LPA $C_{18:1}$. GEF contains 0.08% lysophosphatidylcholine (LPC), 0.03% lysophosphatidylethanolamine (LPE), and 0.13% lysophosphatidylinositols (LPIs), which act as an endocannabinoid GPR55 ligand.[38–41] The order of lysophospholipids in GEF is LPA > LPI > LPC > LPE. GEF also contains about 1% phosphatidic acid (PA) $C_{16:0–18:2}$, 0.5% PA $C_{18:2–18:2}$, and 0.2% PA $C_{16:0–18:1}$, which are now considered second messengers.[42] The majority of lipid components of gintonin or GEF have negative rather than positive or neutral charges (Fig. 1). This might be the reason that gintonin can be separated from other ginseng components through anion exchange chromatography.[25] Polyunsaturated linoleic acid, which is an endogenous GPR40 ligand.[43–46] is the most abundant component of gintonin and GEF. Linoleic acid ($C_{18:2}$) is the main fatty acid component of LPA, other LPLs, and PAs in GEF, raising the possibility that linoleic acid ($C_{18:2}$) is the primary fatty acid compound of LPA, LPL, PA and other lipid synthesis components in ginseng. Thus, gintonin contains three ligands of G protein-coupled receptors such as LPA receptors, GPR40, GPR55, and PA, a second messenger, which can also be also converted into LPAs if they are attacked by phospholipases in the digestive system. Linoleic acid ($C_{18:2}$) is also an essential fatty acid in animals. Linoleic acid ($C_{18:2}$) derived from GEF could also be further transformed into other physiologically active lipid-derived agents as a precursor of arachidonic acid and prostaglandins in animals.[47]

3.1. Bioactive lipid syntheses of gintonin and formation of bioactive complexes with ginseng proteins

Gintonin is a kind of glycolipoprotein in ginseng.[25] Although LPA and other bioactive phospholipid syntheses in ginseng have not been directly demonstrated, they can be inferred from plant LPA and lipid synthetic processes. Plant LPAs and other lysophospholipids can originate from water soluble glycerol 3-phosphate (G3P) by transfer of an acyl group, of which might mostly be linoleic acid ($C_{18:2}$) in ginseng as described above, by glycerol 3-phosphate acyltransferase. Interestingly, most animal LPAs have oleic acid ($C_{18:1}$) or palmitic acid rather than linoleic acid ($C_{18:2}$), as men-

tioned above. Choline, ethanolamine, or an inositol group can be also added to the phosphate group of LPA to produce LPC, LPE or LPI.[48] Interestingly, in gintonin and GEF, the major group added to LPA to produce LPI is inositol. LPAs can be further acylated to form PAs by LPA acyltransferase,[48] of which the second acyl group could also be palmitic ($C_{16:0}$), linoleic ($C_{18:2}$), or oleic acid ($C_{18:1}$) in order of prevalence in GEF (Fig. 1). Thus, in LPA and PA syntheses, the main acyl group will be in the order of linoleic ($C_{18:2}$) $\gg$ palmitic ($C_{16:0}$) > oleic acid ($C_{18:1}$). PAs could be further processed to form other PLs or glycerolipids.[48] Thus, LPAs, and other lysophospholipids like LPIs and PAs can be intermediate metabolites for the synthesis of diverse glycerolipids and PLs in ginseng. Interestingly, these bioactive lipids are isolated as a complex with ginseng proteins such as GLP151 and ginseng major protein, which produces a unique gintonin.[25,30] In addition, it was revealed that the amounts of LPAs in gintonin were much higher than those in other foodstuffs or herbal medicines with an abundance of linoleic ($C_{18:2}$).[25,30] Bioactive lipid components in gintonin and GEF might be derived from hydrolysis of ginseng plasma membrane lipids during organic extraction with high temperature and bind to ginseng proteins. Further study is needed to elucidate the origins of these bioactive lipids in gintonin and why gintonin exists as a complex with ginseng proteins.

3.2. Mode of action of gintonin: involvement of gintonin- or GEF-mediated regulations of adrenergic, cholinergic, glutamatergic, serotonergic receptor regulations, and autophagic stimulation via LPA receptors

It appears that the primary targets on the plasma membrane of gintonin are LPA receptors,[26] since LPAs were first found in gintonin and LPAs are more abundant than other bioactive ligands such as LPIs, LPCs, and LPEs.[37] Recently, it was demonstrated that gintonin linoleic acid and LPI interact with GPR40 and GPR55 to exhibit cellular effects such as insulin secretion and Ca^{2+}-mediated cell migration, respectively.[49] Thus, gintonin contains at least three different ligand complexes for G protein-coupled receptor regulation such as LPA receptors, GPR40, and GPR55 (Fig. 2).[49] Interestingly, although the signaling pathways of GPR40 are different from that of LPA receptors,[49] LPA receptors and GPR55 share common signaling pathways, in that activation of both receptors elicits $[Ca^{2+}]_i$ transients and induces Ca^{2+}-dependent cell migration.[49] Gintonin-mediated activation of GPR55 and LPA receptors is coupled to $[Ca^{2+}]_i$ transients through $G\alpha_{q/11}$ activation-phospholipase C activation-IP_3 formation-$[Ca^{2+}]_i$ transient induction. Thus, gintonin produces second messenger Ca^{2+} via LPA receptors and GPR55. Ca^{2+}, an intracellular mediator of gintonin, initiates a cascade of amplifications inducing further intracellular effects and/or intercellular communications by activation of Ca^{2+}-dependent kinases, receptors, and gliotransmitters, and neurotransmitter release.[18] Recent studies have shown that gintonin-mediated $[Ca^{2+}]_i$ transients in mammalian cells are further directly and indirectly coupled to the regulation of adrenergic, cholinergic, glutamatergic, and serotonergic systems through the release of respective neurotransmitters in the central and peripheral nervous systems (Fig. 2).[50–53] Gintonin enhances brain neurotrophic BDNF and VEGF release and expression.[54,55] Gintonin also stimulates autophagic flux in primary astrocytes.[56] Thus, gintonin-mediated regulations of various receptors, neurotrophic and autophagic systems are further coupled to diverse *in vitro* and *in vivo* biological effects such as synaptic transmission, cell migration, cell proliferation, glycogenolysis, anti-depression, and neurogenesis in neuronal cells (Fig. 2). Furthermore, oral gintonin or GEF treatment is associated with improvement of learning and memory,[51,52] and enhancement of physical stamina via catecholamine-glycogenolysis axis.[50] Gintonin also shows anti-metastatic effects via autotaxin inhibition.[57]

