Akt regulates the expression of MafK, synaptotagmin I, and syntenin-1, which play roles in neuronal function

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Abstract

Background: Akt regulates various cellular processes, including cell growth, survival, and metabolism. Recently, Akt’s role in neurite outgrowth has also emerged. We thus aimed to identify neuronal function-related genes that are regulated by Akt.

Methods: We performed suppression subtractive hybridization on two previously established PC12 sublines, one of which overexpresses the wild-type (WT) form and the other, the dominant-negative (DN) form of Akt. These sublines respond differently to NGF’s neuronal differentiation effect.

Results: A variety of genes was identified and could be classified into several functional groups, one of which was developmental processes. Two genes involved in neuronal differentiation and function were found in this group. v-Maf musculoaponeurotic fibrosarcoma oncogene homolog K (MafK) induces the neuronal differentiation of PC12 cells and immature telencephalon neurons, and synaptotagmin I (SytI) is essential for neurotransmitter release. Another gene, syntenin-1 (Syn-1) was also recognized in the same functional group into which MafK and SytI were classified. Syn-1 has been reported to promote the formation of membrane varicosities in neurons. Quantitative reverse transcription polymerase chain reaction analyses show that the transcript levels of these three genes were lower in PC12 (WT-Akt) cells than in parental PC12 and PC12 (DN-Akt) cells. Furthermore, treatment of PC12 (WT-Akt) cells with an Akt inhibitor resulted in the increase of the expression of these genes and the improvement of neurite outgrowth. These results indicate that dominant-negative or pharmacological inhibition of Akt increases the expression of MafK, SytI, and Syn-1 genes. Using lentiviral shRNA to knock down endogenous Syn-1 expression, we demonstrated that Syn-1 promotes an increase in the numbers of neurites and branches.

Conclusions: Taken together, these results indicate that Akt negatively regulates the expression of MafK, SytI, and Syn-1 genes that all participate in regulating neuronal integrity in some way or another.

Background

Akt (also termed “protein kinase B”) mediates a variety of biological responses to insulin, cytokines, and numerous growth factors. As such, Akt has been well recognized as an important regulator for multiple biological processes, including metabolism, cell size, apoptosis, and cell cycle progression [1]. Recently, the importance of Akt in neuronal functions beyond neuronal protection against apoptotic insults has emerged. Akt was reported to inhibit the neuronal differentiation of hippocampal neural progenitor cells [2] and of PC12 cells [3-5]. Similarly, Akt activity was found to be sustainedly augmented when neurite outgrowth of PC12 cells was inhibited by CSK overexpression [6].

These actions of Akt are evoked by phosphorylating its substrates and thus regulating the activity of proteins and the expression of genes. A number of Akt substrates and Akt-regulated genes have been identified, but these are mostly involved in metabolism, cell size, apoptosis, and cell cycle progression. These include Gsk3, BAD, p21Cip1/WAF1, p27Kip1, and certain transcription factors and transcription factor regulators such as cAMP-response element binding protein (CREB), the FOXO family of Forkhead transcription factors, 1αB kinase, and...
Mdm2 [7-16]. Through these transcription factors and regulators, Akt regulates the transcription of genes that possess anti-apoptotic, pro-survival or pro-apoptotic functions, such as Bcl-2, Bcl-XL, A1, and Fasl. [15,17,18].

Unlike these Akt-regulated genes in apoptosis and survival, however, hardly any genes implicated in neuronal differentiation process have been revealed to be regulated by Akt. Therefore, we sought to find Akt-regulated differentiation genes in rat PC12 pheochromocytoma cells, which are often used as a model of neuronal differentiation. We performed suppression subtractive hybridization (SSH) on two previously established subclonal PC12 cell lines that ectopically express a wild-type (WT) or dominant-negative (DN) form of Akt1 [3]. PC12 (WT-Akt) cells barely differentiate in response to NGF, whereas PC12 (DN-Akt) cells extend their neurites quite well. Approximately seventy genes including v-maf musculoaponeurotic fibrosarcoma oncogene homolog K (MafK), synaptotagmin I (SytI), and syntenin-1 (Syn-1) were recognized as genes expressed at a higher level in cells that express have PC12 (DN-Akt) cells. We demonstrated here that knockdown of Syn-1 decreases the number of neurites per neuritogenic cell and the percentage of branch-bearing neurites, implicating a positive role for Syn-1 in neurite outgrowth. Likewise, MafK and SytI are known to play a role in neuronal differentiation and neurotransmitter release, respectively. By quantitative reverse transcription polymerase chain reaction (RT-PCR) analysis, we confirmed that PC12 (WT-Akt) cells had a lower level of expression of these three genes than PC12 (WT-Akt) cells, and also demonstrated that pharmacological inhibition of Akt causes an increase in the expression of these genes in PC12 (WT-Akt) cells. Therefore, it is alleged that Akt downregulates the expression of MafK, SytI, and Syn-1 genes, all of which are positively related to neuronal function.

