BMB
Reports

Nuclear Akt promotes neurite outgrowth in the early stage of neuritogenesis

Ji Hye Park[1,2,3#], *Sang Bae Lee*[1,#], *Kyung-Hoon Lee*[2,3,*] *& Jee-Yin Ahn*[1,3,*]

[1]Department of Molecular Cell Biology, [2]Department of Anatomy, [3]Center for Molecular Medicine, Samsung Biomedical Research Institute, Sungkyunkwan University School of Medicine, Suwon 440-746, Korea

In addition to its pivotal role in neuronal survival, PI3K/Akt signaling is integral to neuronal differentiation and neurite outgrowth. However, the exact role of Akt in neuronal differentiation is still controversial. Here, we found that nuclear expression of CA-Akt resulted in unusual rapid neurite outgrowth and overexpression of KD-Akt caused multiple dendrite growth without specific axon elongation. Moreover, microarray data revealed that the expression of FOXQ1 expression was about 10-fold higher in cells with nuclear, active Akt than in control cells. Quantitative real-time PCR analysis showed that mRNA levels were upregulated in NLS-CA-Akt cells as compared to KD or EV cells. Furthermore, our FACS analysis demonstrated that overexpression of NLS-CA-Akt accumulate cells in the G1 phase within 24 h, fitting with the rapid sprouting of neuritis. Thus, our data implied that at least in this early time frame, the overexpression of nuclear, active Akt forced cells into neurite development through probably FOXQ1regulation. [BMB Reports 2012; 45(9): 521-525]

INTRODUCTION

Normal development of the nervous system depends on proper neuronal migration, proliferation and the establishment of an organized structure with functional circuits. Developing neurons that fail to make correct synapses are eliminated by apoptosis, and neurons become dependent on neurotrophic factor for survival. The PI3K/Akt pathway has been implicated in preserving neuronal survival (1). In addition to controling neuronal survival, the PI3K-Akt pathway is integral in neuronal differentiation and neurite outgrowth (2, 3).

PI3Ks convert phosphatidylinositol 4,5-bisphosphate (PIP_2) into phosphatidylinositol 3,4,5-triphosphate (PIP_3) (4). PIP_3 contributes to the activation of Akt/protein kinase B, the major effector of PI3K signaling, which can regulate apoptosis through phosphorylation of its substrate or by binding to its substrate, propagating its signal from the cytoplasm to the nucleus. For instance, active Akt promotes cell survival by direct phosphorylation of its cytoplasmic targets (Bad, MDM2, and caspase-9), or phosphorylation of the nuclear target fork head box transcription factor, FOXO1. Active Akt also binds to nuclear targets Ebp1 and B23, enhancing neuronal survival (5, 6). In addition to its pivotal role in neuronal survival, emerging evidence implicates Akt in neurite outgrowth. Akt regulates neurite outgrowth and cytoskeletal rearrangement through the activation of peripherin (7), Girdin (8), and mTOR (2) or the inactivation of GSK3-beta (9). However, the roles of Akt in neurite outgrowth still remain unclear. For example, recent studies in primary neurons demonstrated that Akt is a positive regulator (10-12). Conversely, studies from rat pheochromocytoma (PC) 12 cells, which are sympathetic neuron-like cells, suggest Akt is a negative regulator (13-15) or has no significant effect (16). Moreover, the inhibition of GSK-beta by Akt signaling is critical for the development of axons, but not dendrites, in hippocampal neurons (17). Thus, whether Akt influences differentiation or predominantly enhances survival and as a result, enables greater differentiation, needs to be determined.

Forkhead box Q1 (FOXQ1, also known as HFH1) is a member of the FOX gene family. It is a transcription factor that represses the promoter activity of smooth muscle specific genes (18), and its expression is regulated by Hoxa1 in embryonic stem cells (19). A recent study reported that $p21^{CIP1/WAF1}$ (hereafter called p21), a member of the cip/kip family of cyclin kinase inhibitors, is a downstream target gene of FOXQ1. FOXQ1 binds to and directly increases the transcriptional activity of p21 and its protein expression levels (20).

