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REVIEW

Involvement of Akt in neurite outgrowth

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Abstract The regulation of neuronal differentiation and neurite outgrowth is essential during development of the nervous system and is crucial in developing therapies to promote axon regeneration after nerve injury or in neurodegenerative diseases. The serine/threonine kinase Akt has been well documented to promote neuronal survival. More recently Akt has also been revealed as key mediator of several aspects of neurite outgrowth, including elongation, branching and calibre. Downstream of Akt, several substrates have been identified that are likely to play key roles in Akt-mediated neurite outgrowth, such as glycogen synthase kinase 3β, peripherin, mammalian target of rapamycin and δ-catenin. The physical interaction between Akt and Hsp27, another protein that has been linked with neurite outgrowth, may also be significant in the process of neurite outgrowth. This review will unite and discuss the research to date that has examined the functionality of Akt in neuronal differentiation during development and neurite outgrowth.

Keywords Akt · Differentiation · Glycogen synthase kinase 3β · Heat shock protein 27 (Hsp27) · Neurite outgrowth

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Introduction

The serine/threonine kinase Akt, also known as protein kinase B, plays a central role in mediating cell survival, metabolism and cellular growth responses [reviewed in 1]. In addition, Akt has recently emerged as an important regulator of neurite outgrowth. Three isoforms of Akt (Akt1, Akt2 and Akt3) have been found in mammalian cells, all of which are functional [2–4]. All three isoforms possess a conserved structure consisting of an N-terminal pleckstrin homology (PH) domain, a kinase domain and a C-terminal regulatory domain. Akt is normally inactive in the cell cytosol and can be activated through stimulation of a variety of transmembrane receptors including receptor tyrosine kinases. Akt is the major effector of the phosphatidylinositol 3-kinase (PI3K) signalling pathway, and the best described pathway of Akt activation is through the recruitment of PI3K to the plasma membrane where it binds to activated tyrosine kinase or G protein-coupled receptors. Once activated, PI3K phosphorylates the membrane phospholipid phosphatidylinositol (4,5)-diphosphate (PIP2), converting it to phosphatidylinositol (3,4,5)-triphosphate (PIP3). The accumulation of PIP3 promotes the translocation of Akt to the plasma membrane, where Akt binds to PIP3 via its PH domain, allowing phosphorylation of Thr308 on Akt by phosphoinositide-dependent kinase 1(PDK1) [5, 6]. Maximal activity of Akt requires the additional phosphorylation of Ser473 in the regulatory domain, and the elusive PDK2 responsible for phosphorylation at Ser473 has now been identified as mammalian target of rapamycin (mTor), in a rapamycin-insensitive complex with Sin1 and rictor [7, 8].

Akt has numerous substrates [reviewed in 9], which can be identified by the presence of the consensus motif RXRXXS/T* (*denotes phosphorylation site), where X

may be any amino acid [10]. Akt can influence many cellular functions through activation or inhibition of substrates, such as promoting survival, metabolism, proliferation and differentiation [reviewed in 11].

Developing neurons that do not make correct synaptic connections die by apoptosis, and in the developed brain, post-mitotic neurons become dependent on neurotrophic factors and neurotransmitters for survival. The PI3K-Akt pathway, which is activated by neurotrophins, has emerged as an important survival pathway in neurons. Akt has the ability to regulate apoptosis either indirectly through phosphorylation of transcription factors or by directly phosphorylating proteins that regulate cell survival. For example, activated Akt promotes survival through phosphorylation of transcription factors forkhead/FOXO [12], NF-κB [13, 14] and mdm2 [15] or through phosphorylation of Bcl-2 family members Bad and Bim [16, 17]. In addition to its vital function in cell survival, a role for PI3K/Akt signalling has also been implicated in neuronal differentiation [reviewed in 18], and several aspects of neurite outgrowth, including elongation, calibre and branching, are regulated by Akt. Known targets of Akt that are implicated in neuronal differentiation include glycogen synthase kinase 3β (GSK3β) [19, 20], the mammalian target of rapamycin (mTOR) [21, 22], cyclic AMP response element binding protein (CREB) [23], peripherin [24] and β-catenin [25]. In addition to roles in survival and neurite outgrowth within the nervous system, Akt is also implicated in synaptogenesis [26, 27] and synaptic transmission [28].

