Neurotrophin Receptor Interacting Factor (NRIF) Is an Essential Mediator of Apoptotic Signaling by the p75 Neurotrophin Receptor*

Received for publication, September 10, 2004, and in revised form, December 14, 2004
Published, JBC Papers in Press, January 24, 2005, DOI 10.1074/jbc.M410435200

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The p75 neurotrophin receptor is a pleiotropic signaling molecule that regulates cellular survival, neurite outgrowth, and myelin formation (1). This founding member of the tumor necrosis factor receptor superfamily can directly bind all of the neurotrophins, including nerve growth factor (NGF),1 brain-derived neurotrophic factor (BDNF), and neurotrophin-3 and -4 (NT-3, NT-4), but it also functions as a co-receptor in a variety of protein complexes. p75 can interact with TrkA to form a high affinity neurotrophin binding site (2) and enhance survival signaling (3). It can also associate with the Nogo receptor and Lingo-1 and, upon interaction with myelin proteins, block neurite outgrowth (4, 5), and, together with Sortilin, the neurotrophin receptor interacting factor (NRIF, also known as NRAGE, H20648/ZFP 110) in p75-mediated cell death. NRIF was isolated in a p75 receptor complex as well as the cellular context. For example, sympathetic neurons of superior cervical ganglia undergo a period of programmed cell death during ontogenesis, and NGF, supplied by the tissues innervated, prevents the loss of these neurons through binding to a p75-TrkA complex (7). In contrast, specific activation of p75 (8) or a p75-sortilin complex (6) induces apoptosis in the neurons. Genetic deletion of p75 prevents the normal period of cell death in the developing superior cervical ganglia (8, 9), thus demonstrating the key role of this receptor in regulating the survival of this neuronal population.

How p75 initiates this variety of biological effects is not well understood; however, the stress kinase c-Jun N-terminal kinase (JNK), has been suggested to play a role in mediating this apoptotic signal. Neurotrophin activation of JNK through p75 correlates with the induction of cell death (43) and inhibition of the kinase prevents the receptor from killing (23, 10). Interestingly, c-Jun, the downstream target of the kinase, is not required for the receptor to activate apoptosis (11); however, other JNK substrates have been implicated in the p75 death pathway, including the pro-apoptotic Bel-2 family member Bad (12) and the tumor suppressor p53 (13). p53 was suggested to function in p75 signaling, because expression of the viral p53 inhibitor E1B prevented the receptor from inducing apoptosis in sympathetic neurons and mice lacking p53 show a significant reduction in the normal attrition of sympathetic neurons during development (13).

A number of proteins have also been identified that can bind the intracellular domain of the receptor (1), and several have been implicated in regulating apoptosis, including NRAGE (14), NADE (15) and NRIF (16); however, evidence demonstrating a direct requirement for any of these proteins in p75-mediated apoptosis is lacking. Moreover, the mechanisms through which they regulate cell death pathways remain largely unknown. In the present study, we examine the role of NRIF (neurotrophin receptor interacting factor, also known as ZFP 110) in p75-mediated cell death. NRIF was isolated in a yeast two-hybrid screen and encodes a 94-kDa zinc finger protein of the Krüppel family (16). It was suggested to have role in p75-mediated apoptosis based on the fact that mice lacking nrif display a significant reduction in cell death in the developing retina (16), a phenotype also observed in p75 null mice (17). Despite this in vivo correlative evidence, it remains to be shown that NRIF is directly involved in signaling through the p75 receptor.

Here, we demonstrate that NRIF is required for p75-mediated apoptosis of sympathetic neurons, but is dispensable for cell death after NGF withdrawal. In addition, we establish a link between NRIF and both of the known components of p75 apoptosis signaling, JNK and p53.
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MATERIALS AND METHODS

Cell Culture—Mice lacking nrif were maintained on a 129Sv background and genotyped as previously described (16) and p53−/− mice on a mixed background were genotyped as previously described (18). Sympathetic neurons were isolated from the superior cervical ganglia as described (11). Because the superior cervical ganglia of nrif or p53−/− mice on a mixed background were genotype, the sympathetic neurons were dissociated with 0.25% trypsin and 0.3% collagenase for 30 min at 37 °C. The neuronal cells were removed with a 2-h preplating on uncoated, Falcon 60-mm plates (BD Biosciences). The neurons were cultured on poly-l-ornithine and laminin-coated 4-well slides (Nalge Nunc International) in UMEM (BioWhittaker) supplemented with 3% fetal calf serum (Invitrogen), 2 mM L-glutamine (Invitrogen), and 20 ng/ml NGF (Harlan). The neurons were maintained for 4–5 days in the presence of NGF before being used for survival assays in NGF withdrawal and p75 activation experiments.