Although Akt substrates are identified throughout cells, activated Akt normally translocates to the nucleus or is in the nucleus. To better understand Akt signaling in neurite outgrowth, we employed stably transfected PC12 cells with an inducible form of GFP-myc-nuclear localization signal (NLS)-constitutively active (CA)-Akt, kinase-dead (KD)-Akt, or empty vector (EV) alone (21) and induced differentiation by nerve growth Factor (NGF). We investigated the role of nuclear ac-

*Corresponding author. Jee-Yin Ahn, Tel: +82-31-299-6134; Fax: +82-31-299-6139; E-mail: jeeahn@skku.edu; Kyung-Hoon Lee, Tel: +82-31-299-6070; Fax: +82-31-299-6089; E-mail: leekh@skku.edu
#These authors are contributed equally to this work.
http://dx.doi.org/10.5483/BMBRep.2012.45.9.114

Received 24 May 2012, Revised 4 June 2012, Accepted 4 June 2012

Keywords: Akt, FOXQ1, Neurite outgrowth

tive Akt in neurite outgrowth of PC12 cells.

RESULTS AND DISCUSSION

NGF-mediated neurite outgrowth of PC12 cells is a well-established model for neuronal differentiation studies. To study the contradictory role of Akt in neurite outgrowth of PC12 cells, we employed a Tet-off PC12 cell line that was stably transfected with the inducible form of NLS-CA-Akt, KD-Akt or EV. The expression of Akt constructs was induced by the removal of tetracycline from the culture medium (Fig. 1) and cells were stimulated with NGF (50 ng/ml) for neurite outgrowth. Consistent with the findings of previous studies and our prior report, NGF-treated PC12 cells exhibited slowed division, and underwent neuronal differentiation (22-25). By day 4 of NGF treatment, almost 50% of the mock-transfected EV-PC12 cells evidenced fully extended neuritis, and after 7 days of exposure to NGF, almost all cells (more than 90%) displayed neurite formation (data not shown).

Surprisingly, CA-Akt-expressing PC12 cells exhibited unusually rapid neurite outgrowth, revealing neurite sprouting in 12 h that was more than twice the length of neurites from EV cells, 24 h after NGF stimulation. The longest neurites in CA-Akt cells were observed within 48 h (Fig. 2A and B), suggesting that Akt activation is probably involved in the initial events of neuronal differentiation. In contrast, KD-Akt-expressing cells had notably delayed neurite outgrowth, and neurite length which was much shorter than EV cells (Fig. 2B). This was consistent with previous findings that overexpression of dominant negative-Akt or inhibition of Akt kinase activity by the Akt kinase inhibitor ML-9 or the PI3K inhibitors LY294002 or wortmannin, inhibited neurite outgrowth (14). Moreover, KD-Akt cells showed multiple neurites but no specific elongation of the axon (Fig. 2C and D), reflecting previous reports that constitutively active myristorylated Akt leads to the formation of multiple axons (17). Thus, our data suggested that nuclear Akt caused rapid neurite outgrowth in response to NGF treatment. Its kinase activity was probably involved in regulating neurite outgrowth and axon specification since KD-Akt resulted in multiple examples of neuritis but had no effects on the elongation of the specific axon.

CA-Akt resulted in significant, rapid elongation of neurite outgrowth, while KD-Akt contributed to increased numbers of neuritis without increasing neurite length in early differentiation. As differentiation proceeded, by day 5 of the NGF treatment, no notable differences were seen in neurite length or morphology among EV, CA and KD cells. Our phase contrast data with time-course showed that neurite outgrowth of CA-Akt PC12 cells reached a maximum within 48 h and

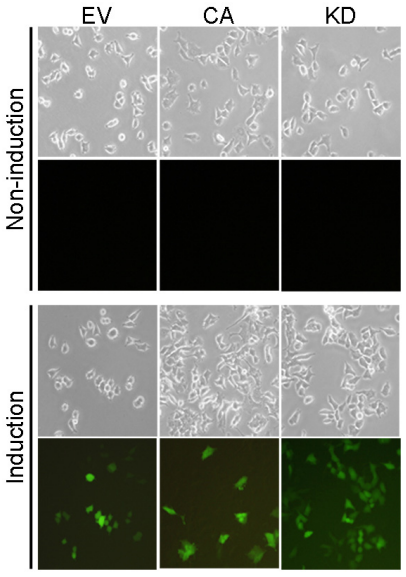

Fig. 1. Induction of tetracycline-regulated, stably transfected Akt cell lines. Each stably transfected cell line was seeded in six-well tissue culture plates, and maintained for one day in an induction medium without tetracycline. Photographs were acquired with a fluorescence microscope (×200) on random fields. Bar = 50 μm.

