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PIKE/Nuclear PI 3-Kinase Signaling in Preventing Programmed Cell Death

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Abstract PI 3-kinase enhancer (PIKE) is a nuclear GTPase that enhances PI 3-kinase (PI3K) activity. Nerve growth factor (NGF) treatment leads to PIKE activation by triggering the nuclear translocation of PLC-γ1, which acts as a physiological guanine nucleotide exchange factor (GEF) for PIKE. PI3K occurs in the nuclei of a broad range of cell types, and various stimuli elicit PI3K nuclear translocation. While cytoplasmic PI3K has been well characterized, little is known about the biological function of nuclear PI3K. Surprisingly, nuclei from 30 min NGF-treated PC12 cells are resistant to DNA fragmentation initiated by the activated cell-free apoptosome, and both PIKE and nuclear PI3K are sufficient and necessary for this effect. Moreover, pretreatment of the control nucleus with PI(3,4,5)P_3 alone mimics the anti-apoptotic activity of NGF by selectively preventing apoptosis, for which nuclear Akt is required but not sufficient. Recently, a nuclear PI(3,4,5)P_3 receptor, nucleophosmin/B23, has been identified from NGF-treated PC12 nuclear extract. PI(3,4,5)P_3/B23 complex mediates the anti-apoptotic effects of NGF by inhibiting DNA fragmentation activity of caspase-activated DNase (CAD). Thus, PI(3,4,5)P_3/B23 complex and nuclear Akt effectors might coordinately mediate PIKE/nuclear PI3K signaling in promoting cell survival by NGF. J. Cell. Biochem. 96: 463–472, 2005.

Key words: PIKE; nuclear PI 3-kinase; PLC-γ1; Akt; PI(3,4,5)P_3; nucleophosmin/B23; CAD

During embryogenesis, the neurons of vertebrate sympathetic and sensory ganglia become dependent on neurotrophic factors, derived from their targets, for survival and maintenance of differentiated functions [Vogel, 1993]. In the development of mammalian nervous system, half of all generated neurons undergo a predetermined program of cell death to adjust the final number of neurons to the number of the target cells they are innervating [Villa et al., 1994].

Nerve growth factor (NGF) is the prototypical member of a family of neurotrophic factors called neurotrophins, which signal cell survival, differentiation, growth cessation, and apoptosis through two cell surface receptors, the Trks and p75NTR (p75 neurotrophin receptor). NGF activates a variety of signaling cascades, including the PI3K/Akt, the Ras/mitogen-activated protein kinase (MAPK), and the cAMP/protein kinase A (PKA) pathways [Hunter, 2000], but the PI 3-kinase (PI3K) pathway is particularly important for mediating neuronal survival under a wide variety of circumstances [Brunet et al., 2001]. NGF, by binding to TrkA receptor, elicits the recruitment of PI3K to the vicinity of the plasma membrane. The catalytic subunit of PI3K generates the D3-phosphoinositol PI(3,4,5)P_3 at the inner surface of the plasma membrane, which in turn leads to the activation of Akt [Vanhaesebroeck and Alessi, 2000]. Akt is recruited to the plasma membrane through the interaction of its pleckstrin homology domain with PIP_3. At the plasma membrane, the activation of Akt is dependent on phosphorylation, which is achieved at least in part by phosphoinositide-dependent protein kinase-1 (PDK1) [Brunet et al., 2001]. The PI3K/Akt pathway is sufficient and, in some cases, necessary for the trophic-factor-induced cell survival of several neuronal cell types [Yao and Cooper,

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1995; Dudek et al., 1997; Miller et al., 1997; Philpott et al., 1997].

Targets of cytoplasmic PI3K have been studied extensively. The lipid products of PI3K in the cytoplasm influence cytoskeletal rearrangements, vesicle transport, cell metabolism, and apoptosis. Nuclear phosphatidylinositols have also been shown to implicate in RNA processing and transport [Boronenkov et al., 1998; Bunney et al., 2000]. In response to NGF treatment, PI3K and its downstream effectors also translocate into the nucleus, however, whether nuclear PI3K cascade plays any role in preventing apoptosis remains elusive. In this review, we will summarize the current understanding of nuclear PI3K signaling and its upstream effector, PI 3-kinase enhancer (PIKE).

