

# APP Regulates NGF Receptor Trafficking and NGF-Mediated Neuronal Differentiation and Survival

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## Abstract

$\beta$ -amyloid precursor protein (APP) is a key factor in Alzheimer's disease (AD) but its physiological function is largely undetermined. APP has been found to regulate retrograde transport of nerve growth factor (NGF), which plays a crucial role in mediating neuronal survival and differentiation. Herein, we reveal the mechanism underlying APP-mediated NGF trafficking, by demonstrating a direct interaction between APP and the two NGF receptors, TrkA and p75NTR. Downregulation of APP leads to reduced cell surface levels of TrkA/p75NTR and increased endocytosis of TrkA/p75NTR and NGF. In addition, APP-deficient cells manifest defects in neurite outgrowth and are more susceptible to  $A\beta$ -induced neuronal death at physiological levels of NGF. However, APP-deficient cells show better responses to NGF-stimulated differentiation and survival than control cells. This may be attributed to increased receptor endocytosis and enhanced activation of Akt and MAPK upon NGF stimulation in APP-deficient cells. Together, our results suggest that APP mediates endocytosis of NGF receptors through direct interaction, thereby regulating endocytosis of NGF and NGF-induced downstream signaling pathways for neuronal survival and differentiation.

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## Introduction

An important pathological hallmark of Alzheimer's disease (AD) is the formation of extracellular senile plaques in the brain, whose major components are  $\beta$ -amyloid ( $A\beta$ ) peptides.  $A\beta$  is proteolytically derived from the  $\beta$ -amyloid precursor protein (APP) through sequential cleavages first by  $\beta$ -secretase (BACE1) and then by the  $\gamma$ -secretase complex [1,2,3]. Extensive evidence demonstrates that overproduction/accumulation of  $A\beta$  in vulnerable brain regions is a primary culprit in AD pathogenesis:  $A\beta$  is neurotoxic and can trigger a cascade of neurodegenerative steps including synaptic dysfunction/loss, formation of intra-neuronal fibrillary tangles, and subsequent neuronal death [4,5].

Full-length APP is a type-I transmembrane protein. After its synthesis in the endoplasmic reticulum, APP is transported along the secretory pathway to the Golgi/trans-Golgi network and the plasma membrane [6,7,8]. Cell surface APP can be internalized for endosomal/lysosomal degradation [9,10]. Although APP has been under great scrutiny since its identification, the physiological functions of APP remain largely undetermined. A role for APP has been suggested in signal transduction, cell adhesion, calcium metabolism, neurite outgrowth and synaptogenesis, etc, all requiring corroboration with *in vivo* evidence [2]. In addition, several studies, including ours, have indicated that APP may play a role in protein trafficking regulation: APP was found to function as

a kinesin-I membrane receptor to mediate axonal transport of BACE1 and PS1 [11,12], though another study failed to verify this result [13]. We recently found that APP regulates cell surface delivery of  $\gamma$ -secretase components but not BACE1 [14]. APP was also shown to interact with high-affinity choline transporter and APP deficiency affected its endocytosis [15]. Another interesting study found that increased doses of APP markedly decrease retrograde transport of nerve growth factor (NGF) and causes degeneration of forebrain cholinergic neurons in a mouse model of Down's Syndrome (DS) [16].

NGF belongs to the neurotrophin family, which plays an important role in regulating development of both the central and peripheral nervous systems [17]. Neurotrophins bind to specific receptor tyrosine kinases (Trks) at the cell surface and activate them. Formation of the ligand-receptor complexes also initiates internalization of the activated receptors into vesicles and these internalized receptors remain activated as long as they are associated with the ligands [18]. Upon binding to its specific receptors, TrkA and p75NTR, NGF can activate a series of downstream signaling events mediating neuronal survival, differentiation, and maintenance. The two major NGF-mediated signaling pathways, PI3K/Akt and MAPK, are involved in neuronal survival and differentiation, respectively [19,20,21]. Since retrograde transport of NGF after endocytosis upon its binding to TrkA/p75NTR was shown to be affected by APP and

the underlying mechanism has not been determined [16], herein we investigate the effects of APP on regulating TrkA/p75NTR trafficking and on the downstream signaling events upon NGF stimulation.

## Materials and Methods

### Cell cultures, transfection and infection

Maintenance of mouse embryonic fibroblast (MEF) cells derived from *APP/APLP2* double knockout and control mice [22], phenochromocytoma PC12 cells [17], and primary neuronal cultures derived from postnatal day 0 mice or embryonic day 17 rat embryos [23], has been previously described. MEF cells were transiently transfected with APP, TrkA, and/or p75NTR plasmids using Lipofectamine 2000 (Invitrogen). Stable downregulation of APP in PC12 cells was achieved by transfection of a pSUPER RNAi vector containing a small hairpin RNA (shRNA) targeting the APP sequence and selection with 200  $\mu$ g/mL G418 [14]. Lentivirus containing the same APP targeting shRNA sequence was used to infect primary rat neurons for APP downregulation. All procedures were performed in accordance with the Guide for Care and Use of Laboratory Animals of the National Institutes of Health and were approved by the Institutional Animal Use and Care Committee of Sanford-Burnham Medical Research Institute.

