View metadata, citation and similar papers at core.ac.uk
brought to you by CORE
provided by Elsevier - Publisher Connector

Biochimica et Biophysica Acta 1846 (2014) 342–352

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbacan

Review

The links between AKT and two intracellular proteolytic cascades: Ubiquitination and autophagy

Masayuki Noguchi *, Noriyuki Hirata, Futoshi Suizu

Division of Cancer Biology Institute for Genetic Medicine, Hokkaido University, Sapporo, Japan

ARTICLE INFO

Article history:
Received 8 May 2014
Received in revised form 29 July 2014
Accepted 30 July 2014
Available online 7 August 2014

Keyword:
AKT
Autophagy
Ubiquitination

ABSTRACT

The serine threonine kinase AKT plays a central role in the regulation of cell survival in a variety of human neoplastic diseases. A series of studies have revealed a connection between AKT signaling and two important protein degradation pathways in mammalian cells: the ubiquitin–proteasome system and autophagy. Two distinct ubiquitination systems have been reported to regulate AKT signaling: K63-linked ubiquitination, which promotes the oncogenic activation of AKT, and K48-linked ubiquitination, which triggers the proteasomal degradation of phosphorylated AKT. Autophagy is an evolutionarily conserved mechanism for the gross disposal and recycling of intracellular proteins in mammalian cells. AKT signaling may play a regulatory role in autophagy; however, the underlying mechanisms have not been fully clarified. Recently, AKT was shown to phosphorylate key molecules involved in the regulation of autophagy. Furthermore, lysosomal co-localization of the AKT–Phafin2 complex is reportedly critical for the induction of autophagy. In this review, we will discuss the connection between AKT, a core intracellular survival regulator, and two major intracellular proteolytic signaling pathways in mammalian cells.

© 2014 The Authors. Published by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/3.0/).

Contents

1. Introduction . . . 343
2. AKT signaling and ubiquitination . . . 345
3. K48-linked ubiquitination of AKT . . . 345
4. K63-linked ubiquitination and AKT kinase activation . . . 346
5. Other ubiquitin modifiers involved in AKT signaling . . . 346
6. PI3K–AKT–mTOR signaling is linked to autophagy . . . 347
7. Lysosomal interaction of AKT with Phafin2. . . . 347
8. Connection to cell death . . . 349
9. Concluding remarks . . . 349
Acknowledgement . . . 350
References. . . . 350

Abbreviations: BRCA1, breast cancer susceptibility gene 1; BRCT, Brca1 C-terminal; CHIP, chaperone-associated ubiquitin ligase; CYLD, cylindromatosis; DAPK, death-associated protein kinase; DUB, deubiquitinating enzyme); EGF, epidermal growth factor; FADD, fas-associated protein with death domain; FGFR1, fibroblast growth factor receptor 1; Hsp90, heat shock protein 90; IAP1/2, inhibitors of apoptosis 1/2; IκB, nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor; IGF-1, insulin-like growth-factor 1; JNK1, c-Jun N-terminal kinase; MULAN, mitochondrial ubiquitin ligase activator of NF-κB; mTOR, mammalian target of rapamycin; Myr, myristoylated; NEDD4, neural precursor cell expressed developmentally down-regulated protein 4; NFκB, nuclear factor-kappa B; PI3K, phosphoinositide 3-kinase; PAK1, p21 protein-activated kinase 1; PAS, pre-autosomal structures; PH, pleckstrin homology; PIAS1, protein inhibitor of activated STAT-1; PTEN, phosphatase and tensin homolog deleted from chromosome 10; RNF8, ring finger protein 8; S6K1, p70 S6 kinase 1; Skp2, SKP1 interacting partner 2; 3-MA, 3-methyladenine; TNF, tumor necrosis factor; TPR, tetratricopeptide repeat; TRAF6, tumor necrosis receptor-associated factor 6; TRAIL, TNF-related apoptosis-inducing ligand; TSC, tuberous sclerosis complex; TTC3, tetratricopeptide repeat domain 3; Ubl, ubiquitin-like; ULK1, Unc-51 like autophagy activating kinase 1; Vps34, vacuolar protein sorting 34

* Corresponding author at: N15, W7, Kita-ku, Sapporo 060–0815, Japan. Tel.: +81 11 706 5069; fax: +81 11 706 7826.
E-mail address: m_noguch@igm.hokudai.ac.jp (M. Noguchi).

http://dx.doi.org/10.1016/j.bbcan.2014.07.013
0304-419X/© 2014 The Authors. Published by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/3.0/).

1. Introduction

The serine threonine kinase AKT, also called protein kinase B (PKB), regulates a range of cellular processes, including cell survival, cell cycle progression, cytoskeletal organization, vesicle trafficking, glucose transport, and platelet function. Deregulation or malfunction of AKT contributes to a wide variety of human diseases including cancers, glucose intolerance, schizophrenia, viral infections, and autoimmune diseases [1–3]. Two major proteolytic pathways are present in mammalian cells: autophagy and the ubiquitin–proteasome system [4–6]. A series of studies has shown that the PI3K (phosphoinositide 3-kinase) –AKT–mTOR (mammalian target of rapamycin) pathway, which mediates anti-apoptotic signaling, may play an important role in the regulation of autophagy and the ubiquitin-proteasomal system in mammalian cells [7–13].

Ubiquitin was originally proposed to deliver tagged proteins to the cellular waste disposal machinery via the 26S proteasome. Ubiquitin, a 76-residue protein, is covalently associated with protein substrates. Protein ubiquitination is mediated by the concerted action of essentially three enzyme families (E1, E2, and E3). Ubiquitin is activated first by a ubiquitin-activating enzyme (E1) through an ATP-dependent reaction to form an E1-thioesterlinkage, and the activated ubiquitin is transferred to a member of the ubiquitin-conjugating enzyme E2 family. A ubiquitin–protein ligase (E3) then mediates the transfer of ubiquitin from E2 to the substrate protein by promoting the formation of an isopeptide bond between the ubiquitin (Ub) carboxyl-terminus and specific lysine side chains on the substrate [14,15]. In the human genome, there are over 500 genes that appear to be E3 ligases, and there are potentially over 1500 molecules that can be targeted by ubiquitin and/or ubiquitin like protein modifications [16]. E3 ligases can generally be classified into two families: RING (really interesting new gene) type E3 ligases, and HECT (homologous to the E6-associated protein C terminus) type E3 ligases. The activity of the ubiquitin system is dependent on the specificity of the E3 ubiquitin ligases [17].

In addition to its control of protein turnover, protein ubiquitination of protein has an additional impact on various cellular functions, including facilitation of cell-surface-receptor turnover and control of gene transcription [15].

Two distinct ubiquitination systems for AKT have been reported: K63-linked ubiquitination of lysine of ubiquitin by TRAF6 (tumor necrosis receptor-associated factor 6) [18], Skp2 (SKP1 interacting partner 2) [12], or NEDD4 (neural precursor cell expressed developmentally down-regulated protein 4) [19], induces AKT activation by promoting plasma membrane translocation and/or nuclear translocation. In contrast, K48-linked ubiquitination of lysine of ubiquitin by BRCA1 (breast cancer susceptibility gene 1) [8], MULAN (mitochondrial ubiquitin

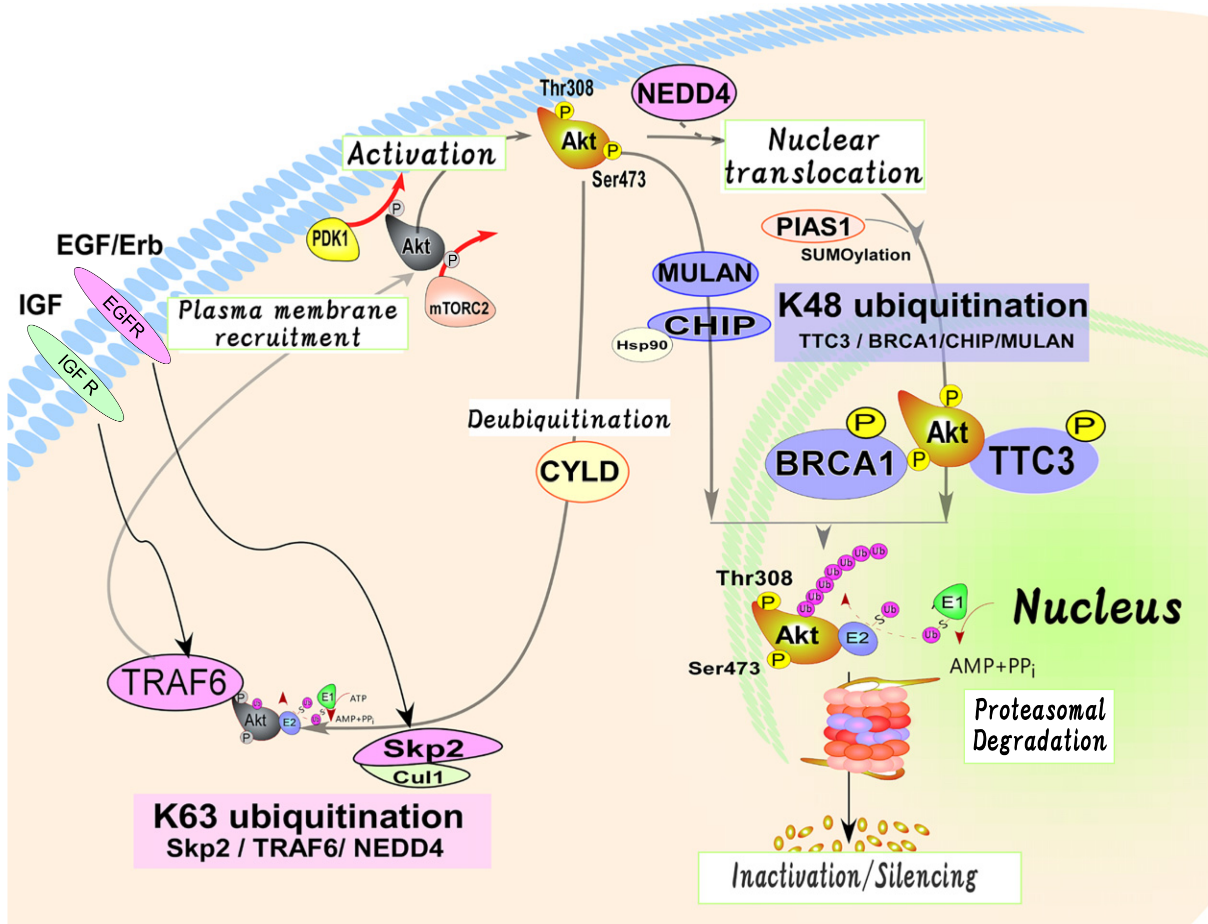

Fig. 1. Two distinct ubiquitination systems regulate AKT and downstream cellular responses. K63-linked ubiquitination by TRAF6/Skp2 induces the translocation of AKT to the plasma membrane, which is important for oncogenic AKT activation. NEDD4 catalyzes K63-linked ubiquitination of phosphorylated AKT to promote its nuclear translocation. K48-linked ubiquitination of AKT by TTC3, CHIP, or MULAN, or BRCA1 promotes proteasomal degradation. PIAS1-mediated SUMOlylation of AKT, which occurs mainly in the nucleus, is also essential for the activation of AKT and downstream responses such as proliferation and tumorigenesis. In contrast, K48-linked ubiquitination mediated by BRCA1 or TTC3 occurs in the nucleus and triggers proteasomal degradation. MULAN- and CHIP-mediated ubiquitination likely to occurs in the cytosol. Moreover, the CYLD deubiquitinase controls the balance between AKT ubiquitination and deubiquitination.

ligase activator of NF-κB) [10], CHIP (chaperon-associated ubiquitin ligase) [20], and TTC3 (tetratricopeptide repeat domain 3) promotes the proteasomal degradation of AKT [9], likely in order to silence AKT kinase activity (Fig. 1).