Rat Schwann cells were isolated from postnatal day 4 rats as described by Carter et al. (19) and maintained in Dulbecco’s modified Eagle’s medium supplemented with 10% fetal calf serum and 2 μM forskolin (Sigma). Cells were transfected with Effectene (QIAGEN) according to the manufacturer’s instructions. When adenovirally infected, cells were split the day before infection and infected with 4.5 × 10^6 plaque-forming units/cell of virus expressing NRIF and GFP bicistronically or GFP alone for mouse embryo fibroblasts or 7.5 × 10^6 plaque-forming units/cell for sympathetic neurons or Schwann cells. At the indicated times, cells were harvested for staining or immunoblotting, as indicated.

Mef2c (MEFs) were maintained in Dulbecco’s modified Eagle’s medium supplemented with 10% fetal calf serum. Cultures were split every 3 days, and all experiments were done with cells that had been passaged fewer than 12 times.

ProNGF Production—ProNGF was generated by transfection of HEK 293 cells with a furin-resistant, His-tagged construct, and the protein was purified using nickel-bead chromatography (Xpress System Protein purification, Invitrogen) as per the manufacturer’s instructions using imidazole for elution, as previously described (21). Mature NGF was similarly produced and used for comparison in all experiments with ProNGF.

Survival Assays—For NGF withdrawal experiments, NGF was removed by washing the cultures twice in Dulbecco’s medium, and once with Dulbecco’s containing an antibody to NGF at 0.1 μg/ml (Chemicon International). The procedure was similar for the p75 activation experiments except that after the anti-NGF wash, the neurons were switched to media containing anti-NGF together with 12.5 mM KCl, to promote survival, with or without 200 ng/ml BDNF (a gift from Regeneron Pharmaceuticals, Inc.). Forty-eight hours after the switch to NGF-free or BDNF-containing media, the cells were fixed in 4% paraformaldehyde and the number of surviving neurons, identified by DAPI staining the nuclei (Vector Laboratories), were counted. The results were normalized to the number of nuclei. The difference in the response to p75 activation between the two genotypes. The NGF-mediated survival advantage between the two genotypes. The NGF-mediated survival advantage was confirmed by terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling staining in some experiments. For proNGF treatment, the neurons were plated directly in media containing 4.6 mM imidazole (used to elute pro- or mature NGF from thenickel beads), 20 ng/ml NGF or proNGF and fixed 20 h later. Schwann cells and MEFs infected with adeno-NRIF or GFP were similarly scored for apoptosis. In the assays done with infected cells, only GFP-expressing cells were quantified. In all cases, at least 100 cells per condition were counted.

NF-κB Activation Assays—Activation of NF-κB was assessed in primary cultures of Schwann cells, 1 or 2 days after isolation, using a luciferase reporter 6XκB-Luc (a gift from Larry Kerr). The cells were transfected with 0.2 μg of 6XκB-Luc reporter and 0.02 μg of Ras sarcoma (Ras) expression vector (used as internal control for transfection efficiency) per well of a 24-well plate using Effectene (QIAGEN) according to the manufacturer’s protocol. After 24 h, the cells were washed twice in serum-free Dulbecco’s modified Eagle’s medium and treated with 100 ng/ml NGF for 4–6 h and lysed in 40 μl of reporter lysis buffer (Promega). Luciferase activity was measured according to the manufacturer’s instructions (Promega) using a luminometer (Monolight 2010, Analytical Luminescence Laboratory). The results were normalized to the basal activity for each treatment and genotype. There was no consistent difference in the basal activity between genotypes, although there was considerable variability.