### Antibodies

Antibodies used in this study included 22C11 recognizing the amino-terminus of APP (Chemicon), 369 recognizing the carboxyl-terminus of APP, different TrkA antibodies (Santa Cruz, Chemicon, and Upstate), and p75NTR antibodies (Abcam and Cell Signaling). Antibodies recognizing Akt, phosphorylated Akt, MAPK, phosphorylated MAPK, and MAP2 were from Cell Signaling.

### Cell surface protein biotinylation

Biotinylation was carried out as previously described [14]. Biotin-labeled cell surface proteins were precipitated with streptavidin-agarose beads (Pierce), subjected to SDS-PAGE, and analyzed by Western blotting with indicated antibodies.

### Co-immunoprecipitation

PC12 cells were lysed with CelLytic M Cell Lysis Reagent (Sigma) along with a protease inhibitor cocktail (Roche). Cell lysates were subjected to immunoprecipitation with the indicated antibodies and rProtein A-sepharose beads (Biochain Institute), followed by Western blotting.

### NGF treatments

To study the endocytosis of NGF, PC12 cells with stable downregulation of APP and control cells were treated with 1 nM quantum dot-labeled NGF (QD-NGF) for 3 h [24]. After a complete wash, cells were fixed, permeabilized, stained with DAPI, and observed under a fluorescent microscope. In addition, cells were treated with 100 ng/mL NGF for different time periods and the levels of phosphorylated and total Akt/MAPK were analyzed by Western blotting.

### NGF receptor endocytosis

To study the endocytosis of p75NTR and TrkA, cells were first incubated with primary antibodies against p75NTR or TrkA at 4°C for 1 h, and then treated with 100 ng/mL NGF at 37°C for 1 h. Cells were then fixed and incubated with a secondary antibody conjugated with Alexa Fluor<sup>®</sup>-594 (for detecting cell

surface proteins) for 1 h. After a complete wash, cells were permeabilized and then incubated with another secondary antibody conjugated with Alexa Fluor-488 (for detecting both cell surface and internalized proteins). Finally, cells were observed under a confocal microscope.

### Neurite outgrowth

The next day after plating of embryonic day 17 rat primary neurons, neurons were infected with APP or scrambled control (SC) RNAi-containing lentivirus for 1 d. These neurons were then treated with or without 100 ng/mL NGF for 5 d, and then fixed, permeabilized, immunostained with MAP2 antibody and fluorescence-labeled secondary antibody, and observed under a fluorescent microscope. The neurite lengths of infected (indicated by GFP fluorescence) neurons (indicated by positive MAP2 staining) were measured for comparison.

### Neuronal death

Neurons derived from postnatal day 0 APP heterozygous mice and rat primary neurons with APP downregulated by RNAi, as well as respective controls, were treated with or without 100 ng/mL NGF for 5 d. These neurons were then treated with 25  $\mu$ M A $\beta$  for 1 d. Samples were stained by propidium iodide. The numbers of dead (indicated by positive PI staining) neurons were counted and compared.

## Results

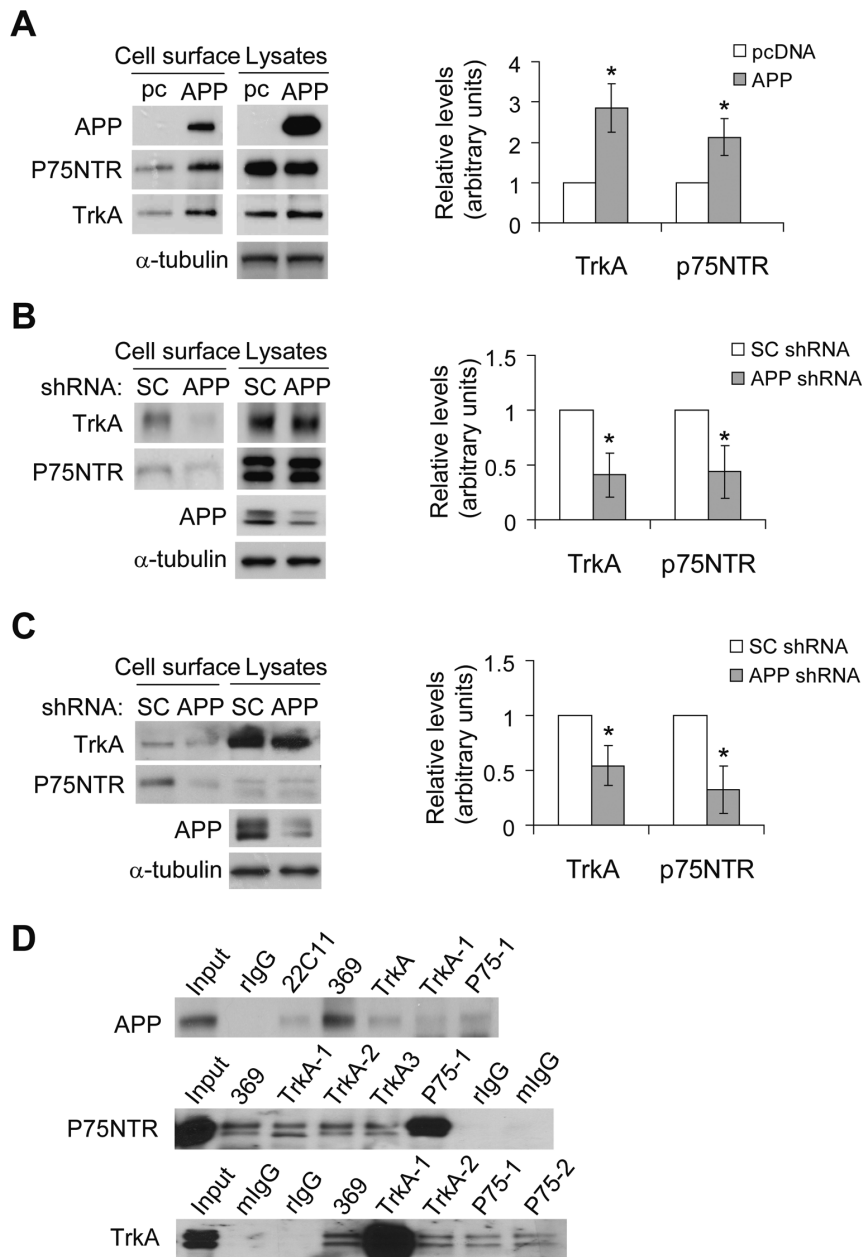
### APP interacts with TrkA and p75NTR and regulates their cell surface accumulation