The detailed *in vivo* effects of gintonin and/or GEF on brain aging, brain aging-related neurodegenerative diseases and autophagic regulations will be described below section. However, other ginseng components, such as ginsenosides and acidic polysaccharides, do not show the same effects as gintonin.[18]

3.3. Anti-brain aging effects of gintonin

Aging-related studies in animals can be tedious, since researchers have to wait for a long time for the animals to grow old enough. Recently, several methods have been developed to induce rapid brain, as well as other organ, aging through treatment with biochemical agents. One of these methods uses D-galactose. D-Galactose has been widely used to induce rapid aging of body organs, including the brain. Because D-galactose-induced aging resembles that in humans, this method is widely used for anti-aging pharmacological agent development in aging model systems.[58–61] The basic molecular mechanisms of D-galactose-induced brain aging are due to the excessive production of reactive oxygen species (ROS) and subsequent ROS-induced mitochondrial dysfunctions in nervous and non-nervous systems. Thus, the D-galactose-induced aging model is more similar to the damaged-related factors of aging theory than the programmed factor theory.[62,63]

The hippocampus is part of brain's limbic system. It plays important roles in the consolidation of diverse cognitive information from short-term memory to long-term memory. Brain aging affects hippocampal functions and induces memory dysfunctions.[64] However, gintonin administration enhances hippocampal-dependent cognitive functions.[54] Gintonin co-administration with D-galactose reversed D-galactose alone-induced hippocampal senescence.[65] Thus, long-term treatment of D-galactose induces hippocampal aging by reducing the hippocampal cell proliferation, differentiation, and maturation observed in human brain aging. Interestingly, hippocampal LPA1 receptor expression is also diminished under D-galactose insult.[65] Co-administration of gintonin with D-galactose reverses D-galactose-induced reduction of cell proliferation, increases differentiation of immature neurons into mature neurons, and increases LPA1 receptor expression in the mouse hippocampus. Gintonin also increases the expression of phosphorylated cyclic adenosine monophosphate response element binding protein (pCREB), which plays an important role in cognitive functions. In addition, in an electrophysiological study, D-galactose decreased the long-term potentiation (LTP) amplitude, which is a basic cellular mechanism that underlies learning and memory.[65] Co-administration of gintonin in D-galactose mice enhanced LTP and restored cognitive functions compared with those in mice treated with D-galactose alone. The LPA and LPA1 receptors play a crucial role in early brain development.[65] Relatively little is known about the role of the LPA1 receptor in the adult brain, as opposed to the developmental brain. In the D-galactose-induced brain aging model, GEF administration recovers a decrease in adult LPA1 receptor expression, which raises the possibility that brain senescence might accompany a decrease in adult hippocampal LPA1 receptor expression levels and that LPA1 receptor restoration by gintonin administration might contribute to the maintenance of hippocampal homeostasis related with cognitive functions under oxidative stress. This concept might be supported by LPA1 receptor null-mice that show cognitive and attention deficits.[12]

3.4. Anti-Alzheimer's disease effects of gintonin

AD represents a severe geriatric brain disease, since it usually occurs in those aged over 65 years. AD occurs in about 10% of people over 65 years and the occurrence dramatically increases in those aged over 80 years.[66] AD severity or AD-related social problems