Immunostaining—Rat Schwann cells were maintained as described above. After transfection with adeno-GFP or GFP-NRIF, cells were fixed in 4% paraformaldehyde, blocked with 10% goat serum in PT (phosphate-buffered saline, 0.1% Triton X-100) and immunostained with antisemur to caspase 3 (a gift from Idun Pharmaceuticals, Inc.) diluted 1:1000 in PT, followed by a biotinylated secondary antibody (Vector Laboratories) and Cy-3 streptavidin. Nuclei were visualized by DAPI staining the nuclei (Vector Laboratories), were counted. The results were normalized to the number of nuclei. The difference in the response to p75 activation between the two genotypes. The NGF-mediated survival

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of the nrif−/− neurons was not different from the nrif+/+ cells (relative to wild type, 110 ± 29% of the nrif−/− neurons were viable after 48 h in the presence of 20 ng/ml NGF) nor was the survival in KCl (relative to wild type, 99 ± 16% of the nrif−/− neurons were viable). In addition, there was no difference in the degree of apoptosis in response to NGF withdrawal between the genotypes (Fig. 2). Thus, although NRIF is required for p75-mediated cell death, it is not required for apoptosis that occurs when neurons are deprived of NGF.

The Activation of JNK by p75 Is Attenuated in the Absence of NRIF—Activation of the stress kinase JNK by p75 has been shown to be necessary for the receptor to induce cell death (10, 22, 23). The mechanism of JNK activation by p75 has not been delineated; however, TRAF6, a member of the tumor necrosis factor receptor associated family (25), and the GTP-binding protein Rac (10) have been implicated as upstream activators. Furthermore, it was recently reported that co-expression of NRIF, TRAF6, and p75 in 293 HEK cells reconstituted JNK activation by neurotrophin (24). Therefore, we evaluated the stimulation of this kinase in neurons from mice lacking nrif. Sympathetic neurons isolated from nrif−/− or wild type mice were cultured in KCl, as for the cell death assays, treated with BDNF for 24 h and the activation of JNK assessed by Western blotting for phospho-JNK. Unlike wild type neurons, there was no JNK activation detectable in those from nrif−/− mice (Fig. 3A). The total level of JNK was not significantly different between the wild type and null neurons (based on normalization to tubulin and a Student’s t test, p = 0.36, n = 4). In addition, we evaluated the stimulation of this kinase by immunostaining for phospho-JNK. Approximately 80% of the wild type neurons were phospho-JNK positive upon p75 activation compared with <20% of the nrif null neurons after p75 activation (Fig. 3B). Thus, the expression of NRIF is required for the stimulation of JNK by p75.

We also investigated p75 regulation of this pathway by immunostaining for the transcription factor c-Jun. c-Jun is a well characterized downstream target of JNK, which up-regulates itself and accumulates in the nucleus following phosphorylation by JNK, thus it is often used to evaluate the activation of the kinase. Surprisingly, we found that the overall level of c-Jun was significantly reduced in the nrif−/− neurons and BDNF treatment still elicited some accumulation of the transcription factor in the nucleus (Fig. 3C). This result suggests that there is a mechanism by which the transcription factor is regulated that is independent of JNK. However, p75-mediated cell death does not require c-Jun (11), thus our results implicating NRIF in the activation of JNK are more relevant to the apoptotic pathway.
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Deletion of nrif Does Not Alter the Ability of p75 to Activate NF-κB—Another signaling system that regulates cellular viability is under the influence of p75 is the NF-κB pathway. This transcription factor was shown to be activated in Schwann cells by NGF binding to p75 and to promote survival (25). Therefore, we evaluated the stimulation of NF-κB by neurotrophins in Schwann cells from nrif+/+ and −/− mice by a luciferase reporter assay (Fig. 4). There was no significant difference between the genotypes in the ability of p75 to activate this transcription factor.

NRIF Expression Induces Apoptosis in Primary Cells—The observation that neurons do not undergo p75-mediated cell death or JNK activation in the absence of nrif suggested that this interactor mediates an apoptotic signal. Previous findings demonstrated that expression of NRIF in cell lines, such as 293 HEK cells, primarily caused cell cycle arrest (26); however, we considered the possibility that NRIF may activate cell death in primary, non-immortalized cells. To test this hypothesis, NRIF was expressed in primary Schwann cells, sympathetic neurons, or mouse embryonic fibroblasts (MEFs). When mouse sympathetic neurons were infected with an adrenovirus expressing GFP and NRIF bicistronically, −45% of the GFP-positive neurons were apoptotic, determined by DAPI staining of condensed or fragmented nuclei (Fig. 5A). In contrast, <10% of these neurons were apoptotic when infected with the GFP virus alone. Similarly, when primary rat Schwann cells or mouse embryonic fibroblasts (MEFs) were infected with the NRIF virus, a significant increase in apoptosis was observed as compared with the GFP control. Similar results were seen in the absence of the adenovirus when the Schwann cells were transiently transfected with NRIF or GFP expression vectors (data not shown). These results demonstrate that expression of NRIF is sufficient to induce cell death in multiple primary cell types.