While the underlying mechanism remains undetermined, it has been shown that APP overexpression impairs the retrograde axonal transport of NGF [16]. Because endocytosis of NGF is the first step for its retrograde transport and NGF endocytosis is mediated by its binding to the NGF receptors, TrkA and p75NTR, at the cell surface, we investigated whether APP can regulate cell surface levels of TrkA and p75NTR. We first overexpressed TrkA and p75NTR individually in APP/APLP2 double knockout MEF cells and then transfected them with APP or control pcDNA. The results showed that the steady state cell surface levels of TrkA and P75NTR were increased by 2.9 and 2.1 folds, respectively, in the presence of APP (Figure 1A). We also generated stable cell lines of rat phenochromocytoma PC12 cells in which the level of APP was downregulated by RNAi and found that these cells had reduced steady state cell surface levels of TrkA (~2.4 folds) and P75NTR (~2.3 folds) (Figure 1B). In addition, downregulation of APP in rat primary neurons by RNAi also drastically reduced steady state cell surface levels of TrkA (~1.8 folds) and p75NTR (~3.1 folds) (Figure 1C). Together these results clearly indicate that APP can regulate cell surface levels of the NGF receptors TrkA and p75NTR.

APP has been reported to be able to interact with p75NTR and TrkA [25,26]. Herein, we carried out co-immunoprecipitation studies and confirmed that APP indeed interacts with p75NTR and TrkA (Figure 1D). Fluorescent immunostaining also showed that APP colocalizes with TrkA and p75NTR (data not shown).

### Downregulation of APP results in increased endocytosis of NGF and NGF receptors

Next, we studied whether APP deficiency affects NGF endocytosis. When PC12 cells with APP stably downregulated by RNAi were treated with QD-NGF for 3 h, the level of endocytosed QD-NGF in these cells was about 2.6 folds higher



**Figure 1. APP regulates cell surface levels of NGF receptors.** (A) APP/APL2 dKO cells were first transfected with p75NTR or TrkA. After splitting equally, cells were transfected with pc DNA (control) or APP. Cell surface proteins were biotinylated, affinity precipitated, and subjected to Western blotting. (B) PC12 cells were stably transfected with APP or scrambled control (SC) shRNA-containing vectors. Cell surface protein levels were analyzed by biotinylation. (C) Rat primary neurons were infected with APP or scrambled control (SC) shRNA-containing lentivirus for 5 d. Cell surface protein levels were analyzed by biotinylation. Protein levels were quantified by densitometry and normalized to those of controls for comparison (set as one arbitrary unit). Error bars indicate SEM. \*:  $P < 0.05$ ,  $n = 3$ . (D) Equal protein amounts of PC12 cell lysates were incubated with rabbit IgG (rIgG), mIgG, APP antibodies (22C11 and 369), different TrkA antibodies (1–3) and P75NTR antibodies (1–2). Immunoprecipitated proteins were subjected to Western blotting.