**Methods**

**Materials**

A potent and specific inhibitor of Akt (AKTi-1/2; naphthyridinone) and the Gsk3β inhibitor TWS119 were purchased from Calbiochem (CA, USA). Actinomycin D was from Sigma (MO, USA). Nerve growth factor (NGF) 2.5 S was obtained from Roche (NJ, USA). G418 was included in the culture media. Where indicated, 100 ng/ml of NGF was added to the cultures in DMEM containing 1% horse serum and 0.5% fetal calf serum.

**SSH**

Cells were treated with 100 ng/ml of NGF for 2 hrs. Total RNA was prepared with the RNeasy kit (Qiagen, CA, USA) and cDNA was synthesized using the SMART™ PCR cDNA Synthesis Kit (BD Biosciences, CA, USA). SSH was carried out with the PCR-Select™ cDNA subtraction kit (BD Biosciences), as described by the manufacturer. cDNAs synthesized from the total RNA of PC12 (WT-Akt) and PC12 (DN-Akt) cells were used as the drivers and testers, respectively. Plasmids encoding subtracted cDNA were subjected to cycle sequencing using the ABI PRISM™ 310 genetic analyzer (Applied Biosystems, CA, USA). The sequences obtained were used for sequence alignment with the National Center for Biotechnology Information, GenBank, using a basic blast search.

**Semi-quantitative and quantitative RT-PCR**

cDNA was synthesized from RNA using the Omniscript RT kit (Qiagen) according to the manufacturer’s protocol. The cDNA mixture was amplified using PCR with specific primers (Table 1), and the resulting products were separated on 2% agarose gel. Quantitative PCR was run using SYBR green PCR mastermix and the LightCycler Real-time PCR Detection System (Roche). The number of amplification steps required to reach the threshold cycle number (Ct) was computed using LightCycler software 4.0 (Roche). To normalize input cDNA, Gapdh was run separately in all experiments under the same conditions. The expression levels of each gene were normalized against Gapdh using the comparative Ct method.

**Inhibitor treatment**

Cells were pretreated with the Akt inhibitor AKTi-1/2 (0.1 - 10 μM) or the Gsk3β inhibitor TWS119 (10 μM) for 3 hrs in DMEM and 1% horse serum and 0.5% fetal calf serum, prior to treatment with 100 ng/ml of NGF for either 10 min or 2 hr. For the transcriptional inhibitor actinomycin D, cells were treated with 100 ng/ml of NGF for 2 hr, followed by 10 μM actinomycin D for 2 hr in the absence of NGF.

**Lentivirus vector and infection**

The lentiviral shRNA vector was constructed in plasmid pLV-TH (Dr. Didier Trono, University of Geneva, Geneva, Switzerland). Target sequences for knockdown of Syn-1 were: shSyn-1#1 (GGATTAGGAGGAGCA- GAT), shSyn-1#2 (GTGAGATCAACGGACAGAA). A nonsilencing control shRNA (shControl) was GCGTGTACATGCCTGTA. Viral stock was generated.
Table 1 Primers used for semi-quantitative RT-PCR

| Gene   | Forward primer                                      | Reverse primer                                      |
|--------|-----------------------------------------------------|-----------------------------------------------------|
| Ect2   | 5'-GAGAGCTGGTGCAATGCT-3'                            | 5'-TCCCTGAGCTGGTGGAT-3'                             |
| Cadm1  | 5'-CCTGGGCAACCTGTCTCAT-3'                           | 5'-CTGCTGCTCTGCTGGTCTCAT-3'                         |
| Rgs4   | 5'-GTGCTCCGGCTGGCCTTC-3'                            | 5'-CATGTCGGGCTGGTCTTC-3'                            |
| Cldn1  | 5'-AACTCTTGGTATGGGAGAC-3'                           | 5'-CAGAGAGCTTGGACGAC-3'                             |
| Knx2   | 5'-CCAAGCAGTGCAGACCA-3'                             | 5'-CCCTCAGCTTGGACGACCA-3'                           |
| SocS5  | 5'-GTTGAGATGACGCAACAG-3'                            | 5'-AATCTCTGAGCGAATTTG-3'                            |
| Pbm1-predicted | 5'-GCTCTGACGGCTTCCATAAG-3 | 5'-AAAGTCGCGATCCACAG-3 |
proteins that are involved in (1) signaling cascades and cell communication, (2) the organization and localization of cellular compartments and components, (3) gene expression, (4) protein metabolism, and (5) developmental processes (Table 2).