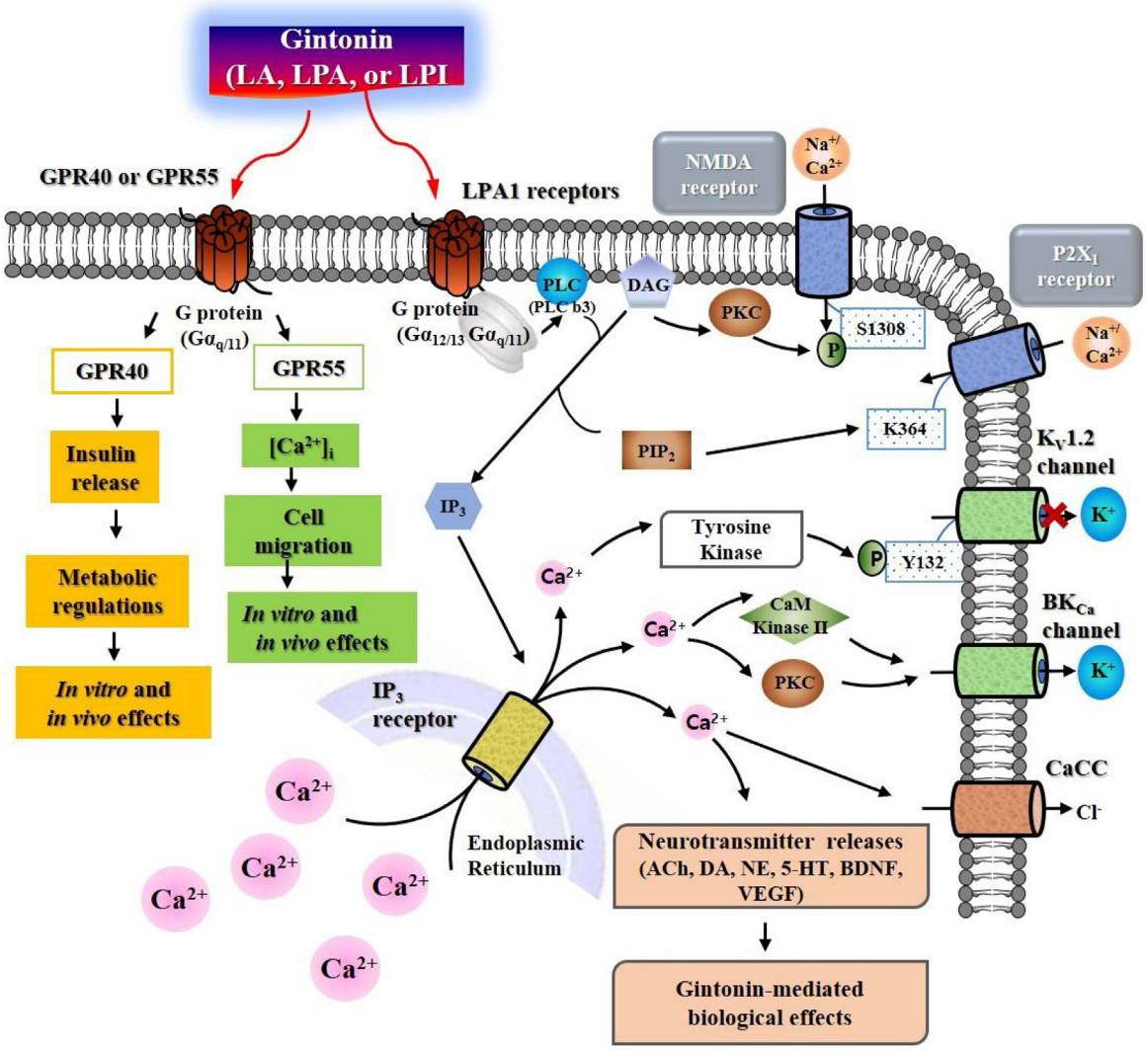

Fig. 2. Signal transduction of ginseng gintonin on the mammalian cell plasma membrane via LPA receptors, GPR40, and GPR55. Gintonin activates LPA GPCRs, GPR40 and GPR55, respectively, which can lead to intracellular responses through the regulations of ion channels and receptors.[18] In nervous system, gintonin-mediated signaling transduction pathway can be also coupled to neurotransmitter (*i.e.*, acetylcholine, dopamine, norepinephrine, and serotonin) release for intercellular communications and to stimulations of BDNF and VEGF releases.[18] The released neurotransmitters and neurotrophic factors further regulate their respective receptors to exhibit ginseng gintonin-mediated *in vivo* biological effects. Thus, *in vivo* biological effects of ginseng gintonin might be achieved via LPA receptors, GPR40, and/or GPR55 and indirectly via activations of respective receptors by ligands released by gintonin treatment. ACh, acetylcholine; DA, dopamine; NE, norepinephrine; 5-HT, serotonin.

in aging societies are due to the fact that therapeutic drugs have not yet been developed, and as such, AD is considered an incurable disease. The number of AD patients is growing every year, and increasing the economic and psychological burdens in many households. The worldwide prevalence of AD is estimated to double and 115 million within the next 20 years.[67] AD is clinically characterized by dysfunction in learning and memory, as well as deterioration of other cognitive and noncognitive mental and motor functions, and whole brain atrophy, which makes social activities impossible.[66,67] The main causes of AD are the long-term accumulations of amyloid-β (Aβ) and tau proteins in the brain. Aβ and tau protein are neurotoxic; it accumulates into aggregates, and induces inflammation and oxidative stresses in the brain. It also induces cell death, destroys synaptic connections among neurons, and finally results in the clinical signs and symptoms of AD.[66–68] A series of events occur in Aβ aggregate formation, from the accumulation of amyloid-β protein precursors (AβPPs) in neurons and their subsequent accumulation, resulting in the death of brain cells and eventually AD. This is the "amyloid cascade hypothesis",[69] although this hypothesis has recently been challenged by the observations that many young adults and older persons without dementia show substantial Aβ plaque accumulations in the brain and that inhibitors of Aβ plaque formation failed to show a therapeutic effect on AD in clinical trials.[70,71]

Gintonin decreases Aβ1–42 release and attenuates Aβ1–42-induced cytotoxicity in SH-SY5Y neuroblastoma cells.[72] In studies of the molecular basis of gintonin-induced anti-AD effects using SH-SY5Y neuroblastoma cells, gintonin stimulated non-amyloidogenic rather than amyloidogenic pathways to produce soluble AβPPα (sAβPPα), which is has neuroprotective and neurotrophic effects, so is beneficial to neurons.[72] Gintonin promotes non-amyloidogenic sAβPPα release via Ca^{2+}-dependent metalloprotease α-secretase activation, and protein-trafficking processes.[72] *In vivo* studies have shown that gintonin has anti-AD effects in short- and long-term Aβ-induced cognitive dysfunction in wild-type animals. As a short-term treatment for 2 weeks, gintonin rescued acute Aβ1–42-induced cognitive dysfunction in mice.[72]