Involvement of Akt in neurite outgrowth

Neurons form functional networks by extending axons and dendrites (collectively termed neurites) that can connect via synapses to other neurons and cells. Many extracellular factors can influence neurite outgrowth, for instance neurotrophins. Neurotrophins, such as nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF) and neurotrophin 3 (NT3), bind to Trk receptor tyrosine kinases, which then activate PI3K-Akt signal transduction [18]. In adult dorsal root ganglion (DRG) neurons, for example, PI3K-Akt signalling is primarily involved in NGF-induced neurite growth via the NGF-specific receptor TrkA [29, 30]. However, it should be noted that signalling pathways other than PI3K-Akt, such as Ras-MAPK, can also be involved in neurite outgrowth but to varying degrees dependent on the cell type. In addition, molecules such as cyclic adenosine monophosphate (cAMP) and octanoic acid (a straight medium-chain fatty acid) are also known to induce neurite outgrowth [31, 32]. There are several aspects of neurite growth in which Akt has been implicated, including neurite elongation, calibre (neurite diameter) and branching.

Neurite elongation

The migration of growth cones, located at the tips of neurites, enables the extension of neurites. Akt has consistently been shown to have a positive influence on neurite elongation in primary neuronal cells (Table 1). A recent example of positive Akt involvement showed that overexpression of constitutively active (CA)-Akt, in both unstimulated and BDNF-stimulated primary hippocampal neurons, strongly enhanced dendritic length compared to wild type (WT)-Akt [33]. Also recently, the PI3K-Akt-GSK pathway has been clearly shown to regulate the early stages of dendrite formation in hippocampal neurons stimulated with hepatocyte growth factor (HGF) [34]. The HGF-induced increase in neurite length was accompanied by Akt activation and pharmacological inhibition of PI3K-Akt with the PI3K inhibitor LY294002 blocked the HGF-enhancement of dendritic length [34]. Direct knockdown of Akt via siRNA blocked neurite outgrowth in adult NGF-responsive DRG neurons, while inhibition of MEK/MAPK had no significant effect [35]. The same group previously reported that pharmacological inhibition of Akt with the phosphatidylinositol ether analog 1L6-hydroxymethyl-chiro-inositol-2-(R)-2-O-methyl-3-O-octadecyl-sn-glycerocarbonate (Akt inhibitor) or of PI3K with LY294002 attenuates neurite outgrowth, analysed in

Table 1 Summary of data reporting the effect of Akt on neurite outgrowth

Cell type	Effect of Akt on neurite outgrowth	Reference(s)
Hippocampal neurons	Positive: enhances dendritic length and complexity, calibre and branching	[33, 34, 54–56]
Dorsal root ganglion neurons	Positive: required for neurite outgrowth, enhanced calibre and branching not length	[35, 36, 43, 53]
Superior nerve ganglion neurons	Positive: induces neurite extension	[37]
Hypoglossal neurons	Positive: enhances regeneration	[41]
Snail neurons	Positive: inhibition reduced neurite outgrowth	[48]
PC12 cells	Positive: increases neurite outgrowth; elongation	[40–43]
	Negative: inhibited neurite outgrowth; reduced branching	[31, 32, 44, 47]
	No significant effect	[46]

response to NGF, laminin or NGF plus laminin [36]. In the nervous system, activating transcription factor 3 (ATF3) is only expressed in injured neurons, and the co-overexpression of ATF3 and MEKK1 was shown induce neurite elongation via Akt activation in superior nerve ganglion neurons and in PC12 cells without NGF stimulation [37]. They also observed that overexpression of ATF3 also inhibited apoptosis induced by MEKK1 overexpression. Interestingly, ATF3 expression induced Hsp27 upregulation, which the authors suggest may directly or indirectly activate Akt and thus inhibit cell death and induce neurite extension [37]. Furthermore, we have recently shown that NGF significantly induces Hsp25 expression at the mRNA and protein level in PC12 cells [38].

