Chapter 3
Classical Signaling Pathways

Hideki Hayashi

Abstract In this chapter, I introduce a number of important aspects of intracellular signaling pathways related to the protection and degeneration in cells, especially neurons, under physiological and pathological conditions. Extracellular stimuli activate intracellular signaling pathways by receptor- and/or channel-mediated manner or simple diffusion across the plasma membrane. A variety of intracellular signaling molecules responses to each extracellular stimulus and reflects the coordinated actions of the cells. While there are many signaling pathways contributing to cellular functions and survival, I will focus on the MAPK/ERK and the Akt pathways in this chapter. The roles of these pathways have been extensively studied in neurons and other cells. However, the contribution and/or the cross talk of these intracellular signaling pathways in the degeneration and protection of the retina remains unclear because each signaling molecule in these pathways has diverse roles under physiological and pathological conditions. I would be happy if this chapter would help the readers to understand the complex and the ingenious regulating mechanisms of the intracellular signaling pathways for the degeneration and protection in neurons of the retina.

Keywords MAPK/ERK • Neuroprotection • PI3K-Akt

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3.1 MAPK/ERK Pathways

Mitogen-activated protein kinases (MAPKs) are serine/threonine kinases conserved in eukaryotes from yeasts to vertebrates and one of the most important enzyme groups, which regulate a large variety of events including development, cell migration, differentiation, metabolism, survival, and degeneration. In mammals, four distinct MAPK subfamilies have been identified (Fig. 3.1): extracellular signal-regulated kinases 1 and 2 (ERK1/2), c-jun N-terminal kinases 1, 2, and 3 (JNK1/2/3), p38 MAPKα, β, γ, and δ, and ERK5. Although MAPKs are not directly phosphorylated by receptor-type tyrosine kinases or receptor-associated protein kinases, a signal transduction cascade composed of three modules in each MAPK subfamily activates MAPKs in series by phosphorylation. The phosphorylation of a specific MAPK is induced by a specific MAP kinase kinase (MAPKK or MAP2K) and a specific MAP kinase kinase kinase (MAPKKK or MAP3K), which phosphorylates MAPKK.

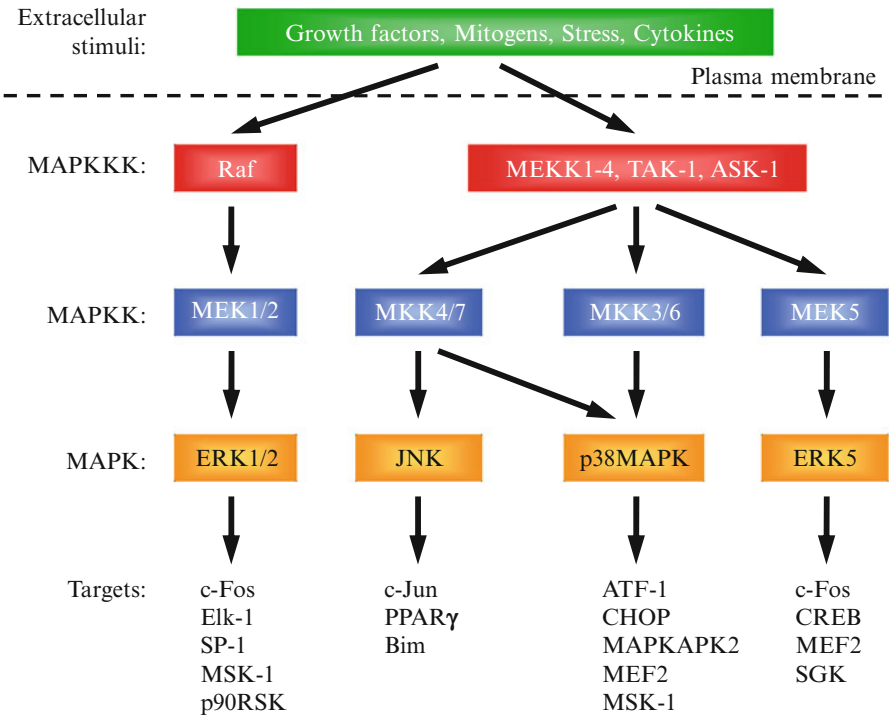

Fig. 3.1 The simplified scheme of the MAPK cascade and its regulations and targets (see text for details)

