PI3K/AKT signaling mediated by G protein-coupled receptors is involved in neurodegenerative Parkinson's disease (Review)

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Received July 29, 2016; Accepted December 12, 2016

DOI: 10.3892/ijmm.2016.2833

Abstract. Parkinson's disease (PD) is a common progressive and multifactorial neurodegenerative disease, characterized by the loss of midbrain dopaminergic neurons. Numerous pathological processes including, inflammation, oxidative stress, mitochondrial dysfunction, neurotransmitter imbalance, and apoptosis as well as genetic factors may lead to neuronal degeneration. Motor deficits in PD are due mostly to the progressive loss of nigrostriatal dopaminergic neurons. Neuroprotection of functional neurons is of significance in the treatment of PD. G protein-coupled receptors (GPCRs) have been implicated in the neuroprotection against PD through the survival of dopaminergic neurons. In addition, phosphatidyl-inositol-3-kinase (PI3K)/AKT signaling has also been demonstrated to be neuroprotective. Knowledge of the mechanisms involved in this cellular protection could be critical for developing treatments to prevent this neurodegenerative disorder. In this review, we highlight the protective roles of the PI3K/AKT signaling pathway in the function of representative serotonin GPCRs. Particular attention is given to the molecular mechanisms of this pathway proposed to explain the favorable effects of food ingredients against neurodegenerative disease.

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Abbreviations: DHA, docosahexaenoic acid; L-DOPA, levodopa; ER, estrogen receptor; GPCRs, G protein-coupled receptors; 5-HT, serotonin or 5-hydroxytryptamine; n-3 PUFA, ω-3 polyunsaturated fatty acid; PD, Parkinson's disease; PI3K, phosphatidyl-inositol-3-kinase; PTEN, phosphatase and tensin homolog; RBD, Ras-binding domain; ROCK, Rho-associated coiled-coil containing protein kinase; ROS, reactive oxygen species; α-syn, α-synuclein.

Key words: G protein-coupled receptor, serotonin, dopamine, neuron, small G protein, phosphoinositide 3-kinase, AKT, phosphatase and tensin homolog, Parkinson's disease

Contents

1. Introduction

Parkinson's disease (PD) is characterized by neuropsychiatric symptoms such as depression and anxiety preceding the onset of motor symptoms (1). Major features of PD include the loss of dopaminergic neurons in the substantia nigra and Lewy body depositions (2). It has been suggested that mitochondrial dysfunction, oxidative stress and oxidative damage underlie the pathogenesis of PD (3). Activity of substantia nigra dopaminergic neurons is critical for striatal synaptic plasticity and associative learning. The degeneration of dopaminergic neurons leads to a disinhibition of the subthalamic nucleus thus increasing excitatory projections to the substantia nigra. In consequence, excessive activity causes excitotoxicity and oxidative stress (3,4). Consequently, intracellular accumulation of filamentous α-synuclein (α-syn) aggregates to form Lewy bodies, a pathologic hallmark of PD (4). Lewy body disease is also a group of neurodegenerative disorders characterized by α-syn accumulation that includes Lewy body dementia and PD symptoms (5). Genetic defects, aging, and environmental toxicants have been recognized as risk factors for the development of these diseases. Although the pathogenesis is still unclear, evidence suggests that oxidative stress plays a central role in progession of the disease. In particular, reactive oxygen species (ROS) may play an important role in inflammatory processes (6). Cellular ROS metabolism is definitely regulated by a variety of proteins involved in the redox mechanism with the phosphatidyl-inositol-3-kinase (PI3K)/AKT signaling pathway (7) (Fig. 1). Accordingly, the PI3K/AKT pathway acts as a pivotal determinant of cell fate regarding

senescence and apoptosis, which is mediated by intracellular ROS generation (7). In addition, ROS activate PI3K/AKT and inactivate phosphatase and tensin homologue deleted on chromosome ten (PTEN) (8,9). High concentrations of ROS may induce cellular damage. However at lower concentrations ROS may act as intracellular secondary messengers. An excess amount of oxidative stress can lead to crushing consequences in the nervous system during aging. Therefore, both acute and chronic neurodegenerative disorders could be mainly a result of oxidative stress (10). ROS regulation and inhibition of the apoptotic pathway thereby protecting cells have been shown to be controlled by the PI3K/AKT signaling pathway (11). The mechanism involved in PI3K/AKT activation exhibits stimuli-specific variations.