PIKE SIGNALING IN THE NUCLEUS

Cytoplasmic PI3K activation requires activated receptor tyrosine kinases (RTKs) (e.g., PDGFR, EGFR, CD28, etc.) or GTPase proteins such as Ras. However, none of these known PI3K activators is present in the nucleus. Stimulation of cells with NGF nevertheless activates nuclear PI3K temporally different from the cytoplasmic counterpart, and consequently leads to nuclear accumulation of 3-phosphorylated phosphoinositide lipids [Neri et al., 1999; Tanaka et al., 1999], suggesting that nuclear PI3K is regulated by unknown effectors. Previously we reported that a novel nuclear GTPase PIKE interacts with PI3K to stimulate its lipid kinase activity [Ye et al., 2000]. Dominant-negative PIKE, in which GTP binding residues lysine 413 and serine 414 are mutated into alanine and asparagines, respectively, prevents the NGF enhancement of nuclear PI3K activity. The interaction between PIKE-S GTPase and PI3K is GTP-dependent—like that between Ras and PI3K [Rodriguez-Viciana et al., 1994; Ye et al., 2000]. Cytoplasmic PI3K activators, such as RTKs and Ras, bind to one of the two PI3K subunits, but PIKE-S directly associates with both p85 and p110 [Ye et al., 2000]. PIKE encodes a 753 amino acid polypeptide and is a brain-specific protein with molecular weight about 90 kDa, predominantly localizing to the nucleus. PIKE possesses three proline-rich domains, a GTPase domain and a PH domain. In PC12 cells, NGF treatment leads to PIKE interactions with 4.1N, a neuronal-specific isoform of erythrocyte membrane cytoskeleton protein 4.1R, which has translocated to the nucleus, fitting with the initial identification of PIKE based on its binding 4.1N in a yeast two-hybrid screen. PI3K translocates to the nucleus following NGF treatment with a time course that resembles the activation of PIKE-S. However, activation of nuclear PI3K by PIKE is inhibited by the NGF-stimulated 4.1N translocation to the nucleus [Ye et al., 2000]. NGF treatment elicits PIKE-S activation by triggering the nuclear translocation of PLC-γ1, which acts as a physiologic guanine nucleotide exchange factor (GEF) for PIKE-S through its SH3 domain [Ye et al., 2002]. Surprisingly, this effect is independent of its lipase catalytic activity.

PHOSPHOLIPASE C-γ1 SUPPRESSES APOPTOSIS

PLC-γ1 is essential for cell proliferation and differentiation. PLC-γ1 contains two SH2 domains, one SH3 domain, and an additional PH domain that is split by the Src homology (SH) domains; these domains are arranged in the order PH(N)-SH2-SH2-SH3-PH(C), where N and C in parentheses denote NH_2- and COOH-terminal locations, respectively. The SH2 domains mediate the association of PLC-γ1 with autophosphorylation sites on activated RTKs, an essential prerequisite to PLC-γ1 tyrosine phosphorylation and activation. Interestingly, the mitogenic activity is not dependent on its phospholipase activity, but requires its SH3 domain [Smith et al., 1994; Huang et al., 1995]. PLC-γ1 does not possess any known nuclear localization signal (NLS) and predominantly localizes to the cytoplasm. Nevertheless, it has been detected in the nucleus [Martelli et al., 1994; Zini et al., 1995; Diakonova et al., 1997; Neri et al., 1998] and localizes to the nucleus in highly transformed and proliferating cell lines (e.g., A431, HeLa, mouse hepatoma MH 22A, and rat Zajdela ascitic hepatoma cells) but not in primary embryo skin or lung fibroblasts. The differential subcellular localizations in normal or highly transformed cell lines might reflect the degree of transformation of the cell type or phase of the cell cycle [Diakonova et al., 1997]. This idea is consistent with the observation of an increased amount of PLC-γ1 in nuclei of regenerating rat liver at 22 h, which suggests that there is a relationship between the S-phase of the cell cycle and

intranuclear localization of PLC-γ1 [Neri et al., 1997].