Autophagy is an evolutionally conserved homeostatic process for intracellular degradation by which intracellular proteins are sequestered in a double-membrane-bound autophagosome and delivered to the lysosome during stress conditions; this process facilitates both degradation and recycling in mammalian cells. The molecular machinery of autophagy co-ordinates diverse aspects of cellular and organismal responses to other dangerous stimuli such as infection [21,22]. Therefore, defective autophagy underlies a wide variety of human disease and physiology including cancer, neurodegeneration, and infectious diseases [5,23,24]. The three best characterized forms of autophagy are chaperon-mediated autophagy, microautophagy, and macroautophagy. Macroautophagy, which was originally referred to simply as

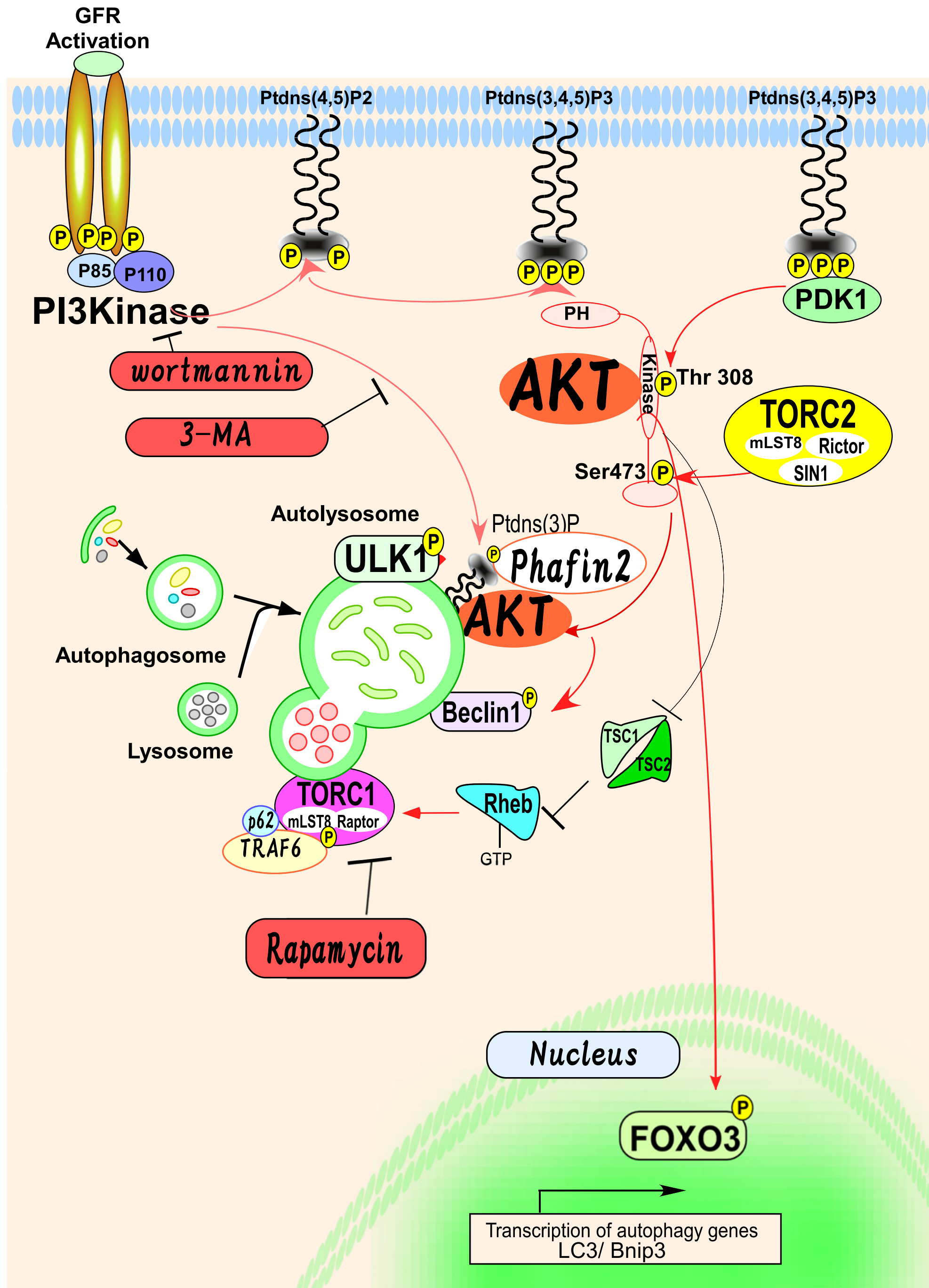

Fig. 2. The PI3K–AKT–mTOR pathway and the induction of autophagy. The PI3K–AKT–mTOR pathway plays an important role in the regulation of autophagy. Activation of AKT suppresses catabolic processes such as autophagy. Pharmacological PI3K inhibitors such as wortmannin or 3-MA inhibit autophagy. In contrast, rapamycin, which inhibits mTORC1, is a potent inducer of autophagy. In addition to acting as a traffic nutrient transporter, mTORC1 signals to the ULK1/ULK2 and Beclin1 complexes to promote autophagosome formation. TRAF6-mediated K63-linked ubiquitination of mTORC1 is important for its translocation to lysosomes and the activation of autophagy. In addition to mTORC1, AKT may also be involved in the regulation of autophagy. Casein kinase II, a kinase upstream of AKT that localizes to the Golgi/lysosome, phosphorylates p62 to promote the autophagic clearance of ubiquitinated proteins. FOXO3, ULK1 (ATG1), and Beclin1 (ATG6) control autophagy as direct substrates of AKT. The finding that translocation of AKT–Phafin2 and p62–TRAF6–mTORC1 to the lysosome is essential for autophagy underscores the significance of the AKT–mTOR axis in regulation of the process.

autophagy, sequesters cytosolic proteins or organelles within double-membrane encapsulated autophagosome; these structures are then fused with lysosomes for degradation, organelle turn-over, and recycling of proteins. Macroautophagy can be both selective and non-selective, and can be subdivided into several subcategories (i.e., mitophagy, ribophagy, or aggrephagy). Recent studies indicate that selective autophagy is also mediated through ubiquitination [25,26]. The role of ubiquitination in selective autophagy provides further evidence of the connection between ubiquitination and autophagy [25,26].

Studies with pharmacological inhibitors suggest that AKT can regulate autophagy [11]. Although the underlying molecular mechanisms are not fully understood, it is clear that ULK1 (Unc-51 like autophagy activating kinase 1, ATG1) and beclin1 (ATG6), two key regulators of autophagy, are substrates for AKT-dependent phosphorylation [11,27]. In addition, AKT is shown to bind to Phafin2 in order to be re-localized to the lysosome during autophagy induction [28]. Furthermore, TRAF6-mediated ubiquitination of p62, an autophagy receptor for ubiquitinated proteins, which is critical for the regulation of apoptosis and autophagy, is important for the lysosomal localization of mTOR, a major downstream effector of AKT that regulates autophagy [29–31] (Fig. 2).

In this review, we summarize the current state of knowledge regarding ubiquitination of AKT and the role of AKT in autophagy. We place this in the context of the two major cellular proteolytic pathways in order to highlight the importance of this signaling network, in particular as regards human diseases.

2. AKT signaling and ubiquitination

AKT belongs to the AGC family of kinases [1,32,33] and contains a pleckstrin homology (PH) domain at its N-terminus. The AKT-PH domain is an electrostatically polarized domain that adopts the same fold and topology as other PH-domains, which consist of a seven-stranded β-sandwich capped by an α-helix [32].

In response to growth factors and other extra-cellular stimuli, AKT is activated by the lipid products [PtdIns (3,4,5)P_3 and its immediate breakdown product PtdIns (3,4)P_2] of phosphoinositide 3′-kinase (PI3K), which phosphorylates the 3-OH position of the inositol core of inositol phospholipids (PtdIns) [34–36]. Structural studies reveal that the binding pocket of PtdIns (1,3,4,5)P_4 [the polar head group of PtdIns (3,4,5)P_3] is within variable loop 1 (VL1, the loop between the β1 and β2 strands) of the AKT PH domain [37–40].

Three AKT isoforms have been identified in the human genome (AKT1, AKT2, and AKT3). These isoforms share a highly conserved PH-domain, which binds to PI(3,4,5)P_3. The interaction is critical for the translocation of AKT to the plasma membrane. Under physiological conditions, AKT1 and AKT2 are ubiquitously expressed, whereas AKT3 expression is restricted, predominantly to heart, kidney, brain, testis, lung, and skeletal muscle [1–3,41]. It is clear that AKT plays a central role in regulating cell survival. AKT phosphorylates the pro-apoptotic BAD protein, which enhance the association of BAD with 14-3-3 proteins. This effectively blocks the death-inducing function of BAD, since it is then unable to interact with and antagonize the anti-apoptotic protein Bcl-2. AKT also activates the IκB (nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor)/NFκB (nuclear factor-kappa B) transcription factor complex by inducing the proteasomal degradation of IκB to inhibit apoptosis. AKT also induces phosphorylation of the FOXO/forkhead family of transcription factors and, consequently, reduces cellular levels of $p27^{Kip1}$. Other AKT substrates include GSK (glycogen synthase kinase), CREB (cyclic AMP response-element binding protein), XIAP (X-linked inhibitor of apoptosis protein), Nur77, $p27^{Kip1}$, MDM2 (murine double minute 2), eNos (endothelial nitric oxide synthase), and WEE1Hu (Wee-like protein kinase) [3]. Due to the diverse roles of AKT as outlined above, deregulation or malfunction of AKT is associated with many human diseases, including cancer, glucose intolerance, schizophrenia, viral infections, and autoimmune disorders [1–3,42].

Activation of the AKT isoforms-1, -2, and -3 is regulated by phosphorylation at two regulatory sites: threonine 308/309/305, which are targeted by PDK1 (phosphoinositide-dependent kinase-1) and serine 473/474/472 which are targeted by the ribosome-associated Rictor–mTOR complex (mTORC2, mammalian target of rapamycin complex 2) [1–3,33,42–45]. Phosphorylation of both these sites is required for maximum kinase activity [1–3,42]. In line with other kinases, the activity of AKT is clearly regulated by both phosphorylation and dephosphorylation [1–3,42]. However, kinases are also targets of ubiquitination and ubiquitin-like modifications, including SUMOylation [16,46–48]. Consistent with this AKT kinase activity can be modified by both lysine 48 (K48)-linked polyubiquitination and lysine 63(K63)-linked polyubiquitination [4,48]. K48-linked ubiquitination is thought to control proteasomal degradation of AKT, thereby effectively shutting off AKT activity and downstream responses. In contrast, K63-linked polyubiquitination is thought to serve as a regulatory signal that promotes plasma membrane recruitment of AKT [47–49]. The diversity of the biological actions that can be controlled by ubiquitination/deubiquitination is due to both the high substrate specificity of the ubiquitin system and the variety of regulatory mechanisms that control the activity of both ligases and deubiquitinases [16,49–52].

