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Tumor Necrosis Factor Receptor-associated Death Domain Protein Is Involved in the Neurotrophin Receptor-mediated Antiapoptotic Activity of Nerve Growth Factor in Breast Cancer Cells*

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The common neurotrophin receptor p75^{NTR} has been shown to initiate intracellular signaling that leads either to cell survival or to apoptosis depending on the cell type examined; however, the mechanism by which p75^{NTR} initiates its intracellular transduction remains unclear. We show here that the tumor necrosis factor receptor-associated death domain protein (TRADD) interacts with p75^{NTR} upon nerve growth factor (NGF) stimulation. TRADD could be immunodetected after p75^{NTR} immunoprecipitation from MCF-7 breast cancer cells stimulated by nerve growth factor. In addition, confocal microscopy indicated that NGF stimulation induced the plasma membrane localization of TRADD. Using a dominant negative form of TRADD, we also show that interactions between p75^{NTR} and TRADD are dependent on the death domain of TRADD, thus demonstrating its requirement for binding. Furthermore, the p75^{NTR}-mediated activation of NF-κB was inhibited by transfection with a dominant negative TRADD, resulting in an inhibition of NGF antiapoptotic activity. These results thus demonstrate that TRADD is involved in the p75^{NTR}-mediated antiapoptotic activity of NGF in breast cancer cells.

Nerve growth factor (NGF)[1] is the archetypal member of the neurotrophin family of proteins (including brain-derived neurotrophic factor, NT-3, and NT-4/5), which display a broad range of survival and trophic activities for neuronal cells (1). In addition to its neurotrophic function, other activities of NGF have been described. For example, NGF can modulate gene expression in monocytes (2), is chemotactic for melanocytes (3), and is motogenic for Schwann cells (4). NGF also stimulates the proliferation of lymphocytes (5), keratinocytes (6), and both prostate (7) and breast cancer (8) cells.

Cellular responses to NGF are elicited via two specific cell surface receptors: TrkA tyrosine kinase receptors (p140^{TrkA}) and the common neurotrophin receptor, p75^{NTR}, which belongs to the tumor necrosis factor (TNF)-receptor gene family. Although NGF binding to p140^{TrkA} is known to activate its kinase domain, thus triggering various downstream Ras signaling pathways such as the mitogen-activated protein kinases, the function of p75^{NTR} and how it signals remain controversial. p75^{NTR} has been shown to regulate the activation of TrkA receptors but is also capable of triggering cellular responses independent of them (9–11). In breast cancer cells, the mitogenic activity of NGF is mediated through activation of p140^{TrkA}, whereas p75^{NTR} is required for its antiapoptotic effect, independent of p140^{TrkA} (12). There is evidence that NGF can both positively and negatively regulate cell death and differentiation, depending on the cell type examined. In some cases, p75^{NTR} is an inducer of apoptosis, even without NGF stimulation (13), whereas in other cases, the activation of p75^{NTR} results in a protection from cell death (14). The mechanism by which p75^{NTR} initiates such signaling is poorly described, but the tumor necrosis factor receptor-associated factor (TRAF) proteins, particularly TRAF2 and TRAF6, as well as the receptor-interacting protein 2 have been shown to interact with p75^{NTR} and differentially modulate both the activation of the transcription factor NF-κB and cell survival in response to NGF (15, 16).

The first protein recruited to TNF-receptor 1 is the TNF-R1-associated death domain protein (TRADD), which serves as a platform to recruit additional mediators such as the TRAF proteins (17). Although the involvement of TRAF proteins in the p75^{NTR} signaling complex has been described, the requirement for TRADD has not been reported. Here we show that TRADD functionally associates with p75^{NTR} in breast cancer cells. The interaction between p75^{NTR} and TRADD is required for activation of NF-κB, which controls the antiapoptotic effect of nerve growth factor.