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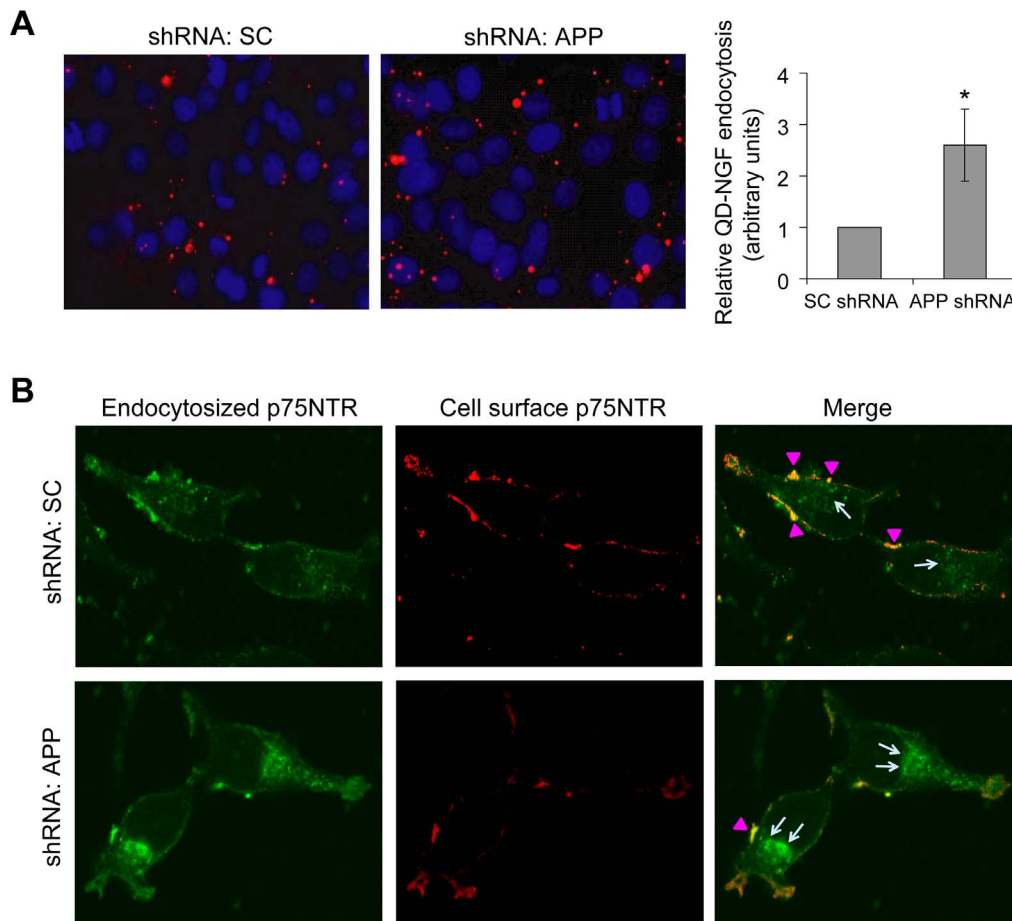
than that in control cells (Figure 2A). These results are consistent with the finding that APP overexpression impairs retrograde transport of NGF [16].

Binding of NGF to its receptors is necessary for its endocytosis. Therefore, we studied whether APP also affects endocytosis of NGF receptors. After cells were treated with NGF for 1 h, we observed considerably more internalized p75NTR in APP downregulated cells than in control cells (Figure 2B). A similar finding was observed for TrkA endocytosis (data not shown).

These results suggest that APP may mediate NGF endocytosis through regulating endocytosis of TrkA/p75NTR.

#### APP deficiency results in increased neuronal differentiation and survival in response to NGF

Since NGF activates a series of downstream signaling events that mediate neuronal survival and differentiation, we investigated whether altering cellular levels of APP affects neuronal survival