3. K48-linked ubiquitination of AKT

TTC3 (tetratricopeptide repeat domain 3), an E3 ubiquitin ligase, was identified as an AKT binding partner through a yeast two-hybrid screen. TTC3 contains an H2-RING finger domain, AKT phosphorylation motifs (TPRSLSAP), tetratricopeptide repeat (TPR) motifs, and two nuclear localization signals [9]. Several lines of evidence indicate that phosphorylation plays a key role in regulating the function of E3 ligases [53]. The interaction of an E3 ligase with a substrate, which is regulated by post-translational modification such as phosphorylation, provides substrate specificity [53]. Indeed, both in vitro and in a cellular context, AKT induces phosphorylation of TTC3 at Ser378. This phosphorylation is an important prerequisite step for AKT ubiquitination. In mammalian cells, TTC3 interacts preferentially with phosphorylated AKT at Thr308 to induce the polyubiquitination and subsequent degradation of AKT. Phosphorylation of TTC3 at Ser378 is required for ubiquitination and proteasomal degradation of AKT and its downstream biological responses [9].

The breast cancer susceptibility gene 1 (BRCA1) plays a key role in mammary tumorigenesis. The BRCA1–BRCT (Brca1 C-terminal) domain binds to phospho-T308 and phospho-S473 AKT and triggers their K48-linked ubiquitination and degradation, presumably in the nucleus. This suggests that BRCA1 is a negative regulator of the AKT pathway and emphasizes the significance of the BRCA1/AKT pathway in tumorigenesis [8]. AKT is also ubiquitinated and negatively regulated by the mitochondrial protein MULAN, an E3 ligase, possibly in the cytosol. MULAN preferentially interacts with and mediates the ubiquitination of phosphorylated AKT, thereby promoting AKT degradation, which suppresses cell proliferation and viability [10]. However, whether MULAN preferentially ubiquitinates phospho-T308 or phospho-S473 was not specified in the study [10].

Furthermore, Hsp90 (heat shock protein 90) reportedly interacts with and stabilizes AKT [54]. CHIP (chaperone-associated ubiquitin ligase), one of the E3 ligases that harbors a TPR (tetratricopeptide repeat) motif, was shown to interact with Hsp90 and control the ubiquitination of AKT [20,55]. CHIP co-regulates the degradation of the Alzheimer's disease-related protein, tau through coordinated interactions. This likely involves AKT, since AKT regulates both tau kinase activity and protein quality control, thus linking several common pathways that contribute to dysfunction in Alzheimer's disease [20]. Since TTC3 also induces proteasomal degradation of phospho-AKT, there may be a pathogenic connection between the TTC3–AKT–eNOS axis and the neurological symptoms of Down syndrome [9]. However, a direct involvement of

TTC3 in the regulation of tau and Alzheimer's disease has yet to be demonstrated.

Interestingly, K48-linked ubiquitination of AKT occurs both in the cytosol (MULAN and CHIP) and in the nucleus (BRCA1 and TTC3) [8–10,20,55]. Because AKT has nuclear (FOXO, Nur77) and cytosolic substrates (BAD, GSK), AKT ubiquitination and proteasomal degradation may differentially affect the phosphorylation of nuclear and cytosolic substrates, thereby controlling the various cellular responses regulated by AKT. However, how these different ubiquitin ligases differentially regulate the AKT is currently unknown and clarification requires further study.

It is noteworthy that unlike phosphorylation of AKT, ubiquitination of AKT has multiple effects in other aspect of enzymatic action. Ubiquitin-dependent AKT degradation is prevented by a translation-coupled phosphorylation event at Thr450 mediated by mTORC2 in a growth factor independent manner. Thus, phosphorylation of AKT at the Turn motif (TM) by mTORC2 in a growth factor independent manner is also reported to control the stability of AKT [56].

4. K63-linked ubiquitination and AKT kinase activation

Ubiquitination also has proteasome-independent functions in endocytosis and signaling. Unlike K48-linked ubiquitination, which signals for proteasomal degradation, K63-linked polyubiquitin chains are thought to serve as a regulatory signal and to provide a scaffold for the assembly of protein kinase complexes [47–49]. TRAF6, HectH9, c-IAP1/2 (inhibitors of apoptosis 1/2), and RNF8 (ring finger protein 8) are all known to mediate K63-linked ubiquitination [57–60].

AKT resides primarily in the cytosol and nucleus. Upon growth factor stimulation, PI3K catalyzes the production of PI(3,4,5)P_3 at the plasma membrane. The PH-domain of AKT binds PI(3,4,5)P_3 with high affinity. However, the molecular mechanisms involved in the translocation of AKT to the plasma membrane and its subsequent activation by PDK1 or mTORC2 are not fully understood. TRAF6, a member of the tumor necrosis factor receptor-associated factor family [61], was shown to promote AKT activation through K63-linked ubiquitination [11] (Fig. 1). In response to insulin-like growth-factor stimulation (IGF-1), TRAF6 ubiquitinates the PH-domain of AKT, which promotes AKT membrane recruitment and phosphorylation. Sequence alignment identified K8 and K14 within the PH-domain of AKT as the primary sites for K63-linked ubiquitination. K63-linked ubiquitination of AKT promotes translocation of AKT to the plasma membrane, where it is phosphorylated by PDK1 and mTORC2 at Thr308 and Ser473, respectively. Ubiquitination does not affect the binding of AKT to PI(3,4,5)P_3, but may cause a conformational change that enables AKT to interact with a putative protein that transports the kinase to the plasma membrane. In addition, TRAF6 acts not only upstream of mTORC1 through p62 during nutrient sensing but also downstream of mTORC1 through the AKT–TSC (tuberous sclerosis complex) axis in response to insulin and growth factors. TRAF6$^{-/-}$ mice exhibit thymic atrophy and disorganogenesis, suggesting that TRAF6-mediated AKT activation primarily affects the immune system [62]. Therefore, blocking TRAF6 may have therapeutic potential in cancer especially in the immune systems. We note, however, that the E3 ubiquitin ligase TRAF6 does not always activate AKT kinase activity [63,64]. TRAF6 inhibits LPS-induced AKT activation in FLT3-ITD-positive MV4-11 AML cells [63]. Activation of the PI3K–AKT pathway is higher in TRAF6-deficient T cells [64]. Consistently, levels of basal phosphorylation of GSK3β are higher in TRAF6 knock down cells [65]. Thus, TRAF6 expression may affect AKT phosphorylation differently in a various cellular environments.

There is also evidence that SCF E3 ligase (Skp2, E3 ligase S-phase kinase associated protein 2) mediates EGF (epidermal growth factor) signaling to AKT via K63-linked ubiquitination [12]. In contrast to TRAF6 E3 ligase, which orchestrates IGF-1-induced AKT ubiquitination and activation [11], the Skp2 SCF complex mediates AKT ubiquitination and membrane recruitment in response to the activation of ErbB receptors by EGF. Furthermore, Skp2 overexpression correlates with AKT activation and breast cancer metastasis [12]. Ubiquitination of AKT by Skp2 or TRAF6, each of which promotes the membrane recruitment of AKT for the subsequent activation, is extracellular growth factor dependent: EGF for Skp2 and IGF for TRAF6, respectively [11,12]. These observations support a model in which different growth factors use distinct E3 ligases to activate AKT. Thus, depending on the context, either Skp2 or TRAF6 can catalyze K63-linked ubiquitination of AKT to control cellular responses.

NEDD4-1(neural precursor cell expressed developmentally down-regulated protein 4) reportedly catalyzes the attachment of K63-linked polyubiquitin chain to AKT in vitro [19]. Thus, AKT joins PTEN (phosphatase and tensin homolog deleted from chromosome 10) [66] and fibroblast growth factor receptor 1 (FGFR1) [67], as a substrate for the NEDD4-1 E3 ligase. AKT ubiquitination by NEDD4-1 positively regulates the nuclear trafficking of the activated form of AKT (p-AKT). Ubiquitinated p-AKT translocates to the perinuclear region, where it is released into the cytoplasm, imported into the nucleus, or coupled to the proteasome for degradation [19]. IGF-1 signaling stimulates the selective ubiquitination of p-AKT (and not total AKT) by NEDD-4 [19]. The AKT (E17K), a constitutively active and oncogenic mutant, is more effectively ubiquitinated by NEDD4-1 and hence more efficiently targeted to the nucleus than that wild-type AKT, suggesting that AKT (E17K) is a better substrate for E3 ligases [19] (Fig. 1).

K63-linked ubiquitination provides a molecular platform for protein–protein interactions involved in signaling activation, protein trafficking, and receptor endocytosis [47–49]. In this regard, it is possible that K63-linked ubiquitination of AKT may increase its affinity for PAK1 (p21 protein-activated kinase 1) [68].

5. Other ubiquitin modifiers involved in AKT signaling

Deubiquitinating enzymes (DUBs) cleave the peptide or isopeptide bond between ubiquitin and a substrate protein to reverse the ubiquitination reaction [48,69]. By removing ubiquitin from proteins, DUBs regulate protein degradation, localization, activation, and interactions. Recently, CYLD (cylindromatosis), which was originally identified as deubiquitinating enzyme in the NF-κB pathway, was identified as a DUB for AKT [70]. Studies of the CYLD knockout mouse and human CYLD mutation in various human cancers indicate that CYLD is a tumor suppressor [71,72]. CYLD interacts directly with AKT and deubiquitinates it in response to growth factor stimulation, thereby allowing E3 ligases back again to induce the ubiquitination and activation of AKT [70]. CYLD may also remove K63-linked ubiquitin from activated AKT, which facilitates K48-linked-ubiquitination and degradation by BRCA1 and/or TTC3. Thus, CYLD could act as a molecular switch for the ubiquitination and deubiquitination of AKT, which in turn would determine its localization and activation during cancer progression [70] (Fig. 1).

Ubiquitin and ubiquitin-like (Ubl) protein modifications affect protein stability, activity, and localization, although the biological consequences of these modifications are less well characterized in comparison to ubiquitin [4,47–49]. In general, kinases are enriched among ubiquitination targets [16]. However, another post-translational modification of AKT, SUMOylation at K273 by PIAS1 (protein inhibitor of activated STAT-1), has also been reported. PIAS1 SUMOylates and activates AKT in the nucleus, although the mechanism behind this is not yet fully understood. However, SUMOylation of AKT may be essential to activate AKT, promote proliferation, and thereby enhance tumorigenic activity [73]. These observations together underscore the physiological significance of the ubiquitin system within the context of the AKT–mTOR axis (Fig. 1). Moreover, indirect involvement of ubiquitination of PI3K–AKT signal is also known to modulate AKT signaling. Parkin, an E3 ligase implicated in the pathogenesis of Parkinson's diseases, is shown to modulate the PI3K–AKT signaling through the ubiquitination and subsequent

endocytosis and trafficking of epidermal growth factor (EGF) receptor (EGFR) without directly interacting with AKT [74].

6. PI3K–AKT–mTOR signaling is linked to autophagy

Growth factor stimulation activates PI3K (phosphoinositide 3-kinase), a lipid kinase, which phosphorylates the 3′-OH group of the inositol ring in inositol phospholipids. Three classes of PI3Ks (class IA, IB, II, and III) are defined by their distinct substrate preferences. Class I PI3Ks are responsible for the production of phosphatidylinositol 3-phosphate (PtdIns(3)P), phosphatidylinositol (3,4)-bisphosphate (PI(3,4)P_2), and phosphatidylinositol (3,4,5)-trisphosphate (PI(3,4,5)P_3. Class III PI3Ks produce PtdIns(3)P from PI by forming a heterodimer with catalytic (Vps34) and regulatory (Vps15/p150) subunits [32,75].