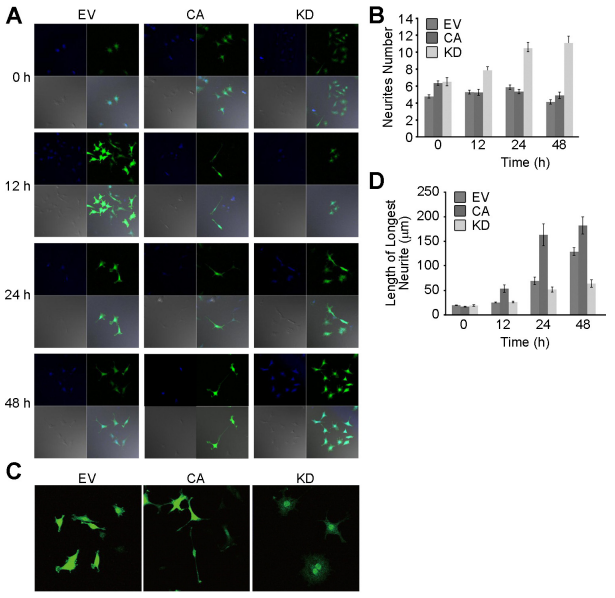

Fig. 2. NLS-CA-Akt mediates rapid neurite outgrowth. Each stably transfected cell line was maintained for 48 h in an induction medium without tetracycline in the presence or absence of 50 ng/ml NGF. (A) Phase contrast microscopy. Photographs were acquired with a confocal microscope (×200) with random fields at the indicated time. Nuclei were counterstained with DAPI (blue). (B) The quantitative analysis of neurite formation. A minimum of five random fields were counted at 48 h under NGF treated conditions. Cells with processes longer than two cell bodies after 48 h were counted as positive. Data represent the average of three independent experiments with standard deviations. (C) Cell images that were obtained by a confocal microscope (×200) after 24 h of NGF induction. (D) The quantitative analysis of neurite numbers.

showed little further elongation at 5 days (Fig. 3A). This indicated that Akt promotion of neurite outgrowth reached a maximum within 48 h and was outpaced by the normal speed of NGF-induced neurite outgrowth. Thus, our data indicated that the positive effects of nuclear, active Akt on neurite elongation occurred only in an early differentiation stage, accelerating neurite outgrowth. This might explain the controversy over Akt differentiation, and suggests a possible critical developmental period for the influence of Akt on neuritogenesis in PC12 cells.

To dissect the cell signal for NLS-CA-Akt induction of rapid neurite outgrowth, we performed cDNA microarray analysis and found that Foxq1 (Forkhead box, subclass q1, previously named HFH-1/hepatocyte nuclear factor 3-homolog 1) mRNA was upregulated in NLS-CA-Akt cells compared to KD or EV cells. Our RT-PCR analysis and real-time PCR analysis showed that Foxq1 mRNA levels in NLS-CA-Akt cells were approximately five-fold higher than in NLS-KD-Akt cells and around 10-fold higher than in EV cells (Fig. 4A and B). A recent study showed that overexpression of FOXQ1 reduces cell proliferation and FOXQ1 directly transactivates gene expression of p21, which functions as a G1 cyclin kinase inhibitor, arresting cells in G1 (20). This raises the possibility that FOXQ1 is a potent downstream mediator of nuclear, active Akt in the initiation of neuronal differentiation. NGF potentiates differentiation of PC12 cells, causing an arrest in the G_0/G_1 phase (26). Therefore, we hypothesized that accumulation of FOXQ1 by overexpression of nuclear Akt enhances p21 expression and subsequently causes cells to arrest in G1, thereby promoting rapid neurite outgrowth. To test this hypothesis, we analyzed early stage cell cycle profiles of NLS-CA-Akt, NLS-KD-Akt and EV cells via flow cytometry. Our results demonstrated that NGF treatment elicited a rapid increase in the G1 population in NLS-CA-Akt cells within 12-24 h whereas we could not detect any defect in the cell cycle with KD or EV cells. Even before NGF exposure, the cells showed more cells in the G1 phase (Fig. 4C). This data implied that at least in this early time frame of neurite development process, overexpression of nuclear, active Akt forced cells into neurite outgrowth, precluding the S phase entry through perhaps FOXQ1 regulation.