Three genes, v-maf musculoaponeurotic fibrosarcoma oncogene homolog K (MafK), Synaptotagmin I (SytI), and Synaptotagmin-1 (Syn-1), in the fifth group of clustering drew our interest because these genes are known or presumed to be associated with neuronal function. MafK is one of the small Maf family proteins. It is expressed in neural tissues of both late embryonal stage and postnatal period, in the embryonic mesoderm, and in mesenchymal and hematopoietic cells [21]. MafK certainly plays a role in differentiation of these specific types of cells; it participates in NGF-promoted neuritogenesis of PC12 cells and immature telencephalon neurons [22], and is required for erythroid differentiation [23]. SytI is abundant in the synaptic vesicles of neurons, where it acts as a calcium sensor and plays a critical role in neurotransmitter release [24,25]. Its expression was shown to be induced in neural crest cultures by BMP4, a factor that evokes norepinephrine differentiation [26,27]. Syn-1 is involved mainly in the regulation of plasma membrane dynamics in neuronal as well as non-neuronal cells [28,29]. Ectopic expression of Syn-1 in neurons was shown to increase the number of neuritic varicosities [30].

We performed quantitative RT-PCR to quantitate the differences between transcript levels in three cell populations: PC12 (WT-Akt), PC12 (DN-Akt), and PC12 (parental). The transcript level of each gene in PC12 (WT-Akt) and PC12 (DN-Akt) cells was presented as a fold change from that of the PC12 (parental) cells. As shown in Fig. 2A, all of the three genes exhibited increased transcript levels in both PC12 (DN-Akt) and PC12 (parental) cells compared to PC12 (WT-Akt) cells. The transcript levels of these genes seem inversely correlated with the Akt activity levels, being highest in PC12 (DN-Akt) and lowest in PC12 (WT-Akt). The SytI transcript was remarkably elevated, with an ~18-fold increase in PC12 (DN-Akt) cells, compared to PC12 (WT-Akt) cells. The fold differences of other gene transcripts were 2.7-4.3. To check whether detected changes in the transcript levels are followed by changes in protein levels, western blot analysis was performed (Fig. 2B). In agreement with the transcript levels, MafK, SytI, and Syn-1 protein levels were also higher in PC (DN-Akt) cells than in PC (WT-Akt) cells.

Effect of an Akt-specific inhibitor on expression of MafK, SytI, and Syn-1 genes in PC12 (WT-Akt) cells

As stated above, our experiments were based on two Akt-manipulated PC12 sublines that turned out to have

### Table 2 A list of representative genes that are upregulated in PC12 (DN-Akt) cells compared to PC12 (WT-Akt) cells

| Functional classification | Gene expression, Nucleic acid metabolism | Functional classification |
|---------------------------|------------------------------------------|---------------------------|
| Signaling cascades, Cell communication | DEAH box polypeptide 15 | Ect2 |
| Epithelial cell transforming sequence 2 oncogene (Ect2) | Polybromo 1_predicted (Pbrm1_predicted) | Methionine adenosyltransferase II, alpha (Mat2a) |
| Regulator of G protein signaling 4 (Rgs4) | v-maf musculoaponeurotic fibrosarcoma oncogene homolog K (MafK) | Development of anatomical structure and nervous system, Developmental process, Secretion |
| Mss4 | RNA binding motif protein 3 | Canx |
| Syntenin 1 (Syn-1) | Phosphatidylinositol glycan anchor biosynthesis, class O | Cadm1 |
| Kinetochore associated 2_predicted (Kntc2) | Protein metabolism | Socs5 |
| Connexin 45 (Cx45) | SytI | MafK |
| Organization and localization of cellular compartments and components | Canx | Syn-1 |
| SytI | SFRS protein kinase 2 | SytI |
| Calnexin (Canx) | Connexin 45 (Cx45) | Ect2 |
| Cell adhesion molecule 1 (Cadm1) | Methionine adenosyltransferase II, alpha (Mat2a) | Development of anatomical structure and nervous system, Developmental process, Secretion |
| Mss4 | DEAH box polypeptide 15 | Canx |
| Syn-1 | Polybromo 1_predicted (Pbrm1_predicted) | Cldnd1 |
| Kntc2 | v-maf musculoaponeurotic fibrosarcoma oncogene homolog K (MafK) | Apool |
| Not clustered | RNA binding motif protein 3 | DEAD box polypeptide 42 |
| Calulin domain containing 1 (Clind1) | Phosphatidylinositol glycan anchor biosynthesis, class O | Apool |
| Similar to golli-interacting protein | Protein metabolism | Apool |
| DEAD box polypeptide 42 | SytI | Apool |
| Apolipoprotein O-like (Apool) | Suppressor of cytokine signaling 5 (Socs5) | Apool |
| Suppressor of cytokine signaling 5 (Socs5) | Sp3 transcription factor | Apool |

**Figure 1 Verification of genes identified from SSH by semi-quantitative RT-PCR**

WT, PC12 (WT-Akt) cells; DN, PC12 (DN-Akt) cells.
PC12 (parental) cells (data not shown). These data, together, indicate that Akt downregulates the levels of *MafK*, *SytI*, and *Syn-1* transcripts.