Accumulating evidence indicates that PLC-γ1 plays a pivotal role in protecting cell from apoptosis induced by oxidative stress and UV irradiation [Lee et al., 1999; Bae et al., 2000]. Treatment of PLC-γ1-deficient MEF with H_2O_2 results in rapid cell death, whereas normal MEF are resistant to the stress [Wang et al., 2001]. Pretreatment of normal MEF with a selective pharmacological inhibitor of PLC-γ1, or inhibitors of inositol trisphosphate receptors and PKC, increases their sensitivity to H_2O_2, whereas treatment of PLC-γ1-deficient MEF with agents capable of directly activating PKC and enhancing calcium mobilization significantly improves their survival. PLC-γ1 is also required for protection from cell death induced by loss of extracellular matrix adhesion or heat stress [Bai et al., 2002; Chattopadhyay and Carpenter, 2002]. Employing stably transfected PC12 cells with inducible form of PLC-γ1, which contains various SH domain truncations, we recently show that deletion of N-SH2 domain or both N- and C-SH2 domains but not SH3 domain abolishes the anti-apoptotic activity of PLC-γ1 by diminishing its lipase activity. Consistently, lipase inactive PLC-γ1 mutant (LIM) also fails to suppress apoptosis. Thus, SH domains in PLC-γ1 might mediate its anti-apoptotic action through regulating the enzymatic activity [Liu and Ye, 2005]. Interestingly, the nuclei from wild-type PLC-γ1 stably transfected cells resist to DNA fragmentation elicited by the activated cell-free apoptosome, whereas enormous DNA cleavage reveals in the nuclei from LIM cells, suggesting that nuclear PLC-γ1 is implicated in programmed cell death as well. Although all these results demonstrate PLC-γ1 play an important role in preventing apoptosis, definitive evidence supporting the anti-apoptotic function of nuclear PLC-γ1 remains elusive.

PIKE PROMOTES CELL SURVIVAL

A database search leads to the identification of PIKE-L, which differs from PIKE-S in containing a 40 kDa C-terminal extension which includes an Arf-GAP and two ankyrin repeat domains (Fig. 1) [Rong et al., 2003]. PIKE-L and PIKE-S are alternatively spliced isoforms. Both PIKE-L and PIKE-S are brain specific. However, whereas PIKE-S occurs in all brain regions examined, PIKE-L is uniquely absent

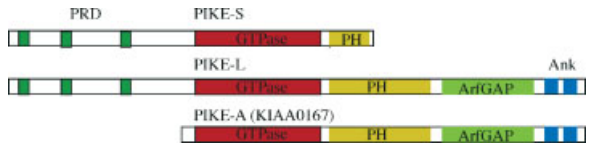

Fig. 1. Diagram of three PIKE isoforms.

from the cerebellum. The subcellular localization of the two proteins differs. PIKE-S is exclusively nuclear, whereas PIKE-L occurs in multiple subcellular fractions and, by immunohistochemistry, is observed throughout the cell body and all neuronal processes.

Sequence analysis results in the discovery that PIKE-L binds to the adaptor protein Homer 1c. Residues 187–190 of PIKE-L have the sequence PKPF, which fit the consensus motif (PxxF) present in proteins that bind to the EVH1 domain of Homer [Xiao et al., 2000]. Mutation of P187 of PIKE-L abolishes binding of PIKE-L to Homer 1c and provides a useful tool to analyze the importance of this binding in various signal cascades. Binding of mGluR I to Homer and thereby PIKE-L occurs only in the case of the group I class of mGluRs, which are the only forms that contain the Homer-ligand PxxF motif. The association of mGluR1 with PIKE-L via Homer 1c suggests that mGluR1 might activate PI3K separately from its well-known activation of PLC. This pathway has been verified by the demonstration that transfection of HEK293 cells with mGluR5 stimulates PI3K activity but mutants of mGluR5 that do not bind Homer fail to activate PI3K [Rong et al., 2003]. Moreover, Homer mutants that do not bind mGluR5 block PI3K activation. In hippocampal cultures, mGluR activation increases PI3K activity, while infection with an adenovirus containing a dominant-negative form of PIKE-L blocks such activation. Finally, mGluR activation fails to stimulate PI3K in the cerebellum, a brain region that is devoid of PIKE-L. A major action of group I mGluRs is prevention of apoptosis, and this appears to be mediated by PIKE-L and PI3K [Rong et al., 2003]. In hippocampal cultures, mGluR activation and PIKE-L transfection block the apoptotic effects of staurosporine and other agents. By contrast, a PIKE-L dominant-negative construct augments apoptosis, and a PIKE construct that cannot bind Homer prevents the anti-apoptotic actions of mGluR activation. Additionally, mGluR activation does not block apoptosis in the cerebellum, which lacks PIKE-L.