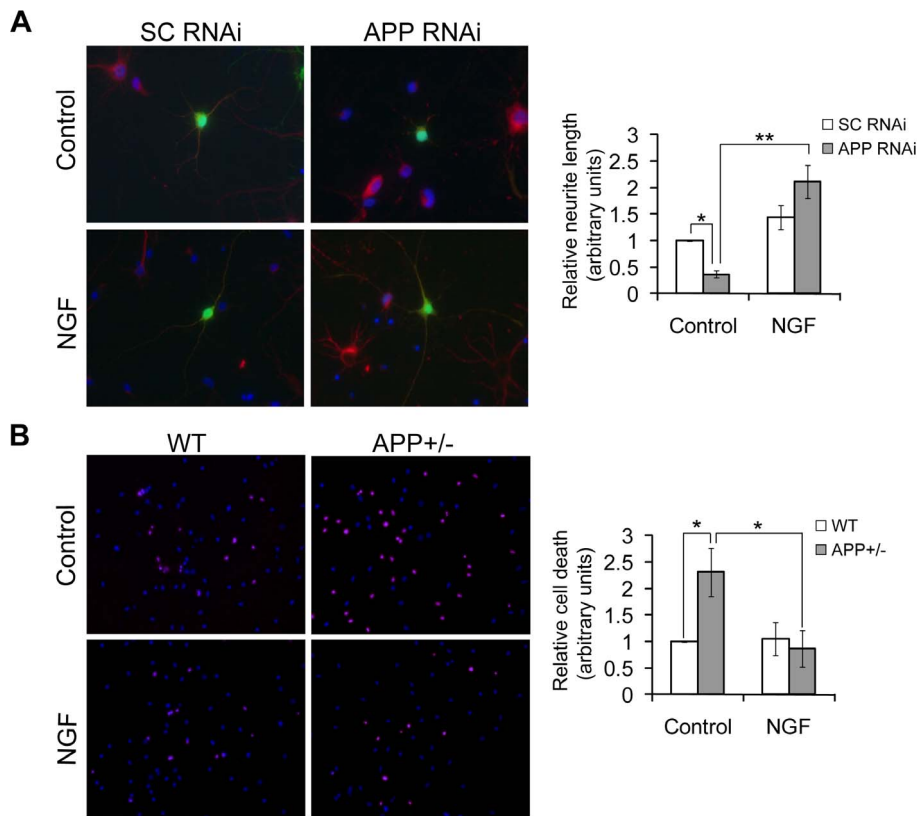


**Figure 2. APP deficiency promotes endocytosis of NGF and NGF receptors.** (A) PC12 cells stably expressing APP shRNA and control cells expressing scrambled control (SC) shRNA were treated with 1 nM QD-NGF for 3 h. After a complete wash, cells were fixed, permeabilized, stained with DAPI, and observed under a fluorescent microscope. Residual QD-NGF (in red) levels were quantified from 5 randomly selected regions with comparable cell numbers. Control was set as one arbitrary unit. \*:  $P < 0.05$ . (B) Cells were first incubated with a p75NTR antibody at 4°C for 1 h, and then treated with 100 ng/mL NGF at 37°C for 1 h. Cells were fixed and incubated with a secondary antibody conjugated with Alexa Fluor®-594 for 1 h. After a complete wash, cells were permeabilized and then incubated with another secondary antibody conjugated with Alexa Fluor-488. Cells were observed under a confocal microscope. Arrows and arrow heads indicate internalized and cell surface p75NTR, respectively. doi:10.1371/journal.pone.0080571.g002

and differentiation. In the absence of exogenous NGF treatment (i.e. at the basal levels), neurite outgrowth (indicative of neuronal differentiation) of rat primary neurons with APP downregulated by RNAi was about 2.7 folds less than that of control cells (Figure 3A), consistent with our previous results that APP-deficient mouse neurons manifest dramatic neuronal outgrowth defects [27]. However, when neurons were treated with NGF for 5 d, neurite outgrowth of APP-downregulated neurons was about 1.5 folds more than that of control cells (Figure 3A), suggesting that APP deficiency results in an increased neuronal differentiation in response to NGF treatment. We also compared neurons' resistance to A $\beta$  neurotoxicity. The results showed that when cells were treated with A $\beta$ , APP heterozygous knockout (+/-) mouse neurons had a much higher death rate (~2.3 folds) than control neurons in the absence of NGF treatments (i.e., at the basal NGF levels) (Figure 3B), but APP+/- neurons had a similar death rate to control neurons upon NGF treatments. These data suggest that APP-deficient cells respond more acutely to NGF-mediated survival signals than control cells.

#### Downregulation of APP enhances the NGF-mediated PI3K/Akt and MAPK pathways

Neuronal survival and differentiation are regulated by the NGF-activated downstream signaling pathways PI3K/Akt and MAPK, respectively [19,20,21]. Herein, we found that when cells were treated with NGF, phosphorylation of both Akt and MAPK for their activation was dramatically elevated (Figure 4): in control cells, NGF treatments for 1, 3 and 5 d promoted Akt phosphorylation for 1.7, 1.6 and 1.4 folds, respectively, and promoted MAPK phosphorylation for 3.8, 2.5 and 2.4 folds, respectively; while in APP downregulated cells, NGF treatments for 1, 3 and 5 d promoted Akt phosphorylation for 2.8, 2.9 and 2.4 folds, respectively, and promoted MAPK phosphorylation for 8.7, 6.6 and 4.6 folds, respectively. However, when we compared the change of Akt and MAPK phosphorylation in control and in APP downregulated cells, we noticed that the increased levels of both Akt and MAPK phosphorylation were much higher in APP downregulated cells than in control cells (Figure 4), which is consistent with the more significant survival and differentiation responses to NGF in these cells.



**Figure 3. APP deficiency impairs neurite outgrowth and neuronal survival at basal levels, but promotes neurite outgrowth and neuronal survival more acutely upon NGF stimulation.** (A) The day after plating embryonic day 17 rat primary neurons, neurons were infected with APP or scrambled control (SC) RNAi-containing lentivirus for 1 d. These neurons were then treated with or without 100 ng/mL NGF for 5 d, fixed, permeabilized, immunostained with MAP2 antibody and a fluorescently-labeled secondary antibody, and observed under a fluorescent microscope. Infected cells were indicated by GFP fluorescence (in green) and neurons were indicated by positive MAP2 staining (in red). The neurite lengths of infected neurons (>100) were measured for comparison. (B) Primary neurons from postnatal day 0 wild type (WT) and APP heterozygous (+/-) mice were treated with or without 100 ng/mL NGF for 5 d. These neurons were then treated with 25  $\mu$ M A $\beta$  for 1 d. After staining with propidium iodide (in red) and DAPI (in blue), the numbers of dead neurons (>300) were counted for comparison. Controls were set as one arbitrary unit. Error bars indicate SEM. \*:  $P < 0.05$ , \*\*:  $P < 0.01$ . doi:10.1371/journal.pone.0080571.g003