Since the overall level of a gene transcript is determined by transcription and mRNA stability, we investigated whether the elevated levels of gene transcripts in PC12 (DN-Akt) cells are due to increased mRNA stability in these Akt-downregulated cells. Gene transcript decay assays employing the transcriptional inhibitor actinomycin D revealed that the mRNA stability of *MafK*, *SytI*, and *Syn-1* does not differ between PC12 (WT-Akt) and PC12 (DN-Akt) cells (data not shown). For this reason, the different levels of transcripts for these genes in PC12 (WT-Akt) and PC12 (DN-Akt) cells appear to reflect altered levels of transcription between the two cell populations.

**Effect of a Gsk3β inhibitor on expression of MafK, SytI, and Syn-1 genes in PC12 (DN-Akt) cells**

Several transcription factors have been shown to be regulated by Akt and its downstream effectors. Gsk3β is one of the major molecules downstream of Akt. It phosphorylates and regulates transcription factors such as CREB and NF-kB [34]. We tested whether the Gsk3β pathway is involved in Akt-induced downregulation of transcription for the genes examined here. PC12 (DN-Akt) cells were treated with TWS119, a Gsk3β inhibitor [35]. Before examining the effect of this inhibitor on the transcript levels of the genes, we first wanted to determine whether this inhibitor would work actively as a Gsk3β inhibitor under our experimental conditions. To this end, we assayed the level of β-catenin, because β-catenin is another well-characterized substrate of Gsk3β and undergoes degradation upon phosphorylation [36]. As shown in Fig. 4A, TWS119 treatment of PC12 (DN-Akt) cells resulted in an increase in the level of β-catenin, indicating that TWS119 actively inhibits Gsk3β. Under these conditions, the transcript levels of *MafK* and *Syn-1* were significantly lowered after treatment with this Gsk3β inhibitor, while that of *SytI* was not notably changed (Fig. 4B). This result suggests that the reduction of *MafK* and *Syn-1* expression by Akt occurs, at least in part, through Gsk3β inhibition.

**Knockdown of Syn-1 decreases the neurite complexity in PC12 cells**

We took an shRNA approach to knock down Syn-1 in PC12 (DN-Akt) cells. Cells were transfected with shControl, shSyn-1#1, or shSyn-1#2. By semiquantitative and quantitative RT-PCR and western blot analysis, we found an efficient knockdown of Syn-1 transcript and protein in cells transfected with shSyn-1#2 as compared with shControl-transfected cells (Fig. 5). We thus analyzed several aspects of neurite outgrowth in PC12 (DN-Akt) cells.
Akt) cells transfected with shSyn-1#2. It was microscopically observed that cells transfected with this shRNA possessed less elaborate neurites compared to control cells (Fig. 6A). The number of neurites originating from the cell body of neuritogenic cells was decreased in cells where Syn-1 was knocked down (Fig. 6B). Cells transfected with shSyn-1 had 1.74 ± 0.15 whereas control cells had 2.80 ± 0.20 neurites per cell on the 5th day of NGF treatment. Another neurite outgrowth feature was also observed to be altered by Syn-1 knockdown. As for the neurite number, neurite branching was significantly reduced in cells transfected with shSyn-1 as compared with that of control cells (Fig. 6C). 64.5% of neurites from the control cells had at least one branch point, but only 26.5% of Syn-1-knockdown cell neurites displayed branching. Unlike these effects on the neurite network, however, the number of cells bearing at least one neurite was not altered noticeably by Syn-1 knockdown (Fig. 6D). These data demonstrate that Syn-1 does not affect neurite initiation but rather it promotes neurite profusion and branching in PC12 cells.