The essential role of Vps34 (vacuolar protein sorting 34, a class III PI3K) in autophagy has been established largely through the use of the pharmacological inhibitors wortmannin and 3-methyladenine (3-MA), which suppress autophagy [76–78]. A study of Vps34-null cells demonstrated that this protein plays an essential role in autophagy in the liver and heart [79]. In budding yeast and unicellular eukaryotes, Vps34 regulates vesicle trafficking [80]. In yeast, the nucleation and assembly of the initial phagophore membrane requires Vps34, the myristoylated serine/threonine kinase Vps15 (p150 in mammalian cells), Atg14 (Barker or mAtg14 in mammalian cells), and Atg6/Vps30 (Beclin1 in mammalian cells) [81]. In multicellular organisms and higher eukaryotes, a number of growth factor receptors activate the catalytic p110 subunit of class IA PI3Ks (p110α/p110β, 110δ). In mammalian cells, Vps34 is also involved in nutrient signaling to mTOR [82].

The PI3K–AKT–mTOR pathway, which mediates anti-apoptotic signaling, may play an important role in the regulation of macroautophagy, the most prevalent form of autophagy in mammalian cells [26,83–85] (Fig. 2). Pharmacological inhibitors of the PI3K–AKT–mTOR pathway are known to modulate the induction of autophagy. Pan-PI3K inhibitors, such as wortmannin or LY294002, which is known to suppress the production of both PtdIns(3)P and PI(3,4,5)P_3, inhibit macroautophagy in isolated rat hepatocytes [78]. By contrast, 3-MA (a class III PI3K inhibitor), which specifically inhibits the production of PtdIns(3)P, is widely utilized as an autophagy inhibitor [28,86]. Rapamycin, an mTORC1 inhibitor, is widely used to induce autophagy [87]. Class I PI3K products block the autophagic pathway and distinct classes of PI3K are involved in signaling pathways that control macroautophagy [77]. Inhibition of PI3K or AKT synergistically sensitizes malignant glioma cells to rapamycin [88]. These complexities are partly explained by the possibilities of the cross inhibition of class I vs. class III PI3K products. A constitutively active form of AKT suppressed the induction of autophagy by rapamycin, whereas dominant negative AKT promoted autophagy induced by treatment with rapamycin and LY294002 [88]. Inhibition of AKT synergistically sensitizes malignant cells to rapamycin, thus promoting autophagy, and sensitizes PTEN-null tumors to lysotropic reagents [89].

Since activation of PI3K–AKT underlies the pathogenesis of various cancers [1–3], it is imperative that we understand how modulation of PI3K–AKT and the subsequent changes in autophagy might affect cancer progression. Some lessons may come from observations that-AKT activation is suggested to prevent the induction of autophagy in order to preserve cell viability and promote normal cell growth [7,13,90]. Activation of AKT potentiates anabolic processes, including protein translation and cell growth, and suppresses catabolic processes such as autophagy [13]. For example, constitutive AKT activity inhibits the TSC complex; this leads to an increase in Rheb, a small GTPase that activates mTORC1, and as a consequence, suppresses autophagy. Furthermore, AKT suppresses the retrograde degeneration of dopaminergic axons by inhibiting macroautophagy [91]. Conversely, PTEN, a negative regulator of AKT, stimulates autophagy [13,90] (Fig. 2).

7. Lysosomal interaction of AKT with Phafin2

Another investigation offers additional evidence of the involvement of AKT in autophagy regulation. By yeast two-hybrid screening, Phafin2 (EAPF or PLEKHF2), a lysosomal protein with a unique structure of N-terminal PH domain and C-terminal FYVE (Fab 1, YOTB, Vac 1, and EEA1) domain, was found to interact with AKT [28]. These conserved motifs place Phafin2 in a family of proteins known to induce caspase-independent apoptosis via the lysosomal-mitochondrial pathway [92]. Previously, Phafin2 was suggested to control receptor trafficking through the endosome [93]. AKT translocates with Phafin2 to the lysosome in a PtdIns(3)P-dependent manner after induction of autophagy. Lysosomal accumulation of the AKT–Phafin2 complex and subsequent induction of autophagy are lysosomal PtdIns (3)P-dependent events, and the formation of this complex at lysosome is a critical step in induction of autophagy via interaction with PtdIns (3)P [28]. These observations also suggest that the regulation of lysosomal localization of AKT affects autophagy induction (Fig. 3).

Autophagy also implicated in tumor progression [5,23,24,94,95]. In order to elucidate the role of Phafin2 in the regulation of tumorigenesis, we have examined whether Phafin2 overexpression affects tumor growth.

In this regard, our preliminary observations suggested that overexpression of wild type Phafin2, but not PtdIns(3)P interaction-defective-Phafin2, inhibited RAS-induced and TCL1-induced transformation. Although precise mechanisms for the involvement of autophagy in tumorigenesis remain to be clarified in further studies, these observations are consistent with the precedent reports that regulation of autophagy is indeed involved in the tumor progression [94,95].

PtdIns(3)P is highly enriched on early endosomes and in the internal vesicles of multivesicular endosomes [96]. These findings clarify the molecular mechanism by which cytosolic AKT translocates to the lysosome to phosphorylate its substrates and control autophagy induction.

3-MA, a widely utilized pharmacological inhibitor for autophagy, is known to inhibit enzymatic action of class III PI3K activity, which suppresses production of PtdIns(3)P [77,87]. Using 3-MA, we showed that PtdIns(3)P-dependent lysosomal translocation of the AKT–Phafin2 complex was required for induction of autophagy [28].

Experiments in lower eukaryotes also support a role for AKT in autophagy [6,97]. Based on structural similarities, the yeast Sch6 gene is a candidate AKT ortholog [98–100]. In this regard, it has been suggested that Sch9 kinase may regulate autophagy in response to change in nutrient availability [98,99]. Notably, both negative [101] and positive [102,103] roles of the mTORC1 substrate S6K1 (p70 S6 kinase 1) in autophagy have been reported.

The physiological roles of AKT in the regulation of autophagy are further supported by experiments in mammalian cells [27,104]. ULK1 (ATG1), a serine threonine kinase related to UNC-51 kinase in Caenorhabditis elegans, induces autophagy by phosphorylating Beclin1 and activating Vps34 [104]. AKT phosphorylates ULK1 at Ser774 in vitro, providing further support that AKT is involved in the regulation of autophagy [27].

Furthermore, FOXO3, member of the O subclass of the forkhead family of transcription factors, is an AKT substrate that regulates the cell death machinery [105]. FoxO3 coordinately activates protein degradation by the autophagic/lysosomal and proteasomal pathways in atrophying muscle cells [106]. In hematopoietic stem cells under starvation conditions, FOXO3 activity is required for induction of a gene expression program that induces autophagy in order to mitigate the energy crisis and promote cell survival [107]. Because rapamycin cannot induce autophagy in skeletal muscle, the cellular salvage pathway relies more on AKT–FOXO signaling, rather than on the AKT–mTOR axis. In this regard, it is of note that FOXO protein stability is regulated via Skp2/MDM2-mediated-ubiquitination and proteasomal degradation [108].

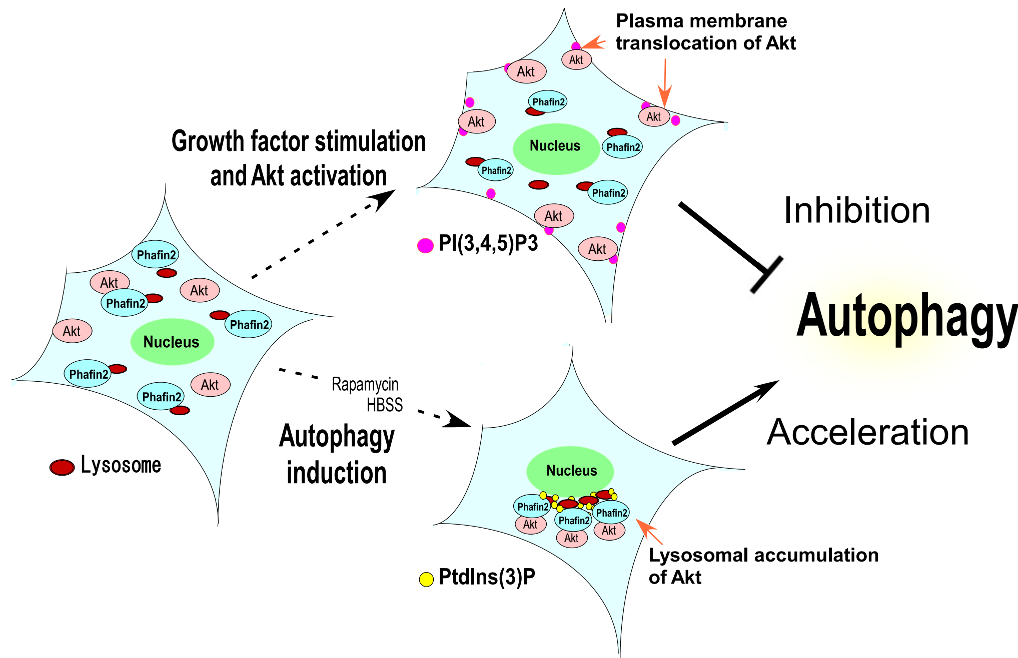

Fig. 3. Spatial control of AKT determines the fate of autophagy. In response to growth factor stimulation, class I PI3Ks produce PI(3,4,5)P_3 at the plasma membrane. The PH-domain of AKT interacts with PI(3,4,5)P_3 to promote the translocation of AKT to the plasma membrane, where it is activated by PDK1 and/or mTORC2. Upon autophagy induction, class III PI3Ks produce PtdIns(3)P at the lysosomal/autophagosomal membrane, which recruits proteins necessary for the biogenesis of the membrane-bound compartment. The lysosomal protein Phafin2, which contains PtdIns(3)P binding modules, associates with AKT to promote its lysosomal translocation. These observations suggest a model in which the intracellular spatial regulation of AKT at lysosome affects autophagy induction: AKT targeted to the plasma membrane via PI(3,4,5)P_3 inhibits autophagy, while AKT targeted to the lysosome via PtdIns(3)P with Phafin2 promotes autophagy.

Given the above observations, it is clear that the relationship between PI3K–AKT signaling and induction of autophagy is complex (Table 1).

Pan-PI3K inhibitors, such as wortmannin and LY294002, are shown to inhibit autophagy in isolated rat hepatocytes [78]. It is also reported that an increase of class I PI3K products or stimulating enzymatic activity of class I PI3K to produce class I PI3K products can reduce macroautophagy [77]. In contrast, an increase in class III PI3K products stimulates macroautophagy in human colon cancer HT-29 cells [77].

Table 1
The roles of the PI3K–AKT–mTORC signal in the regulation of autophagy and apoptosis.
The intracellular PI3K–AKT–mTOR signal is known to be important for the regulation of cell survival. Accumulating evidence supported the direct involvement of the PI3K–AKT–mTOR signal in the regulation of apoptosis and autophagy in mammalian cells. Class I PI3K activation, but not class III PI3K activation, inhibited autophagy induction [77]. In contrast, suppression of class I PI3K/class III PI3K inhibited autophagy induction [77]. Activation of AKT inhibits induction of autophagy [13,90], whereas dominant negative AKT promoted autophagy [88–91]. Consistently, AKT/mTORC1 inhibitors promote both apoptosis and autophagy [13,87]. It is well established that pan-PI3K inhibitors (wortmannin or LY294002) are known to inhibit production of PI(3,4,5)P_3, and hence inhibit AKT kinase activity [1–3,32,75]. Therefore, full picture of the PI3K–AKT–mTOR signal for the molecular regulation of apoptosis vs. autophagy is not completely clarified. Further studies will be required to fully understand the role of the PI3K–AKT–mTOR signal and the additional signals that control the induction of autophagy vs apoptosis in different cellular environment in mammalian cells.