G protein-coupled receptors (GPCRs) are a large class of molecules involved in signal transduction across cell membranes, which are the most common targets of neuro-pharmacological drugs in the central nervous system (12,13). Stimulation of GPCRs leads to activation of heterotrimeric G proteins and their intracellular signaling pathways. In addition to the signaling via heterotrimeric G proteins, GPCRs can also signal by interacting with various small G proteins to regulate downstream effector pathways (14). Some small G proteins can associate directly with GPCRs, and often modulate the GPCR signaling network. It is becoming clear that these GPCRs are not only activated by authentic agonists but that they also exhibit agonist-independent intrinsic activity. In addition, a hallmark of GPCRs is their ability to recognize and respond to chemically diverse ligands, which efficiently activate PI3K/AKT signaling in numerous cell types (Fig. 1). As mentioned above, the PI3K/AKT signaling pathway transduces a signal regulating a wide range of events involved in cell survival and metabolism. Defective regulation of the PI3K/AKT pathway has been linked to several diseases including cancer, diabetes, atherosclerosis and neurodegenerative diseases (15,16) (Fig. 1). Knowledge concerning the interplay between GPCRs and PI3K/AKT may contribute to improved treatment and prevention of these diseases. However, regulation of the interplay appears to be complex. Some PI3Ks can be activated by binding of the regulatory subunit to specific tyrosine-phosphorylated domains in cell surface receptors. In addition, Ras family proteins are important direct activators of PI3Ks, interacting via an amino-terminal Ras-binding domain (RBD) (17,18). Different PI3Ks could also be activated in a receptor-specific manner and by distinct GTPases of the Ras and Rho families (19). This review summarizes current understanding of therapeutic GPCRs and PI3K/AKT signaling for neurodegenerative diseases such as PD. We also address the behavioral relevance of GPCRs and PI3K/AKT signaling in PD.

2. Relationship between cell surface GPCRs and PI3K/AKT signaling via small GTPases

GPCRs are integral membrane proteins that regulate intracellular secondary messenger levels via the coupling of activation by extracellular stimuli. For example, activation of GPCRs starts a series of molecular events leading to GPCR kinase-mediated receptor phosphorylation (20). In addition, GPCRs can stimulate Ras and activate class I PI3Ks depending on RasGEF and RasGRP4 (21). Several effector molecules for the small GTPases support cancer cell migration and invasion by regulating the PI3K/AKT signaling pathway (22). Furthermore, it has been shown that Rit and Rin subfamily Ras-related small GTPases are associated with neuronal disorders such as PD (23). Members of the Rho GTPase family have important roles in regulating several aspects of cytoskeleton-based functions, including cell migration, proliferation, and apoptosis. The Rho-associated coiled-coil containing protein kinase (ROCK) is a serine/threonine kinase and a major downstream effector of Rho GTPases (24). ROCK enhances actin/myosin contraction (25). Furthermore, ROCK activates caspase-dependent apoptosis signaling cascades (26). PTEN has been identified as a ROCK substrate that is also involved in cell death and survival (27,28). ROCK phosphorylates PTEN and stimulates its phosphatase activity. PTEN is a negative regulator of the PI3K/AKT pathway by dephosphorylating the inositol 3'-phosphate group, which has important roles in cell survival and apoptosis (Fig. 2). PTEN decreases the AKT phosphorylation levels induced by ROCK activation. Accordingly, ROCK appears to be involved in regulation of PI3K/AKT signaling. Hence, inhibition of ROCK activation attenuates apoptosis (29). Furthermore, the Rho/ROCK/PTEN pathway may be a key regulatory step in cell transformation, and thus plays an essential role in Ras-induced tumorigenesis (30). In mammals, four isoforms of the type I PI3K catalytic p110 subunits have been identified (Fig. 2). Activated Ras molecules bind directly to an N-terminal RBD on p110 to appropriately activate lipid kinase activity of PI3K following AKT activation (31). There are three known AKT isoforms which play critical and diverse roles in cells. A type of GPCR agonist could trigger the pro-survival AKT signaling pathway and protect neurons (32) (Fig. 2). Notably, a novel role for AKT has been found in maintaining neuronal serotonin (5-HT) receptor function (33). In addition, 5-HT activates the PI3K/AKT signaling pathway in several cancer cell lines (34). Growing evidence suggests their possible roles in the pathogenesis and treatment of PD (35,36). The serotonergic system may play a significant role in the pathogenesis of PD.