Discussion
In this study, we identified novel Akt-regulated genes, *MafK*, *SytI*, and *Syn-1*. We showed that the transcript levels of these three genes were lower in PC12 (WT-
Akt) cells than in PC12 (DN-Akt) cells, but increased following treatment with an Akt inhibitor. Taken together, these results indicate that Akt downregulates the expression of MafK, SytI, and Syn-1. MafK and SytI are involved in certain aspects of neuronal function. Inhibition of MafK expression has been reported to suppress neurite generation in PC12 cells treated with NGF and in primary immature neuronal cells [22], and mice deficient in MafK and MafG suffer from neurological dysfunction [37]. SytI is a synaptic vesicle protein in neurons. It acts as a calcium sensor and plays a role in neurotransmitter release [24,25]. Syn-1 interacts with a plethora of proteins via its PDZ domains and regulates transmembrane-receptor trafficking, tumor-cell metastasis, and probably neuronal-synaptic function [29]. The neuronal action of Syn-1 has been somewhat speculative and is based on the following findings. Syn-1 binds to several proteins implicated in axon outgrowth, fasciculation, and/or guidance, including Unc51.1 [38], ephrin receptors [39] and neurofascin [40]. Moreover, Syn-1 was shown to be expressed maximally in periods of intense growth and synapse formation during neuron development and its ectopic overexpression increased the number of short dendritic varicosities in neurons [30]. Here, we examined whether Syn-1 could affect the outgrowth of neurites. We transfected PC12 (DN-Akt) cells with a hairpin siRNA directed against Syn-1. This shRNA effectively reduced the endogenous level of Syn-1. We showed that a decreased Syn-1 level led to a decrease in the number of neurites and the percentage

![Figure 4](image-url)
of branch-bearing neurites. Unlike these results, however, overexpression of Syn-1 in mature cultured hippocampal neurons was reported not to affect the number of branches [30]. This discrepancy may be due to the different levels of Syn-1 signaling, resulting from decreased or elevated expression of Syn-1, or reflect differences between mature hippocampal cells and neuroblast PC12 cells. When PC12 (DN-Akt) and PC12 (parental) cells were compared, it was also noticed that PC12 (parental) cells have fewer neurite branch points than PC12 (DN-Akt) cells (data not shown). These results suggest that Syn-1 has a role in producing profuse neurites. Though Syn-1 has been implicated in neuronal membrane varicosities [30], its Namikawa et al. 2000 K. Namikawa, M. Honma, K. Abe, M. Takeda, K. Mansur, T. Obata, A. Miwa, H. Okado and H. Kiyama, Akt/protein kinase B prevents injury-induced motoneuron death and accelerates axonal regeneration, J. Neurosci. 20 (2000), pp. 2875-2886. View Record in Scopus | Cited By in Scopus (108)effect on neurite branching has not been described.

There have been conflicting reports regarding the role of Akt in neuronal outgrowth/differentiation. Several studies have reported a negative impact of Akt upon neuronal differentiation [2-6], while others published opposite results [41,42]. The reason for this discrepancy is not known. The direction of Akt’s effect on neuronal differentiation probably depends on the state/context of cells. In fact, signaling molecules have been shown to induce different phenotypes, depending on the cellular stage/context. For instance, phosphoinositide 3-kinase has been shown to be required for NGF-induced differentiation of PC12 cells [43], however the same results were not observed by other research group [44]. It may be that the same signaling molecule has different sensitivity to its interacting partners under different cell contexts, producing different outcomes. It has been reported that Ras responds only to phosphoinositide 3-kinase of the basal rather than stimulated state under certain circumstances [45]. Another example is Akt and Raf-MEK-MAPK pathways. Akt interacts with and inhibits the Raf-MEK-MAPK pathway [46], but this interaction occurs only in certain stages of cell differentiation [47]. The strength and duration of a molecule’s signaling can be affected by its interacting proteins, which themselves also have changing activity and duration. Therefore, the signaling strength and duration of Raf-MAPK, which mediates neuronal differentiation when activated sustainedly [48,49], could be controlled by Akt. It has been suggested that a high level of Akt activity inhibits cell differentiation, whereas low Akt activation levels may be permissive or necessary for cell differentiation [2]. Furthermore, depending on the different level of Raf-MAPK, even the same level of Akt activity would produce different outcomes.

How Akt regulates neuronal outgrowth/differentiation has not been resolved. In the present study, we demonstrate that Akt downregulates the expression of several genes of neuronal functions, including \textit{MafK}, \textit{Syt1}, and \textit{Syn-1}, and some other genes such as \textit{Canx} and \textit{Cadm1} (Table 2). It can be envisioned from these results that Akt-mediated downregulation of these genes of neuronal
function is relevant to the diminished manifestation of neuronal phenotype in cells overexpressing Akt. Akt is often found activated in some neuroblastomas [50,51] as well as many other human cancers [52,53]. Neuroblastomas with hyperactive Akt or with low potential to differentiate in response to neurotrophins show poor prognosis [50,54]. There might be a causal connection between the Akt activity and the differentiation ability in these neuroblastomas. We observed that incubation of PC12 (WT-Akt) cells with the Akt inhibitor AKTi-1/2 restored their neurite outgrowth responsiveness to NGF (Fig. 3B). In this respect, a use of an Akt inhibitor like AKTi-1/2 would be advantageous in that it can not only possess the cytotoxic effect but can also lead the cells to respond to the differentiation effect of neurotrophins.