	Activity	Apoptosis	Autophagy
Class I PI3K	↑	↓	↓
	↓	↑	↓
Class III PI3K	↑	ND	↑
	↓	↑	↓
AKT	↑	↓	↓
	↓	↑	↑
mTORC1	↓	↑	↑

The effect of class I PI3K inhibitors on autophagy is very context dependent, partly because the specificity of inhibition of crossreactivity. For example, class I PI3K products are known to activate AKT [13,90,91] and thus likely to expect to block autophagy. Pan-PI3K inhibitors inhibit production of PI(3,4,5)P_3, and thereby block AKT kinase activity [1–3,32,75]. Their usage would thus be expected to increase autophagy. However, pan-PI3K inhibitors such as wortmannin and LY294002 inhibit the production of not only PI(3,4,5)P_3 but also PtdIns(3)P [86], and therefore, it is logical that this would suppress induction of autophagy.

The ability of class I and III inhibitors to block autophagy may be linked to the AKT–Phafin2 complex, since PtdIns(3)P-dependent lysosomal localization of the AKT–Phafin2 complex is required for induction of autophagy [28]. It is also plausible that class I PI3K products/activation to inhibit autophagy induction [77] is partly due to the consequence of activation of AKT.

Although a prevailing view is that AKT activation blocks autophagy [13,90,91], the elimination of AKT by siRNA can also paradoxically inhibit the process [28].

Furthermore, induction of AKT kinase activity is also stimulated by the mTORC1 inhibitor [109,110], yet this compound robustly induces autophagy [44,111,112]. In this scenario, induction of AKT in response to mTORC1 inhibition is probably due to release of the feedback inhibition from mTORC1 towards PI3K e.g. via IRS1.

Presumably, the high levels of AKT phosphorylation in rapamycin-treated cells may not be sufficient to reverse the mTORC1 inhibition. Notably, prolonged treatment with rapamycin inhibits AKT kinase activity [113], a finding supported by the presence of TSC-independent mTORC2 regulation [114]. Thus, the precise effect of rapamycin treatment in mammalian cells is heavily context-dependent.

Consistent with the role of mTOR in yeast, nutrition-dependent regulation of mTOR is thought to be a major determinant of autophagy in mammalian cells. mTORC1 is regulated through the activity of a class I

PI3K that promotes the membrane binding and subsequent activation of AKT and PDK1, which subsequently blocks autophagy [7,84,85,101]. In the absence of growth factors, mTOR is inactivated, which relieves its inhibitory effect on autophagy. AKT inhibits the GTPase-activating protein complex TSC1–TSC2, leading to stabilization of RhebGTP, which mediates signaling to mTOR, a key regulator of autophagy [84]. A TSC-independent mechanism for autophagy regulation has also been proposed [114].

In mammalian cells, hVps34 is also involved in nutrient signaling to mTOR [82]. mTOR inhibition by starvation or rapamycin treatment induces the recruitment of multiple Atg proteins to PAS (pre-autosomal structures) to initiate autophagosome formation. Inactivation of mTORC1 by rapamycin stimulates autophagy in the presence of nutrients, suggesting that mTOR blocks autophagy [115].

Recently, it was shown that TRAF6 together with p62 binds to and induces K63-linked ubiquitination of mTOR [29] (Fig. 2). In this scenario, mTOR ubiquitination by the p62–TRAF6 complex is central to the regulation of cell growth, tumor transformation, and autophagy. TRAF6 activates mTORC1 and inhibits autophagy through p62 and the K63-linked polyubiquitination of mTOR [29,31]. Interfering with mTOR ubiquitination or with the p62–TRAF6 interaction can impair mTORC1 activation in response to nutrients, and has an important impact on the control of cell proliferation and transformation [29]. Hence, TRAF6-mediated ubiquitination is important for the lysosomal translocation and activation of mTORC1, which modulates autophagy and nutrient sensing [29]. Recently, insulin stimulation and nutrient signaling were shown to be critical for controlling the localization of the mTOR and TSC complex at lysosomes [116].

8. Connection to cell death

There are three major types of cell death: apoptotic (type I cell death), autophagic (type II cell death), and necrotic (type III cell death) [117,118]. Autophagy is in some cases survival mechanism that maintains the balance between the manufacture of cellular components and the breakdown of damaged or unnecessary organelles and other cellular constituents. However, autophagy also kills cells under certain conditions, in a process called autophagic cell death; this involves pathways and mediators different from those of apoptosis [117,118]. In autophagic cell death, cells die with an autophagic morphology and have little or no association with phagocytosis, in contrast with apoptosis [118]. Instead, a characteristic feature of autophagic cell death is the appearance of vacuoles (autophagosomes) that are used for self-degradation. Autophagic cell death appears to be accompanied by 'classical' autophagy, although it does not depend on this process [118].

Attention has turned to cross-talk regulation between anti-apoptotic pathways and the induction of autophagy [7,117,119,120]. Given that AKT signaling regulates the cell death and survival machinery, it will be interesting to examine how AKT signaling integrates into the cell death and survival machinery through autophagy. Proapoptotic signals such as TRAIL (TNF-related apoptosis-inducing ligand) [121], TNF (tumor necrosis factor) [122], and FADD (fas-associated protein with death domain) [123] induce autophagy. Conversely, anti-apoptotic signaling pathways such as the class I PI3K/AKT/mTOR axis suppress autophagy [124]. Consistent with this notion, activation of AKT, a major downstream effector of PI3K, which promotes cell survival, is an inhibitor of autophagy [7,13,90].

The precise nature of the cross-talk between cell death and survival via AKT signaling remains undetermined. In one report, AKT inactivation with small molecule inhibitors did not appreciably induce apoptosis, but markedly increased autophagy [89]. It has been reported that AKT and autophagy cooperate to promote the survival of drug-resistant glioma. The combined inhibition of PI3K and mTOR with PI-103 triggers autophagy without activating AKT (primarily in PTEN mutant cells) [125]. Anti-apoptotic protein Bcl-2, a major effector of AKT signaling that maintains the mitochondrial outer membrane potential to regulate the cell survival machinery, inhibits Beclin1-dependent autophagy [126]. The autophagy protein Atg12 associates with anti-apoptotic Bcl-2 family members to promote mitochondrial-mediated apoptosis [111]. Autophagy induced by suberoylanilide hydroxamic acid inhibits AKT and upregulates Beclin1 [127]. In response to ceramide or starvation, the Beclin1 binding partner and inhibitor Bcl-2 are phosphorylated by JNK1 (c-Jun N-terminal kinase). Hyperphosphorylated Bcl-2 dissociates from Beclin1, which is then able to induce autophagy. In addition, phosphorylation of Beclin1 on T119 by DAPK (death-associated protein kinase) also reduces the Bcl-2–Beclin1 interaction and activates autophagy. Thus, phosphorylation of autophagy-related proteins is an additional aspect of autophagy regulation [6]. Notably, AKT regulates autophagy and tumorigenesis through Beclin1 phosphorylation [11]. This may be due to AKT-dependent regulation of the Beclin1/vimentin interaction, which modulates intermediate filament formation, the consequent suppression of autophagy, and stimulates transformation.

FOXO3, a critical cell survival regulator downstream of AKT [105], functions in parallel with the mTOR pathway through AKT to control autophagy [6,107]. Phosphorylated FOXO3 controls the transcription of autophagy-related genes including LC3 and Bnip3 (BNIP3 subfamily of BH3-only proteins), which mediates the effect of FOXO3 on autophagy [128]. Activation of AKT induces Skp2/MDM2-mediated ubiquitination and proteasomal degradation of FOXO [129]. Furthermore, AKT phosphorylates BAD, which subsequently increases the association of BAD with 14-3-3 proteins, which prevents it from interacting with the anti-apoptotic protein Bcl-2 [1–3]. Therefore, AKT–BAD–Bcl-2–Beclin1–FOXO axis may underlie the regulation of autophagic versus apoptotic cell death.

9. Concluding remarks

AKT signaling plays a central role in many biological processes including cell proliferation and apoptosis. There is an increasing number of AKT binding partners, each of which can modulate AKT activity [130, 131]. A series of studies has revealed a connection between AKT and two important protein degradation pathways, ubiquitination and autophagy. Upon growth factor stimulation, AKT translocates to the plasma membrane via K63-linked ubiquitination. Thereafter, two critical kinases mediate the activation of AKT: PDK1 phosphorylates AKT on T308, and mTORC2 phosphorylates AKT on S473. SUMOlylation of AKT by PIAS1 is also important for AKT activation and downstream biological responses such as proliferation and tumorigenesis. In contrast, K48-linked ubiquitination by BRCA1, TTC3, or MULAN primarily results in the proteasomal degradation of AKT and thus silences AKT kinase activity. In addition to the ubiquitination of mTOR by TRAF6 and FOXO3 by Skp2/MDM2 [6,29,129], several other ubiquitination substrates have been identified, each of which is firmly at the intersection between ubiquitination and autophagy [25,26]. The lysosomal localization of AKT, which is regulated by the phosphatidylinositol [PtdIns(3)P]-dependent localization of Phafin2 to the lysosome, is an essential step in the regulation of autophagy. It is plausible that during the course of autophagy induction, AKT may phosphorylate autophagy regulatory molecules at the lysosome. Casein kinase II, which is upstream of AKT activation at the Golgi/lysosome [132], phosphorylates p62 at S403. This phosphorylation regulates the selective autophagic clearance of ubiquitinated proteins [30], perhaps as a failsafe mechanism for those which were not efficiently degraded by proteasomes. Because activation of AKT at the plasma membrane inhibits the induction of autophagy, the intracellular localization of AKT may affect the cellular responses of the proteolytic cascade (Fig. 3).

Conjugation of K63-linked ubiquitin chains is important for AKT membrane localization and oncogenic activation/phosphorylation. Defects in K48-linked ubiquitination resulting from BRCA1 mutation, for example, may enhance the overall activity of AKT. This directly implicates AKT ubiquitination control as a critical step in its oncogenic

activity. Since ubiquitination plays an important role in AKT activation, we suggest that small molecule inhibition of this pathway is an attractive strategy for treatment of human diseases. For example, an inhibitor of K63-linked AKT ubiquitination should inhibit plasma membrane recruitment of AKT and block its activation. Alternatively, activation of TTC3 or other ligases that mediate K48-linked ubiquitination of AKT could also inhibit the kinase. Overall, the finding that AKT signaling contributes to the regulation of autophagy and ubiquitination offers new therapeutic targets for human diseases caused by the dysregulation of autophagy such as infection, cancer, and neurodegenerative diseases.

Acknowledgement

MN is supported by the Takeda Science Foundation and the Naito Foundation.