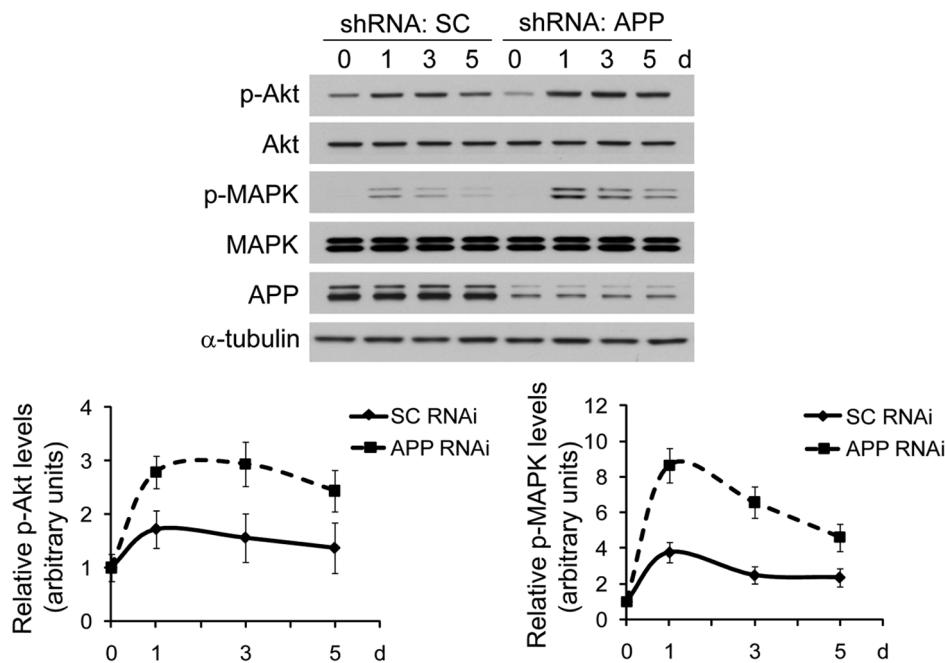
## Discussion

Although its detailed physiological/pathological function remains largely undetermined, APP is crucially involved in AD as the precursor of A $\beta$ . In addition, a reduced availability of NGF has also been found to contribute to AD: an impairment of NGF maturation from its precursor proNGF causes the vulnerability of cholinergic neurons in AD [28,29,30]; deprivation of NGF leads to AD-like pathologies such as A $\beta$  accumulation/deposition, tau hyperphosphorylation, synaptic dysfunction and memory deficits in mice [31,32]; and administration of NGF can ameliorate A $\beta$  pathologies and prevent memory deficits in AD animal models [33,34]. Recent studies have suggested a correlation between APP processing/A $\beta$  accumulation and NGF/NGF receptor mediated signaling pathways. For example, our present study, as well as others' has shown that APP can interact with both TrkA and p75NTR [25,26]. One study suggested that the interaction between APP and TrkA requires the tyrosine residue at APP position 682 (Y682, numbering based on APP695 isoform) [26]. APP-Y682 has been shown to be important for the function and processing of APP [35]. Interestingly, overexpression of TrkA has been found to be associated with both phosphorylation of APP-Y682 and alteration of APP processing [36]. In addition, there are reports showing that NGF can affect APP expression and

localization [37,38,39]. On the other hand, APP can regulate activation of the NGF/TrkA signaling pathway, the subcellular distribution of TrkA and the sensitivity of neurons to the trophic action of NGF [26]. Increased levels of APP also markedly decreases retrograde transport of NGF and causes degeneration of forebrain cholinergic neurons in a mouse model of DS [16]. However, the detailed molecular pathways linking APP and NGF/NGF receptor signaling have yet to be fully clarified.

Herein, we have found that APP deficiency results in a significant decrease in cell surface levels of the two NGF receptors, TrkA and p75NTR. Because APP has been shown to mediate intracellular trafficking of certain proteins [11,12,14,15], one possibility is that APP can also regulate intracellular trafficking of TrkA and p75NTR through its interaction with these receptors. Therefore, an increase in the APP level could result in more TrkA/p75NTR at the cell surface and thus inhibit NGF endocytosis, whereas a decrease in the APP level could facilitate endocytosis of NGF upon its binding to TrkA and p75NTR. Indeed, our data have shown that endocytosis of TrkA/p75NTR, as well as endocytosis of NGF, is drastically higher in APP-downregulated cells than in control cells.

Upon binding NGF, cell surface receptors are activated and trigger a series of downstream signaling pathways, such as PI3K/Akt and MAPK, which mediate neuronal survival and differen-



**Figure 4. APP deficiency enhances activation of Akt and MAPK upon NGF treatment.** PC12 cells stably expressing APP shRNA and control cells expressing scrambled control (SC) shRNA were treated with 100 ng/mL NGF for the indicated time periods. Equal protein amounts of cell lysates were subjected to Western blotting to measure phosphorylation (p)/activation of Akt and MAPK. Protein levels were quantified by densitometry and normalized to those of controls for comparison (set as one arbitrary unit). Error bars indicate SEM, n = 3. doi:10.1371/journal.pone.0080571.g004

tiation, respectively [19,20,21]. Herein, we have found that upon NGF treatment, Akt and MAPK phosphorylation/activation is much higher in APP-downregulated cells than in control cells. This is probably attributed to an increased endocytosis of NGF-receptor complexes in APP-downregulated cells and these complexes remain active as long as the ligand keeps associated with the receptors [18]. Moreover, more extensive activation of Akt and MAPK signaling pathways in APP-deficient neurons facilitates their differentiation and survival in response to NGF: although APP-deficient neurons have significant defects in neurite outgrowth and are highly susceptible to neurotoxicity-induced neuronal death when compared to control cells, as shown in our results (Figure 3) and described previously [27], these neurons have comparable neurite outgrowth and A $\beta$ -induced death rates to those of control cells.