3. Serotonin stimulates GPCRs and PI3K/AKT signaling in cells

Human 5-HT receptor is a seven-transmembrane-domain GPCR, which activates adenylyl cyclase constitutively upon agonist activation (37). A pharmacological model for GPCR activation is the ternary complex model in which GPCR exists in an equilibrium of dynamic conformational states (38). Through the GPCR, 5-HT activates the PI3K/AKT and MAPK signaling pathways (34), which is an important intermediate signaling process in the behavioral functions of 5-HT receptors (39) (Fig. 1). 5-HT also functions as an angiokine to promote angiogenesis (40). In endothelial cells, 5-HT also activates PI3K/AKT signaling via GPCRs similar to that observed with VEGF (40). It has been apparent that the interaction of 5-HT and dopamine plays a key role in the behavior. 5-HT and dopamine levels decrease with age (41). In addition, 5-HT has been postulated as a key neuromodulator and neurotransmitter involved in aggression and stress. 5-HT

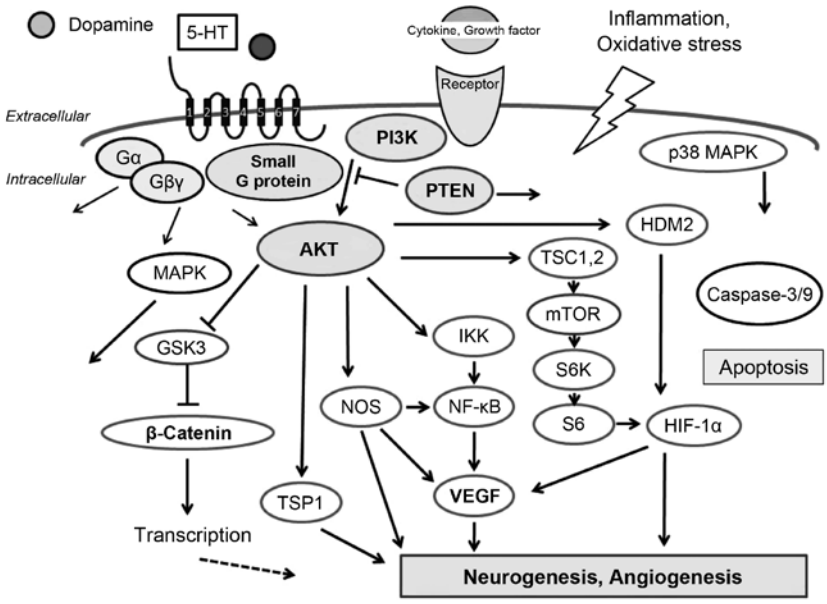

Figure 1. Schematic depiction and overview of GPCR/PI3K/AKT/PTEN signaling in an extracellular 5-HT response is shown. Example of molecules known to act on the GPCR/PI3K/AKT/PTEN signaling pathway are also shown. An arrow (↑) indicates stimulation whereas a hammerhead (⊤) indicates inhibition. Note that some critical pathways have been omitted for clarity. GPCR, G protein-coupled receptor; PI3K, phosphoinositide 3-kinase; PTEN, phosphatase and tensin homolog; 5-HT, serotonin.