References

[1] B.D. Manning, L.C. Cantley, AKT/PKB signaling: navigating downstream, Cell 129 (2007) 1261–1274.
[2] D.P. Brazil, Z.Z. Yang, B.A. Hemmings, Advances in protein kinase B signalling: AKTion on multiple fronts, Trends Biochem. Sci. 29 (2004) 233–242.
[3] M. Noguchi, T. Obata, F. Suizu, Regulation of the PI3K–Akt Network: current status and a promise for the treatment of human diseases, Curr. Signal. Ther. 3 (2008) 138–151.
[4] M. Hochstrasser, Origin and function of ubiquitin-like proteins, Nature 458 (2009) 422–429.
[5] N. Mizushima, B. Levine, A.M. Cuervo, D.J. Klionsky, Autophagy fights disease through cellular self-digestion, Nature 451 (2008) 1069–1075.
[6] C. He, D.J. Klionsky, Regulation mechanisms and signaling pathways of autophagy, Annu. Rev. Genet. 43 (2009) 67–93.
[7] J.J. Lum, D.E. Bauer, M. Kong, M.H. Harris, C. Li, T. Lindsten, C.B. Thompson, Growth factor regulation of autophagy and cell survival in the absence of apoptosis, Cell 120 (2005) 237–248.
[8] T. Xiang, A. Ohashi, Y. Huang, T.K. Pandita, T. Ludwig, S.N. Powell, Q. Yang, Negative regulation of AKT activation by BRCA1, Cancer Res. 68 (2008) 10040–10044.
[9] F. Suizu, Y. Hiramuki, F. Okumura, M. Matsuda, A.J. Okumura, N. Hirata, M. Narita, T. Kohno, J. Yokota, M. Bohgaki, C. Obuse, S. Hatakeyama, T. Obata, M. Noguchi, The E3 ligase TTC3 facilitates ubiquitination and degradation of phosphorylated Akt, Dev. Cell 17 (2009) 800–810.
[10] S. Bae, S.Y. Kim, J.H. Jung, Y. Yoon, H.J. Cha, H. Lee, K. Kim, J. Kim, I.S. An, H.D. Um, I.C. Park, S.J. Lee, S.Y. Nam, Y.W. Jin, J.H. Lee, S. An, Akt is negatively regulated by the MULAN E3 ligase, Cell Res. 22 (2012) 873–885.
[11] R.C. Wang, Y. Wei, Z. An, Z. Zou, G. Xiao, G. Bhagat, M. White, J. Reichelt, B. Levine, Akt-mediated regulation of autophagy and tumorigenesis through Beclin 1 phosphorylation, Science 338 (2012) 956–959.
[12] C.H. Chan, C.F. Li, W.L. Yang, Y. Gao, S.W. Lee, Z. Feng, H.Y. Huang, K.K. Tsai, L.G. Flores, Y. Shao, J.D. Hazle, D. Yu, W. Wei, D. Sarbassov, M.C. Hung, K.I. Nakayama, H.K. Lin, The Skp2-SCF E3 ligase regulates Akt ubiquitination, glycolysis, herceptin sensitivity, and tumorigenesis, Cell 149 (2012) 1098–1111.
[13] J.J. Lum, R.J. DeBerardinis, C.B. Thompson, Autophagy in metazoans: cell survival in the land of plenty, Nat. Rev. Mol. Cell Biol. 6 (2005) 439–448.
[14] M. Hochstrasser, Evolution and function of ubiquitin-like protein-conjugation systems, Nat. Cell Biol. 2 (2000) E153–E157.
[15] C.M. Pickart, M.J. Eddins, Ubiquitin: structures, functions, mechanisms, Biochim. Biophys. Acta 1695 (2004) 55–72.
[16] Y. Merbl, P. Refour, H. Patel, M. Springer, M.W. Kirschner, Profiling of ubiquitin-like modifications reveals features of mitotic control, Cell 152 (2013) 1160–1172.
[17] R. Verma, R. Oania, J. Graumann, R.J. Deshaies, Multiubiquitin chain receptors define a layer of substrate selectivity in the ubiquitin–proteasome system, Cell 118 (2004) 99–110.
[18] W.L. Yang, J. Wang, C.H. Chan, S.W. Lee, A.D. Campos, B. Lamothe, L. Hur, B.C. Grabiner, X. Lin, B.G. Darnay, H.K. Lin, The E3 ligase TRAF6 regulates Akt ubiquitination and activation, Science 325 (2009) 1134–1138.
[19] C.D. Fan, M.A. Lum, C. Xu, J.D. Black, X. Wang, Ubiquitin-dependent regulation of phospho-AKT dynamics by the ubiquitin E3 ligase, NEDD4-1, in the insulin-like growth factor-1 response, J. Biol. Chem. 288 (2013) 1674–1684.
[20] C.A. Dickey, J. Koren, Y.J. Zhang, Y.F. Xu, U.K. Jinwal, M.J. Birnbaum, B. Monks, M. Sun, J.Q. Cheng, C. Patterson, R.M. Bailey, J. Dunmore, S. Soresh, C. Leon, D. Morgan, L. Petrucelli, Akt and CHIP coregulate tau degradation through coordinated interactions, Proc. Natl. Acad. Sci. U. S. A. 105 (2008) 3622–3627.
[21] H.W. Virgin, B. Levine, Autophagy genes in immunity, Nat. Immunol. 10 (2009) 461–470.
[22] N. Mizushima, M. Komatsu, Autophagy: renovation of cells and tissues, Cell 147 (2011) 728–741.
[23] D.J. Klionsky, The molecular machinery of autophagy: unanswered questions, J. Cell Sci. 118 (2005) 7–18.
[24] B. Levine, N. Mizushima, H.W. Virgin, Autophagy in immunity and inflammation, Nature 469 (2011) 323–335.
[25] C. Kraft, M. Peter, K. Hofmann, Selective autophagy: ubiquitin-mediated recognition and beyond, Nat. Cell Biol. 12 (2010) 836–841.
[26] D.J. Klionsky, Autophagy: from phenomenology to molecular understanding in less than a decade, Nat. Rev. Mol. Cell Biol. 8 (2007) 931–937.
[27] M. Bach, M. Larance, D.E. James, G. Ramm, The serine/threonine kinase ULK1 is a target of multiple phosphorylation events, Biochem. J. 440 (2011) 283–291.
[28] M. Matsuda-Lennikov, F. Suizu, N. Hirata, M. Hashimoto, K. Kimura, T. Nagamine, Y. Fujioka, Y. Ohba, T. Iwanaga, M. Noguchi, Lysosomal interaction of akt with phafin2: a critical step in the induction of autophagy, PLoS ONE 9 (2014) e79795.
[29] J.F. Linares, A. Duran, T. Yajima, M. Pasparakis, J. Moscat, M.T. Diaz-Meco, K63 polyubiquitination and activation of mTOR by the p62–TRAF6 complex in nutrient-activated cells, Mol. Cell 51 (2013) 283–296.
[30] G. Matsumoto, K. Wada, M. Okuno, M. Kurosawa, N. Nukina, Serine 403 phosphorylation of p62/SQSTM1 regulates selective autophagic clearance of ubiquitinated proteins, Mol. Cell 44 (2011) 279–289.
[31] J. Moscat, M.T. Diaz-Meco, p62 at the crossroads of autophagy, apoptosis, and cancer, Cell 137 (2009) 1001–1004.
[32] L.C. Cantley, The phosphoinositide 3-kinase pathway, Science 296 (2002) 1655–1657.
[33] S. Wullschleger, R. Loewith, M.N. Hall, TOR signaling in growth and metabolism, Cell 124 (2006) 471–484.
[34] B. Vanhaesebroeck, D.R. Alessi, The PI3K-PDK1 connection: more than just a road to PKB, Biochem. J. 346 (Pt 3) (2000) 561–576.
[35] M. Frech, M. Andjelkovic, E. Ingley, K.K. Reddy, J.R. Falck, B.A. Hemmings, High affinity binding of inositol phosphates and phosphoinositides to the pleckstrin homology domain of RAC/protein kinase B and their influence on kinase activity, J. Biol. Chem. 272 (1997) 8474–8481.
[36] S.R. James, C.P. Downes, R. Gigg, S.J. Grove, A.B. Holmes, D.R. Alessi, Specific binding of the Akt-1 protein kinase to phosphatidylinositol 3,4,5-trisphosphate without subsequent activation, Biochem. J. 315 (Pt 3) (1996) 709–713.
[37] D. Auguin, P. Barthe, C. Royer, M.H. Stern, M. Noguchi, S.T. Arold, C. Roumestand, Structural basis for the co-activation of protein kinase B by T-cell leukemia-1 (TCL1) family proto-oncoproteins, J. Biol. Chem. 279 (2004) 35890–35902.
[38] C.C. Milburn, M. Deak, S.M. Kelly, N.C. Price, D.R. Alessi, D.M. Van Aalten, Binding of phosphatidylinositol 3,4,5-trisphosphate to the pleckstrin homology domain of protein kinase B induces a conformational change, Biochem. J. 375 (2003) 531–538.
[39] K.M. Ferguson, J.M. Kavran, V.G. Sankaran, E. Fournier, S.J. Isakoff, E.Y. Skolnik, M.A. Lemmon, Structural basis for discrimination of 3-phosphoinositides by pleckstrin homology domains, Mol. Cell 6 (2000) 373–384.
[40] J. Yang, P. Cron, V.M. Good, V. Thompson, B.A. Hemmings, D. Barford, Crystal structure of an activated Akt/protein kinase B ternary complex with GSK3-peptide and AMP-PNP, Nat. Struct. Biol. 9 (2002) 940–944.
[41] I. Sanidas, C. Polytarchou, M. Hatziapostolou, S.A. Ezell, F. Kottakis, L. Hu, A. Guo, J. Xie, M.J. Comb, D. Iliopoulos, P.N. Tsichlis, Phosphoproteomics screen reveals Akt isoform-specific signals linking RNA processing to lung cancer, Mol. Cell 53 (2014) 577–590.
[42] M. Noguchi, F. Suizu, Regulation of AKT by phosphorylation of distinct threonine and serine residues, Adv. Med. Biol. 47 (2012) 139–162.
[43] D.D. Sarbassov, D.A. Guertin, S.M. Ali, D.M. Sabatini, Phosphorylation and regulation of Akt/PKB by the rictor-mTOR complex, Science 307 (2005) 1098–1101.
[44] R. Zoncu, A. Efeyan, D.M. Sabatini, mTOR: from growth signal integration to cancer, diabetes and ageing, Nat. Rev. Mol. Cell Biol. 12 (2011) 21–35.
[45] V. Zinzalla, D. Stracka, W. Oppliger, M.N. Hall, Activation of mTORC2 by association with the ribosome, Cell 144 (2011) 757–768.
[46] D. Komander, M.J. Clague, S. Urbe, Breaking the chains: structure and function of the deubiquitinases, Nat. Rev. Mol. Cell Biol. 10 (2009) 550–563.
[47] D. Mukhopadhyay, H. Riezman, Proteasome-independent functions of ubiquitin in endocytosis and signaling, Science 315 (2007) 201–205.
[48] F.E. Reyes-Turcu, K.H. Ventii, K.D. Wilkinson, Regulation and cellular roles of ubiquitin-specific deubiquitinating enzymes, Annu. Rev. Biochem. 78 (2009) 363–397.
[49] D. Komander, M. Rape, The ubiquitin code, Annu. Rev. Biochem. 81 (2012) 203–229.
[50] O. Kerscher, R. Felberbaum, M. Hochstrasser, Modification of proteins by ubiquitin and ubiquitin-like proteins, Annu. Rev. Cell Dev. Biol. 22 (2006) 159–180.
[51] R.L. Welchman, C. Gordon, R.J. Mayer, Ubiquitin and ubiquitin-like proteins as multifunctional signals, Nat. Rev. Mol. Cell Biol. 6 (2005) 599–609.
[52] E. Reinstein, A. Ciechanover, Narrative review: protein degradation and human diseases: the ubiquitin connection, Ann. Intern. Med. 145 (2006) 676–684.
[53] T. Hunter, The age of crosstalk: phosphorylation, ubiquitination, and beyond, Mol. Cell 28 (2007) 730–738.
[54] A.D. Basso, D.B. Solit, G. Chiosis, B. Giri, P. Tsichlis, N. Rosen, Akt forms an intracellular complex with heat shock protein 90 (Hsp90) and Cdc37 and is destabilized by inhibitors of Hsp90 function, J. Biol. Chem. 277 (2002) 39858–39866.
[55] A.J. Ramsey, L.C. Russell, S.R. Whitt, M. Chinkers, Overlapping sites of tetratricopeptide repeat protein binding and chaperone activity in heat shock protein 90, J. Biol. Chem. 275 (2000) 17857–17862.
[56] V. Facchinetti, W. Ouyang, H. Wei, N. Soto, A. Lazorchak, C. Gould, C. Lowry, A.C. Newton, Y. Mao, R.Q. Miao, W.C. Sessa, J. Qin, P. Zhang, B. Su, E. Jacinto, The mammalian target of rapamycin complex 2 controls folding and stability of Akt and protein kinase C, EMBO J. 27 (2008) 1932–1943.
[57] S. Adhikary, F. Marinoni, A. Hock, E. Hulleman, N. Popov, R. Beier, S. Bernard, M. Quarto, M. Capra, S. Goettig, U. Kogel, M. Scheffner, K. Helin, M. Eilers, The ubiquitin ligase HectH9 regulates transcriptional activation by Myc and is essential for tumor cell proliferation, Cell 123 (2005) 409–421.