EXPERIMENTAL PROCEDURES

Materials—Cell culture reagents were provided by Bio-Whittaker except insulin (Organon), transferrin (Sigma), and fibronectin (Falcon-Biocoat). Recombinant NGF and other neurotrophins (BDNF, NT-3, and NT-4) were purchased from R&D Systems. C2 ceramide analogue (*N*-acetyl-D-sphingosine), Hoechst 33258, and electrophoresis reagents were from Sigma. The ExGen 500 transfectant and the Opti-MEM were from Euromedex. Protein A-agarose was purchased from Transduction Laboratories (Lexington, KY). The mouse monoclonal IgG1 anti-NGF receptor p75^{NTR} antibody was from Upstate Biotechnology, Inc. (Lake Placid, NY). The goat and rabbit polyclonal antibodies (IgG) raised against TRADD and TRAF2, respectively, were purchased from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA), and the corresponding sec-

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[1] The abbreviations used are: NGF, nerve growth factor; BDNF, brain-derived neurotrophic factor; p75^{NTR}, p75-neurotrophin receptor; TNF, tumor necrosis factor; TRAF, tumor necrosis factor receptor-associated factor; TRADD, TNF receptor-associated death domain protein; CMV, cytomegalovirus; PBS, phosphate-buffered saline.

ondary antibodies were from Sigma. The mouse monoclonal anti-Myc antibody was from Jackson Laboratories. Polyclonal goat anti-rabbit IgG and donkey anti-goat IgG, both coupled to Alexa 568, and polyclonal goat anti-mouse IgG coupled to Alexa 488 were purchased from Molecular Probes, Inc. (Eugene, OR). Full-length TRADD cDNA or truncated cDNA lacking the sequence coding the death domain (TRADDΔDD:Δ195–312) were cloned into a PRK5-derived vector containing a Myc tag. To obtain the Myc tag (MASMEQKLISEEDLGIP), the oligonucleotides 5′-GGATGGCATCAATGGAGCAGAAGCTTATCTCAGAGGAAGATCTGGGAATACCGTGC-3′ and 5′-GGCCGCACGGTATTCCCAGATCTTCCTCTGAGATAAGCTTCTGCTCCATTGATGCCATCCTGCA-3′ were annealed and inserted in *Pst*I/*Not*I-digested PRK5 (Pharmingen). Human TRADD cDNA was obtained by PCR with the oligonucleotides 5′-TGCGGCCGCATGGCAGCTGGGCAAA-3′ and 5′-TGCGGCCGCTAGGCCAGGCCGCCATT-3′ and was cloned in pCRII-TOPO vector (Invitrogen). This insert was recovered after *Not*I digestion and was cloned in PRK5 tag-Myc digested by *Not*I. Truncated TRADD cDNA (TRADDΔDD:Δ195–312) was cloned in PRK5 tag-Myc vector with the same strategy. The oligonucleotides used were 5′-TGCGGCCGCATGGCAGCTGGGCAAA-3′ and 5′-GCGGCCGCGGCGGCGGCGGCTTCA-3′. A p75^{NTR} dominant negative, coding only the first 276 amino acids (corresponding to the extracellular and plasma membrane region) of human p75^{NTR} was cloned into an episomic vector under CMV promoter control; the membrane association has been assessed in Madin-Darby canine kidney cells using immunocytochemistry. The activation of the NF-κB pathway was assessed using a pNF-κB luciferase construct (Stratagene), which contains five tandem repeats of consensus NF-κB binding site sequence. The control was realized with pLuc-MCS from Stratagene. pCMVβ-galactosidase vector was from Clontech.

Cell Culture and Apoptosis Measurement—The MCF-7 breast cancer cell line was obtained from the American Type Culture Collection and routinely grown as monolayers. Cells were maintained in minimal essential medium (Earle's salts) supplemented with 20 mM Hepes, 2 g/liter sodium bicarbonate, 2 mM L-glutamine, 1% non-essential amino acids, 5 μg/ml insulin, 10% fetal calf serum, 40 units/ml penicillin/streptomycin, and 50 μg/ml gentamycin. Cells were grown at 37 °C in a humidified atmosphere of 5% CO_2.

Apoptosis of MCF-7 cells was induced by treatment with ceramide analogue C2 (10 μM for 24 h), previously described as a proapoptotic agent for human breast cancer cells (18, 19). For determination of the proportion of cells in apoptosis, cells were fixed with cold methanol (−20 °C) for 10 min and washed twice with phosphate-buffered saline (PBS) before staining with 1 μg/ml Hoechst 33258 for 10 min in the dark at room temperature. Cells were then washed with PBS and mounted using Glycergel (Dako). The apoptotic cells exhibiting condensed and fragmented nuclei were counted with an Olympus-BH2 fluorescence microscope over randomly selected fields. A minimum of 1000 cells were examined for each condition, and the results are expressed as a percentage of apoptotic cells observed of the total number of cells counted.