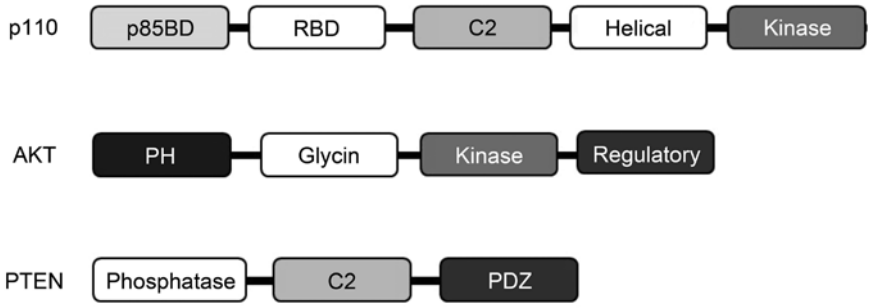

Figure 2. Schematic depiction representing the primary domain structures of human PI3K p110 subunit, AKT, and PTEN proteins. Note that the sizes of protein are modified for clarity. PI3K, phosphatidyl-inositol-3-kinase; PTEN, phosphatase and tensin homolog; p85BD, p85 binding domain; RBD, Ras-binding domain; C2, C2 domain, a protein structural domain involved in targeting proteins to cell membranes; Helical, helical structured domain; Kinase, kinase domain; PH, pleckstrin homology domain; Glycin, glycin-rich domain; Regulatory, regulatory domain; phosphatase, phosphatase domain; PDZ, a common structural domain in signaling proteins (PSD95, Dlg and ZO-1).

receptors may control dopaminergic neuron activity in a region-dependent manner. Thus, alterations in 5-HT release and a loss of serotonergic neurons may be linked to PD symptoms. Recent studies are focusing on agents involving neurotransmitters including 5-HT receptors. In addition, among a variety of proteins included in the GPCR family, serotonin 5-HT receptors are attractive as important biological targets of PD (42). It has been shown that the role of small GTPases of the Rho family in morphogenic signaling linked to 5-HT in neurons may control neuronal morphology and motility (43). 5-HT receptors are widely distributed in the central nervous system, especially in the brain region and are essential for learning and cognition (44). Among them, the basal ganglia are an extremely organized network of subcortical nuclei including the striatum and substantia nigra, which play a key role in many functions such as emotion, cognition, and motor control. These regions are critically involved in neurodegenerative diseases including PD and Lewy body disease (45,46). Serotonergic neurons of the dorsal raphe nucleus are excited at a steady rate during waking (47). Certain hallucinogens, antipsychotics, and antidepressants function by targeting the 5-HT receptor in addition to endogenous 5-HT. Through its traditional activity as a GPCR and ligand-gated ion channel, the neurotransmitter 5-HT has a complicated function in the modulation of brain information processing. In addition, it can be speculated that local microinjection of 5-HT would affect activity of the corresponding neurons (48). 5-HT can also exert intricate effects on the activity of midbrain dopaminergic neurons mediated by its various receptor subtypes. Dopamine-containing neurons in the brain receive an obvious innervation from 5-HT originating in the raphe nuclei of the brainstem (49). Therefore, the significant role of 5-HT in central dopamine dysfunction has been shown (50). Principal control of the interaction between 5-HT and dopamine-containing neurons in the brain appears to be mutually inhibitory. When dopamine innervation in

the striatum is critically low, the serotonergic system plays an important role in the development of idiopathic PD (51). Patients with PD frequently develop dementia, which is associated with neocortical deposition of α-syn in Lewy bodies and Lewy neurites (52). Widespread deficits in serotonergic and dopamine innervation of neocortical and basal ganglia regions have been demonstrated in advanced PD (53). 5-HT has major roles in brain diseases involving abnormal mood and cognition. Studies show that 5-HT receptor-antagonists have antipsychotic and antidepressant properties, whereas agonist ligands possess cognition-enhancing and hallucinogenic properties. In addition, the effects of a rapid reduction in 5-HT function have shown a reduction in cognitive status in dementia with Lewy bodies (54). Consequently, antidepressants may be useful in treating depression in PD (55).