[58] M.J. Bertrand, S. Milutinovic, K.M. Dickson, W.C. Ho, A. Boudreault, J. Durkin, J.W. Gillard, J.B. Jaquith, S.J. Morris, P.A. Barker, cIAP1 and cIAP2 facilitate cancer cell survival by functioning as E3 ligases that promote RIP1 ubiquitination, Mol. Cell 30 (2008) 689–700.
[59] M.S. Huen, R. Grant, I. Manke, K. Minn, X. Yu, M.B. Yaffe, J. Chen, RNF8 transduces the DNA-damage signal via histone ubiquitylation and checkpoint protein assembly, Cell 131 (2007) 901–914.
[60] A. Sorrentino, N. Thakur, S. Grimsby, A. Marcusson, V. von Bulow, N. Schuster, S. Zhang, C.H. Heldin, M. Landstrom, The type I TGF-beta receptor engages TRAF6 to activate TAK1 in a receptor kinase-independent manner, Nat. Cell Biol. 10 (2008) 1199–1207.
[61] L. Deng, C. Wang, E. Spencer, L. Yang, A. Braun, J. You, C. Slaughter, C. Pickart, Z.J. Chen, Activation of the IkappaB kinase complex by TRAF6 requires a dimeric ubiquitin-conjugating enzyme complex and a unique polyubiquitin chain, Cell 103 (2000) 351–361.
[62] T. Akiyama, S. Maeda, S. Yamane, K. Ogino, M. Kasai, F. Kajiura, M. Matsumoto, J. Inoue, Dependence of self-tolerance on TRAF6-directed development of thymic stroma, Science 308 (2005) 248–251.
[63] U. Schnetzke, M. Fischer, A.K. Kuhn, B. Spies-Weisshart, E. Zirm, A. Hochhaus, J.P. Muller, S. Scholl, The E3 ubiquitin ligase TRAF6 inhibits LPS-induced AKT activation in FLT3-ITD-positive MV4-11 AML cells, J. Cancer Res. Clin. Oncol. 139 (2013) 605–615.
[64] C.G. King, T. Kobayashi, P.J. Cejas, T. Kim, K. Yoon, G.K. Kim, E. Chiffoleau, S.P. Hickman, P.T. Walsh, L.A. Turka, Y. Choi, TRAF6 is a T cell-intrinsic negative regulator required for the maintenance of immune homeostasis, Nat. Med. 12 (2006) 1088–1092.
[65] K. Yoon, E.J. Jung, S.R. Lee, J. Kim, Y. Choi, S.Y. Lee, TRAF6 deficiency promotes TNF-induced cell death through inactivation of GSK3beta, Cell Death Differ. 15 (2008) 730–738.
[66] X. Wang, L.C. Trotman, T. Koppie, A. Alimonti, Z. Chen, Z. Gao, J. Wang, H. Erdjument-Bromage, P. Tempst, C. Cordon-Cardo, P.P. Pandolfi, X. Jiang, NEDD4-1 is a proto-oncogenic ubiquitin ligase for PTEN, Cell 128 (2007) 129–139.
[67] A. Persaud, P. Alberts, M. Hayes, S. Guettler, I. Clarke, F. Sicheri, P. Dirks, B. Ciruna, D. Rotin, Nedd4-1 binds and ubiquitylates activated FGFR1 to control its endocytosis and function, EMBO J. 30 (2011) 3259–3273.
[68] M. Higuchi, K. Onishi, C. Kikuchi, Y. Gotoh, Scaffolding function of PAK in the PDK1-Akt pathway, Nat. Cell Biol. 10 (2008) 1356–1364.
[69] S.M. Nijman, M.P. Luna-Vargas, A. Velds, T.R. Brummelkamp, A.M. Dirac, T.K. Sixma, R. Bernards, A genomic and functional inventory of deubiquitinating enzymes, Cell 123 (2005) 773–786.
[70] W.L. Yang, G. Jin, C.F. Li, Y.S. Jeong, A. Moten, D. Xu, Z. Feng, W. Chen, Z. Cai, B. Darnay, W. Gu, H.K. Lin, Cycles of ubiquitination and deubiquitination critically regulate growth factor-mediated activation of Akt signaling, Sci. Signal. 6 (2013) ra3.
[71] S.C. Sun, Deubiquitylation and regulation of the immune response, Nat. Rev. Immunol. 8 (2008) 501–511.
[72] R. Massoumi, Ubiquitin chain cleavage: CYLD at work, Trends Biochem. Sci. 35 (2010) 392–399.
[73] R. Li, J. Wei, C. Jiang, D. Liu, L. Deng, K. Zhang, P. Wang, Akt SUMOylation regulates cell proliferation and tumorigenesis, Cancer Res. 73 (2013) 5742–5753.
[74] L. Fallon, C.M. Belanger, A.T. Corera, M. Kontogiannea, E. Regan-Klapisz, F. Moreau, J. Voortman, M. Haber, G. Rouleau, T. Thorarinsdottir, A. Brice, P.M. van Bergen En Henegouwen, E.A. Fon, A regulated interaction with the UIM protein Eps15 implicates parkin in EGF receptor trafficking and PI(3)K-Akt signalling, Nat. Cell Biol. 8 (2006) 834–842.
[75] J.A. Engelman, J. Luo, L.C. Cantley, The evolution of phosphatidylinositol 3-kinases as regulators of growth and metabolism, Nat. Rev. Genet. 7 (2006) 606–619.
[76] G. Juhasz, J.H. Hill, Y. Yan, M. Sass, E.H. Baehrecke, J.M. Backer, T.P. Neufeld, The class III PI(3)K Vps34 promotes autophagy and endocytosis but not TOR signaling in Drosophila, J. Cell Biol. 181 (2008) 655–666.
[77] A. Petiot, E. Ogier-Denis, E.F. Blommaart, A.J. Meijer, P. Codogno, Distinct classes of phosphatidylinositol 3′-kinases are involved in signaling pathways that control macroautophagy in HT-29 cells, J. Biol. Chem. 275 (2000) 992–998.
[78] E.F. Blommaart, U. Krause, J.P. Schellens, H. Vreeling-Sindelarova, A.J. Meijer, The phosphatidylinositol 3-kinase inhibitors wortmannin and LY294002 inhibit autophagy in isolated rat hepatocytes, Eur. J. Biochem. 243 (1997) 240–246.
[79] N. Jaber, Z. Dou, J.S. Chen, J. Catanzaro, Y.P. Jiang, L.M. Ballou, E. Selinger, X. Ouyang, R.Z. Lin, J. Zhang, W.X. Zong, Class III PI3K Vps34 plays an essential role in autophagy and in heart and liver function, Proc. Natl. Acad. Sci. U. S. A. 109 (2012) 2003–2008.
[80] Y. Yan, J.M. Backer, Regulation of class III (Vps34) PI3Ks, Biochem. Soc. Trans. 35 (2007) 239–241.
[81] Y. Zhong, Q.J. Wang, X. Li, Y. Yan, J.M. Backer, B.T. Chait, N. Heintz, Z. Yue, Distinct regulation of autophagic activity by Atg14L and Rubicon associated with Beclin 1-phosphatidylinositol-3-kinase complex, Nat. Cell Biol. 11 (2009) 468–476.
[82] J.M. Backer, The regulation and function of class III PI3Ks: novel roles for Vps34, Biochem. J. 410 (2008) 1–17.
[83] K. Lindmo, H. Stenmark, Regulation of membrane traffic by phosphoinositide 3-kinases, J. Cell Sci. 119 (2006) 605–614.
[84] D.J. Klionsky, Autophagy, Curr. Biol. 15 (2005) R282–R283.
[85] A.J. Meijer, P. Codogno, Regulation and role of autophagy in mammalian cells, Int. J. Biochem. Cell Biol. 36 (2004) 2445–2462.
[86] Y.T. Wu, H.L. Tan, G. Shui, C. Bauvy, Q. Huang, M.R. Wenk, C.N. Ong, P. Codogno, H. M. Shen, Dual role of 3-methyladenine in modulation of autophagy via different temporal patterns of inhibition on class I and III phosphoinositide 3-kinase, J. Biol. Chem. 285 (2010) 10850–10861.
[87] N. Mizushima, T. Yoshimori, B. Levine, Methods in mammalian autophagy research, Cell 140 (2010) 313–326.
[88] H. Takeuchi, Y. Kondo, K. Fujiwara, T. Kanzawa, H. Aoki, G.B. Mills, S. Kondo, Synergistic augmentation of rapamycin-induced autophagy in malignant glioma cells by phosphatidylinositol 3-kinase/protein kinase B inhibitors, Cancer Res. 65 (2005) 3336–3346.
[89] M. Degtyarev, A. De Maziere, C. Orr, J. Lin, B.B. Lee, J.Y. Tien, W.W. Prior, S. van Dijk, H. Wu, D.C. Gray, D.P. Davis, H.M. Stern, L.J. Murray, K.P. Hoeflich, J. Klumperman, L. S. Friedman, K. Lin, Akt inhibition promotes autophagy and sensitizes PTEN-null tumors to lysosomotropic agents, J. Cell Biol. 183 (2008) 101–116.
[90] S. Arico, A. Petiot, C. Bauvy, P.F. Dubbelhuis, A.J. Meijer, P. Codogno, E. Ogier-Denis, The tumor suppressor PTEN positively regulates macroautophagy by inhibiting the phosphatidylinositol 3-kinase/protein kinase B pathway, J. Biol. Chem. 276 (2001) 35243–35246.
[91] H.C. Cheng, S.R. Kim, T.F. Oo, T. Kareva, O. Yarygina, M. Rzhetskaya, C. Wang, M. During, Z. Talloczy, K. Tanaka, M. Komatsu, K. Kobayashi, H. Okano, N. Kholodilov, R.E. Burke, Akt suppresses retrograde degeneration of dopaminergic axons by inhibition of macroautophagy, J. Neurosci. 31 (2011) 2125–2135.
[92] W. Chen, N. Li, T. Chen, Y. Han, C. Li, Y. Wang, W. He, L. Zhang, T. Wan, X. Cao, The lysosome-associated apoptosis-inducing protein containing the pleckstrin homology (PH) and FYVE domains (LAPF), representative of a novel family of PH and FYVE domain-containing proteins, induces caspase-independent apoptosis via the lysosomal-mitochondrial pathway, J. Biol. Chem. 280 (2005) 40985–40995.
[93] N.M. Pedersen, C. Raiborg, A. Brech, E. Skarpen, I. Roxrud, H.W. Platta, K. Liestol, H. Stenmark, The PtdIns3P-binding protein Phafin 2 mediates epidermal growth factor receptor degradation by promoting endosome fusion, Traffic 13 (2012) 1547–1563.
[94] A. Takamura, M. Komatsu, T. Hara, A. Sakamoto, C. Kishi, S. Waguri, Y. Eishi, O. Hino, K. Tanaka, N. Mizushima, Autophagy-deficient mice develop multiple liver tumors, Genes Dev. 25 (2011) 795–800.
[95] Y. Wei, Z. Zou, N. Becker, M. Anderson, R. Sumpter, G. Xiao, L. Kinch, P. Koduru, C.S. Christudass, R.W. Veltri, N.V. Grishin, M. Peyton, J. Minna, G. Bhagat, B. Levine, EGFR-mediated Beclin 1 phosphorylation in autophagy suppression, tumor progression, and tumor chemoresistance, Cell 154 (2013) 1269–1284.
[96] D.J. Gillooly, I.C. Morrow, M. Lindsay, R. Gould, N.J. Bryant, J.M. Gaullier, R.G. Parton, H. Stenmark, Localization of phosphatidylinositol 3-phosphate in yeast and mammalian cells, EMBO J. 19 (2000) 4577–4588.
[97] I. Vivanco, C.L. Sawyers, The phosphatidylinositol 3-Kinase AKT pathway in human cancer, Nat. Rev. Cancer 2 (2002) 489–501.
[98] F. Madia, M. Wei, V. Yuan, J. Hu, C. Gattazzo, P. Pham, M.F. Goodman, V.D. Longo, Oncogene homologue Sch9 promotes age-dependent mutations by a superoxide and Rev1/Polzeta-dependent mechanism, J. Cell Biol. 186 (2009) 509–523.
[99] M. Kaeberlein, R.W. Powers III, K.K. Steffen, E.A. Westman, D. Hu, N. Dang, E.O. Kerr, K.T. Kirkland, S. Fields, B.K. Kennedy, Regulation of yeast replicative life span by TOR and Sch9 in response to nutrients, Science 310 (2005) 1193–1196.
[100] P. Fabrizio, F. Pozza, S.D. Pletcher, C.M. Gendron, V.D. Longo, Regulation of longevity and stress resistance by Sch9 in yeast, Science 292 (2001) 288–290.
[101] E.F. Blommaart, J.J. Luiken, P.J. Blommaart, G.M. van Woerkom, A.J. Meijer, Phosphorylation of ribosomal protein S6 is inhibitory for autophagy in isolated rat hepatocytes, J. Biol. Chem. 270 (1995) 2320–2326.
[102] R.C. Scott, O. Schuldiner, T.P. Neufeld, Role and regulation of starvation-induced autophagy in the Drosophila fat body, Dev. Cell 7 (2004) 167–178.
[103] X. Zeng, T.J. Kinsella, Mammalian target of rapamycin and S6 kinase 1 positively regulate 6-thioguanine-induced autophagy, Cancer Res. 68 (2008) 2384–2390.
[104] R.C. Russell, Y. Tian, H. Yuan, H.W. Park, Y.Y. Chang, J. Kim, H. Kim, T.P. Neufeld, A. Dillin, K.L. Guan, ULK1 induces autophagy by phosphorylating Beclin-1 and activating VPS34 lipid kinase, Nat. Cell Biol. 15 (2013) 741–750.
[105] A. Brunet, A. Bonni, M.J. Zigmond, M.Z. Lin, P. Juo, L.S. Hu, M.J. Anderson, K.C. Arden, J. Blenis, M.E. Greenberg, Akt promotes cell survival by phosphorylating and inhibiting a Forkhead transcription factor, Cell 96 (1999) 857–868.
[106] J. Zhao, J.J. Brault, A. Schild, P. Cao, M. Sandri, S. Schiaffino, S.H. Lecker, A.L. Goldberg, FoxO3 coordinately activates protein degradation by the autophagic/lysosomal and proteasomal pathways in atrophying muscle cells, Cell Metab. 6 (2007) 472–483.
[107] M.R. Warr, M. Binnewies, J. Flach, D. Reynaud, T. Garg, R. Malhotra, J. Debnath, E. Passegue, FOXO3A directs a protective autophagy program in haematopoietic stem cells, Nature 494 (2013) 323–327.
[108] H. Huang, D.J. Tindall, Regulation of FOXO protein stability via ubiquitination and proteasome degradation, Biochim. Biophys. Acta 1813 (2011) 1961–1964.
[109] K.E. O'Reilly, F. Rojo, Q.B. She, D. Solit, G.B. Mills, D. Smith, H. Lane, F. Hofmann, D.J. Hicklin, D.L. Ludwig, J. Baselga, N. Rosen, mTOR inhibition induces upstream receptor tyrosine kinase signaling and activates Akt, Cancer Res. 66 (2006) 1500–1508.
[110] X. Wang, P. Yue, Y.A. Kim, H. Fu, F.R. Khuri, S.Y. Sun, Enhancing mammalian target of rapamycin (mTOR)-targeted cancer therapy by preventing mTOR/raptor inhibition-initiated, mTOR/rictor-independent Akt activation, Cancer Res. 68 (2008) 7409–7418.
[111] A.D. Rubinstein, M. Eisenstein, Y. Ber, S. Bialik, A. Kimchi, The autophagy protein Atg12 associates with antiapoptotic Bcl-2 family members to promote mitochondrial apoptosis, Mol. Cell 44 (2011) 698–709.
[112] P. Nicklin, P. Bergman, B. Zhang, E. Triantafellow, H. Wang, B. Nyfeler, H. Yang, M. Hild, C. Kung, C. Wilson, V.E. Myer, J.P. MacKeigan, J.A. Porter, Y.K. Wang, L.C. Cantley, P.M. Finan, L.O. Murphy, Bidirectional transport of amino acids regulates mTOR and autophagy, Cell 136 (2009) 521–534.
[113] D.D. Sarbassov, S.M. Ali, S. Sengupta, J.H. Sheen, P.P. Hsu, A.F. Bagley, A.L. Markhard, D.M. Sabatini, Prolonged rapamycin treatment inhibits mTORC2 assembly and Akt/PKB, Mol. Cell 22 (2006) 159–168.