NF2 is a tumor suppressor, however, the molecular mechanism accounting for this effect remains obscure. Recently, we show that merlin exerts its activity by inhibiting PI3K, through binding to PIKE-L [Rong et al., 2004]. Wild-type merlin, but not patient-derived mutant (L64P), binds PIKE-L and inhibits PI3K activity. This suppression of PI3K activity results from merlin disrupting the binding of PIKE-L to PI3K. In addition, merlin suppression of PI3K activity as well as schwannoma cell growth is abrogated by a single PIKE-L point mutation (P187L) which cannot bind merlin, but can still activate PI3K. Knocking down PIKE-L with RNA interference abolishes merlin's tumor suppressive activity. Our findings that merlin specifically binds to PIKE-L and abrogates PIKE effects on PI3K provides further evidence that merlin acts as a tumor suppressor by antagonizing the PIKE/PI3K pathway. The discovery that merlin regulates cell growth through PI3K/Akt signaling pathways is intriguing in light of the established relationship between other FERM-containing proteins and apoptosis [Kondo et al., 1997; Gautreau et al., 1999]. Although most previous studies of merlin function have focused on the ability of merlin to reduce cell proliferation, merlin expression can also result in increased cell death [Shaw et al., 1998]. Transduction of merlin into human schwannoma cells was found to decrease cell growth by inducing apoptosis [Schulze et al., 2002]. PI3K/Akt pathway plays an essential role in promoting cell survival in various cell types. In this fashion, merlin expression would result in decreased PIKE-induced PI3K activity, decreased Akt activation, culminating in increased cell death (Fig. 2).

The chromosome 12q13-15 region is frequently amplified in human sarcomas and brain tumors [Smith et al., 1992; Reifenberger et al., 1994]. 12q13-15 amplicon contains two separate core regions, one containing *MDM2* and the other containing *CDK4* [Berner et al., 1996; Elkahloun et al., 1996; Reifenberger et al., 1996]. Blast search reveals the *PIKE* gene is localized at 12q13.3 adjacent to *CDK4* [Collins, 1995; Elkahloun et al., 1997]. In our evaluation of *PIKE* amplification in human glioblastoma multiforme, we have identified a new *PIKE* form (PIKE-A), which was originally identified in the human genome sequencing effort as *KIAA0167* [Nagase et al., 1996], and was independently identified by Liu and collaborators [Xia et al., 2003]. Gene structure analysis reveals that PIKE-A results from different transcription initiation site from that of PIKE-L and -S. PIKE-A contains the GTPase, PH, ArfGAP and two Ankyrin repeats domains present in PIKE-L but lacks the PRD containing N-terminus, which binds protein 4.1N, PI3K, and PLC-γ1. PIKE-L and -A isoforms-regulated signaling cascades are summarized in Figure 2.

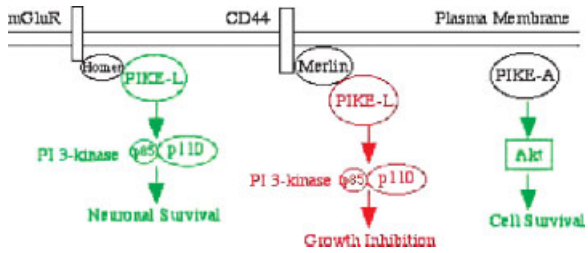

Fig. 2. PIKE-L and -A signaling. Dimerized Homer through its EVH1 domain binds PIKE-L and mGluR I via the "Homer binding motif." mGluR I agonists trigger the formation of Homer/PIKE-L complex and activate PI3K, enhancing neuronal survival (Left). PIKE-L also couples PI3K to merlin, a CD44 receptor-associated protein. Merlin interacts with PIKE-L on its N-terminus and sequesters PI3K from binding to PIKE-L, resulting in inactivation of PI3K and suppression of cell growth (middle). PIKE-A, lacking PI3K binding motif in PIKE-L, associates with active Akt and enhances its kinase activity, promoting cell survival (right).