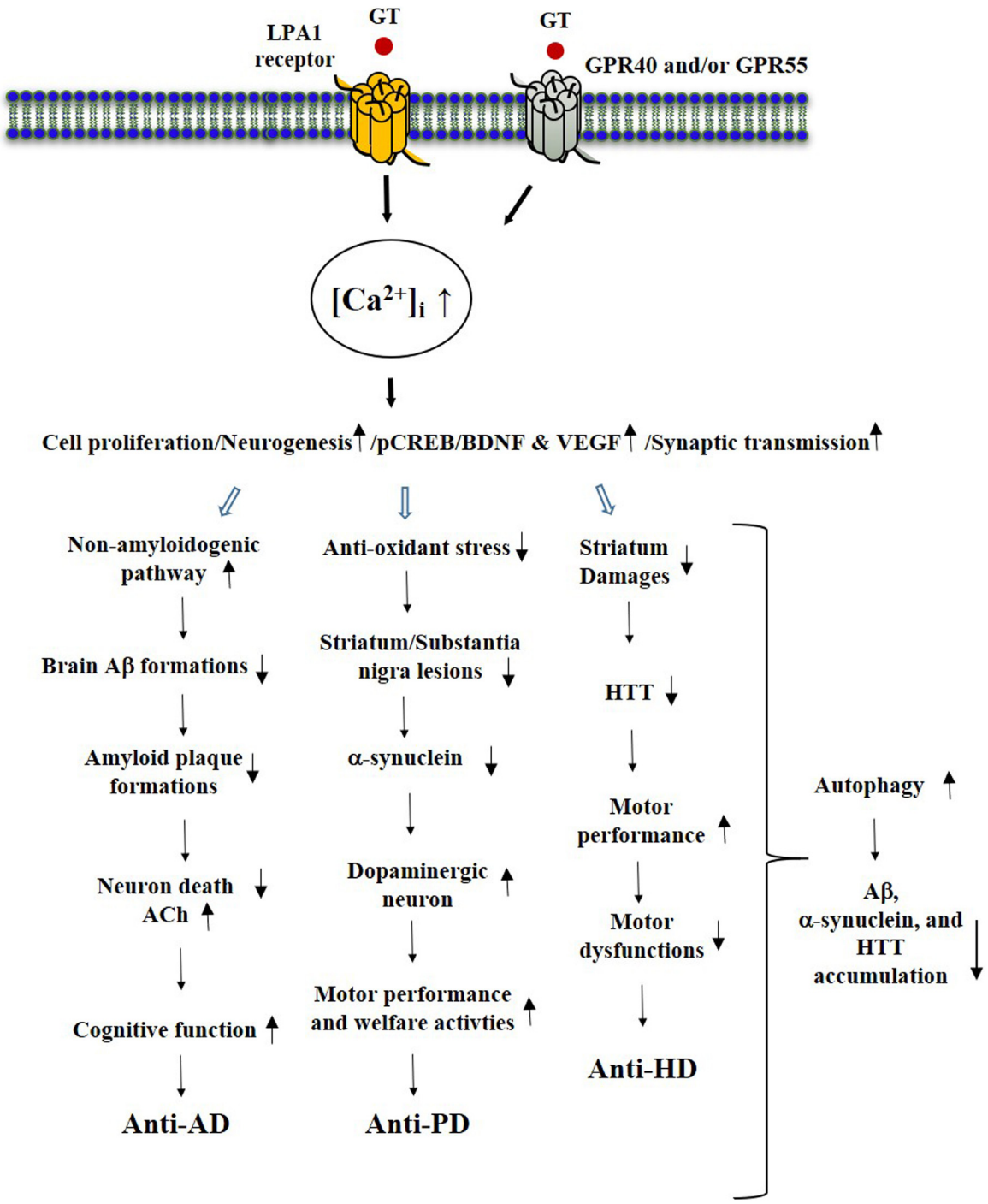

Fig. 3. Effects of gintonin and GEF on geriatric brain diseases. Long-term oral administration of ginseng gintonin attenuates geriatric brain diseases. The common mode of action of gintonin against neurodegenerative diseases is LPA receptors activation and anti-apoptosis, anti-inflammation, anti-oxidative stress, and autophagic stimulations. GPR40 and GPR55 might be involved in geriatric brain diseases but not yet clearly demonstrated as much as LPA and LPA1 receptor. HTT, huntingtin.

In transgenic AD animal models, which are widely used to model brain senescence-related AD induction, it usually takes 9 months to observe memory dysfunctions and amyloid plaque accumulation in the cortex and hippocampus. In the brain aging AD model, long-term oral administration of gintonin for 3 months significantly reduced amyloid plaque deposition in the cortex and hippocampus, and rescued short- and long-term memory and spatial working memory impairments.[72] Long-term oral administration of gintonin also significantly showed anti-neuroinflammatory effects by reducing microglial Iba-1 expression in the cortex and hippocampus.[72] Thus, the gintonin-mediated anti-AD effects inhibit Aβ formation, decrease microglial activation, and increase beneficial sAβPPα via LPA1 receptor signaling pathways. This results in decreased amyloid plaque formation/accumulation, as well as decreased neuroinflammation in the brain, and recovery of brain function, resulting in enhancement of cognitive functions. This was the first indication that ginseng gintonin intake can prevent Aβ formation and amyloid plaque accumulation in the brains of aged AD model animals (Fig. 3).

3.5. Gintonin effects on hippocampal cholinergic systems in AD animal models

In addition to the amyloid-induced AD hypothesis, another hypothesis has been circulating for four decades. This is that AD is due to a loss of cholinergic innervations in the cerebral cortex in aged populations. In fact, brain acetylcholine concentration also declines with brain aging and in AD patients.[73] Brain cholinergic dysfunctions including muscarinic and/or nicotinic acetylcholine receptor-related synaptic malfunctions and decreases in brain acetylcholine concentrations are correlated with cognitive impairment. This leads to the "cholinergic hypothesis of AD", since Aβ accumulation induces death of cholinergic neurons, resulting in decreases in brain acetylcholine concentrations.[74,75] Even in normal animals, administration of cholinergic antagonists, such as scopolamine, induces transient cognitive deficits,[76] indicating that the brain's cholinergic system is important in cognitive functions in animals and humans. This hypothesis is supported by the development and clinical application of a cholinesterase inhibitor. Acetylcholinesterase inhibitors, such as donepezil, are also clinically approved, although these drugs only partially alleviate AD symptoms without additional AD therapy.[77]