The evidence gleaned from primary neuronal cells clearly suggests a positive role for Akt in neurite extension; however, in the neuronal-like PC12 cell line there are conflicting data. NGF induces PC12 cells to form a sympathetic neuron-like phenotype, developing neuronal morphology characterised by neurite outgrowth that requires PI3K signalling activity [39]. The PI3K-Akt signalling cascade promotes neurite elongation in NGF-stimulated PC12 cells [40], and CA-Akt overexpression spontaneously induced neurite elongation in PC12 cells [41, 42] and also greatly enhanced outgrowth stimulated by NGF [42]. Furthermore, overexpression of dominant negative (DN)-Akt or inhibition of Akt activity by the kinase inhibitor ML-9 or PI3K inhibition with LY294002 and wortmannin inhibited neurite outgrowth [42]. In support of this, although the evidence is somewhat circumstantial, inhibition of integrin linked kinase (ILK) or overexpression of DN-ILK in PC12 cells and DRG neurons prevents the activation of downstream targets Akt and GSK3β, blocking neurite length and frequency per cell, in the absence of cell death [43]. Interestingly, phospho-Akt (and also its PH domain when expressed ectopically) is localised at the tips of growth cones, which supports a role for Akt in the regulation of neurite elongation [44]. In addition, it has been shown that the proline-rich inositol polyphosphate 5-phosphatase (PIPP) hydrolyses PIP3 and negatively regulates Akt phosphorylation at serine-473 [45]. PIPP is localised to the plasma membrane of undifferentiated PC12 cells but upon NGF treatment PIPP becomes concentrated at the growth cone and neurite shaft [45]. Overexpression of WT-PIPP significantly reduced neurite elongation but not initiation, whilst RNAi interference of PIPP protein expression generated hyper-elongation of neurites (the latter being prevented by PI3K inhibition). Increased phospho-Ser473-Akt and phospho-Ser9-GSK3β occurred at the growth cone following targeted depletion of PIPP, indicating PIPP as a negative regulator of the PI3K pathway, and demonstrating the importance of spatial distribution of phospho-Ser473-Akt and phospho-Ser9-GSK3β signalling for neurite elongation [45].

Conversely, some studies using PC12 cells have indicated that selective activation of PI3K-Akt signalling may not result in neurite outgrowth. In one study, PI3K was shown to provide only a minor contribution to neuritogenesis; its activation and downstream signalling to Akt were suggested to promote cell survival instead [46]. Additionally, the involvement of Akt was negligible during octanoic acid-induced neurite outgrowth in PC12 cells [31]. Increased βIII-tubulin expression was evident in PC12 cells treated with octanoic acid and also upregulation of the p38 MAPK, ERK and c-Jun N-terminal kinase (JNK) pathways occurred, but not Akt phosphorylation. Furthermore, the PI3K inhibitor wortmannin had little effect on neurite outgrowth, compared to strong inhibition by p38 MAPK and ERK inhibitors [31]. In support of this, NGF- or cAMP-induced neurite outgrowth in PC12 cells was increased in cells expressing DN-Akt [32, 47] but prevented in those cells overexpressing WT-Akt [47]. The results suggested that Akt may negatively regulate neurite outgrowth by overriding the growth-arresting effect of these inducers of neurite outgrowth. Interestingly, a study investigating growth cone motility observed that use of the potent Akt inhibitor significantly reduced neurite outgrowth in snail neurons in vitro, although filopodial elongation was increased [48]. The authors suggest that this dual effect of inhibition of PI3K-Akt signalling is reminiscent of growth cone searching behaviour. Therefore, perhaps Akt can promote neurite extension already underway but inhibits filopodia on growth cones. This combination of effects may help to explain the degree of varied results with Akt and neurite outgrowth in the literature.

Some studies have focused on the role of PI3K rather than Akt in neurite outgrowth; however, as a key downstream effector of PI3K, these studies likely implicate Akt involvement. The application of NGF to distal axons of sympathetic neurons increases PI3K signalling in distal axons and cell bodies, accompanying neurite elongation [49]. Another study has shown that PI3K signalling is necessary for growth cone chemotropism in NGF-induced Xenopus spinal neurons expressing rat TrkA [50]. In addition, PI3K has been shown to be required for glial cell derived neurotrophic factor (GDNF)-stimulated neurite outgrowth of primary dopaminergic neurons [51], and indeed our group has observed severely reduced neurite outgrowth using LY294002 prior to NGF treatment of PC12 cells [38]. In the absence of NGF, overexpression of CA-PI3K in PC12 cells generated the formation of neurite-like processes that were inhibited by wortmannin, though neurites were not completely formed as they lacked an

accumulation of F-actin and GAP43 at the growth cone [52]. However, due to the enhancement of microtubule bundling, microtubule organisation could be the key event for elongation in the cells expressing active PI3K [52]. Conversely, in an early study, pre-incubation of NGF stimulated PC12 cells with wortmannin and also the overexpression of an inactive mutant p85 subunit of PI3K strongly inhibited neurite outgrowth [39]. Wortmannin was also found to cause the collapse of mature neurites [39], although this effect may result from the toxicity of the drug.