The cell death induced by NRIF expression involved activation of caspase 3 and cytochrome c release from the mitochondria. As depicted in Fig. 5B, 24 h after transfecting rat Schwann cells with GFP-NRIF, the GFP-positive cells typically displayed a condensed or fragmented nucleus and were reactive to an antibody that recognizes the activated form of caspase 3. In many situations, it has been found that release of cytochrome c from the mitochondria precedes the activation of caspase 3. To determine if NRIF-mediated apoptosis involved cytochrome c release, Schwann cells were infected with adenovirus expressing NRIF or GFP, subjected to subcellular fractionation and the heavy membrane fraction, which is enriched with mitochondria, was evaluated for cytochrome c content by immunoblotting. At both 18 and 24 h (data not shown) after infection we observed a decrease in the amount of cytochrome c in the mitochondria from NRIF-expressing cells relative to those infected with adeno-GFP (Fig. 5C). Taken together, we conclude that NRIF is a pro-apoptotic molecule that induces the release of cytochrome c from the mitochondria and activates caspase 3.

Given that p75 was not able to maximally activate JNK in the absence of NRIF, we considered the possibility that NRIF induced cell death by activating this kinase; however, over expression of NRIF in MEFs or Schwann cells (data not shown) did not significantly increase the levels of phospho-JNK (Fig. 5D). Thus, NRIF is necessary, but not sufficient for stimulating the kinase, as was previously suggested based on ectopic expression of NRIF in 293 HEK cells (24).

Both p75- and NRIF-mediated Apoptosis Are p53-Dependent—The tumor suppressor p53 has also been implicated in p75-mediated apoptosis, based on the ability of the inhibitor E1B to prevent the receptor-induced death (13). To directly determine whether the neuronal apoptosis induced by p75 activation requires p53, sympathetic neurons were isolated from p53+/+ and −/− mice and subjected to NGF withdrawal or p75 activation by BDNF, as above. Interestingly, although the p53−/− neurons were totally resistant to cell death induced by p75 activation (Fig. 6A), they underwent apoptosis as well as the wild type following NGF removal (Fig. 6B). Thus, these results demonstrate that, like NRIF, p53 is required for p75-mediated apoptosis but is dispensable for cell death occurring after NGF deprivation.

To determine whether p53 was also required for NRIF-mediated cell death in these neurons, we cultured sympathetic neurons from p53 littermates and infected them with the GFP- or NRIF-expressing adenovirus and scored the number of apoptotic nuclei 48 h later. While the p53+/+ neurons underwent apoptosis when infected with NRIF, neurons derived from the −/− mice did not die when NRIF was ectopically expressed (Fig. 6C). Thus, both p75- and NRIF-induced apoptosis require p53.

DISCUSSION

The p75 neurotrophin receptor has been shown to function as a key regulator during the development of the mammalian nervous system. It is required for naturally occurring cell death in several neuronal populations, including sympathetic neurons in the superior cervical ganglia (8), cholinergic neurons in the basal forebrain (27), and neuronal precursors in the developing retina and spinal cord (28). This receptor is also induced by a wide variety of insults in both the peripheral and central nervous system (29) and in several models it is responsible for the resulting cell death. For example, p75 mediates loss of hippocampal neurons following seizure (44), cortical neurons after severance of the cortico-spinal tract (30), and oligodendrocytes in response to spinal cord lesions (31).