3.1.1 ERK1/2

ERK1 (MAPK3) and ERK2 (MAPK1) are 90 % identical in primary sequence [1] and are expressed in all tissues with various extents. ERK1/2 are phosphorylated by MAPKK1/2 (MEK1/2), which are activated by Raf-1, B-Raf, or other MAPKKK proteins in the Ras-Raf-MEK-ERK cascade. A double phosphorylation at threonine and tyrosine residues of ERK1/2 by MEK1/2 is required for maximum activation. In most cases, the activation of the ERK1/2 cascade is initiated by receptor-type tyrosine kinases, G-protein-coupled receptors, and ion channels at the plasma membrane. An inactive Ras-GDP is converted into an active Ras-GTP by guanine nucleotide exchange factors. The conversion of Ras-GDP to Ras-GTP is stimulated by growth factor receptors such as vascular endothelial growth factor (VEGF) receptor, epidermal growth factor receptor, and insulin-like growth factor receptor [2]. In addition to these growth factor receptors, tropomyosin-sensitive receptor kinases (Trks), which are the high-affinity receptors for neurotrophins such as nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), and neurotrophin 3 and neurotrophin 4, also activate the Ras-Raf-MEK-ERK pathway [3]. The ERK1/2 pathway has a large number of downstream targets including transcription factors such as Elk-1 and c-Fos and protein kinases such as p90 ribosomal S6 kinase and mitogen- and stress-activated protein kinase-1 (MSK-1). Thus, changes of ERK1/2 activity will cause intricate and profound effects on the cell function and survival.

BDNF is a well-known neurotrophin as a neuroprotective molecule against optic nerve injury and optic neuropathy. Nakazawa et al. [4] reported that an intravitreal injection of BDNF, but not NGF and neurotrophin 3, protected rat retinal ganglion cells (RGCs) from optic nerve transection-induced cell death via signaling pathways involving ERK as well as PI3K. It has also been reported that tears in normal tension glaucoma patients contain less BDNF than those in normal subjects [5]. Thus, the administration of BDNF and/or stimulation of the TrkB, a receptor of BDNF, -ERK pathway has been considered as one of the neuroprotective strategies in potential glaucoma therapies.

7,8-Dihydroxyflavone, a member of the flavonoid family, is a selective and a bioactive high-affinity TrkB agonist, which inhibits kainic acid-induced apoptosis in the hippocampus, decreases infarct volumes induced by the transient middle cerebral artery occlusion, and reduces the neurotoxicity of the dopaminergic toxicant 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine in mice [6]. It has been recently reported that the 7,8-dihydroxyflavone promoted the survival signaling pathways of ERK as well as Akt and protected RGCs and the RGC-5 cell line, a retinal neuronal precursor cell line, from oxidative stress and excitotoxicity [7].

Zhou et al. [8] performed an intravitreal injection of recombinant adeno-associated virus to transduce RGCs with genes encoding a constitutively active form of MEK1, an upstream activator of ERK1/2. This treatment induced in vivo activation of ERK1/2 in the retina at 4 weeks after injection of adeno-associated

virus and significantly increased RGC soma and axon survival at 5 and 7 weeks after ocular hypertension surgery in a rat glaucoma model. This study demonstrated that the gene therapy by the adeno-associated virus to transduce RGCs efficiently improved the RGC survival in ocular hypertensive insult, and the ERK1/2 pathway performed an important role in the protection of RGCs from neurodegeneration induced by glaucoma.

If the ERK signaling pathway plays an important role in the neuronal survival, an inhibition of this pathway would cause a survival defect in neurons. Methylprednisolone, a synthetic glucocorticoid, has been used in the therapy of neurological autoimmune central nervous system diseases. Diem et al. [9] determined the effect of high-dosage methylprednisolone therapy, which is the standard approach for acute inflammation of the optic nerve, on the survival of RGCs in an animal model of severe optic neuritis. This study demonstrated that the administration of methylprednisolone significantly exacerbated RGC apoptosis caused by myelin oligodendrocyte glycoprotein-induced experimental autoimmune encephalomyelitis, and the mechanism of the aggravated RGC apoptosis was an inhibition of ERK1/2 phosphorylation by the steroid treatment.