PIKE-S binds and activates nuclear PI3K, which a portion of the signaling cascade that acts through the mitogenic protein kinase Akt. However, PIKE-A lacks the N-terminal domain that binds to PI3K. Instead, immunoprecipitation assay in human cancer cell lines reveals that Akt co-precipitates with PIKE-A in two human cancer cell lines, NGP-127 and CRL-2061, which overexpress endogenous PIKE-A [Ahn et al., 2004b]. Co-precipitation is not observed in a third cell line U87MG, which does not overexpress PIKE-A. Moreover, PIKE-A specifically interacts with active but not inactive Akt in a guanine nucleotides-dependent manner. In alignment with this observation, Akt mutant with threonine 308 and serine 473 to alanine fails to bind to PIKE-A even stimulated the transfected cells with IGF-1, indicating that Akt phosphorylation might play a role in mediating its association with PIKE-A. Truncation and co-immunoprecipitation assay demonstrate that the GTPase domain of PIKE-A directly interacts with the C-terminal regulatory and partial catalytic domains of Akt. Accordingly, PIKE-A robustly enhances Akt kinase activity but not PI3K, consistent with the observation that PIKE-A amplification

correlates with the elevated Akt activity in human cancer cells. Akt plays an essential role in promoting cell survival. Consequently, overexpression of wild-type PIKE-A evidently resists apoptosis; by contrast, dominant-negative PIKE-A mutant (K83AS84N) enhances the apoptotic effects of staurosporine and other agents. Further, knocking down PIKE-A leads to marked increase of cell death. Akt kinase activity couples to both PIKE-A expression levels and cell death status [Ahn et al., 2004a]. Collectively, all these findings support that PIKE promotes cell survival by augmenting PI3K/Akt signaling cascade.

NUCLEAR PI 3-KINASE INHIBITS APOPTOSIS

PI3K and Akt predominantly locate in the cytoplasm, but they also occur in the nucleus, or translocate to there upon stimulation [Borgatti et al., 1997; Lu et al., 1998; Marchisio et al., 1998; Bavelloni et al., 1999; Neri et al., 1999; Tanaka et al., 1999]. Nuclear PI3K has been shown to implicate in RNA processing and transport [Boronenkov et al., 1998; Bunney et al., 2000]. NGF elicits translocation of PI3K and its downstream effectors into the nucleus, however, the biological functions of nuclear PI3K remain elusive. The hypothesis that nuclear PI3K protects DNA fragmentation during programmed cell death appears attractive. If it is correct, then nuclei from PC12 cells treated with NGF might resist DNA fragmentation elicited by the activated in vitro cell-free apoptotic solution, consisting of HEK 293 cell cytosol supplemented with purified active caspase-3 [Liu et al., 1996, 1997]. As expected, DNA fragmentation is evident in 0 and 10 but not 30 min or 1 h NGF-treated samples, indicating that 30 min to 1 h NGF treatment activates pathways inhibiting caspase-3 activated DNA fragmentation factor/caspase-activated DNase (DFF40/CAD). PARP cleavage also displays a similar temporal pattern. In addition, this rapid effect does not need new protein synthesis, since preincubation of PC12 cells with transcription and translation inhibitors does not alter the protective actions of NGF. Therefore, NGF triggers a rapid protective action against both active caspase and CAD, independent of transcription and translation. Strikingly, DNA fragmentation inhibitory activity selectively occurs in the nuclear but not cytosolic fractions from NGF-treated PC12 cells, underscoring specific anti-apoptotic machinery is activated in the nucleus upon NGF stimulation.