In an effort to determine the molecular mechanism by which this receptor signals cell death, a number of proteins have been identified that bind to the intracellular domain of p75. Several of these have been implicated in the apoptotic pathway, including NRAGE (14), NADE (15), tumor necrosis factor receptor-associated factor 2 (TRAF2) (32), and 6 (TRAF6) (33) and NRIF (16); however, these studies have relied primarily on ectopic, overexpression of the given p75 interactor. NRIF was previously implicated in p75-mediated apoptosis based on the phenotypic similarity between NRIF and p53. In an effort to determine the molecular mechanism by which this receptor signals cell death, a number of proteins have been identified that bind to the intracellular domain of p75. Several of these have been implicated in the apoptotic pathway, including NRAGE (14), NADE (15), tumor necrosis factor receptor-associated factor 2 (TRAF2) (32), and 6 (TRAF6) (33) and NRIF (16); however, these studies have relied primarily on ectopic, overexpression of the given p75 interactor. NRIF was previously implicated in p75-mediated apoptosis based on the phenotypic similarity between NRIF and p53. In an effort to determine the molecular mechanism by which this receptor signals cell death, a number of proteins have been identified that bind to the intracellular domain of p75. Several of these have been implicated in the apoptotic pathway, including NRAGE (14), NADE (15), tumor necrosis factor receptor-associated factor 2 (TRAF2) (32), and 6 (TRAF6) (33) and NRIF (16); however, these studies have relied primarily on ectopic, overexpression of the given p75 interactor. NRIF was previously implicated in p75-mediated apoptosis based on the phenotypic similarity between NRIF and p53. In an effort to determine the molecular mechanism by which this receptor signals cell death, a number of proteins have been identified that bind to the intracellular domain of p75. Several of these have been implicated in the apoptotic pathway, including NRAGE (14), NADE (15), tumor necrosis factor receptor-associated factor 2 (TRAF2) (32), and 6 (TRAF6) (33) and NRIF (16); however, these studies have relied primarily on ectopic, overexpression of the given p75 interactor. NRIF was previously implicated in p75-mediated apoptosis based on the phenotypic similarity between NRIF and p53.
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**Fig. 3.** p75 stimulation of the JNK pathway is attenuated in nrif−/− sympathetic neurons. A, sympathetic neurons from nrif+/+ and −/− mice were cultured in 20 ng/ml NGF for 4 days, rinsed, and refed with media containing a neutralizing antibody to NGF and 12.5 mM KCl with or without 200 ng/ml BDNF. After 24 h, the cells were lysed and immunoblotted using JNK and phospho-JNK antibodies. A representative blot from four experiments is shown in the left panel and quantitation by densitometry of phospho-JNK to total JNK is shown in the right panel (n = 3, white bars are KCl and black bars are in the presence of BDNF). B, sympathetic neurons from nrif+/+ and −/− mice were cultured as above, treated with or without 200 ng/ml BDNF for 20 h, the cells were then fixed and immunostained using an antibody to phospho-JNK and DAPI to identify healthy nuclei. In the left panel representative photomicrographs of wild type (+/+) and nrif null (−/−) neurons immunostained for phospho-JNK are depicted. The right panel shows quantitation of the percentage of phospho-JNK positive neurons. White bars indicate KCl treatment, and black bars indicate the addition of BDNF. The mean is depicted (n = 2). C, sympathetic neurons were cultured and treated with BDNF as described above, fixed, and immunostained using an antibody to c-Jun and DAPI to visualize healthy nuclei. Quantitation of the percentage of neurons with nuclear c-Jun is depicted. White bars indicate KCl treatment, and black bars indicate the addition of BDNF. The mean ± S.E. is depicted (n = 5).

**Fig. 4.** The activation of NF-κB by p75 is unaffected in nrif−/− Schwann cells. Schwann cells were isolated from wild type and nrif null mice at postnatal day 2–4 and transfected with an NF-κB luciferase reporter 24 h later. One day after transfection, cells were treated in serum-free media with 100 ng/ml NGF or left untreated for 4–6 h. Lysates were collected and luciferase activity measured. All values were normalized to control cells (in serum-free media alone). Gray bars indicate control, and black bars indicate NGF treatment. The mean is depicted (n = 4 for wild-type, n = 2 for null).

nrif−/− and p75−/− mice in the developing retina (16), yet a direct role for this interacting protein in signaling from p75 was not established. Here we demonstrate that NRIF is a pro-apoptotic molecule required for p75 to induce cell death in sympathetic neurons. Our results indicate that the activation of JNK, but not NF-κB, by the receptor requires NRIF. In addition, we show that both NRIF and p75 induce apoptosis through a p53-dependent mechanism.

NRIF, originally identified in a yeast two-hybrid screen, was suggested to function in p75 signaling based on its ability to bind the receptor (16, 26) and the fact that both nrif−/− and p75−/− mice have reduced levels of apoptosis in the embryonic retina (16). Moreover, the expression pattern of p75 and NRIF during murine development is overlapping, although NRIF is more widely expressed suggesting it has a role beyond p75 signaling (16, 34). This is also reflected in the fact that the deletion of nrif in the BL6 strain of mice is embryonic lethal around day 11, a phenotype not observed in the p75 null mice (16). The mechanism by which NRIF functioned, however, remained unclear.