In this study, we demonstrated the effect of nuclear, active Akt in regulating FOXQ1 function in PC12 cell differentiation, and accelerating early neurite outgrowth, providing a possible explanation for conflicting observations on the role of Akt in neuronal differentiation.

The FOX gene family of transcription factors is conserved throughout evolution (27). In this family, FOXOs are known to control cell proliferation and cell survival upon growth factor stimulation. A recent finding showed that FOXO is required for axon growth and to inhibit dendritic length (28). However, the involvement of FOXQ1 in neurite outgrowth has not yet

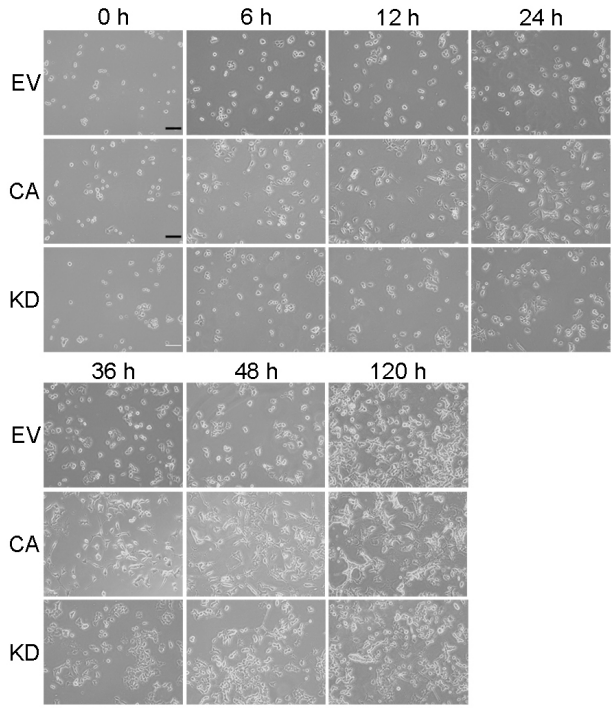

Fig. 3. Promotion by CA-Akt of neurite outgrowth is limited to early times. Induced conditions of CA-, KD-Akt and EV cells were maintained with NGF for five days. Phase-contrast images were obtained at the indicated times. Bar = 50 μm.

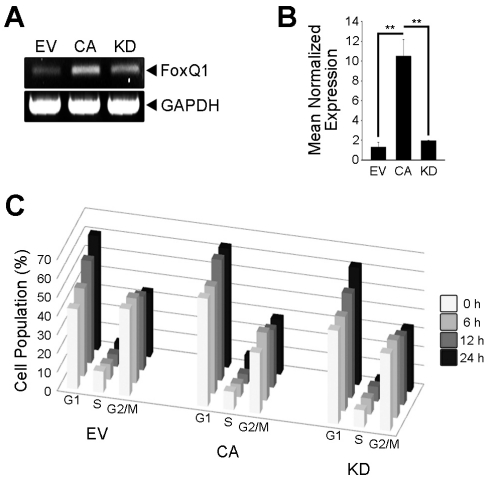

Fig. 4. NLS-Akt regulates rapid neurite outgrowth though FOXQ1 and p21 (A) RT-PCR with CA-Akt, KD-Akt and EV cells. After 24 h of NGF (100 ng/ml), induced cells were harvested and cDNA synthesized. RT-PCR was performed with extracted total RNA using FOXQ1 specific primers. (B) Real time PCR with FOXQ1 specific primers used SYBR Green PCR master mix. (C) FACS analysis of cell lines were maintained for 24 h and treated with NGF as indicated. Cells were stained with PI for FACS analysis. Cell cycle phase distribution is shown.

been considered. Thus, our findings raise the possibility that at least in early neurite outgrowth of PC12 cells, FOXQ1 is required to initiate neurite outgrowth, although the specific mechanism by which Akt enhances FOXQ1 expression and regulates its function remains undefined.