Overall, the majority of PI3K data indicate that it has a positive effect on neurite outgrowth, providing further support regarding a role for Akt in neurite elongation.

Neurite branching

Branching occurs when a growth cone splits, thus creating a branch point. Neurite branch formation is essential for the successful integration of neurons, and there is evidence that PI3K-Akt signalling may play an important role in neurite branching. Overexpression of Akt increased distal branching of embryonic DRG neurons by 2.6-fold without neurotrophin stimulation [53]. When these neurons were treated with NGF, an average of 32.5 branch points per cell developed in CA-Akt cells compared to 7.7 in control transfected cells, strongly suggesting a positive effect for Akt in branching [53]. One study in particular examined in depth the effect of PI3K signalling on dendrite morphology: hippocampal neurons were transfected with several known effectors of PI3K: Akt, Rac1 or Arf6, yet of these only Akt was able to increase dendritic complexity and soma size [54]. Dendritic complexity was reduced by RNAi knockdown of the Akt substrate mTOR, suggesting that the Akt-mTOR pathway is the primary mediator of PI3K regulated dendritic branching [54]. In support, a recent study revealed increased branching of hippocampal neurons overexpressing CA-Akt compared with WT-Akt [33].

Conversely, the positive involvement of the PI3K-Akt pathway in neurite branch formation has been contradicted. It has been suggested that Akt may instead play a suppressive role in NGF-treated PC12 cells as the overexpression of CA-Akt reduced branching [44]. Furthermore, DN-Akt increased branching, although interestingly enhanced neurite elongation was observed. The authors also showed a 2.5-fold increase in branching when NGF-treated PC12 cells were treated with the PI3K inhibitor LY294002, as compared with NGF treatment alone. The NGF-induced phosphorylation of Akt at Thr308 was effectively blocked by the inhibitor. pAkt was absent from some of the small immature branches that, considering the apparent suppressive effect of Akt on branching, may aid the development of branches [44].

Other aspects of neurite outgrowth

During neuronal development, many neurites extend from the cell body. One neurite will differentiate to form an axon, whilst the remainder form dendrites. PI3K-Akt signalling and additionally the Raf-MEK pathway have been shown to be necessary for axon growth in embryonic sensory neurons [53]. The overexpression of CA-Akt in sensory neurons in vitro increased axon calibre by 1.9-fold to 2.3-fold, but had no effect on axon length [53]. Thus, the requirement of Akt was confirmed for axon calibre, but neither overexpression of PI3K or Akt induced significant axon lengthening. Instead, activated Raf-1 was found to generate axon elongation similar to that induced by NGF. In addition, the PI3K-Akt-mTOR pathway has been shown to control soma and dendrite calibre, while a coordinated activation with the Ras-MAPK signalling pathway was necessary for increasing dendritic complexity in dissociated postnatal hippocampal CA1/CA3 neuronal cultures [55]. Hippocampal neurons overexpressing CA-Akt or CA-PI3K showed a consistent increase in dendritic calibre, and furthermore, the total branching tips and number of primary dendrites were significantly increased [55]. An increase in axon calibre may have implications for neurotransmission with, for example, greater calibre enabling increased protein transport.

With regard to neuronal injury, overexpression of CA-Akt has also enabled accelerated motor axon regeneration after axotomy in vivo with adult hypoglossal neurons [41]. Interestingly, adult motor-neurons were shown to have highly elevated levels of phospho-Akt in response to injury after axotomy, whereas phospho-Akt levels decreased (and additionally Akt1 mRNA levels were lowered) in injured neonate motoneurons [41]. The lack of Akt activation in neonates may explain why axotomy induces neuronal death in the neonate [41] and demonstrates the pro-survival capacity of Akt in adult neurons. Also of interest is that CA-Akt or PI3K overexpression has also been shown to induce the formation of multiple axons in hippocampal neurons [56].

The evidence accumulated to date with primary neuronal cells indicates that Akt has a positive role in neurite outgrowth and could be a useful target for neuronal regeneration in adult neurons. It is also possible that Akt plays a larger role in aspects of neurite outgrowth other than elongation, such as branching and calibre, than current data implies as most studies have only examined neurite elongation, and thus, far greater research has accrued with respect to this aspect of neurite morphology.