In an *in vitro* study, gintonin stimulated acetylcholine release from cultured mouse hippocampal neural progenitor cells (NPCs). Gintonin-mediated stimulation of acetylcholine release achieved via $[Ca^{2+}]_i$ transients through LPA receptor signaling pathways and gintonin treatment also increased the expression of choline acetyltransferase (ChAT), an enzyme involved with acetylcholine synthesis.[52] Thus, gintonin increases acetylcholine release from neurons after enhancement by acetylcholine synthesis. In *in vivo* studies, oral administration of GEF for 3 weeks significantly attenuated scopolamine-induced memory impairments. In addition, oral administration of gintonin also significantly attenuated Aβ-induced cholinergic dysfunctions by increasing ChAT activity and decreasing acetylcholine esterase (AChE) activity, and finally an increase in brain acetylcholine concentration. In the transgenic AD mouse model described above,[52] oral administration of gintonin for 3 months also attenuated AD-related hippocampal cholinergic impairment by increasing cholinergic neurons. Thus, gintonin recovers the brain hippocampal cholinergic system damaged by amyloid plaque accumulations in an AD animal model by enhancing acetylcholine release, increasing brain ChAT expression, and decreasing brain AChE expression. Gintonin-mediated cholinergic regulation contributes to the amelioration of aging-related AD.[52] This was the second indication that gintonin has anti-AD effects (Fig. 3).

3.6. Gintonin effects on hippocampal neurogenesis in an AD animal model

It was believed for a long time that adult brain neurogenesis does not occur and that recovery of brain neurons is irreversible after brain damage induced by stroke-induced hypoxia or neurodegenerative disease like AD. However, recent studies have demonstrated that adult neurogenesis including neuronal differentiation, migration and maturations can be observed in limited areas of the brain such as the hippocampus and third ventricle.[77] Now it is believed that adult brain neurogenesis can occur indefinitely.[78] Currently, adult brain neurogenesis has received much attention for its clinical applications, since many animal studies have shown that adult animal brain neurogenesis can be applied to restore cognitive function, and treat stroke, depression, and neurodegenerative diseases.[79] The LPA and LPA1 receptors are mainly involved in embryonic brain development with diverse functions in cell proliferation, morphological changes, and migration to target areas of the brain.[80,81] Thus, the LPA1 receptor is abundantly expressed during prenatal stages and gradually decreases postnatally.[81,82] Recent studies have shown that the LPA1 receptor is also involved in adult brain functions such as cognition, depression, emotion, neurogenesis, mental diseases, and social activity.[83] In addition to gintonin's ability to inhibit β-amyloid plaque formation in the cortex and hippocampus, restore β-amyloid-induced cognitive dysfunction, and restoration the cholinergic system in an AD animal model, Kim et al.[12,84] showed that gintonin stimulates neuronal cell proliferation, *in vitro* and *in vivo*, in the mouse hippocampus. In an *in vitro* study, gintonin treatment increased 5-bromo-2′-deoxyuridine (BrdU) incorporation into hippocampal NPCs. Gintonin increased NeuN-positive neurons, which are biomarkers of mature neurons. However, the actions of gintonin were blocked by an LPA1/3 receptor antagonist and a Ca^{2+} chelator, showing that gintonin-mediated hippocampal neuronal cell proliferation and maturation was achieved via the Ca^{2+}-dependent signaling pathway, probably with LPA1/3 receptor regulation. An *in vivo* study using adult wild-type animals and aging-related AD model animals, 3-month oral administration of GEF also increased hippocampal BrdU incorporation, which indicates an increase in neuronal cell proliferation, in both adult wild-type and transgenic AD mice.[84] This evidence supports that long-term oral gintonin administration induces adult brain neurogenesis in normal animals and even AD model animal.[84,65] This was the third indication that gintonin has anti-AD effects; this time *via* brain neurogenesis (Fig. 3). Collectively, in AD animal models, the gintonin-mediated anti-AD effects include (1) activation of non-amyloidogenic pathways, resulting in the formation of beneficial sAβPPα rather than neurotoxic Aβ; (2) restoration of the brain cholinergic system by increasing ChAT and decreasing AChE activity; and (3) stimulation of hippocampal neurogenesis in both adult wild-type and AD model animals.[52,72,84]

3.7. Effects of gintonin on motor performance

Impaired motor functions or movement disorders in elderly people in aging societies are another important manifestation of aging-related brain diseases. Motor functions of aged individuals become slow and unnatural; walking becomes more difficult and posture becomes slouched.[85,86] Gintonin has been proven preclinically to enhance motor functions through peripheral LPA receptor regulation. Lee et al.[50] compared rota-rod motor performance in fasting mice between a control (saline) vehicle group and an oral gintonin-treated group, and found that gintonin increased blood glucose via liver glycogenolysis and enhanced motor activity, compared with the saline vehicle group. The molecular mechanisms of gintonin-mediated enhancement of motor performance with increased blood glucose include that gintonin treatment first increases catecholamines such as epinephrine and norepinephrine from the adrenal gland of the kidney.[50] The released catecholamines interact with the liver β-adrenergic receptor to initiate liver glycogenolysis and increase blood glucose. Thus, increased blood glucose through liver β-adrenergic receptor activation might act as an energy source for motor performance even in the fasting state, indicating the possibility that gintonin can act as an invigorator for physical stamina in elderly people.

3.8. GEF protects brain dopaminergic neurons in a Parkinson's disease (PD) animal model

PD is a disease that affects motor function and is the second-most prevalent and age-associated neurodegenerative disease after AD.[87] Symptoms of PD include tremors, muscular rigidity, akinesia, bradykinesia, postural instability, and abnormal gait.[88] These symptoms are caused by insufficient dopamine production in the substantia nigra and striatum due

to the selective death of dopaminergic neurons.[88–90] In an animal study using 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), which impairs dopaminergic neurons in the substantia nigra and striatum through ROS formation and induces symptoms similar to human PD such as motor dysfunctions,[91] oral administration of GEF increased the survival rate of these mice.[92,93] Choi et al.[92] and Jo et al.[93] showed that oral GEF administration in a PD mouse model significantly ameliorated the effects of neurological deficits such as motor performance, as measured with pole and rotarod tests, and social welfare activities in nest building tests. GEF administration also increased spontaneous locomotive activity that was measured by travel distance, compared to MPTP alone.[93] Amelioration of neurological deficits by GEF in PD animal models was supported by immuno-histochemical assays showing a reduction in the loss of tyrosine hydroxylase-positive neurons, microglial activation, and activation of inflammatory mediators (interleukin-6, tumor necrosis factor, and cyclooxygenase-2). GEF administration also inhibits phosphorylation of mitogen-activated protein kinases and nuclear factor-kappa B signaling pathways.