Several lines of evidence support that nuclear PI3K accounts for the protective effect. First, incubation of the nuclei from PC12 cells, pretreated with NGF for 30 min, with the specific PI3K inhibitors Wortmannin and LY294002 but not control vehicle solvent DMSO reveal demonstrable DNA fragmentation, indicating that nuclear PI3K is necessary for NGF to protect DNA from cleaving by CAD or other endonucleases. Second, the nuclei from PC12 cells infected by constitutively active (CA) PI3K, are resistant to internucleosomal cleavage regardless of NGF treatment. In contrast, marked DNA fragmentation is observed in dominant-negative PI3K infected nuclei in spite of NGF treatment. Third, knocking down PI3K in PC12 cells provokes enormous DNA fragmentation in the isolated nuclei whether the cells are treated with NGF or not [Ahn et al., 2004c]. Thus, nuclear PI3K is sufficient and necessary for protecting DNA from fragmentation by NGF. It has been shown before that NGF and other agents promote survival of serum-deprived PC12 cells and sympathetic neurons even when macromolecular synthesis is blocked [Rukenstein et al., 1991]. Inhibition of macromolecular synthesis does not interfere with the ability of NGF to block DNA fragmentation, supporting posttranslational influences of NGF on DNA fragmentation and cell survival, consistent with a proposed protein/phosphorylation-driven pathway [Batistatou and Greene, 1991]. The transcription/translation-independent resistance to the activated apoptosome displayed by NGF-treated PC12 cells implicates this pathway as a molecular mechanism for the anti-apoptotic action of NGF.

NUCLEAR PHOSPHATIDYLINOSITOL LIPIDS

Phosphatidylinositol lipids occur in the nucleus of various types of cells, and mediate a broad range of nuclear processes including chromatin structure, pre-mRNA splicing, cell cycle progression, and nuclear response of DNA damage [Zini et al., 1995; Neri et al., 1998; Gillooly et al., 2000; Yokogawa et al., 2000; Gozani et al., 2003]. PI(3,4,5)P_3 and PI(3,4)P_2 promote cell survival by activating Akt, which phosphorylates components of the apoptotic machinery. High concentrations of PI(4,5)P_2 directly inhibit caspases 8 and 9. Moreover,

$PI(4,5)P_2$ and $PI(3,4,5)P_3$ but not $PI(3,4)P_2$ bind and inhibit caspase-3 [Mejillano et al., 2001]. Thus, the resistance to the activated apoptosome in nuclei from NGF-treated PC12 cells might involve nuclear phosphatidylinositol lipids. However, preincubation of a variety of phosphatidylinositol lipid vesicles with the activated cell-free apoptotic solution fails to block the apoptotic cleavage of DFF45 and Lamin A/C. DNA fragmentation assay reveals evidently cleaved DNA, indicating that none of the phosphatidylinositol lipids directly inhibits the activated caspase or CAD. The observation that nuclear PI3K is critical for mediating NGF anti-apoptotic action, combined with the failure of $PI(3,4)P_2$ and $PI(3,4,5)P_3$ to inhibit CAD-initiated DNA fragmentation, indicates that downstream signals from nuclear receptors of $PI(3,4,5)P_3$ but not lipids themselves exert the anti-apoptotic activity. Preincubation of a variety of phosphoinositol lipids with control nuclei, and followed by DNA fragmentation assay after removal of the lipids demonstrates that $PI(3,4,5)P_3$ pretreatment prevents DFF45/ICAD and lamin A/C cleavage in the control nuclei. Consistent with these observations, $PI(3,4,5)P_3$ pretreatment protects control nuclear DNA from degradation, whereas $PI(3,4)P_2$, $PI(4,5)P_2$, and PI-3-P fail, suggesting that $PI(3,4,5)P_3$ can mimic NGF's effect and account for the anti-apoptotic actions of NGF in the nucleus.