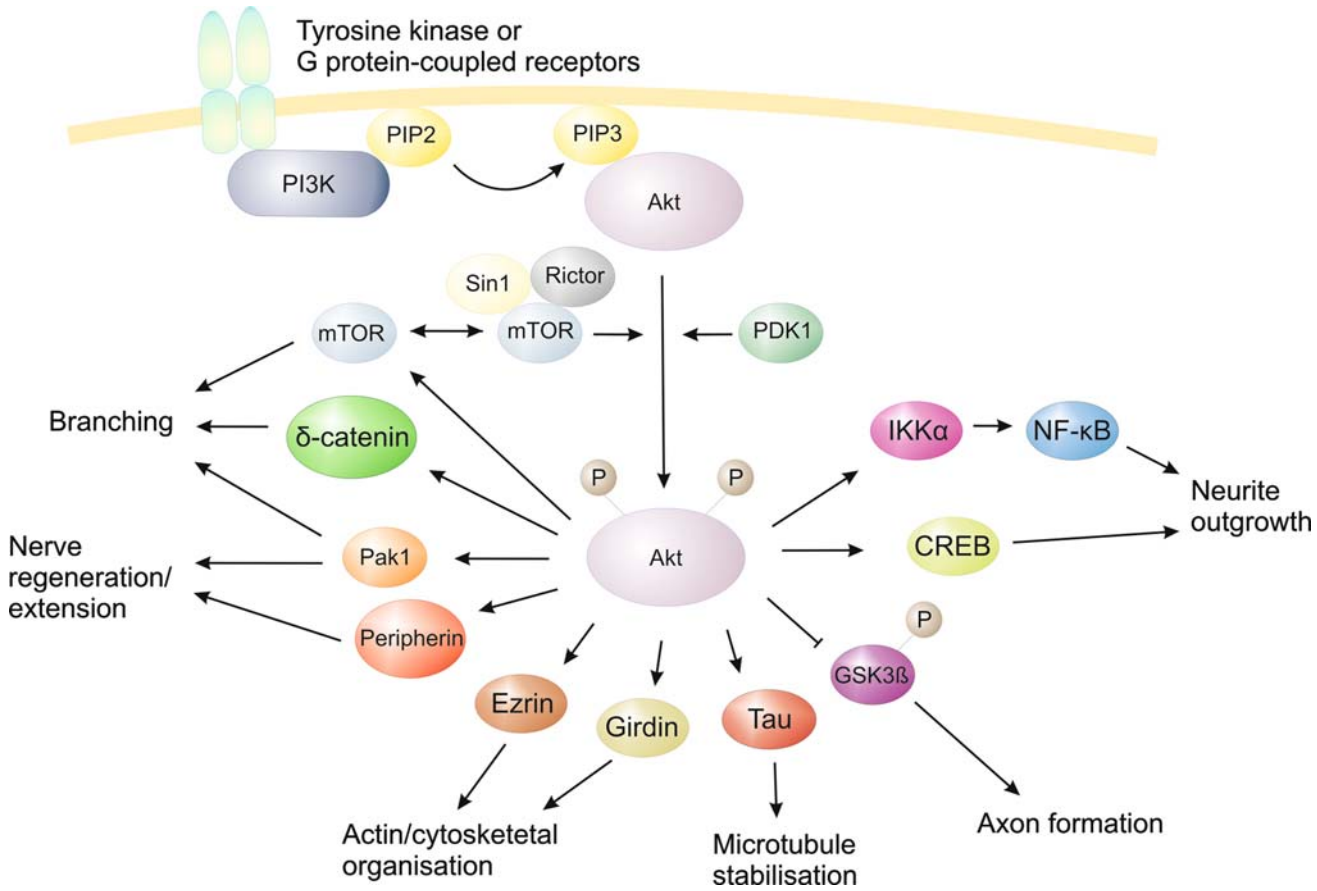

Fig. 1 Targets of Akt that may influence neurite outgrowth. Akt is phosphorylated by PI3K signalling following activation of G protein-coupled or tyrosine kinase receptors at the plasma membrane. Once activated, Akt can subsequently phosphorylate a number of substrates that are involved in aspects of neurite outgrowth/cytoskeletal rearrangement to activate or, in the case of GSK3β, inactivate them