Perron and Bixby [10] investigated that N-cadherin, laminin, and basic fibroblast growth factor (bFGF) promoted neurite outgrowth in embryonic chick retinal neurons and then found that the neurite outgrowth induced by these proteins required the activation of ERK1/2, but not p38 MAPK. It has also been reported that bFGF modulates axon extension and growth cone guidance of RGC in *Xenopus laevis* via the ERK as well as the phospholipase C pathways, but not the phosphatidylinositol 3-kinase (PI3K) pathway [11]. Furthermore, activated leukocyte cell adhesion molecule (ALCAM) expresses only in extending axons of RGCs and plays important roles of extension and guidance of RGC axons in the early stage of developing retina. The translation of ALCAM occurs in axonal growth cones of chick RGCs and requires the activity of the ERK1/2 pathway [12]. Thus, the ERK1/2 signaling pathway not only plays key roles in neuron survival but also regulates the axon growth of RGCs.

3.1.2 JNK

JNK1/2/3 (MAPK8/9/10), also known as stress-activated protein kinase (SAPK) γ/α/β, respectively, are activated by MAPKK4 and MAPKK7, also known as MKK4 and MKK7, respectively (Fig. 3.1). MKK4 and MKK7 lead to the activation of JNKs by simultaneous phosphorylation at a threonine and a tyrosine residue in protein kinase subdomain of JNK. Although MKK7 specifically activates JNKs, MKK4 can also activate p38 MAPKs. Downstream targets of JNKs include transcription factors such as c-jun and FOXO4, nuclear hormone receptors such as peroxisome proliferator-activated receptor-γ and the retinoic acid receptor,

cytosolic proteins such as insulin receptor substrate-1 and the E3 ligase Itch, and mitochondrial proteins of the Bcl-2 family such as Bad and Bim [13]. Therefore, it is considered that JNKs mediate many nuclear, cytosolic, and mitochondrial responses in developmental, physiological, and pathological conditions.

JNK1 and JNK2 are present in a wide variety of tissues, but JNK3 is primarily expressed in neurons of the central and peripheral nervous system [14]. Knockout mice in *Jnk1*, *Jnk2*, or *Jnk3* survive without apparent structural abnormalities, and *Jnk1*/*Jnk3* or *Jnk2*/*Jnk3* double-deficient mice also survive normally. However, *Jnk1*/*Jnk2* double deficiency leads the dysregulation of apoptosis in brain development and then causes embryonic lethality, indicating that JNK1 and JNK2 play a crucial role in the regulation of apoptosis during the CNS development [15]. In further details, although $Jnk1^{-/-}$ mice survive normally, they show a considerable reduction of the phosphorylation of microtubule-associated protein 2, the cytoskeletal protein, and exhibit axonal and dendritic degeneration in the brain [16]. JNK2 deficiency in mice did not show significant morphological changes but, nevertheless, caused impairment of long-term potentiation in the hippocampus during development [17]. JNK3 knockout attenuates the activation of the proapoptotic caspase cascade and leads to a strong resistance to cerebral hypoxic-ischemic injury compared to wild-type mice [18].

In retinal explants, JNK activation caused by calcium overload can induce a caspase-dependent apoptosis of photoreceptors by an increase of the BH3-only protein Bim_{EL}, which is a proapoptotic Bcl-2 family member [19]. In addition, Fernandes et al. [20] demonstrated that JNK signaling was activated in RGCs following optic nerve crush in mice, and double deficiency of *Jnk2* and *Jnk3* strongly protected RGCs from axon injury-induced death. Dual leucine zipper kinase identified by a high-throughput RNA interference screen has been shown as a critical kinase for JNK activation and cell death in primary RGCs and a rodent model of optic neuropathy. Furthermore, the small-molecule kinase inhibitor tozasertib decreases the phosphorylation of MKK7 and JNK, which are the downstream targets of dual leucine zipper kinase, and then protects RGCs from experimental glaucoma and traumatic optic neuropathy [21]. The D-form of JNK inhibitor 1, a synthetic cell-permeable JNK pathway inhibitor, protected RGCs from chronic ocular hypertension-induced [22] and *N*-methyl-D-aspartate (NMDA)-induced neurodegeneration [23]. Agmatine, an endogenous polyamine synthesized by the decarboxylation of L-arginine, suppresses the phosphorylation of JNK and nuclear factor-kappa B, but not ERK1/2 and p38 MAPK, caused by hypoxic damage and then protects RGC-5 cells from apoptotic death [24].