In apoptosis-related evaluation regarding the reduced number of intact substantia nigra and striatum cells after MPTP exposure, the expression of Bax, the main regulatory factor in the process of proapoptotic cell death by inducing mitochondrial pore formation in the developing cerebellum, was enhanced, After GEF co-administration with MPTP, fewer Bax-immunoreactive cells were observed, suggesting that the MPTP-mediated Bax protein induction was repressed by GEF co-treatment. Meanwhile, anti-apoptotic Bcl2 was reduced by MPTP, while GEF co-treatment increased Bcl2 expression[92] These results demonstrate the neuroprotective effects of GEF in the MPTP-exposed mouse substantia nigra and striatum in a PD animal model.[4] Activation of nuclear factor erythroid 2-related factor 2/heme oxygenase-1 (Nrf2/HO-1) is a potential therapeutic target for brain neurodegeneration, since they are activated to protect cells under ROS insults. GEF administration enhances Nrf2/HO-1 expression in the mouse substantia nigra and striatum.[92,93]

In addition to damage to dopaminergic neurons in the substantia nigra and striatum, another characteristic of PD is the accumulation of α-synuclein-containing inclusion bodies within dopaminergic neurons. These inclusions are known as Lewy bodies.[94] Accumulations of α-synuclein in Lewy bodies are caused by impaired axonal transport.[95] GEF treatment to neurons reduces the accumulation of α-synuclein.[93] The molecular mechanisms in GEF-mediated amelioration in the MPTP-induced PD animal model are achieved via multiple pathways such as LPA receptor regulation, anti-apoptosis, anti-inflammation, and anti-oxidant stress.[92,93] Gintonin administration also increased LPA1/3 receptor gene expression in the substantia nigra and striatum in MPTP-treated mice. However, co-administration of the LPA1/3 receptor antagonist blocked GEF-mediated increase of the LPA1/3 receptor gene expression and attenuated increases in tyrosine hydroxylase and Nrf2 protein expression in the substantia nigra and striatum,[92] showing the involvement of LPA receptors in GEF-mediated anti-PD (Fig. 3).

3.9. GEF protects brain striatal neurons in a Huntington's disease (HD) animal model

Huntington's disease (HD), also known as Huntington's chorea, is an inherited brain disorder. The genetic mutation responsible for HD is an abnormal expansion of a CAG trinucleotide repeat in the *HTT* gene that encodes for huntingtin (HTT).[96–98] Symptoms of HD usually begin between 30 and 50 years of age.[96] The damaged brain area includes the basal ganglia.[97] The earliest symptoms are a general lack of coordination of movement and an unsteady gait, often followed by abnormal mood and/or mental abilities.[98,99] As the disease advances, uncoordinated, jerky body movements become more apparent and worsen, until coordinated movement becomes difficult and the person is unable to talk.[99] The general symptoms become similar to PD when HD worsens,[99] and the person's mental abilities generally decline, becoming dementia-like.[100] Patients usually die 20 years after onset and death results from fatal aspiration of pneumonia.[99]

Jang et al.[101] demonstrated that GEF mitigates the neurological symptoms and immuno-histochemical impairments of the striatum in an animal model of HD. They used two *in vitro* and *in vivo* methods to demonstrate the anti-HD effects of GEF. One used 3-nitropropionic acid (3-NPA), which is produced in fungus-contaminated corn and induces an irreversible inhibition of complex II in the mitochondria, impairing cellular respiration and energy metabolism by inhibiting succinate dehydrogenase (SDH) in specific brain sites including the striatum and cortex, resulting in similar symptoms to human HD.[101] In a 3-NPA model of HD, GEF attenuated mitochondrial dysfunction. *i.e.*, succinate dehydrogenase and MitoSOX activities, apoptosis, microglial activation, and mRNA expression of inflammatory mediators (*i.e.*, IL-1β, IL-6, TNF-α, COX-2, and iNOS) in the striatum after 3-NPA-intoxication.[101] However, administration of GEF increases the Nrf2 signaling pathway and inhibits mitogen-activated protein kinases (MAPKs) and the nuclear factor-κB (NF-κB) signaling pathway.[101] The other models used ST*Hdh* cells, which produce aggregated huntingtin protein when exposed to H_2O_2 and damage neurons,[102] and an *in vivo* adeno-associated viral (AAV) vector-infected mouse model of HD, which also overproduces aggregated huntingtin protein. In both these *in vitro* and *in vivo* models, GEF reduced mutant HTT aggregates, protected ST*Hdh* cells, reduced cell death, and reduced aggregates N171-82Q-mutant HTT in the striatum. GEF has beneficial effects in a chemical-induced HD model with antioxidant and anti-inflammatory activities through brain LPA1 receptor regulation. In addition, gintonin also exerts neuroprotective effects in ST*Hdh* cells and an AAV vector-infected model of HD by reducing mutant HTT.[101] Thus, gintonin exhibits anti-HD activities in a chemical model using 3-NPA, an ST*Hdh* cell model and AAV vector-infected mutant model of HD. Taken together, gintonin or GEF shows anti-neurodegenerative activities in animal models of AD, PD, and HD. The common molecular mechanisms by which gintonin exerts anti-brain aging and anti-neurodegenerative effects in diseases such as AD, PD, and HD include anti-apoptotic, anti-inflammatory, and anti-oxidant effects via LPA receptor regulations[12,52,65,84,92,93,101] (Fig. 3).