Most p21 functions are in proliferating cells. For example, an inhibitor treatment of Cdk and Akt mediated phosphorylation of p21 cause cytoplasmic translocation of p21, attenuating its cell cycle arresting function in human endothelial cell proliferation (29). However, similar to many cell cycle-related proteins that possess distinct functions in postmitotic neuronal cells, p21 is also implicated in a neuroprotection mechanism that is regulated by glucocorticoids (30). This suggests that p21 might act differently in postmitotic cells that are not required to enter the cell cycle. Unlike proliferating cells, in neuronal differentiation, as FOXQ1 binds to the p21 promoter and enhances its activity, the accumulation of FOXQ1 might produce excess p21. In this case, nuclear Akt would fail to remove p21 from the nucleus and subsequently turn on neurite outgrowth. Our findings suggest this possible scenario for the initiation of neurite outgrowth by nuclear, active Akt.

MATERIALS AND METHODS

Cell cultures

PC12 cells were maintained in medium A (Dulbecco's modified Eagle's medium with 10% fetal bovine serum, 5% horse serum, and 100 units of penicillin/streptomycin) at 37°C in 5% CO_2 (31). EV control and Myc-CA-, KD-Akt stably transfected PC12 cells (Tet-off cell line) were cultured in medium B (100 μg/ml hygromycin B, 100 μg/ml G418, 2 μg/ml tetracycline in medium A). Transfected genes were induced by culturing in medium C (medium B without 2 μg/ml tetracycline) for 24 h. For differentiation, cells (1×10^4) were treated with 50 ng/ml of NGF for an appropriate induction time.

Phase contrast microscopy and measurements of neurite outgrowth

Cells (1×10^4) were seeded in six-well culture plates. After one day, the medium was exchanged with an inducing medium. After 24 h, 50 ng/ml NGF was administered. Cells were incubated as indicated and considered differentiated if they had neuritis at least twice the length of the cell diameter. For phase contrast microscopy, cells were photographed and scored under an Axiovert 100 microscope (Carl Zeiss, Germany). Neurite processes longer than two cell bodies were counted as neurite, and a minimum of five random fields were photographed in each case. The length of neurites was measured using the Axio Vision program.

Flow cytometry assay

Cells (1×10^6) were fixed with 70% ethanol at −20°C for 24 h and were washed with PBS, and resuspended in 100 μl phosphate/citrate buffer (0.2 M Na_2HPO_4, 0.1 M citrate, pH 7.5) for 30 min at RT. After washing with PBS, samples were stained with Propidium iodide (PI) solution (20 μg/ml PI, 20 μg/ml RNase in PBS) for 30 min at RT and analyzed with a FACSCalibur system (Becton Dickinson, USA).

Quantitative PCR analysis

Cells were maintained in an inducing medium for more than 24 h and stimulated with NGF (100 ng/ml) for differentiation. Cells were harvested and RNA extracted for cDNA synthesis (cDNA-Transcriptor High Fidelity cDNA Synthesis Kit, Roche) (32). For RT-PCR, primers were Forward: CAAGACGATCCCG AGG TGA, Backward: CGCGCAGCACCTTGAACGA. For real-time PCR, primers were Forward: ACGCTGGCCGAGATCA AC, Backward: CCGTCGGCGAAGGTGTATT.

Acknowledgements

We thank Dr. Il-Sun Kwon for help with induction of stably transfected PC12 cells. This research was supported by a grant (NI-1:22-2009-00-010-00) from the Brain Research Center of the 21st Century Frontier Research Program funded by the Ministry of Education, Science and Technology, Republic of Korea to J.Y. Ahn and by a grant (2010-0024380) from by the Ministry of Education, Science and Technology, Republic of Korea to K.H. Lee.