Signalling mechanisms downstream of Akt

Akt phosphorylates or interacts with a number of proteins that may positively influence the development of neuronal morphology (Fig. 1). In particular the serine/threonine kinase GSK3β, is a key Akt substrate [57]. Activated Akt phosphorylates GSK3β at Ser-9 to inactivate its kinase activity [58]; thus, GSK3β activity is inversely correlated with that of PI3K-Akt signalling activity [19]. Phosphorylation of GSK3β was induced by overexpression of WT- and CA-Akt in PC12 cells, and was lower in DN-Akt-expressing cells, whilst considerable GSK3β phosphorylation by Akt and neurite outgrowth occurred spontaneously without NGF treatment [42]. Other studies have also observed GSK3β phosphorylation following Akt activation, and subsequently neurite outgrowth [42, 45]. In HGF-treated hippocampal neurons, Akt activation was shown to phosphorylate GSK3β (inactivating it) and consequently reduce phosphorylation of microtubule-associated protein 2 (MAP2), which can promote microtubule polymerisation and dendrite elongation when dephosphorylated [34]. PI3K (and indirectly Akt) inhibition with LY294002 also suppressed GSK3β phosphorylation, blocking the HGF-enhancement of dendritic length [34]. Inactive Ser9-GSK3β has also been shown to accumulate at the distal end of axon growth cones, which may promote axonal elongation through regulation of microtubule assembly, via the microtubule plus end binding protein adenomatous polyposis coli (APC) [59, 60]. During neuronal differentiation, the establishment of neurite polarity into axons and dendrites is essential. GSK3β relays signals from Akt (and phosphatase and tensin homolog deleted on chromosome 10 (PTEN)) to play a pivotal role in the establishment and maintenance of neuronal polarity [60]. GSK3β is critical for the specification of axon/dendrite fate via its regulation of collapsin response mediator protein-2 (CRMP-2) [61]. Interestingly, in injured rat hypoglossal motor neurons, overexpression of CRMP-2 accelerates nerve regeneration [62]. CRMP-2 seems to promote axon specification and neurite extension through reorganisation of actin filaments, regulation of microtubule assembly, endocytosis of adhesion molecules and also axonal protein trafficking [reviewed in 63]. The phosphorylation of GSK3β also occurs during Wnt signalling [64] and may be significant as

Wnt-7a has been proposed to induce axon and growth cone remodelling in mossy fibres [65]. Overexpression of CA-GSK3β inhibits axon formation, whilst reduction by hairpin siRNA or inhibitors (SB415286, SB216763 and lithium chloride) induces the formation of multiple axons [59, 61]. Thus, GSK3β is a key downstream substrate of Akt in neurite development.

Akt also directly phosphorylates a number of proteins that interact with the cytoskeleton. The microtubule-associated protein tau, which stabilises neuronal microtubules, is phosphorylated in vitro by Akt (Ksiezak-Reding et al. 2003), providing a further mechanistic target. Akt has been shown to regulate actin organisation and cell motility via the protein Girdin [66]. Another substrate of Akt, Ezrin, promotes actin binding and cytoskeletal organisation [67], which is necessary for neuronal development. The enhanced branching generated by overexpression of Akt in embryonic sensory neurons may result from an interaction between Akt and the small G-protein Rac1, which is able to regulate the actin cytoskeleton [53], since Akt has been shown to regulate Rac1 in vivo [68]. Recently, the neuronal intermediate filament protein peripherin has been implicated as a novel substrate for Akt [24]. Akt interacts with the head domain of peripherin in HEK 293T cells and induction of phosphorylated peripherin was observed in nerve-injured hypoglossal motor neurons, promoting regeneration, as well as in PC12 cells overexpressing CA-Akt [24].

The PI3K–Akt pathway is well known to regulate the mTOR pathway, and mTOR has been shown to be a direct substrate of Akt [21, 22]. PI3K-Akt-mTOR signalling has been shown to promote growth and branching in hippocampal neurons, where RNA interference of mTOR decreased both the number and complexity of branches [54]. A study investigating growth cone regeneration revealed that inhibition of mTOR, which is highly expressed in the CNS, resulted in reduced growth cone regeneration following axotomy of CNS and PNS axons at embryonic, newborn and adult stages [69]. However, the reduction was less pronounced than with p38 MAPK (upstream of Hsp27 activation) inhibition by SB208530, although interestingly, an additive effect was observed following inhibition of both mTOR and p38 MAPK [69].