3.10. A role of gintonin-mediated autophagy stimulation in neurodegenerative diseases

Autophagy is a type of intracellular recycling program that removes damaged or old cellular organelles, and protein aggregates or mis-folded protein components.[103] In animal brain experiments, autophagy-related proteasome activity decreases in an age-dependent manner more predominantly in neurons than in glial cells.[104] The autophagy-related gene *Atg* is involved in normal autophagy, and deletion or mutation of this gene induces disturbances in cellular homeostasis, leading to neurodegenerative diseases.[105] Thus, if autophagy is dysregulated in the aging brain, defective proteins and damaged organelles cannot be clearly removed from neurons.[106–108] Accumulation of waste proteins mainly in neurons leads to neuropathological conditions, since they are more vulnerable to the toxicity exerted by misfolded proteins[103] and have lower capacity to combat inflammation and/or oxidative stress.[109] Thus, autophagy dysfunction is a common feature of most neurodegenerative diseases in the aged population with AD.[110,111] PD,[112] and HD[113,114] Formation of abnormal protein aggregates composed of Aβ amyloid[115] and tau

proteins result in AD.[116] Lewy body α-synuclein aggregates result in PD,[117] and HTT protein aggregates result in HD.[118] Thus, depending on the brain disease, the aggregated proteins in the brain differ from each other. The aggregated proteins in AD, PD, and HD can cause synaptic impairment, damage to organelles, neuronal cell death, and finally, clinical symptoms.[109]

It was reported recently that stimulation of astrocytic autophagy might be beneficial for neuroprotection in age-related neurodegenerative diseases.[119] Astrocytes can mediate the clearance of Aβ through its uptake and degradation.[120] In addition, astrocytes are involved in Aβ uptake and degradation[121,122] Regarding PD, it has been observed that α-synuclein, the main component of Lewy body inclusions, accumulates in astrocytes, and correlates with the extent of neuronal loss.[123,124] Furthermore, α-synuclein released from neurons is taken up by astrocytes.[124–126] In the HD brain, disease progression correlates with an increase in reactive astrocytes, and HTT, which contains polyglutamine, also accumulates in astrocytes.[127,128] Loss of the glutamate transporter GLT1, which is involved in astrocytic autophagy, is a common characteristic found in human HD brain and animal models[129,130] Thus, uptake or removal of neurodegenerative disease-related proteins through astrocytic autophagy point to the contribution of astrocytes in neurodegenerative diseases.

In a study of gintonin's role in brain autophagy, Rahman et al.[56] demonstrated that treatment of gintonin to cortical astrocytes upregulated the autophagy marker proteins LC1-I and LC3-II. Gintonin-mediated marker protein increases were blocked by LPA1/3 receptor antagonists, showing that gintonin-mediated enhancement of autophagy marker protein was achieved via G protein-coupled LPA receptor regulation. Gintonin-mediated autophagy also increased the formation of LC3 puncta and the signaling pathway for gintonin-mediated enhancement of autophagy, including AMPK-mTOR pathways, since pretreatment with an AMPK inhibitor, compound C, inhibited LC3-II and LC3 puncta expression, whereas treatment with a mammalian target of rapamycin (mTOR) inhibitor, rapamycin, further enhanced LC3-II and LC3 puncta expression.[131] However, gintonin-mediated autophagy enhancements were significantly blocked by an autophagy inhibitor, such as 3-methyladenine, and knockdown of *beclin-1*, *Atg5*, and *Atg7* gene expression, which are necessary and key elements for vesicle initiation, nucleation, and elongation through phagophore formation.[132] Deletion of the essential autophagic genes *ATG7*[105] and *ATG5*[106] can sufficiently induce the formation of cytoplasmic inclusions and neurodegeneration in neurons, indicating that normal autophagy is necessary for the homeostasis of neurons. When astrocytes were pretreated with a lysosomotropic agent, E-64d/peps A or bafilomycin A1, gintonin-mediated autophagy significantly increased the levels of LC3-II along with the formation of LC3 puncta, also indicating a role for astrocytes in autophagy. Gintonin-mediated enhancement of autophagic flux led to an increase in lysosome-associated membrane protein 1 and degradation of ubiquitinated p62/SQSTM1. Thus, gintonin could be a candidate as an astrocytic autophagy enhancer via the LPA1 receptor-AMPK-mTOR-mediated signaling pathway (Fig. 4).

Astrocytes are a type of brain glial cell and constitute about 20% of all glial cells. They play a housekeeping role in the brain and have diverse functions such as glycogen storage for energy supply to neurons, BBB formation, uptake of excitatory neurotransmitter released from neurons, and synaptic transmission regulation through the formation of tripartite synapses with neurons.[120,133] In addition to the *in vitro* and *in vivo* neuroprotective effects of gintonin, we showed in previous reports that gintonin stimulates gliotransmitter release, astrocytic glycogenolysis, and formation of vascular endothelial growth factor (VEGF) in hypoxic astrocytes.[134–136] Gintonin also stimulates cortical astrocytic autophagy.[56] Although there is currently no direct evidence regarding whether gintonin-mediated stimulation of autophagy in the brain is linked to the amelioration of neurodegeneration (Fig. 4), it seems that gintonin-mediated stimulation of brain astrocytic autophagy could be an attractive therapeutic target for aging-related brain diseases.