Increasing evidence supports that the JNK isoforms play important roles as capable mediators of proapoptotic signaling events under developmental and pathological conditions [25, 13]. Therefore, it has been considered that isoform-specific inhibitors for JNKs are beneficial for therapeutic use in neurodegenerative diseases including glaucoma.

3.1.3 p38 MAPK

The p38 MAPKα/β/γ/δ (MAPK14/11/12/13) are activated by MKK3 and MKK6 (Fig. 3.1). p38 MAPKγ is known as ERK6 and SAPK3, and p38 MAPKδ is also termed as SAPK4. In addition to the MKK3 and MKK6, p38 MAPKs can also be phosphorylated by MKK4, an upstream kinase of JNK, as shown in Fig. 3.1 depicting the cross talk between the p38 MAPK and the JNK pathways, and they share a substantial proportion of substrates. Therefore, p38 MAPKs and JNKs are often co-activated by the same upstream regulators and/or stresses and then demonstrate the synergic regulation for the downstream targets [26, 27]. Many transcription factors have been shown as downstream substrates of p38 MAPKs including CCAAT/enhancer binding protein β (C/EBPβ), C/EBP homologous protein (CHOP), myocyte enhancer factors 2A and 2C, activating transcription factors 1, 2, and 6 (ATF1/2/6), and p53 [28]. Other substrates of p38 MAPKs are MAP kinase-activated protein kinase 2 (MAPKAPK2 or MK2) and MSK-1. MAPKAPK2 collaborates with p38 MAPKs for activating various substrates such as heat shock protein 27 [29], tyrosine hydroxylase [30], cAMP response element-binding protein (CREB), and ATF1 [31]. MSK-1 is activated by p38 MAPKs and ERK1/2 and contributes to the activation of CREB [32].

Although p38 MAPKα is expressed ubiquitously in human tissues, p38 MAPKβ is abundantly expressed in the brain. p38 MAPKγ and p38 MAPKδ are expressed at high level in skeletal muscle and gland tissues, respectively [33]. Deficiency of *p38 Mapkα* results in embryonic lethality at E10.5–12.5 because of defective placental development by abnormal angiogenesis in the embryo itself and the visceral yolk sac [34]. Xing et al. [35] have reported that cortical neurons in coculture with microglia isolated from p38 MAPKα conditional knockout mice were protected from lipopolysaccharide-induced neurodegeneration. p38 MAPKα conditional knockout microglia secreted much less tumor necrosis factor-α (TNF-α), the pro-inflammatory cytokine, in response to lipopolysaccharide compared to wild-type microglia. Beardmore et al. [36] demonstrated that *p38 Mapkβ*$^{-/-}$ mice were viable and exhibit no obvious phenotype and concluded, with additional experiments, that p38 MAPKα was the main isoform of p38 MAPKs contributing to the immune response. Knockout mice in *p38 Mapkγ*, *p38 Mapkδ*, or *p38 Mapkγ/δ* were also viable and fertile without apparent health problem [37]. In addition, the knockout of p38 MAPKγ or p38 MAPKδ or both kinases did not alter the expression or the activity of the other isoforms.