Together, our results show that APP interacts with TrkA/p75NTR, thereby regulating cell surface levels of TrkA/p75NTR and their endocytosis, as well as endocytosis of NGF, and affecting

the NGF-mediated signaling cascades for neuronal survival and differentiation. Consistently, APP has been implicated in critical neuronal functions such as synapse formation, growth cone outgrowth and axon guidance [2,3]. Hence dysregulated NGF signaling cascades following APP impairment may lead to the pathogenic states, including AD and DS.

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## Author Contributions

Conceived and designed the experiments: YWZ HX. Performed the experiments: YWZ YC YL YZ. Analyzed the data: YWZ FFL HX. Contributed reagents/materials/analysis tools: FFL. Wrote the paper: YWZ HX.

## References

- Zhang YW, Thompson R, Zhang H, Xu H (2011) APP processing in Alzheimer's disease. *Mol Brain* 4: 3.
- Zheng H, Koo EH (2011) Biology and pathophysiology of the amyloid precursor protein. *Mol Neurodegener* 6: 27.
- Zheng H, Koo EH (2006) The amyloid precursor protein: beyond amyloid. *Mol Neurodegener* 1: 5.
- Hardy JA, Higgins GA (1992) Alzheimer's disease: the amyloid cascade hypothesis. *Science* 256: 184–185.
- Hardy J, Selkoe DJ (2002) The amyloid hypothesis of Alzheimer's disease: progress and problems on the road to therapeutics. *Science* 297: 353–356.
- Xu H, Sweeney D, Wang R, Thinakaran G, Lo AC, et al. (1997) Generation of Alzheimer beta-amyloid protein in the trans-Golgi network in the apparent absence of vesicle formation. *Proc Natl Acad Sci U S A* 94: 3748–3752.
- Hartmann T, Bieger SC, Bruhl B, Tienari PJ, Ida N, et al. (1997) Distinct sites of intracellular production for Alzheimer's disease A beta40/42 amyloid peptides. *Nat Med* 3: 1016–1020.
- Greenfield JP, Tsai J, Gouras GK, Hai B, Thinakaran G, et al. (1999) Endoplasmic reticulum and trans-Golgi network generate distinct populations of Alzheimer beta-amyloid peptides. *Proc Natl Acad Sci U S A* 96: 742–747.
- Nordstedt C, Caporaso GL, Thyberg J, Gandy SE, Greengard P (1993) Identification of the Alzheimer beta/A4 amyloid precursor protein in clathrin-coated vesicles purified from PC12 cells. *J Biol Chem* 268: 608–612.
- Caporaso GL, Takei K, Gandy SE, Matteoli M, Mundigl O, et al. (1994) Morphologic and biochemical analysis of the intracellular trafficking of the Alzheimer beta/A4 amyloid precursor protein. *J Neurosci* 14: 3122–3138.
- Kamal A, Stokin GB, Yang Z, Xia CH, Goldstein LS (2000) Axonal transport of amyloid precursor protein is mediated by direct binding to the kinesin light chain subunit of kinesin-I. *Neuron* 28: 449–459.
- Kamal A, Almenar-Queralt A, LeBlanc JF, Roberts EA, Goldstein LS (2001) Kinesin-mediated axonal transport of a membrane compartment containing beta-secretase and presenilin-1 requires APP. *Nature* 414: 643–648.