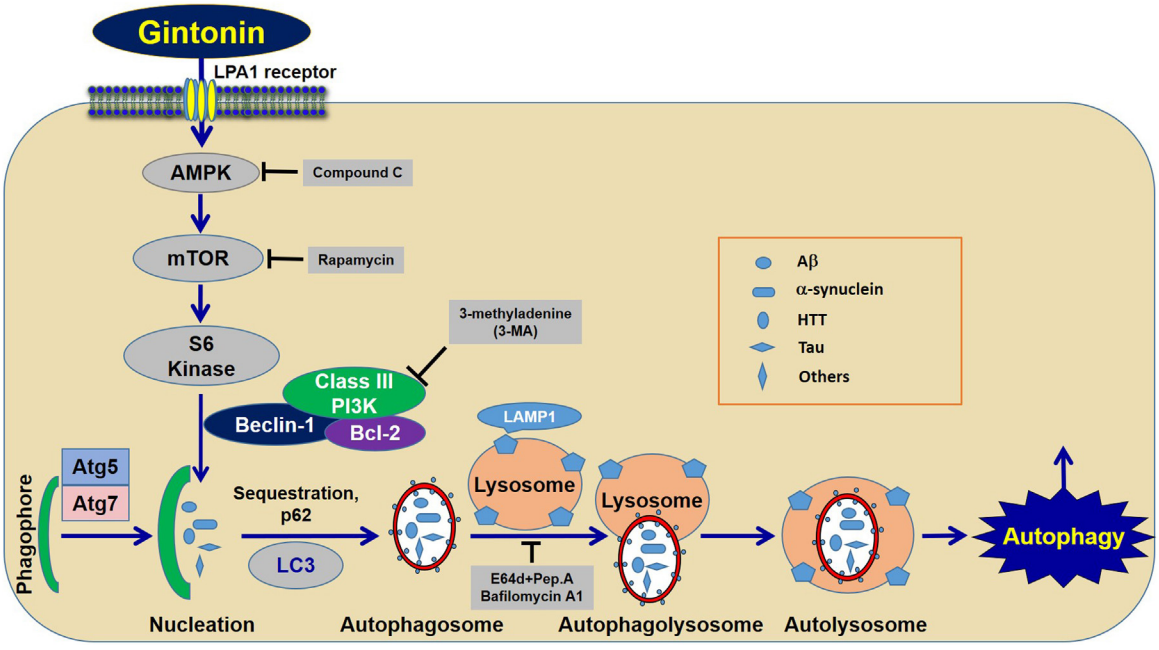

Fig. 4. Signaling pathway for gintonin-mediated autophagic influx via LPA1 receptor. Gintonin stimulates astrocytic autophagy via down-streams of autophagic processes.[56] Stimulations of autophagy flux by gintonin might play a role for housekeeper by cleaning brain toxic wastes such as Aβ, α-synuclein, HTT and others, although it requires more studies to show that gintonin-mediated astrocytic autophagy is coupled to *in vivo* anti-neurodegenerative diseases.

3.11. Development of medicinal food or natural medicines using ginseng gintonin is necessary for elderly people in aging societies

Most well-known traditional medicines contain ligands that act on the GPCR or ligand-gated ion channels in the plasma membrane of animal cells. Many of these medicines treat various types of diseases. In fact, GPCR-related drugs account for more than half of those in pharmaceutical markets. For example, morphine and morphine derivatives, which are derived from the herbal poppy, are widely used as anti-nociceptive agent through the regulations of endogenous opioid receptors.[137] Another recent example, ω-conotoxins, which are peptides isolated from Cone snails and inhibit voltage-gated Ca^{2+} channels with high affinity, are commercialized as specific painkillers for chronic pain.[138] Ginseng usually does not show many side effects, even when taken in the long-term, compared to other traditional medicines. Ginseng saponins (or ginsenosides) were first isolated from ginseng but they did not show a role as a receptor ligand of GPCR or ligand-gated ion channels, since they did not show selectivity in receptors and ligand-gated ion channels, but rather interactions with plasma membrane receptors or ligand-gated ion channels in a non-specific and ambiguous manner and with very low affinity.[17]

Recently, gintonin was isolated from ginseng and gintonin acts as an exogenous ligand for the LPA receptors, GPR40 and GPR55.[49] Gintonin attenuates aging related-neurodegenerative diseases such as AD, PD, and HD,[12,91,92,100] and stimulates autophagy in neuronal cells[56] and is a good candidate for ginseng-derived functional foods or natural medicines against aging-related brain diseases. This is important, since AD, PD, and HD are becoming increasingly more prevalent in our aging society, and dramatically increasing compared with other chronic diseases. These diseases impose huge psychological burdens on families and economic burdens on society, especially in countries with aging societies. Gintonin (or GEF) will be first the ginseng product to target GPCRs such as the LPA receptors GPR40 or GPR55, for brain rejuvenation in senior citizens. Now, further trials are necessary to shift the paradigm of ginseng-related product production from old-fashioned products using dried ginseng root itself or simple ginseng extract to more refined- and disease targeted-oriented ginseng product production.

4. Conclusion

Recent preclinical studies have shown that gintonin and/or GEF ameliorates brain aging-related neurodegenerative disease such as AD, PD, and HD. The mode of actions underlying anti-neurodegenerative diseases by gintonin and/or GEF treatment might be derived from (1) increases in brain BDNF and VEGF expression and increases of acetylcholine and dopamine synthesis enzymes; (2) stimulations of hippocampal and striatal neurogenesis; (3) attenuations of brain apoptosis due to oxidative stress by increasing Nrf2/HO-1 and neuro-inflammation by inhibition of ROS formation and inflammatory cytokine production in brain; (4) restoration of the BBB damaged by oxidative stress and neuroinflammation; and (5) gintonin-mediated stimulation of cortical astrocytic autophagy. These beneficial effects gintonin might provide a molecular bases to inhibit accumulation or facilitation of clean-up of Aβ, α-synuclein or HTT. In the future, further clinical investigations might be required to examine the efficacy, safety or side effects, and tolerability for the prevention and attenuation of brain aging and neurodegenerative diseases.

Author contributions

Conceptualization: SHC, RL, SMN, DGK and SYN. Methodology: SHC, RL, SMN and DGK. Formal analysis: SHC, RL, SMN and DGK. Writing-original draft: SHC, SYN. Writing-review & editing: SHC, IHC, HCK, YC, HR, and SYN. Supervision: SHC, IHC, HCK, YC, HR, and SYN.

Conflicts of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be SHR construed as potential conflicts of interest.

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Ethical statement

Not applicable.

Data availability

The data for this review will be made available upon request.

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