4. Augmentation of the neuroprotective effects of the serotonin/GPCR/PI3K/AKT signaling pathway in PD

Therapeutic neuroprotective agents are currently receiving increased attention for the treatment of PD patients (32). For example, regrowth of axons within the adult nigrostriatal projection which is prominently affected in PD can be achieved by activation of PI3K/AKT signaling (56). In an attractive therapeutic approach, a GPCR and its agonist could trigger the pro-survival PI3K/AKT signaling pathway and protect neurons in *in vivo* and *in vitro* models against neuronal toxicity. Hence, treatment with an AKT inhibitor was found to block the neuroprotective effect (57). Medicinal Chinese herbs and its active ingredients may play various neuroprotective roles, including antioxidant, radical-scavenging, anti-inflammatory, and antiapoptotic activity (Fig. 3). For example, curcumin, which is a major active polyphenol component extracted from the rhizomes of *Curcuma longa*, has been reported to exert neuroprotective effects in an experimental model of PD (58). Curcumin ameliorates dopamine neuronal oxidative damage via activation of the PI3K/AKT pathway (58) (Fig. 3). The effects of curcumin may also be related to increased expression of PTEN (59). In addition, curcumin similarly protects cardiomyocytes against high glucose-induced apoptosis via the PI3K/AKT signaling pathway (60). Danshensu, a main hydrophilic component of the Chinese Materia Medica Radix Salviae Miltiorrhizae, has ROS scavenging and antioxidant activities via activation of the PI3K/Akt signaling pathway (61). Puerarin, an active component of *Pueraria montana* var. lobata is well-known for its anti-oxidative and neuroprotective activities via modulation of the PI3K/AKT pathway (62). In addition, a novel synthetic squamosamide derivative from a Chinese herb has been shown to have neuroprotective effects by activating the PI3K/AKT signaling pathway in experimental PD models (63). *Eucommia ulmoides* Oliv. bark attenuates oxidative stress through activation of PI3K/AKT, thereby protecting cells from neuronal cell death (64). Tyrosol exerted a neuroprotective effect via activation of the PI3K/AKT signaling pathway in a model of PD (65). A series of oxicam non-steroidal anti-inflammatory drugs have been shown to be neuroprotective via activation of the PI3K/AKT signaling pathway (66). *N*-acetyl-5-hydroxytryptamines may also attenuate oxidative cytotoxicity via activation of PI3K/AKT-dependent signaling (67). Furthermore, previous studies have shown the neuroprotective effects of pramipexole-induced hypothermia via the PI3K/AKT signaling pathway (68). *Drynaria fortunei*, a Polydiaceae plant, exerts its cell protective effects via the PI3K/AKT pathway (69). IGF-1 was found to protect the nigrostriatal pathway in a progressive PD model (70). This protection may be preceded by activation of the pro-survival PI3K/AKT signaling cascades. Guanosine was found to protect glial cells via the PI3K/AKT signaling pathway (71). In contrast, gallic acid, a polyphenol found in numerous fruits and vegetables particularly in hickory nuts, downregulates AKT phosphorylation but promotes PTEN expression (71).