13. Lazarov O, Morfini GA, Lee EB, Farah MH, Szodorai A, et al. (2005) Axonal transport, amyloid precursor protein, kinesin-1, and the processing apparatus: revisited. *J Neurosci* 25: 2386–2395.
14. Liu Y, Zhang YW, Wang X, Zhang H, You X, et al. (2009) Intracellular trafficking of presenilin 1 is regulated by beta-amyloid precursor protein and phospholipase D1. *J Biol Chem* 284: 12145–12152.
15. Wang B, Yang L, Wang Z, Zheng H (2007) Amyloid precursor protein mediates presynaptic localization and activity of the high-affinity choline transporter. *Proc Natl Acad Sci U S A* 104: 14140–14145.
16. Salehi A, Delcroix JD, Belichenko PV, Zhan K, Wu C, et al. (2006) Increased APP expression in a mouse model of Down's syndrome disrupts NGF transport and causes cholinergic neuron degeneration. *Neuron* 51: 29–42.
17. York RD, Molliver DC, Grewal SS, Stenberg PE, McCleskey EW, et al. (2000) Role of phosphoinositide 3-kinase and endocytosis in nerve growth factor-induced extracellular signal-regulated kinase activation via Ras and Rap1. *Mol Cell Biol* 20: 8069–8083.
18. Bergeron JJ, Di Guglielmo GM, Baass PC, Authier F, Posner BI (1995) Endosomes, receptor tyrosine kinase internalization and signal transduction. *Biosci Rep* 15: 411–418.
19. Yao R, Cooper GM (1995) Requirement for phosphatidylinositol-3 kinase in the prevention of apoptosis by nerve growth factor. *Science* 267: 2003–2006.
20. Qiu MS, Green SH (1992) PC12 cell neuronal differentiation is associated with prolonged p21ras activity and consequent prolonged ERK activity. *Neuron* 9: 705–717.
21. Zhang Y, Moheban DB, Conway BR, Bhattacharyya A, Segal RA (2000) Cell surface Trk receptors mediate NGF-induced survival while internalized receptors regulate NGF-induced differentiation. *J Neurosci* 20: 5671–5678.
22. Zhang YW, Wang R, Liu Q, Zhang H, Liao FF, et al. (2007) Presenilin/gamma-secretase-dependent processing of beta-amyloid precursor protein regulates EGF receptor expression. *Proc Natl Acad Sci U S A* 104: 10613–10618.
23. Zhang X, Li F, Bulloj A, Zhang YW, Tong G, et al. (2006) Tumor-suppressor PTEN affects tau phosphorylation, aggregation, and binding to microtubules. *Faseb J* 20: 1272–1274.
24. Cui B, Wu C, Chen L, Ramirez A, Bearer EL, et al. (2007) One at a time, live tracking of NGF axonal transport using quantum dots. *Proc Natl Acad Sci U S A* 104: 13666–13671.
25. Fombonne J, Rabizadeh S, Banwait S, Mehlen P, Bredesen DE (2009) Selective vulnerability in Alzheimer's disease: amyloid precursor protein and p75(NTR) interaction. *Ann Neurol* 65: 294–303.
26. Matrone C, Barbagallo AP, La Rosa LR, Florenzano F, Ciotti MT, et al. (2011) APP is phosphorylated by TrkA and regulates NGF/TrkA signaling. *J Neurosci* 31: 11756–11761.
27. Han P, Dou F, Li F, Zhang X, Zhang YW, et al. (2005) Suppression of cyclin-dependent kinase 5 activation by amyloid precursor protein: a novel excitoprotective mechanism involving modulation of tau phosphorylation. *J Neurosci* 25: 11542–11552.
28. Cuello AC, Bruno MA, Bell KF (2007) NGF-cholinergic dependency in brain aging, MCI and Alzheimer's disease. *Curr Alzheimer Res* 4: 351–358.
29. Cuello AC, Bruno MA (2007) The failure in NGF maturation and its increased degradation as the probable cause for the vulnerability of cholinergic neurons in Alzheimer's disease. *Neurochem Res* 32: 1041–1045.
30. Cabeza C, Figueroa A, Lazo OM, Galleguillos C, Pissani C, et al. (2012) Cholinergic abnormalities, endosomal alterations and up-regulation of nerve growth factor signaling in Niemann-Pick type C disease. *Mol Neurodegener* 7: 11.
31. Cattaneo A, Capsoni S, Paoletti F (2008) Towards non invasive nerve growth factor therapies for Alzheimer's disease. *J Alzheimers Dis* 15: 255–283.
32. Houeland G, Romani A, Marchetti C, Amato G, Capsoni S, et al. (2010) Transgenic mice with chronic NGF deprivation and Alzheimer's disease-like pathology display hippocampal region-specific impairments in short- and long-term plasticities. *J Neurosci* 30: 13089–13094.
33. Capsoni S, Marinelli S, Ceci M, Vignone D, Amato G, et al. (2012) Intranasal "painless" human Nerve Growth Factor [corrected] slows amyloid neurodegeneration and prevents memory deficits in App X PS1 mice. *PLoS One* 7: e37555.
34. Tian L, Guo R, Yue X, Lv Q, Ye X, et al. (2012) Intranasal administration of nerve growth factor ameliorate beta-amyloid deposition after traumatic brain injury in rats. *Brain Res* 1440: 47–55.
35. Matrone C (2013) A new molecular explanation for age-related neurodegeneration: The Tyr682 residue of amyloid precursor protein. *Bioessays* 35: 847–852.
36. Tarr PE, Contursi C, Roncarati R, Novello C, Ghersi E, et al. (2002) Evidence for a role of the nerve growth factor receptor TrkA in tyrosine phosphorylation and processing of beta-APP. *Biochem Biophys Res Commun* 295: 324–329.
37. Binnington JC, Kalisch BE (2007) Nitric oxide synthase inhibitors modulate nerve growth factor-mediated regulation of amyloid precursor protein expression in PC12 cells. *J Neurochem* 101: 422–433.
38. Milward EA, Papadopoulos R, Fuller SJ, Moir RD, Small D, et al. (1992) The amyloid protein precursor of Alzheimer's disease is a mediator of the effects of nerve growth factor on neurite outgrowth. *Neuron* 9: 129–137.
39. Fukuyama R, Chandrasekaran K, Rapoport SI (1993) Nerve growth factor-induced neuronal differentiation is accompanied by differential induction and localization of the amyloid precursor protein (APP) in PC12 cells and variant PC12S cells. *Brain Res Mol Brain Res* 17: 17–22.