[114] P. Dalle Pezze, A.G. Sonntag, A. Thien, M.T. Prentzell, M. Godel, S. Fischer, E. Neumann-Haefelin, T.B. Huber, R. Baumeister, D.P. Shanley, K. Thedieck, A dynamic network model of mTOR signaling reveals TSC-independent mTORC2 regulation, Sci. Signal. 5 (2012) ra25.
[115] T. Noda, Y. Ohsumi, Tor, a phosphatidylinositol kinase homologue, controls autophagy in yeast, J. Biol. Chem. 273 (1998) 3963–3966.
[116] S. Menon, C.C. Dibble, G. Talbott, G. Hoxhaj, A.J. Valvezan, H. Takahashi, L.C. Cantley, B.D. Manning, Spatial control of the TSC complex integrates insulin and nutrient regulation of mTORC1 at the lysosome, Cell 156 (2014) 771–785.
[117] D.R. Green, L. Galluzzi, G. Kroemer, Mitochondria and the autophagy-inflammation-cell death axis in organismal aging, Science 333 (2011) 1109–1112.
[118] G. Kroemer, L. Galluzzi, P. Vandenabeele, J. Abrams, E.S. Alnemri, E.H. Baehrecke, M. V. Blagosklonny, W.S. El-Deiry, P. Golstein, D.R. Green, M. Hengartner, R.A. Knight, S. Kumar, S.A. Lipton, W. Malorni, G. Nunez, M.E. Peter, J. Tschopp, J. Yuan, M. Piacentini, B. Zhivotovsky, G. Melino, Classification of cell death: recommendations of the Nomenclature Committee on Cell Death 2009, Cell Death Differ. 16 (2009) 3–11.
[119] G. Kroemer, B. Levine, Autophagic cell death: the story of a misnomer, Nat. Rev. Mol. Cell Biol. 9 (2008) 1004–1010.
[120] B. Levine, J. Yuan, Autophagy in cell death: an innocent convict? J. Clin. Invest. 115 (2005) 2679–2688.
[121] K.R. Mills, M. Reginato, J. Debnath, B. Queenan, J.S. Brugge, Tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) is required for induction of autophagy during lumen formation in vitro, Proc. Natl. Acad. Sci. U. S. A. 101 (2004) 3438–3443.
[122] J. Prins, E. Ledgerwood, P. Ameloot, P. Vandenabeele, P. Faraco, N. Bright, S. O'Rahilly, J. Bradley, Tumour necrosis factor induced autophagy and mitochondrial morphological abnormalities are mediated by TNFR-I and/or TNFR-II and do not invariably lead to cell death, Biochem. Soc. Trans. 26 (1998) S314.
[123] J. Thorburn, F. Moore, A. Rao, W.W. Barclay, L.R. Thomas, K.W. Grant, S.D. Cramer, A. Thorburn, Selective inactivation of a Fas-associated death domain protein (FADD)-dependent apoptosis and autophagy pathway in immortal epithelial cells, Mol. Biol. Cell 16 (2005) 1189–1199.
[124] D. Gozuacik, A. Kimchi, Autophagy as a cell death and tumor suppressor mechanism, Oncogene 23 (2004) 2891–2906.
[125] Q.W. Fan, C. Cheng, C. Hackett, M. Feldman, B.T. Houseman, T. Nicolaides, D. Haas-Kogan, C.D. James, S.A. Oakes, J. Debnath, K.M. Shokat, W.A. Weiss, Akt and autophagy cooperate to promote survival of drug-resistant glioma, Sci. Signal. 3 (2010) ra81.
[126] S. Pattingre, A. Tassa, X. Qu, R. Garuti, X.H. Liang, N. Mizushima, M. Packer, M.D. Schneider, B. Levine, Bcl-2 antiapoptotic proteins inhibit Beclin 1-dependent autophagy, Cell 122 (2005) 927–939.
[127] Q. Cao, C. Yu, R. Xue, W. Hsueh, P. Pan, Z. Chen, S. Wang, M. McNutt, J. Gu, Autophagy induced by suberoylanilide hydroxamic acid in Hela S3 cells involves inhibition of protein kinase B and up-regulation of Beclin 1, Int. J. Biochem. Cell Biol. 40 (2008) 272–283.
[128] C. Mammucari, G. Milan, V. Romanello, E. Masiero, R. Rudolf, P. Del Piccolo, S.J. Burden, R. Di Lisi, C. Sandri, J. Zhao, A.L. Goldberg, S. Schiaffino, M. Sandri, FoxO3 controls autophagy in skeletal muscle in vivo, Cell Metab. 6 (2007) 458–471.
[129] D.R. Plas, C.B. Thompson, Akt activation promotes degradation of tuberin and FOXO3a via the proteasome, J. Biol. Chem. 278 (2003) 12361–12366.
[130] M. Noguchi, V. Ropars, C. Roumestand, F. Suizu, Proto-oncogene TCL1: more than just a coactivator for Akt, FASEB J. 21 (2007) 2273–2284.
[131] K. Du, P.N. Tsichlis, Regulation of the Akt kinase by interacting proteins, Oncogene 24 (2005) 7401–7409.
[132] D.P. Ponce, J.L. Maturana, P. Cabello, R. Yefi, I. Niechi, E. Silva, R. Armisen, M. Galindo, M. Antonelli, J.C. Tapia, Phosphorylation of AKT/PKB by CK2 is necessary for the AKT-dependent up-regulation of beta-catenin transcriptional activity, J. Cell. Physiol. 226 (2011) 1953–1959.