The mechanism of the downregulation of MafK, SytI, and Syn-1 genes by Akt remains unclear. However, the increased expression of MafK and Syn-1 genes in PC12 (WT-Akt) cells upon pharmacological inhibition of Gsk3β (Fig. 4) indicates that Gsk3β somehow regulates the transcription factors for these two genes. Gsk3β phosphorylates several transcription factors, including AP-1, CREB, HSF-1, NFAT, C/EBP, and NF-kB [34], Ngn2 [55], and Smad3/4 [56]. The functional consequence of this interaction with Gsk3β differs among transcription factors. While most are inhibited by Gsk3β, the transcriptional activity of C/EBP and Ngn2 is increased by Gsk3β. Since Akt phosphorylates and inhibits Gsk3β, the transcriptional activity of C/EBP and Ngn2 might be downregulated in PC12 (WT-Akt) cells but upregulated in PC12 (DN-Akt) cells. In this

**Figure 6** Knockdown of endogenous Syn-1 by shRNA decreases the numbers of neurites and branches. PC12 (DN-Akt) cells were infected with lentiviral shSyn-1#2 or shControl. (A) Fluorescent microscopic photographs of cells treated with NGF for 5 days. Scale = 50 μm. (B) The number of neurites originating from the neuritogenic cell body. (C) The percentage of branch-bearing neurites. (D) The percentage of neuritogenic cells among GFP-positive cells. Averages and standard deviations are derived from more than ten fields of view. * P < 0.001 and # P < 0.01 (n = 10-15, mean ± SD).
regard, it remains to be determined whether MafK and Syn-1 gene expressions are altered by expressing the active or dominant-negative (or silencing) form of C/EBP and Ngn2 in PC (WT-Akt) and PC (DN-Akt) cells, respectively. Our work indicates that the Gsk3β pathway does not appear to be implicated in the decreased expression of SytI in PC12 (WT-Akt). Expression of SytI in sympathetic neurons has been shown to be induced by BMP4 [27], and BMP4 signaling is known to be mediated by Smad proteins and MAPKs [57,58]. Therefore, it can be suggested that decreased expression of SytI in PC12 (WT-Akt) cells might be due to the Akt-mediated inhibition of the Raf-MAPK pathway. Alternatively, Akt might directly affect the transcription factor responsible for SytI expression. This could be Brn3, because this is the only transcription factor identified so far that could increase the expression of SytI in neuronal cells [59], and interestingly it has the preferred phosphorylation motif of Akt [31].

Conclusion
Using PC12 cells expressing wild-type or dominant-negative Akt and also using a pharmacological inhibitor of Akt, we demonstrate that Akt can negatively affect the expression of MafK, SytI, and Syn-1 genes. MafK and SytI have been known to positively affect neuronal differentiation or neurotransmitter release. As for Syn-1, we observed here that Syn-1 has a role in producing profuse neurites. We also show that treatment with Akt inhibitor resulted in an improvement of neurite outgrowth. In summary, Akt regulates the expression of MafK, SytI, and Syn-1, which are all neuronal function-related genes.

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Authors’ contributions
YR performed PCR experiments and drafted the manuscript. BJ performed all experiments. CYS helped in experiments and participated in the writing of the manuscript. EUOP aided in designing the experiments. CGK helped BJ in experiments. SY designed the study, coordinated the study, and participated in the writing of the manuscript.

Competing interests
The authors declare that they have no competing interests.