p38 MAPKα in the nuclei of RGCs was activated after optic nerve transection [38]. The maximum phosphorylation of p38 MAPKα was detected 1 day after axotomy and then gradually decreased. Intravitreal administration of SB203580, an inhibitor of p38 MAPKα and p38 MAPKβ, protected RGCs from axotomy-induced cell death. Furthermore, the administration of MK801, an inhibitor of NMDA receptor, increased the number of survived RGC and also decreased p38 MAPK activation in a dose-dependent manner. This study indicated that RGC death induced after optic nerve damage was mediated through NMDA receptor and

then the p38 MAPK signaling pathway. Manabe et al. [39] reported that retinal injury caused by intravitreal injection of NMDA increased the phosphorylation of p38 MAPK and Akt in the ganglion cells and inner nuclear layers. Then, SB203580 rescued RGCs, whereas an inhibitor of the PI3K-Akt pathway, LY294002, exacerbated RGC death after NMDA injection. The authors concluded that the p38 MAPK pathway is proapoptotic and the PI3K-Akt pathway is antiapoptotic in RGCs against NMDA injury. Intravitreal injection of NMDA also upregulates thioredoxin interacting protein, an endogenous inhibitor of thioredoxin, and decreases thioredoxin activity [40]. Elevated thioredoxin interacting protein leads to the increase of oxidative stress, release of inflammatory cytokines such as TNF-α and IL-1β, and activation of the p38 MAPK and JNK pathways. In response to this neurotoxicity, treatment with verapamil, a calcium channel blocker and an inhibitor of thioredoxin interacting protein, attenuated these effects caused by the upregulation of thioredoxin interacting protein. It has been reported that phosphorylated p38 MAPK and JNK were detected in the nuclei of RGCs and amacrine cells and phosphorylated ERK1/2 were found in Müller cells after retinal ischemia induced by an elevation of intraocular pressure [41]. Inhibiting activation of p38 MAPK or ERK1/2, but not JNK, markedly protected retinal cells and restored retinal function from ischemic damage in the retina.

As described above, p38 MAPKs are considered as proapoptotic kinases of neurons and some other cell types in many cases. However, p38 MAPK is activated by the induction of ischemic tolerance, which is a phenomenon that a brief ischemia reduces the lethal damage of subsequent prolonged ischemia, in the gerbil hippocampus, and SB203580 attenuates the ischemic tolerance effect [42]. Intravitreal injection of bFGF shows the neuroprotective effect by elevating phosphorylated p38 MAPK, ERK1/2, and CREB in Müller glia against an NMDA-induced retinal damage [43]. Thus, the roles of p38 MAPKs in neurodegeneration or protection are highly dependent on stimuli, cell types, and other conditions.

3.1.4 ERK5

ERK5 (MAPK7) is activated in response to growth factors including epidermal growth factor, NGF, and BDNF, oxidative stress, and cytokines via the dual phosphorylation at threonine and tyrosine of its TEY sequence by MEK5 (Fig. 3.1). MEK5 is the only identified upstream kinase of ERK5 at present [44]. ERK5 is also known as a big MAPK (BMK) named due to a high molecular weight (a predicted molecular mass of 98 kDa) compared to ERK1/2 [45]. Substrates of ERK5 are serum- and glucocorticoid-inducible kinase (SGK) and the transcription factors such as c-Myc, c-Fos, NF-κB, Sap1a, CREB, and the family of the myocyte enhancer factor 2 (MEF2A, MEF2C, and MEF2D) [46]. Since inhibitors of MEK1/2 such as U0126 and PD98056, which have been used as specific inhibitors for MEK1/2, can also inhibit MEK5, data using these inhibitors should be reconsidered with a role of the MEK5-ERK5 pathway [47].

Deficiency of *Erk5* results in embryonic lethality at days 9.5–10.5 because of severe defects of cardiovascular development and angiogenesis [48], whereas neuron-specific knockout of *Erk5* shows normal development in two different transgenic lines of mice [49]. On the other hand, the expression of a dominant-negative form of ERK5 blocked the protective effect of BDNF from serum withdrawal-induced apoptosis in primary rat embryonic cortical neurons [50]. Thus, it has been considered that the ERK5 signaling pathway contributes to at least the neurotrophin-mediated neuroprotective effect.