GPCRs also mediate physiological functions fundamental for survival (72). Docosahexaenoic acid (DHA), an ω-3 polyunsaturated fatty acid (n-3 PUFA), modulates neuronal cell membrane properties thereby affecting the behavior of GPCRs (Fig. 3). Evidence suggests that DHA affects GPCR function by modulating oligomerization (73). A marked susceptibility of DHA has been found for the interaction with dopamine D2 receptors, which leads to an increased rate of receptor oligomerization (73). The effect of DHA on oligomerization is purely kinetic (73). In addition, levels of DHA are frequently decreased in several neuropsychiatric disorders (74,75). Accordingly, membrane n-3 PUFA plays a key role in GPCR signaling, which may have important implications for neuropsychiatric conditions such as PD. Furthermore, DHA treatment protects hippocampal neurons by increasing AKT phosphorylation (76). The sweet taste receptors T1R2 and T1R3 are GPCRs. Saccharin treatment rapidly stimulates the phosphorylation of AKT and downstream targets (77). As mentioned above, the receptors of 5-HT and dopamine are GPCRs. While dietary restriction maintains healthy behavioral funcioning at least partially by sustaining a high 5-HT level, elevating the 5-HT level improves these behaviors during aging (41). Fluoxetine is a widely used antidepressant drug, which upregulates phosphorylation of AKT (78). Essential amino acid L-tryptophan is critical for the synthesis of 5-HT, consequently maintaining an entry-way for augmenting brain 5-HT levels by means of consuming a tryptophan-enriched diet. Accordingly, experimental animals fed a tryptophan-enriched diet exhibited higher serotonergic activity (79). Clary sage oil could be developed as a therapeutic agent for patients with depression, and the antidepressant-like effect of clary oil is closely associated with modulation of the dopamine pathway (80). After inhalation of clary sage oil, cortisol levels in menopausal women were significantly decreased while the 5-HT concentration was significantly increased (81). 5-HT receptor antagonists were found to change behavioral performance through inhibition of PI3K/AKT (82).

Estradiol was previously found to have an antidepressant-like effect. The antidepressant-like effect of estradiol is due to estrogen receptor (ER)β activation, whereas blockade of the effect of an SSRI by estradiol was mediated by ERα. Estradiol shows a potential slowing of 5-HT clearance mediated by ERβ (83). Maintaining a level of endogenous estrogen in females may prevent women from developing PD (84). Tocotrienols, members of the vitamin E family, have antioxidant properties. Tocotrienols are favorable candidates for neuroprotection in the pathogenesis of PD, and exhibit not only antioxidant properties but also signal-mediated action

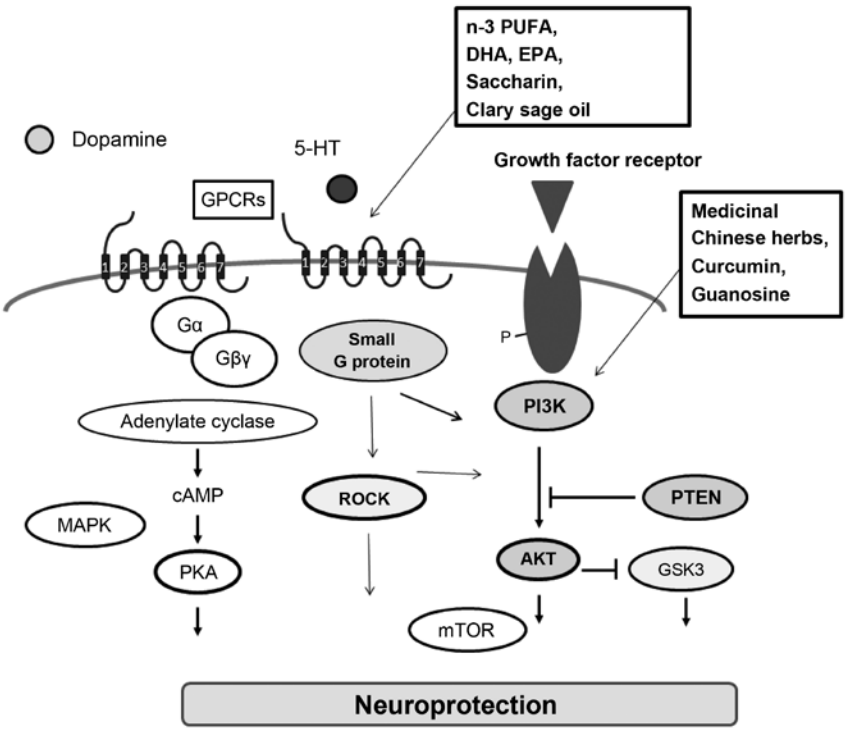

Figure 3. Several modulators linked to the GPCR/PI3K/AKT/PTEN pathway are shown, whose potential molecular targets may be based on the predominant sites. An arrow (↑) indicates stimulation whereas a hammerhead (⊤) indicates inhibition, suggesting implication of GPCR/PI3K/AKT/PTEN modulators for the therapy of PD via neuronal protection. Note that various critical drugs have been omitted for clarity. GPCR, G protein-coupled receptor; PI3K, phosphoinositide 3-kinase; PTEN, phosphatase and tensin homolog; PD, Parkinson's disease.