Transcription factors are also among Akt's targets, such as the cAMP response element-binding protein (CREB), which Akt is reputed to phosphorylate [23]. The CREB-activating transcription factor 1 (ATF1) heterodimer has been implicated to be involved in cAMP-induced neurite extension in a subclone of PC12 cells (PC12D) [70]. Furthermore, CREB was found to play a key role in the neural cell adhesion molecule (NCAM) stimulated axonal outgrowth of PC12-E2 cells [71]. A transcription factor that Akt indirectly activates is nuclear factor-κB (NF-κB), which has been shown by several groups to regulate neurite outgrowth [72–74], and the phosphorylation of IκB kinase α (IKKα) by Akt is necessary for the activation of NF-κB [75].

Other Akt substrates include the Akt substrate p21 activated-kinase 1 (Pak1), which has been implicated in the neurite extension of PC12 cells [76], and may also have a role in enhanced branching [53]. In a contrasting study, where CA-Akt decreased and DN-Akt increased branch formation in NGF-treated PC12 cells, the inhibition of neither Rac, mTOR nor GSK3β (other effectors of PI3K) promoted branch formation [44]. PI3K and Akt are required for the effects of the extracellular matrix protein Reelin on the organisation of the cortical plate, but Akt's downstream targets mTOR and GSK3β are not [77]. On the other hand, the effect of Reelin on the dendritic growth and branching of hippocampal neurons is mediated by mTOR, and not GSK3β [77]. Akt1 has also been shown to phosphorylate δ-catenin, which is a brain-specific member of the p120(ctn) subfamily of armadillo/β-catenin proteins that plays important roles in neuronal development [78]. Overexpression of δ-catenin induces the branching of dendrite-like processes in both NIH 3T3 fibroblasts and primary hippocampal neurons [79], and E18 hippocampal neurons overexpressing δ-catenin demonstrate enhanced arborisation of dendrites and maturation of dendritic spines [79]. Thus, there are already a considerable number of Akt substrates identified through which neurite outgrowth can be influenced, and potentially there are many more yet to be revealed.

Interaction between Akt and Hsp27

In recent years, a physical relationship between Akt and the small heat shock protein 27 (Hsp27) has become apparent, which may play a role in the maintenance of Akt activity during neuritogenesis (Fig. 2). An immuno-precipitable complex between Hsp27 and Akt in spinal motor neurons following nerve injury indicates that these two proteins can physically interact in neuronal cells [80]. The complex has been shown to additionally include p38 mitogen-activated kinase (p38 MAPK) [81, 82], and in neutrophil cells the p38 MAPK substrate MAPK-activated protein kinase-2 (MAPKAPK2) is also present [83–85]. MAPKAPK2 is not required for the physical interaction between Hsp27 with Akt, although it is proposed that MAPKAPK2 mediates the incorporation of p38 into the Hsp27/Akt complex [85]. The phosphorylation of Hsp27 by Akt or MAPKAPK2 is suggested to cause dissociation of Hsp27 from Akt both in vitro and in vivo [84], and dissociation of Hsp27 into dimers and monomers upon phosphorylation is likely to be the reason why Hsp27 no longer forms a part of the

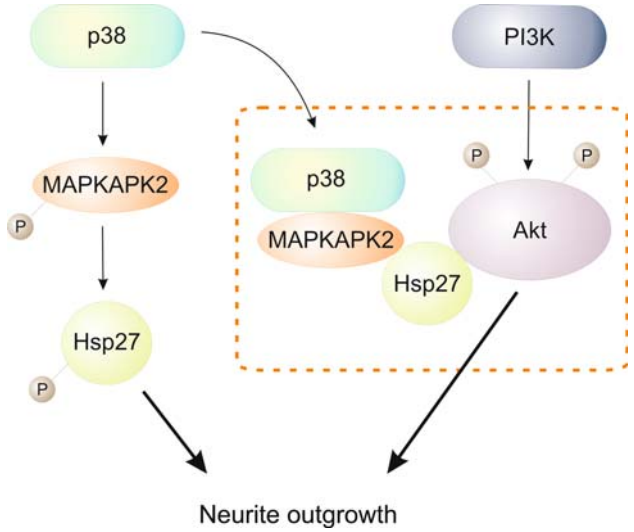

Fig. 2 Akt forms a complex containing Hsp27. Hsp27 is phosphorylated by MAPKAPK2 in the p38 MAPK pathway and has been shown to increase neurite outgrowth. The binding of Hsp27 to Akt is thought to help stabilise Akt, and p38 and MAPKAP2 may also be present. Phosphorylation of Hsp27 may result in its dissociation from the complex