Cell Transfection—Transfection assays were carried out for 3 h in Opti-MEM transfection medium using ExGen 500 reagent, as described by the manufacturer. To analyze the interaction of TRADD with p75^{NTR} by Western blotting, MCF-7 cells were transfected with either 1 μg of PRK5 control vector or Myc-TRADD- or Myc-TRADDΔDD-carrying vector. Cells were then grown for 24 h in 10% fetal calf serum minimal essential medium, serum-rinsed (2 h), and then incubated with or without 200 ng/ml NGF for 24 h. To analyze NF-κB activation, cells were co-transfected with 0.2 μg of pCMVβ-galactosidase, 1.2 μg of NF-κB-luciferase reporter, and 0.5 μg of expression plasmids encoding either the wild type TRADD, the mutant TRADDΔDD, or the mutant p75^{NTR}. Cells were then grown in fetal calf serum minimal essential medium, serum-rinsed, and incubated in the presence of 10 μM C2 with or without 200 ng/ml NGF for another 24 h. For the study of the NGF antiapoptotic effect, MCF-7 cells were co-transfected with 0.6 μg of green fluorescent protein-carrying vector with either 0.8 μg of the PRK5 control vector or wild type TRADD- or dominant negative TRADDΔDD-carrying vector. Cells were fixed with paraformaldehyde 4% (4 °C, 30 min), and the percentage of apoptosis in green fluorescent protein-stained cells was determined as described above.

Immunoprecipitation and Western Blot Analysis—The interaction of TRADD with p75^{NTR} was assessed after NGF stimulation by immunoprecipitation and Western blotting. Time course analysis was monitored at 5, 10, 15, and 30 min; dose dependence was assessed for 5, 50, 100, and 200 ng/ml. The effect of other neurotrophins (BDNF, NT-3, and NT-4) was tested at concentrations of 50 and 100 ng/ml, after 10 min of stimulation. Cells were washed with PBS and lysed in the following buffer: 150 mM NaCl, 50 mM Tris, pH 7.5, 1% Nonidet P-40, 1 mM sodium orthovanadate, 1 mM phenylmethylsulfonyl fluoride, 1 mM sodium pyrophosphate, 10 μg/ml leupeptin and aprotinin, and 1 μg/ml pepstatin. Cell lysate (1.5 mg) was precleared with protein A-agarose (10 μl/250 μl, 60 min, 4 °C). After centrifugation (10,000 × g, 2 min), the supernatant was incubated with mouse monoclonal anti-p75^{NTR} (20 μl/500 μl) at 4 °C for 2 h. The immunocomplexes were precipitated with protein A-agarose beads (10 μl, 1 h, 4 °C), which were then pelleted by centrifugation (10,000 × *g*, 2 min). The pellet was rinsed three times with lysis buffer and boiled for 5 min in Laemmli buffer. After SDS-PAGE and electroblotting, nitrocellulose membranes (Schleicher & Schuell) were blocked with 3% bovine serum albumin. Membranes were then incubated with appropriate antibodies (1 μg/ml anti-p75^{NTR}, 1:500 anti-TRADD) overnight at 4 °C, rinsed, and incubated with appropriate secondary antibodies diluted at 1:500 for 2 h at room temperature. To assess the requirement of TRADD death domain interactions with p75^{NTR}, endogenous p75^{NTR} was immunoprecipitated from 1.5 mg of total protein extracted from MCF-7 cells transfected with 1 μg of Myc-tagged-TRADD, Myc-tagged-TRADDΔDD, or PRK5 empty vector. Immunoblotting was done as described above with 1:500 anti-Myc antibody. Membranes were washed extensively at room temperature, and the antibody complexes were visualized using the ECL system (Amersham Biosciences) with Eastman Kodak Co. X-Omat AR film.