following ERβ/PI3K/AKT signaling (85). Related activation of ERβ may reduce the progression of PD by precluding α-syn accumulation (86). The α-syn, an intrinsically disordered presynaptic 14 kDa protein whose fibrillation is a critical step in the pathogenesis of PD, affects serotonergic neuronal projections within the hippocampus (87). Inhibition of α-syn fibrillation is brought about by a polyphenolic acid known as caffeic acid in a dose-dependent manner (88). Blocking PI3K/AKT signaling prevents the expression of α-syn and attenuates neuroprotection (63). The inhibitory activity of caffeic acid against α-syn fibrillation may guide in the planning of novel therapeutic treatments for PD.

5. Perspectives

Environmental exposures to toxic mediators such as ROS may lead to neurodegenerative disorders that have shared clinical findings with PD. It is critical to develop strategies to ensure that healthy neurons remain alive following ROS attack without using intricate medications. The precise identity and functional prototypes of molecular intermediates leading to neuronal mortality remain to be deciphered. Recently, traditional Chinese medicinal herbs have become popular as new approaches for the prevention and treatment of PD and/or other neurodegenerative diseases (Fig. 4). Functioning of the PI3K/AKT pathway may ensure that neuro-defense is active in order to render neuroprotection by preventing apoptosis and neuro-inflammation. Herbs may facilitate the above process. In addition, the recent development of selective ligands for 5-HT receptors will not only allow a detailed understanding of this protection but will lead to the development of new treatment strategies, appropriate for neurodegenerative disorders including PD. However, any therapeutic approach that limits itself to drugs against a single pathological process is invalid. Accordingly, combinations with various pharmacological properties are likely to be more effective. We believe that increased knowledge of the molecular details of the nature of the GPCR/PI3K/AKT signaling interaction may lead to

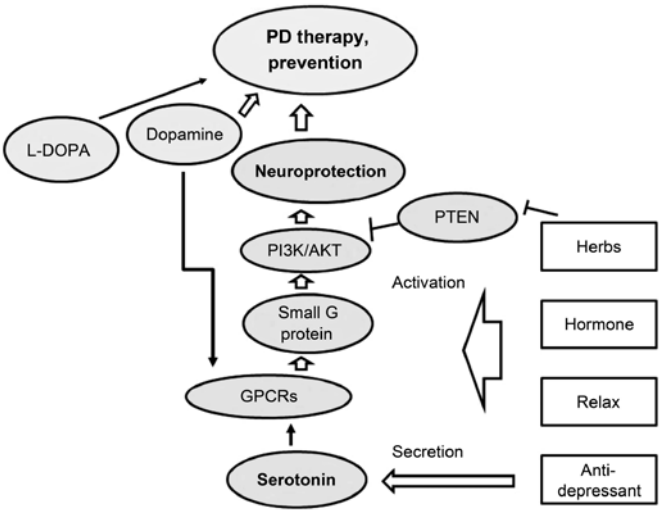

Figure 4. Simplified diagram indicating the possibilities of PD treatment and/or prevention. Several herbs, relax flavor, antidepressants, and/or hormones may contribute to the improved neuroprotection via GPCR/PI3K/AKT signaling. Note that some critical events have been omitted for clarity. PD, Parkinson's disease; GPCR, G protein-coupled receptor; PI3K, phosphoinositide 3-kinase; L-DOPA, levodopa; PTEN, phosphatase and tensin homolog.

better insight into the overall understanding of the function of GPCRs in neurodegenerative disease. Future studies should focus on the availability of novel treatments to improve the therapeutic efficacy in this field.

Acknowledgements

The present study was supported by JSPS KAKENHI (grant nos. 24240098 and 26-12035).

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