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References
1. Yang ZZ, Tschopp O, Bauduy A, Dumbler M, Hynx D, Hemmings BA: Physiological functions of protein kinase B/Akt. Biochem Soc Trans 2004, 32(Pt 2):350-354.
2. Pelletier J, O’Neill A, Schaffer DV: PI3K/Akt and CREB regulate adult neural hippocampal progenitor proliferation and differentiation. Dev Neurobiol 2007, 67(10):1348-1361.
3. Bang OS, Park EK, Yang SI, Lee SR, Franke TF, Kang SS: Overexpression of Akt inhibits NGF-induced growth arrest and neuronal differentiation of PC12 cells. Cell. J Cell Sci 2001, 114(Pt 8):81-88.
4. Pipper A, Dikic I, Lutz MP, Leser J, Kroenerberger B, Eleer C, Kramer H, Muller-Esterl W, Zeuzem S: Cyclic AMP induces transactivation of the receptors for epidermal growth factor and nerve growth factor, thereby modulating activation of MAP kinase, Akt, and neurite outgrowth in PC12 cells. J Biol Chem 2002, 277(46):43623-43630.
5. Higuchi M, Onishi K, Masayama N, Gotoh Y: The phosphatidylinositols-3 kinase (PI3K)-Akt pathway suppresses neurite branch formation in NGF-treated PC12 cells. Genes Cells 2003, 8(8):657-669.
6. Dey N, Howell BW, De PK, Durden DL: CSK negatively regulates nerve growth factor induced neuronal differentiation and augments AKT kinase activity. Exp Cell Res 2005, 307(1):1-14.
7. Cross DA, Alessi DR, Cohen P, Andjelkovich M, Hemmings BA: Inhibition of glycogen synthase kinase-3 by insulin mediated by protein kinase B. Nature 1995, 378(6559):785-789.
8. del Peso L, Gonzalez-Garcia M, Page C, Herrera R, Nunez G: Interleukin-3-induced phosphorylation of BAD through the protein kinase Akt. Science 1997, 278(5338):687-689.
9. Datta SR, Dudek H, Tao X, Masters S, Fu H, Gotoh Y, Greenberg ME: Akt phosphorylation of BAD couples survival signals to the cell-intrinsic death machinery. Cell 1997, 91(2):231-241.
10. Rossig L, Jadidi AS, Urbich C, Barford C, Zeiher AM, Dimmeler S: Akt-dependent phosphorylation of p21(Cip1) regulates PCNA binding and proliferation of endothelial cells. Mol Cell Biol 2001, 21(16):5644-5657.
11. Zhou BP, Liao Y, Xia W, Sps0n B, Lee MH, Hung MC: Cytoplasmic localization of p21(Cip1)/WAF1 by Akt-induced phosphorylation in HER-2/neu-overexpressing cells. Nat Cell Biol 2001, 3(3):249-252.
12. Liang J, Zubovitz J, Petrossi T, Kotchetkov R, Connor MK, Han K, Lee JH, Carallo S, Catzavelos C, Beniston R, Fransen E, Slingerland JM: PKB/Akt phosphorylates p27, impairs nuclear import of p27 and opposes p27-mediated G1 arrest. Nat Med 2002, 8(10):1153-1160.
13. Shin I, Yakes FM, Rojo F, Shin NY, Bakin AY, Baselaña J, Artega CL, PKB/Akt mediates cell-cycle progression by phosphorylation of p27(Kip1) at threonine 157 and modulation of its cellular localization. Nat Med 2002, 8(10):1145-1152.
14. Du K, Montminy M: CREB is a regulatory target for the protein kinase Akt/PKB. J Biol Chem 1998, 273(49):32377-32379.
15. Brunet A, Bonni A, Zigmond MJ, Lin ME, Luo F, Hu LS, Anderson MJ, Arden KC, Blenis J, Greenberg ME: Akt promotes cell survival by phosphorylating and inhibiting a Forkhead transcription factor. Cell 1999, 96(5):857-868.
16. Kops GJ, de Ruiter ND, De Vries-Smits AM, Powell DR, Bos JL, Burgering BM: Direct control of the Forkhead transcription factor AFX by protein kinase B. Nature 1999, 398(6728):630-634.
17. Putzazethins S, Nestorova A, Sable MC, Heidenreich KA, Boxer LM, Heasley LE, Reusch JE: Akt/protein kinase B up-regulates Bcl-2 expression through cAMP-response element-binding protein. J Biol Chem 2000, 275(15):10761-10766.
18. Khothnan A, Tindell C, Law I, Bae D, Bennett B, Nel AE: The NF-kappa B cascade is important in Bcl-xL expression and for the anti-apoptotic effects of the CD28 receptor in primary human CD4+ lymphocytes. J Immunol 2000, 164(4):1743-1754.
19. Lynch KL, Gerona RR, Kielar DM, Martens S, McMahon HT, Martin TF: Synaptotagmin-1 utilizes membrane bending and SNARE binding to drive fusion pore expansion. Mol Biol Cell 2008, 19(12):5993-5103.
20. Dennis G Jr, Sherman BT, Hosack DA, Yang J, Gao W, Lane HC, Lempicki RA: DAVID: Database for Annotation, Visualization, and Integrated Discovery. Genome Biol 2003, 4(5):R3.
21. Mottohashi I, Igarashi K, Onodera K, Takahashi S, Ohtani H, Nakafuku M, Nishizawa M, Engel JD, Yamamoto M: Mesodermal- vs. neuronal-specific expression of MafK is elicited by different promoters. Genes Cells 1996, 1(2):223-236.