Immunofluorescent Labeling and Confocal Microscopy—Cells, cultured onto glass coverslips coated with collagen, were treated for 15 min with NGF (200 ng/ml). Cells were washed twice with PBS and then fixed with PBS containing 4% paraformaldehyde for 30 min at room temperature. After washing with ammonium chloride (10 min at room temperature), cells were permeabilized with 0.05% saponin in PBS (10 min) and blocked for 10 min in permeabilizing buffer (PBS containing 2% BSA and 0.05% saponin). Cells were then successively incubated for 1 h, at room temperature, with primary antibodies (anti-p75 and then anti-TRADD). After five washes with permeabilizing buffer, cells were incubated with both the secondary antibodies (30 min at 37 °C at a 1:100 dilution). Cells were washed twice with permeabilizing buffer, twice with PBS plus 2% BSA, and then with PBS alone. Slides were mounted using Vectashield mounting medium (Vector Laboratories).

Fluorescence-stained slides were examined under a Leica (TCS NT) laser-scanning confocal microscope comprising a krypton/argon laser. Simultaneous two-channel recording was performed. Frame scanning was performed at ×1000 magnification, and a single optical section was collected per field.

NF-κB Activity Measurement—After 24 h of NGF treatment, transfected cells (TRADD, TRADDΔDD, PRK5, NF-κB-Luc, pLuc-MCS, and pCMVβ-galactosidase) were harvested with a reporter lysis buffer (Promega). NF-κB activity was determined using a luciferase assay kit (Promega) and measured with a luminometer (Lumat 9501; Berthold). The induction of NF-κB was calculated by assessing the luciferase expression by luminometer in cells expressing NF-κB promoter element construct or control vector (pLuc-MCS from Stratagene) lacking the NF-κB binding sequences. NF-κB activity was normalized with the pCMVβ control vector coding the β-galactosidase protein.

RESULTS

Interaction of TRADD with p75^{NTR}—To investigate possible interactions between p75^{NTR} and TRADD, MCF-7 cell lysates were prepared from NGF-treated cells followed by immunoprecipitation with p75^{NTR} antibody and Western blotting with TRADD antibody. The results (Fig. 1) indicate that TRADD co-immunoprecipitates with p75^{NTR} when cells have been stimulated with NGF. NGF concentration of 5–50 ng/ml was sufficient to induce the interaction between p75^{NTR} and TRADD; a clear dose-dependent effect was observed (Fig. 1*A*). A study in which TRADD immunoprecipitates were Western blotted with p75^{NTR}, was performed at 100 ng/ml NGF. The time course study (Fig. 1*B*) indicated that p75^{NTR}/TRADD interaction can be detected as soon as 5 min of NGF stimulation; the maximum effect was obtained after 10 min of stimulation. Other neurotrophins (BDNF, NT-3, NT-4) also mediate p75^{NTR}/TRADD interaction (Fig. 1*C*); 50 ng/ml was a sufficient dose for each tested neurotrophin. Confocal microscopy analysis revealed that TRADD shifted to a plasma membrane localization upon NGF stimulation (Fig. 1*D*). This co-localization was demon-