The amino acid sequence of NRIF includes a putative C2H2, or Krüppel type zinc finger domain, which are DNA binding modules, as well as a KRAB domain, which typically act as transcription repressor domains (35). Thus, the structure of NRIF suggests that it regulates gene transcription, which was further supported by evidence that ectopic expression in 293 HEK cells results in a significant portion of NRIF being localized to the nucleus (16, 24). However, the results presented here indicate that NRIF functions in mediating p75 activation of JNK, which is required for the receptor to induce cell death. Thus, this protein may serve multiple roles in p75 signaling.

Recently, another p75 interactor, TRAF6, was shown to be essential for the receptor to induce activation of JNK and the subsequent apoptosis (36). TRAF6 also transduces the signal to NF-κB and JNK for several other receptors, such as IL-1β, RANK, and CD40, through a mechanism involving its oli-
gomerization (37). Interestingly, NRIF was shown to bind to TRAF6, and the interaction of these two proteins enhanced TRAF6 activation of JNK (24). Moreover, co-expression of NRIF, TRAF6, and p75 in 293 HEK cells reconstituted the ability of NGF to stimulate JNK, whereas neither intracellular protein alone was sufficient (24). It was suggested that NRIF...
may facilitate TRAF6 stimulation of downstream pathways through oligomerization, similar to what has been shown for the TRAF6 interactor TIFA (45). In agreement with these results, we found that in the absence of NRIF, p75 was unable to activate the kinase (Fig. 3), although no JNK activation was observed upon overexpression of NRIF alone (Fig. 5D). Thus, NRIF appears to serve as a facilitator for p75 regulation of this pathway, presumably through its interaction with TRAF6.

It should be noted that in addition to NRIF and TRAF6 there have been other signaling proteins implicated as having a role in p75 signaling to JNK, including the GTP-binding protein Rac (10) and the MAGE homolog, NRAGE (14). Whether these proteins form a multicomponent signaling complex is not clear; however, it has been reported that interleukin-1 signals through a pathway involving IRAK-1, TRAF6, and Rac1 (38). These authors focused on the activation of NF-κB and demonstrated that dominant negative Rac could block TRAF6 signaling, yet dominant negative TRAF6 could also block a Rac-driven response; thus, supporting the notion of a signaling complex involving both of these proteins and possibly others.

Several reports have indicated that JNK activation is required for p75 to induce apoptosis. Inhibiting the kinase pharmacologically (23) or by expressing a dominant negative JNK construct in oligodendrocytes (10) or in sympathetic neurons (36) prevented cell death through p75. We also find that, in our neuronal cultures from nrif/-/- mice, we observed some increase in nuclear c-Jun despite the lack of JNK and cell death induction. Thus, there may be an alternative mechanism, independent from the cell death pathway, for regulating this transcription factor.

Several alternative substrates of JNK have been proposed as mediating cell death in response to p75, including Bcl-2 family members Bad (12) and Bim (46) and the transcription factor p53 (13). Our findings further support a role for p53, because we show that p53/-/- neurons are resistant to BDNF. In contrast, p53 was not essential for cell death induced by with-
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drawal of NGF, corroborating a previous report (39). Because the naturally occurring apoptosis in the superior cervical ganglia is significantly reduced in mice lacking p53 (13), our results suggest that p75-mediated cell death plays a significant role in the normal loss of neurons during development. Miller and colleagues (8) have also proposed such a role for p75 signaling based on the attenuated cell death in the superior cervical ganglia of p75−/− mice and the ability of deleting p75 to rescue the massive loss in neurons that occurs in the absence of TrkA, the NGF tyrosine kinase receptor responsible for survival signaling (40).

In addition to its role in p75 signaling, we found that p53 was required for apoptosis induced by ectopic expression of NRIF (Fig. 6C). This result provided an explanation for the ability of NRIF to activate a cell death program without stimulating JNK. Moreover, because JNK can phosphorylate and activate p53 (41, 42), the requirement for p53 in NRIF-mediated apoptosis in the absence of JNK activation indicates that there may be two pathways activated by p75 that converge on p53, one dependent on the stress kinase and one independent through NRIF.

The mechanism by which NRIF affects p53 function remains to be determined; however, we found that MEFs from p19<sup>−/−</sup> mice are also resistant to cell death induced by ectopic NRIF expression (data not shown). p19<sup>−/−</sup> is a well-established upstream activator of p53 that is induced following oncogenic stress (47). Thus, future studies will focus on how p75 and NRIF regulate cell death through the p19/Arf/p53 pathway.

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