REFERENCES

1. Brunet, A., Datta, S. R. and Greenberg, M. E. (2001) Transcription-dependent and -independent control of neuronal survival by the PI3K-Akt signaling pathway. *Curr. Opin. Neurobiol.* **11**, 297-305.
2. Jaworski, J., Spangler, S., Seeburg, D. P., Hoogenraad, C. C. and Sheng, M. (2005) Control of dendritic arborization by the phosphoinositide-3'-kinase-Akt-mammalian target of rapamycin pathway. *J. Neurosci.* **25**, 11300-11312.
3. Jiang, H. and Rao, Y. (2005) Axon formation: fate versus growth. *Nat. Neurosci.* **8**, 544-546.
4. Vanhaesebroeck, B., Leevers, S. J., Ahmadi, K., Timms, J., Katso, R., Driscoll, P. C., Woscholski, R., Parker, P. J. and Waterfield, M. D. (2001) Synthesis and function of 3-phosphorylated inositol lipids. *Annu. Rev. Biochem.* **70**, 535-602.
5. Ahn, J. Y., Liu, X., Liu, Z., Pereira, L., Cheng, D., Peng, J., Wade, P. A., Hamburger, A. W. and Ye, K. (2006) Nuclear Akt associates with PKC-phosphorylated Ebp1, preventing DNA fragmentation by inhibition of caspase-activated DNase. *EMBO J.* **25**, 2083-2095.
6. Lee, S. B., Xuan Nguyen, T. L., Choi, J. W., Lee, K. H., Cho, S. W., Liu, Z., Ye, K., Bae, S. S. and Ahn, J. Y. (2008) Nuclear Akt interacts with B23/NPM and protects it from proteolytic cleavage, enhancing cell survival. *Proc. Natl. Acad. Sci. U.S.A.* **105**, 16584-16589.
7. Konishi, H., Namikawa, K., Shikata, K., Kobatake, Y., Tachibana, T. and Kiyama, H. (2007) Identification of peripherin as a Akt substrate in neurons. *J. Biol. Chem.* **282**, 23491-23499.
8. Enomoto, A., Murakami, H., Asai, N., Morone, N., Wata-