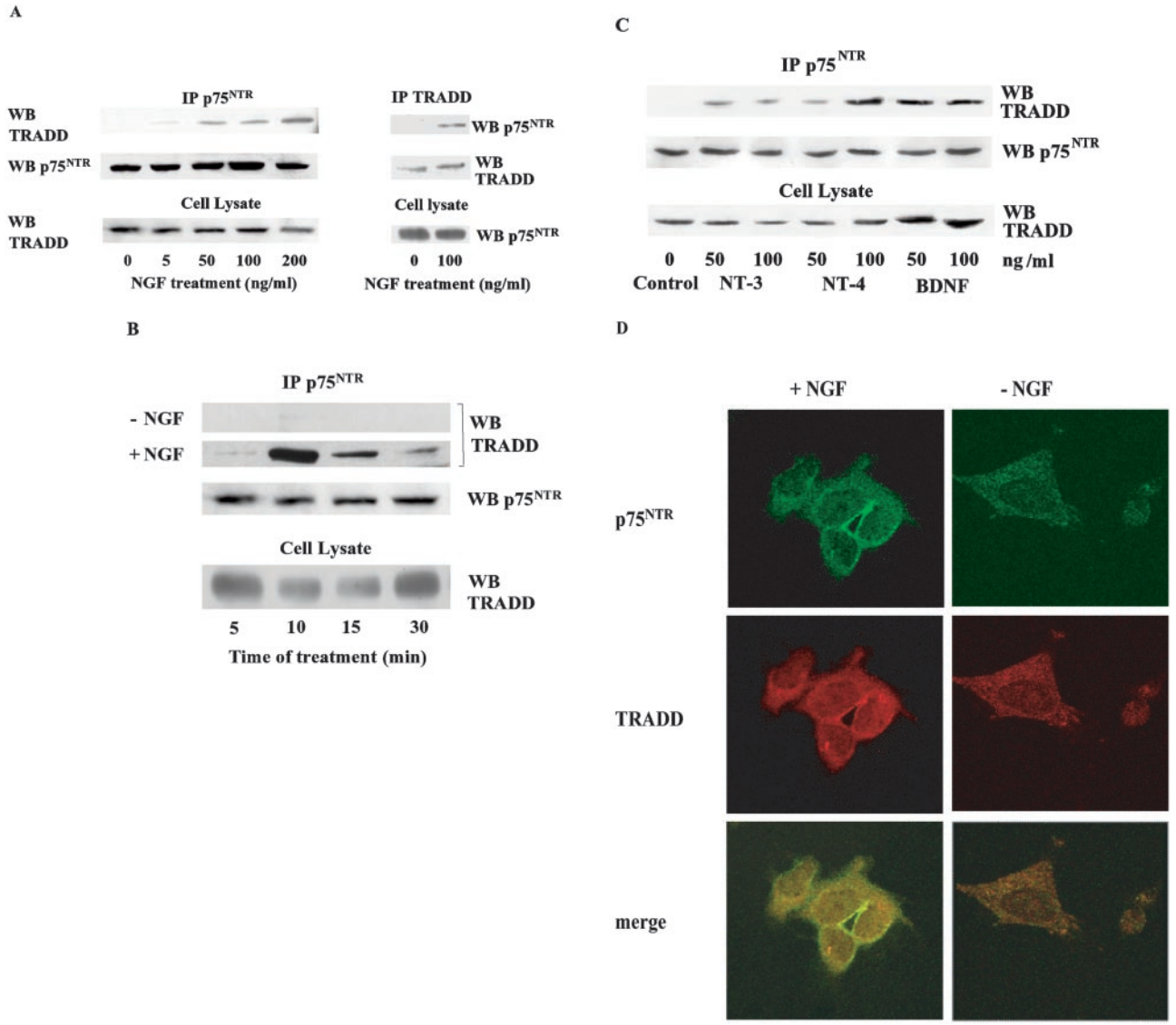

FIG. 1. **Interaction of TRADD with p75^{NTR}.** *A*, dose dependence. Immunoblot detection of TRADD associated with p75^{NTR} is shown. MCF-7 cells were cultured in 10% fetal calf serum-containing medium before serum starvation for 24 h. Cells were then stimulated or not with 5, 50, 100, and 200 ng/ml NGF for 10 min. Total proteins were extracted, 1.5 mg was immunoprecipitated with anti-p75^{NTR} (or anti-TRADD) and immunoblotted (Western blotting (*WB*)) against anti-p75^{NTR} and anti-TRADD. NGF stimulation enhanced the association of endogenous TRADD with endogenous p75^{NTR} receptor. Similar results were obtained in three separate experiments. *B*, time course of p75^{NTR}/TRADD interaction. Immunoprecipitation with anti- p75^{NTR} followed by immunoblot against anti-p75^{NTR} and anti-TRADD were performed after 5, 10, 15, and 30 min of stimulation by 100 ng/ml NGF. *C*, stimulation of p75^{NTR}/TRADD interaction by BDNF, NT-3, and NT-4. Immunoprecipitations and Western blotting were realized as described above. *D*, fluorescence confocal micrographs showing paraformaldehyde-fixed MCF-7 cells treated with or without NGF. Cellular localization of p75^{NTR} and TRADD was assessed using secondary antibodies directed against anti-p75^{NTR} and anti-TRADD coupled to Alexa 488 (*green*) and Alexa 568 (*red*), respectively. NGF induced a shift of TRADD to the plasma membrane.

strated by the appearance of a merged fluorescence at the plasma membrane. The nuclear translocation of TRADD has recently been reported (20), and in order to investigate this possibility in the case of NGF stimulation, a measurement of the intensity of staining for TRADD in both the cytoplasm and the nucleus has been performed using the LSM-Image Browser (Zeiss) software. Our results show that the ratio of nuclear to cytoplasmic staining does not vary significantly upon NGF stimulation (data not shown). Altogether, these results indicate a p75^{NTR} interaction with TRADD, which is regulated by NGF; in addition, no interaction between TRADD and TrkA was ever detected (data not shown). To further confirm the p75^{NTR}/TRADD interaction, MCF-7 cells were transfected with a vector coding Myc-TRADD or Myc-TRADDΔDD, the latter a dominant negative form lacking the death domain (Fig. 2*A*), and the association was determined as above. The results (Fig. 2*B*) demonstrated that the association between p75^{NTR} and TRADD requires the death domain of TRADD, since its deletion clearly impairs the association.