The phosphorylation of ERK5 is increased in dorsal root ganglion neurons in response to peripheral inflammation, suggesting that ERK5 may contribute to the development of inflammatory pain [51]. Watson et al. [52] demonstrated that a retrogradely transported TrkA signal initiated by NGF at distal axons activated ERK5 at the cell bodies of dorsal root ganglion neurons, induced nuclear translocation of ERK5 and the phosphorylation of CREB, and promoted neuron survival.

Wu et al. [53] demonstrated that the knockdown of *Erk5* by siRNA increased VEGF expression and angiogenesis and constitutively active MEK5 adenovirus causing sustained ERK5 activation decreased VEGF expression in glucose-exposed human microvascular endothelial cells. In addition, the reduction of ERK5 protein and increase of VEGF mRNA were found in the retinas of streptozotocin-induced diabetic rats. It has also been reported that increased fibronectin production, one of the characteristic features of diabetic retinopathy, decreased ERK5 expression in the retinae of diabetic rats [54]. In experimental glaucoma with chronic intraocular pressure elevation, only Brn3a among three Brn3 proteins, Brn3a, b, and c, showed a significant reduction with severe nerve damage, and the expression of *Erk5* mRNA, but not *Erk1/2* mRNA, demonstrated a positive correlation with a severity of optic nerve damage [55]. These studies indicate that ERK5 may perform important roles in the retina under pathological conditions such as diabetic retinopathy and glaucoma. However, further investigations have been required to define the roles of ERK5 in the retina and RGCs under physiological and pathological conditions.

3.2 PI3K-Akt Pathway

Akt is a 57 kDa serine/threonine kinase and activated by PI3K. Akt1, 2, and 3 are also known as protein kinase B (PKB) α, β, and γ, respectively. A catalytic domain of Akt/PKB is considerably similar to protein kinases A and C. Akt1/PKBα and Akt2/PKBβ are ubiquitously expressed with various extents, whereas Akt3/PKBγ is more restricted to the brain and testis [56]. Akt is rapidly activated by various growth factors such as platelet-derived growth factor (PDGF), epidermal growth factor, bFGF, insulin, and NGF. Then, Akt activation stimulated by the growth factor is inhibited by a PI3K inhibitor such as wortmannin and by a dominant-negative form of PI3K [57]. However, PI3K does not directly activate Akt. PI3K phosphorylates membrane phospholipids such as phosphatidylinositol 4-phosphate

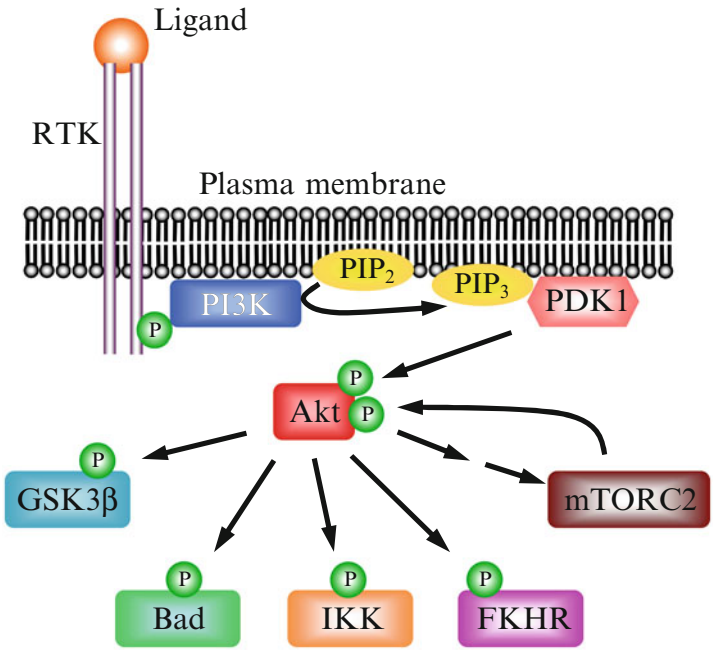

Fig. 3.2 A schematic model for the classic Akt pathway and its substrates (see text for details). *RTK* receptor tyrosine kinase, *PI3K* phosphatidylinositol 3-kinase, *PIP2* PtdIns(4,5)P2, *PIP3* PtdIns(3,4,5)P3, *PDK1* phosphoinositide-dependent protein kinase 1, *GSK3β* glycogen synthase kinase 3β, *IKK* inhibitor of κB kinase, *FKHR* forkhead transcription factor, *mTORC2* mammalian target of rapamycin complex 2