Employing $PI(3,4,5)P_3$ column and NGF-treated PC12 nuclear extracts, B23 is recently identified as a nuclear $PI(3,4,5)P_3$ binding protein [Ahn et al., 2005]. The protective activity remains in the supernatant following $PI(4,5)P_2$ depletion, whereas it is substantially diminished after $PI(3,4)P_2$ depletion, and completely eliminated by $PI(3,4,5)P_3$ depletion, indicating $PI(3,4)P_2$ or $PI(3,4,5)P_3$ nuclear binding target B23 is involved in DNA protection. B23 is a major nucleolar phosphoprotein that plays an essential role in ribosome biogenesis [Hingorani et al., 2000]. B23 interacts with a variety of proteins including nucleolin [Li et al., 1996], cell-cycle related protein p120 [Valdez et al., 1994], HIV-1 Rev protein [Fankhauser et al., 1991], and tumor suppressor ARF, which promotes B23 polyubiquitination and proteosomal degradation [Itahana et al., 2003; Bertwistle et al., 2004]. Overexpression of B23 induces cell cycle arrest in normal fibroblasts, whereas in cells lacking p53 it promotes S-phase entry. Conversely, knocking down B23 inhibits the processing of preribosomal RNA and induces cell death [Itahana et al., 2003]. Moreover, B23 is cleaved by active caspase-3, which may influence its anti-apoptotic action [Chou and Yung, 2001]. Purification from nuclear extract also demonstrates that B23 contributes to DNA fragmentation inhibitory activity. Immunodepletion of B23 from nuclear extracts or knockdown B23 by shRNA in PC12 cells abolishes NGF-provoked protective effect, whereas overexpression of B23 in PC12 cells prevents apoptosis. Compared to the spectrum of proteins associated with the lipid-conjugated beads, B23 exhibited NGF-dependent binding. In vitro binding assay with $PI(3,4,5)P_3$-conjugated beads reveals that binding occurs at 30 min, which partially decays at 60 min. The interaction between B23 and $PI(3,4,5)P_3$ temporally couples to the protective effect elicited by NGF. In the presence of $PI(3,4,5)P_3$, B23 directly binds CAD, and the N-terminus of B23 is dispensable for this activity. By contrast, B23 mutants, that cannot associate with $PI(3,4,5)P_3$, fail to bind CAD and are unable to prevent DNA fragmentation. Together, these findings support that the nuclear $PI(3,4,5)P_3$/B23 complex regulates the anti-apoptotic activity of NGF in the nucleus [Ahn et al., 2005].

NUCLEAR Akt PREVENTS CELL DEATH

Cytoplasmic Akt antagonizes apoptotsis both prior to the release of cytochrome c, by regulating Bcl-2 family member activity and mitochondrial function, and subsequent to the release of cytochrome c, by regulating components of the apoptosome. In addition to its effects on the cytoplasmic apoptotic machinery, Akt also regulates apoptosis by modulating the expression of apoptotic genes in the nucleus. For example, Akt controls a major class of transcription factors—the Forkhead box transcription factor by phosphorylating FOXOs (Forkhead box, group O) and inhibiting their ability to induce the expression of death genes [Biggs et al., 1999; Brunet et al., 1999; Kops and Burgering, 1999]. Recently Akt has also been shown to promote survival in hippocampal neurons by inhibiting the activity of the tumor suppressor p53, which mediates the expression of death genes, including the pro-apoptotic Bcl-2 family member BAX [Yamaguchi and Wang, 2001]. Akt blocking apoptosis also involves the

inhibition of Nur77, a transcription factor implicated in T-cell receptor-mediated apoptosis [Masuyama et al., 2001]. In addition to its function as a suppressor of critical death genes, under some circumstances activation of the Akt survival pathway also triggers the expression of survival genes by phosphorylating CREB and IKK [Du and Montminy, 1998; Ozes et al., 1999; Romashkova and Makarov, 1999]. In addition to growth factor mediating Akt nuclear localization, gender also appears to regulate Akt subcellular distribution [Camper-Kirby et al., 2001]. For example, young women possess higher levels of nuclear-localized phospho-Akt(473) relative to comparably aged men or postmenopausal women. Both localization of phospho-Akt(473) in myocardial nuclei of sexually mature female mice versus males and Akt kinase activity in nuclear extracts of hearts from female mice versus males are elevated. Consistently, cytosolic localization of phospho-forkhead transcription factor is also increased in female relative to male mice. Consequently, nuclear-targeted Akt significantly elevates levels of phospho-Akt and kinase activity and inhibits apoptosis as effectively as plasma membrane myristolated-Akt in hypoxia-induced cell death [Shiraishi et al., 2004].