22. Toroczk B, Angelastro JM, Greene LA. The basic region and leucine zipper transcription factor MafK is a new nerve growth factor-responsive immediate early gene that regulates neurite outgrowth. J Neurosci 2002, 22(20):8971-8980.
23. Franczec C, Pondereous-Jazat V, Augery-Bouquet Y, Robert-Lezene J. NF-E2p18/mafK is required in DMSO-induced differentiation of Friend erythroleukemia cells by enhancing NF-E2 activity. Leukemia 1997, 11(2):273-280.
24. Geppert M, Goda Y, Hammer RE, Li C, Rosahl TW, Stevens CF, Sudhof TC. Synaptotagmin I: a major Ca2+ sensor for transmitter release at a central synapse. Cell 1994, 79(4):717-727.
25. Sudhof TC, Rizo J. Synaptotagmins: C2-domain proteins that regulate membrane traffic. Neuron 1996, 17(3):379-388.
26. Reissmann E, Emisberger U, Francis-West PH, Rueger D, Brickell PM, Rohrer H. Involvement of bone morphogenetic protein-4 and bone morphogenetic protein-7 in the differentiation of the adrenergic phenotype in developing sympathetic neurons. Development 1996, 127(2):2079-2088.
27. Patzek H, Reissmann E, Stanke M, Boby JL, Emisberger U. BMP growth factors and Phox2 transcription factors can induce synaptotagmin I and neuromodulin I during sympathetic neuron development. Mech Dev 2001, 108(1-2):149-159.
28. Zimmermann P, Tomatis D, Rosas M, Grootjans J, Leenaerts I, Degeest G, Reekmans G, Coomans C, David G. Characterization of syntenin, a syndecan-binding PDZ protein, as a component of cell adhesion sites and microfilaments. Mol Biol Cell 2001, 12(2):339-350.
29. Beekman JM, Coffier PJ. The ins and outs of syntenin, a multifunctional intracellular adaptor protein. J Cell Sci 2006, 121(Pt 9):1349-1355.
30. Hirbec H, Martin S, Henley JM. Syntenis is involved in the developmental regulation of neuronal membrane architecture. Mol Cell Neurosci 2005, 28(4):737-746.
31. Datta SR, Brunet A, Greenberg ME. Akt regulates the expression of MafK, a transcription factor that regulates neurite outgrowth. Mol Cell Biol 1999, 19(6):4279-4288.
32. Brunet A, Datta SR, Greenberg ME: The ins and outs of syntenin, a multifunctional intracellular adaptor protein. J Cell Sci 2006, 121(Pt 9):1349-1355.
33. She QB, Chandarlapaty S, Ye Q, Lobo J, Haskell KM, Leander KR, DeFeo-Jones D, Huber HE, Rosen N. Breast tumor cells with PI3K mutation or HER2 amplification are selectively addicted to Akt signaling. Cancer Res 2007, 67(2):935-945.
34. Johnson JS, Segerstrom L, Orrego A, Elmamn S, Henriksson M, Kagedal B, Elsborg S, Sveinbjornsson B, Kogner P. Inhibitors of mammalian target of rapamycin downregulate MYCN protein expression and inhibit neuroblastoma growth in vitro and in vivo. Oncogene 2008, 27(20):2910-2922.
35. Vivanco I, Sawyers CL: The phosphatidylinositol 3-Kinase AKT pathway in human cancer. Nat Rev Cancer 2002, 2(7):491-500.
36. Carpentier JD, Faber AL, Horn C, Doono GP, Biggs SL, Robbins CM, Hostetter G, Bogulavskiy S, Moses TY, Savage S, Uhrin M, Lin A, Du J, Qian YW, Zecker DJ, Tucker-Kellogg G, Touchman J, Patel K, Mousseaux E, Bittner M, Schvitz R, Lai MH, Blanchard KL, Thomas JE: A transforming mutation in the pleckstrin homology domain of AKTI in cancer. Nature 2007, 448(7152):439-444.
37. Nakagawara A, Arima-Nakagawara M, Scavarda NJ, Azar CG, Cantor AB, Brodeur GM: Association between high levels of expression of the TRK gene and favorable outcome in human neuroblastoma. N Engl J Med 1993, 328(13):847-854.
38. Ma YC, Song MR, Park JP, Henry Ho HY, Hu L, Kurtev MV, Zieg J, Ma Q, Pfaff SL, Greenberg ME: Regulation of motor neuron specification by phosphoantigen of neurothein 2. Neuron 2008, 58(1):65-77.
39. Lang MH, Chuang DM: Differential roles of glycosyn synthase kinase-3 isomers in the regulation of transcriptional activation. J Biol Chem 2008, 283(14):10362-10368.
40. Kawabata M, Inamura T, Miyazono K: Signal transduction by bone morphogenetic proteins. Cytokine Growth Factor Rev 1996, 7(1):49-61.
41. Shibuya H, Iwata H, Masuyama N, Gotoh Y, Yamaguchi K, Irie K, Matsumoto K, Nishida E, Ueno N: Role of TAC1 and TAF1 in BMP signaling in early Xenopus development. EMBO J 1998, 17(14):1019-1028.
42. Latchman DS: POU family transcription factors in the nervous system. J Cell Physiol 1999, 179(2):126-133.

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