nabe, T., Kawai, K., Murakumo, Y., Usukura, J., Kaibuchi, K. and Takahashi, M. (2005) Akt/PKB regulates actin organization and cell motility via Girdin/APE. *Dev. Cell* **9**, 389-402.
9. Zhou, F. Q., Zhou, J., Dedhar, S., Wu, Y. H. and Snider, W. D. (2004) NGF-induced axon growth is mediated by localized inactivation of GSK-3beta and functions of the microtubule plus end binding protein APC *Neuron*. **42**, 897-912.
10. Kimura, K., Hattori, S., Kabuyama, Y., Shizawa, Y., Takayanagi, J., Nakamura, S., Toki, S., Matsuda, Y., Onodera, K. and Fukui, Y. (1994) Neurite outgrowth of PC12 cells is suppressed by wortmannin, a specific inhibitor of phosphatidylinositol 3-kinase. *J. Biol. Chem.* **269**, 18961-18967.
11. Mills, J., Digicaylioglu, M., Legg, A. T., Young, C. E., Young, S. S., Barr, A. M., Fletcher, L., O'Connor, T. P. and Dedhar, S. (2003) Role of integrin-linked kinase in nerve growth factor-stimulated neurite outgrowth. *J. Neurosci.* **23**, 1638-1648.
12. Namikawa, K., Honma, M., Abe, K., Takeda, M., Mansur, K., Obata, T., Miwa, A., Okado, H. and Kiyama, H. (2000) Akt/protein kinase B prevents injury-induced motoneuron death and accelerates axonal regeneration. *J. Neurosci.* **20**, 2875-2886.
13. Bang, O. S., Park, E. K., Yang, S. I., Lee, S. R., Franke, T. F. and Kang, S. S. (2001) Overexpression of Akt inhibits NGF-induced growth arrest and neuronal differentiation of PC12 cells. *J. Cell. Sci.* **114**, 81-88.
14. Higuchi, M., Onishi, K., Masuyama, N. and Gotoh, Y. (2003) The phosphatidylinositol-3 kinase (PI3K)-Akt pathway suppresses neurite branch formation in NGF-treated PC12 cells. *Genes Cells* **8**, 657-669.
15. Ooms, L. M., Fedele, C. G., Astle, M. V., Ivetac, I., Cheung, V., Pearson, R. B., Layton, M. J., Forrai, A., Nandurkar, H. H. and Mitchell, C. A. (2006) The inositol polyphosphate 5-phosphatase, PIPP, Is a novel regulator of phosphoinositide 3-kinase-dependent neurite elongation. *Mol. Biol. Cell* **17**, 607-622.
16. Ashcroft, M., Stephens, R. M., Hallberg, B., Downward, J. and Kaplan, D. R. (1999) The selective and inducible activation of endogenous PI 3-kinase in PC12 cells results in efficient NGF-mediated survival but defective neurite outgrowth. *Oncogene*. **18**, 4586-4597.
17. Jiang, H., Guo, W., Liang, X. and Rao, Y. (2005) Both the establishment and the maintenance of neuronal polarity require active mechanisms: critical roles of GSK-3beta and its upstream regulators. *Cell* **120**, 123-135.
18. Hoggatt, A. M., Kriegel, A. M., Smith, A. F. and Herring, B. P. (2000) Hepatocyte nuclear factor-3 homologue 1 (HFH-1) represses transcription of smooth muscle-specific genes. *J. Biol. Chem.* **275**, 31162-31170.
19. Martinez-Ceballos, E., Chambon, P. and Gudas, L. J. (2005) Differences in gene expression between wild type and Hoxa1 knockout embryonic stem cells after retinoic acid treatment or leukemia inhibitory factor (LIF) removal. *J. Biol. Chem.* **280**, 16484-16498.
20. Kaneda, H., Arao, T., Tanaka, K., Tamura, D., Aomatsu, K., Kudo, K., Sakai, K., De Velasco, M. A., Matsumoto, K., Fujita, Y., Yamada, Y., Tsurutani, J., Okamoto, I., Nakagawa, K. and Nishio, K. (2010) FOXQ1 is overexpressed in colorectal cancer and enhances tumorigenicity and tumor growth. *Cancer Res.* **70**, 2053-2063.
21. Ahn, J. Y., Rong, R., Liu, X. and Ye, K. (2004) PIKE/nuclear PI 3-kinase signaling mediates the antiapoptotic actions of NGF in the nucleus. *EMBO J.* **23**, 3995-4006.
22. Buchkovich, K. J. and Ziff, E. B. (1994) Nerve growth factor regulates the expression and activity of p33cdk2 and p34cdc2 kinases in PC12 pheochromocytoma cells. *Mol. Biol. Cell*. **5**, 1225-1241.
23. Burstein, D. E. and Greene, L. A. (1982) Nerve growth factor has both mitogenic and antimitogenic activity. *Dev. Biol*. **94**, 477-482.
24. Ignatius, M. J., Chandler, C. R. and Shooter, E. M. (1985) Nerve growth factor-treated, neurite-bearing PC12 cells continue to synthesize DNA. *J. Neurosci*. **5**, 343-351.
25. Nguyen Tle, X. and Ahn, J. Y. (2007) Lipase inactive mutant of PLC-gamma1 regulates NGF-induced neurite outgrowth via enzymatic activity and regulation of cell cycle regulatory proteins. *J. Biochem. Mol. Biol*. **40**, 888-894.
26. Rudkin, B. B., Lazarovici, P., Levi, B. Z., Abe, Y., Fujita, K. and Guroff, G. (1989) Cell cycle-specific action of nerve growth factor in PC12 cells: differentiation without proliferation. *EMBO J.* **8**, 3319-3325.
27. Arden, K. C. (2008) FOXO animal models reveal a variety of diverse roles for FOXO transcription factors. *Oncogene*. **27**, 2345-2350.
28. Christensen, R., de la Torre-Ubieta, L., Bonni, A. and Colon-Ramos, D. A. (2011) A conserved PTEN/FOXO pathway regulates neuronal morphology during C. elegans development. *Development* **138**, 5257-5267.
29. Rossig, L., Jadidi, A. S., Urbich, C., Badorff, C., Zeiher, A. M. and Dimmeler, S. (2001) Akt-dependent phosphorylation of p21(Cip1) regulates PCNA binding and proliferation of endothelial cells. *Mol. Cell Biol*. **21**, 5644-5657.
30. Harms, C., Albrecht, K., Harms, U., Seidel, K., Hauck, L., Baldinger, T., Hubner, D., Kronenberg, G., An, J., Ruscher, K., Meisel, A., Dirnagl, U., von Harsdorf, R., Endres, M. and Hortnagl, H. (2007) Phosphatidylinositol 3-Akt-kinase-dependent phosphorylation of p21 (Waf1/Cip1) as a novel mechanism of neuroprotection by glucocorticoids. *J. Neurosci*. **27**, 4562-4571.
31. Kwon, I. S., Lee, K. H., Choi, J. W. and Ahn, J. Y. (2010) PI(3,4,5)P3 regulates the interaction between Akt and B23 in the nucleus. *BMB Rep.* **43**, 127-132.
32. Cho, S. A., Seo, M. J., Ko, J. Y., Shim, J. H., Yoo, J., Kim, J. H., Kim, S. Y., Ryu, N. K., Park, E. Y., Lee, H. W., Lee, Y. S., Bahk, Y. Y. and Park, J. H. (2010) Up-regulation of Idh3α causes reduction of neuronal differentiation in PC12 cells. *BMB Rep.* **43**, 369-374