NGF stimulates phosphorylated Akt to translocate to the nucleus of PC12 cell [Borgatti et al., 2003]. Upon NGF treatment, the nuclei from PC12 cells infected by control, wild-type or CA Akt adenovirus are resistant to internucleosomal cleavage. In contrast, evident DNA fragmentation is observed in dominant-negative Akt infected nuclei in spite of NGF treatment, indicating nuclear Akt is required for NGF-mediated anti-apoptotic signaling. However, without NGF treatment all the nuclei display demonstrable DNA degradation even infected with CA Akt, suggesting cytosolic Akt activation alone is not sufficient to inhibit DNA cleavage. Similar results are also obtained with NLS-tagged Akt stably transfected PC12 cells, although partial protection is observed in the nuclei from CA NLS-Akt-CA cells [Ahn et al., 2004c]. Thus, these observations indicate that nuclear Akt is necessary but not sufficient to mediate the anti-apoptotic action of NGF. Presumably, B23/PI(3,4,5)P_3 complex cooperatively antagonizes apoptosis with nuclear Akt and its downstream effectors. PIKE signaling cascade blocking DNA fragmentation is depicted in Figure 3.

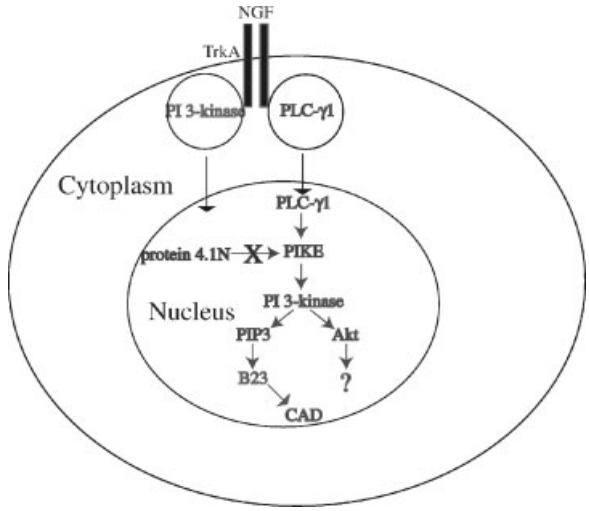

Fig. 3. PIKE signaling in prevention of programmed cell death in the nucleus. NGF, by binding to TrkA receptor, elicits the recruitment of both PI3K and PLC-γ1 to the vicinity of the plasma membrane. The activated PI3K and PLC-γ1 translocate into the nucleus. PLC-γ1 acts as a GEF and stimulates PIKE activation through its SH3 domain. The GTP bound PIKE associates with nuclear PI3K and provokes its activation. The nuclear translocated protein 4.1N inhibits nuclear PI3K activation by sequestering PI3K from PIKE. The catalytic subunit of PI3K generates the D3-phosphoinositol PI(3,4,5)P_3, which in turn leads to the activation of Akt in the nucleus. PI(3,4,5)P_3 binds B23 and this complex directly associates with CAD, blocking its DNA fragmentation activity. Presumably, nuclear Akt also exerts anti-apoptotic effect through its downstream nuclear targets.

SUMMARY

The discovery that PIKE/nuclear PI3K signaling mediates the anti-apoptotic actions of NGF demonstrates that nuclear PI3K protects DNA from internucleosomal cleavage during apoptosis, suggesting a continuity of survival-promoting effect from cytoplasmic to nuclear PI3K. Its anti-apoptotic activity is probably mediated through nuclear PI(3,4,5)P_3. Identification of B23 as a nuclear PI(3,4,5)P_3 receptor confirms this finding, since the complex exerts its inhibitory activity against DNA fragmentation by directly suppressing CAD. However, nuclear localized active Akt also plays a critical role in this action. The downstream nuclear effectors of Akt and nuclear B23/PI(3,4,5)P_3 complex might synergistically mediate the rapid anti-apoptotic activity of NGF. Characterization of the downstream targets of nuclear Akt will further our understanding of the molecular mechanisms of NGF-promoted neuronal cell survival.

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