(PtdIns(4)P) and phosphatidylinositol 4,5-bisphosphate (PtdIns(4,5)P_2), thereby producing phosphatidylinositol 3,4-bisphosphate (PtdIns(3,4)P_2) and phosphatidylinositol 3,4,5-trisphosphate (PtdIns(3,4,5)P_3). The binding of the pleckstrin homology (PH) domain of Akt to PtdIns(3,4)P_2 and/or PtdIns(3,4,5)P_3 is the driving force to recruit Akt to the plasma membrane [58]. Akt has two major phosphorylation sites at Thr308 in the kinase domain and Ser473 in the carboxy-terminal tail. These phosphorylation sites are phosphorylated by distinct kinases. Phosphoinositide-dependent protein kinase 1 (PDK1) is responsible for the phosphorylation of Thr308 [59], and Akt at Ser473 is phosphorylated by the mammalian target of rapamycin (mTOR) complex 2 [60, 61], referred to as PDK2 (Fig. 3.2). On the one hand, the phosphatase and tensin homologue deleted on chromosome ten (PTEN) negatively regulates the PI3K-Akt pathway by dephosphorylating PtdIns(3,4,5)P_3 to PtdIns(4,5)P_2 [62, 63].

It has been reported that elevated intracellular cAMP by pharmacological reagent like forskolin can activate Akt, whereas this effect was not blocked by the PI3K inhibitor wortmannin [64]. Wortmannin also did not prevent heat-shock-induced Akt activation [65]. Thus, while the activation of PI3K is still considered as

the main factor for Akt activation, several alternative pathways stimulating Akt may exist.

Studies in Akt knockout mice have shown the various important roles of Akt in mammals. Deficiency of Akt2 induced severe insulin resistance and diabetes [66]. Insulin action, which stimulates glucose uptake in muscle, fat, and several other tissues, was markedly decreased, resulting in the attenuation of whole-body glucose disposal. In contrast to Akt2 knockout mice, Akt1 knockout mice were normal with insulin-dependent glucose metabolism. However, these mice demonstrated embryonic and postnatal defects in growth, and then this defect was sustained in adulthood [67]. In addition, deficiency of Akt1 increased neonatal mortality and shortened life span [68]. Akt1/Akt2 double-knockout neonates were severely growth deficient with about a half of a birth weight of their wild-type or double-heterozygous littermates. These mice died shortly after birth with impaired skin development, skeletal muscle atrophy, and delayed bone development [69]. On the other hand, Akt3-deficient mice exhibited normal growth and glucose metabolism. However, brain size and weight of adult $Akt3^{-/-}$ mice were markedly decreased by approximately 25 % because of cell size and cell number, suggesting a critical role of Akt3 in postnatal brain development of mammals [70]. It has been reported that Akt1/Akt3 double-knockout mice were embryonic lethal and the lethality could be attributed to placental insufficiency, cardiovascular anomalies, and impaired nervous system development [71].

Akt regulates a variety of biological functions [56] such as glucose and glycogen metabolisms [72], angiogenesis [73], cell cycle [74], and also cell survival. Since this chapter focuses on intracellular signaling pathways of degeneration and protection, the following section describes about the roles of Akt in cell survival. Akt has numerous downstream substrates related to apoptosis and survival, for instance, glycogen synthase kinase 3β (GSK3β), inhibitor of κB kinase (IKK), forkhead transcription factor (FKHR), and the Bcl-2 family protein Bad.