complex [85]. However, the overexpression of Hsp27 in PC12 cells has been shown to maintain Akt phosphorylation, and taken together with the complex formation, it is possible that Akt may be stabilised in an active conformation and protected from dephosphorylation by Hsp27 [81]. Indeed, when COS-7 cells are treated with H_2O_2, Akt is activated gradually, and the association of Hsp27 with Akt increases concurrently with the enhancement of Akt activity [80]. This potential stabilisation may be beneficial in maintaining the activity of Akt during processes such as neurite outgrowth. After sciatic nerve axotomy in murine spinal motor neurons, the signalling pathways of Akt, Hsp27 and p38 MAPK proteins were activated [82]. p38 MAPK was shown to be required for the induction of Hsp27 expression, although only Akt and Hsp27 formed a complex. Four days after axotomy a marked increase in p38, Akt and Hsp27 was detected in the distal area of regenerating nerves, suggesting a role for Akt and Hsp27 in motor neuron regeneration [82]. Hsp27 itself has been suggested to play a role in neurite outgrowth [reviewed in 86] but, as with Akt, the data are somewhat controversial as some groups (including our own) have found Hsp27 is not necessary for neuronal differentiation of olfactory neurons, P19 embryonic carcinoma or PC12 cells [38, 87, 88]. The variation in requirement for Hsp27 in neuronal differentiation of different cell types could be due to the variation in signalling pathways activated by different inducers of neurite outgrowth, such as NGF and laminin. Numerous connections between both Hsp27 and Akt in neuronal differentiation have been identified, and the exact relevance, and although the ultimate consequence of such connections remains unclear, a cooperative role between these proteins is a possibility and should be investigated further.

Discussion/concluding remarks

The neuroprotective function of Akt has been well documented [reviewed in 89], and evidence gleaned from primary neuronal cells indicates that Akt also plays a key role in neurite outgrowth. However, research with the neuronal-like PC12 cells has been more controversial. This may be due to differences between PC12 cells used within different research groups as their phenotype can differ depending on the source and passage number as they readily accumulate mutations. For example, our group possesses PC12 cells from three sources, and we have found that they exhibit notable morphological differences in neurites following NGF-induction of neurite outgrowth. Thus, PC12 cells are perhaps a less than satisfactory model system for the investigation of the molecular biology of neurite outgrowth. Although studies have indicated Akt to be an important protein in several aspects of neurite outgrowth, the intricate signalling that regulates neuritogenesis is not clear as the precise roles played by PI3K-Akt signalling in the development of neurite morphology currently remain undefined. For instance, the inhibition of GSK3β by Akt has been shown to be necessary for the development of axons but not dendrites in hippocampal neurons [59]. A co-operative role in neurite outgrowth may exist between Akt and cytoskeletal-interacting proteins such as Hsp27, and perhaps co-expression of Akt with Hsp27 may yield more consistent results in PC12 cells. It would be interesting to determine whether greater neurite outgrowth occurred in primary cells with co-expression compared to Hsp27 or Akt alone. Akt-mediated Hsp27 phosphorylation promotes intracellular redistribution of Hsp27 and protein interaction in granular keratinocyte differentiation [90], which supports the idea of a close relationship between Akt and Hsp27 during differentiation in general. The authors suggest that Akt may be able to modulate the chaperone functionality of Hsp27 and its interaction with the cytoskeleton during differentiation. It is interesting that Akt (and Hsp27) is at the intersection of neuronal survival and differentiation. However, the close link between pro-survival effects and differentiation fate results in the emergence of a grey convergent area, potentially affecting clarity of results. An important question might be: is Akt genuinely influencing differentiation or predominantly enhancing survival and as a result enabling greater differentiation?

Novel Akt substrates continue to be identified, such as the neuron-specific intermediate filament peripherin [24], which

will help to clarify mechanisms through which Akt exerts its effect on neurite outgrowth. Further research is required to elucidate the role of this multifunctional protein during all aspects of neurite outgrowth, and a possible co-operation with Hsp27 should not be overlooked. It is also of interest to note that Akt dysfunction has been proposed to play a central role in autism spectrum disorder (ASD), which is a common neurodevelopmental disorder [reviewed in 91]. Thus, the manipulation of Akt activity has potential to be therapeutically beneficial, particularly for injury models and neurodegenerative disorders such as Alzheimer's and Parkinson's disease, due to the anti-apoptotic nature of this protein and its positive influence on neurite outgrowth.

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