Akt inactivates GSK3β by phosphorylating it at Ser9. GSK3β contributes to the regulation of glycogen synthesis [75] and the canonical Wnt signaling pathway in phosphorylating β-catenin [76]. β-Catenin phosphorylated by GSK3β is degraded by ubiquitin-proteasome system, but non-phosphorylated β-catenin acts as a transcription factor and plays key roles in cell survival [77]. Endo et al. [78] demonstrated that activated Akt markedly phosphorylated/inactivated GSK3β in the hippocampus after transient global cerebral ischemia and the inhibition of the Akt/GSK3β signaling pathway by a PI3K inhibitor facilitated ischemic injury. Then, the authors conclude that the activation of the Akt/GSK3β pathway may regulate the survival of vulnerable neurons after ischemia. It has also been reported that PDGF-CC, one of the PDGF family members, protects cortical neurons from middle cerebral artery occlusion-induced ischemic injury and rescues RGCs from optic nerve crush or intravitreal injection of NMDA-induced neuronal death [79]. This study mechanistically showed that PDGF-CC induced the phosphorylation of GSK3β at Ser9 with significant Akt activation.

In response to stress signals such as oxidative stress and endoplasmic reticulum stress, apoptosis signal-regulating kinase 1 (ASK1) can initiate the JNK-dependent

proapoptotic signaling. Puckett et al. [80] have shown that insulin-like growth factor 1 (IGF-1) induced ASK1 inactivation by phosphorylating it at Ser967 with Akt activation. This process was mediated by Akt-activated IKK forming a complex with and phosphorylating ASK1 at Ser967, suggesting that the Akt/IKK pathway can counteract stress signal initiated by the ASK1/JNK proapoptotic pathway.

It has been reported that advanced glycation end product or TNF-α induced apoptosis in human retinal pericytes through the activation of the proapoptotic transcription factor FKHR via p38 MAPK and JNK [81]. In this study, Akt inhibitor facilitated the FKHR-mediated apoptosis in the retina. In addition, the Akt survival signal inactivating FKHR was inhibited by PTEN activation during the photoreceptor cell death in the *rd* mouse strain, the well-characterized animal model of retinitis pigmentosa [82]. These studies indicate the importance of the Akt/FKHR pathway in retinal cell survival.

Akt inhibits the mitochondrion-mediated proapoptotic function of Bad by phosphorylating it [83]. RGCs of goldfish can regenerate after optic nerve injury. Koriyama et al. [84] have demonstrated that IGF-1 was upregulated 2–3 days after optic nerve injury and then Akt activity and phosphorylation of Bad were increased. Subsequently, an increased level of Bcl-2 and reduced activity of caspase 3 were observed. In addition, IGF-1 induced Akt phosphorylation and neurite growth in cultured goldfish retina. In contrast to goldfish, rat RGCs die by apoptosis after optic nerve injury. The level of IGF-1 in the retina was decreased 1–2 days prior to the parallel decrease of phosphorylations of Akt and Bad by optic nerve injury. The administration of IGF-1 induced Akt phosphorylation and improved RGC survival in vitro and in vivo [85]. In the rat glaucoma model with chronic elevation of intraocular pressure, the phosphorylation levels of Akt, Bad, and CREB in the retina were simultaneously increased 1 week after the injury and decreased thereafter compared to normal intraocular pressure control, suggesting important roles of Akt at the early stage of injury in control of cell death or survival [86]. Furthermore, advanced glycation products caused cell death in rat retinal tissue cultures, primary neuron cultures, and retinal neuron cell lines (R28). Erythropoietin protected these retinal cells by enhancing Bad phosphorylation and Bcl-xl expression and reducing Bax expression via ERK- and Akt-dependent signaling pathways [87]. In addition, the Ser473 kinase for Akt mTOR complex 2 is also indirectly regulated by Akt [60, 61] (Fig. 3.2) and contributes to cell survival. Nitric oxide can protect neurons from apoptosis through the PI3K-Akt pathway [88]. In cultured retinal neurons, Akt phosphorylation at Thr308 and Ser473 induced by the nitric oxide donor *S*-nitroso-*N*-acetylpenicillamine was blocked by the mTOR inhibitor KU0063794, indicating the participation of the mTOR complex 2 in the antiapoptotic PI3K-Akt process [89].

As described above, Akt mediates antiapoptotic processes via various molecules and performs crucial roles in not only neuroprotection but also other biological functions such as glucose metabolism, development, and cell fate in neurons and other types of cells.

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