TRADD Is Required for p75^{NTR}-induced Activation of NF-κB—Stimulation of p75^{NTR} is known to activate the transcription factor NF-κB in breast cancer cells so that it translocates to the nucleus (12). Since TRADD is known to be a mediator of TNFR1-induced NF-κB activation (17), it was important to determine whether TRADD was also involved in the activation of NF-κB mediated through p75^{NTR}. The results (Fig. 3) demonstrated that TRADD wild type control vector strongly enhanced the NGF-mediated induction of NF-κB. Furthermore, this induction was inhibited by p75^{NTR} dominant negative (lacking the cytoplasmic tail) or by TRADDΔDD dominant negative, thus demonstrating the involvement of TRADD in the activation of NF-κB in breast cancer cells.

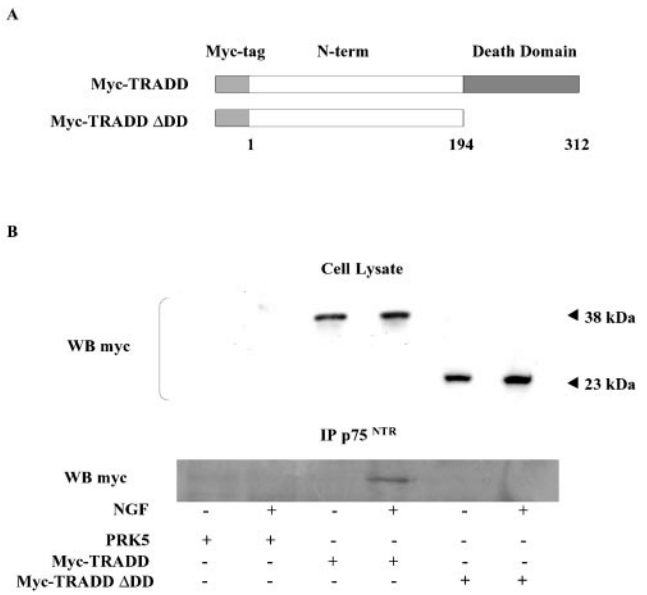

FIG. 2. **p75^{NTR}/TRADD interaction requires the death domain of TRADD.** Subconfluent MCF-7 cells were transiently transfected with 1 μg of expression plasmid coding several constructs (*A*): Myc-tagged TRADD, Myc-tagged TRADDΔDD, or control vector PRK5. Transfection was followed by stimulation with NGF (200 ng/ml) for 24 h before immunoprecipitation of endogenous p75^{NTR} from 1.5 mg of cell lysate and immunoblot against anti-Myc (*B*). Control to assess the levels of expression of the transfected products was performed with anti-Myc immunoblot of total cell lysate.

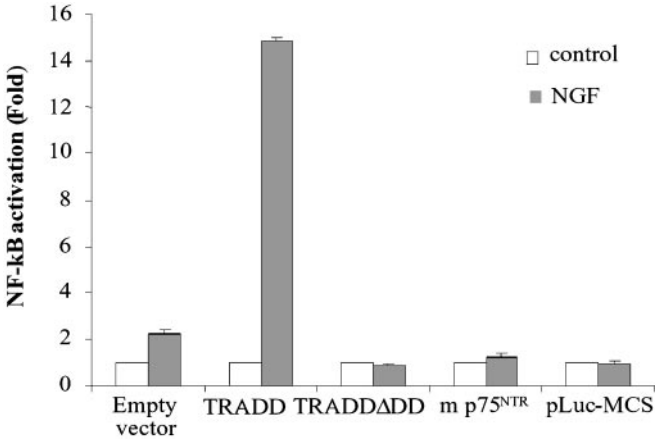

FIG. 3. **TRADD is required for NF-κB activation by NGF.** MCF-7 cells were co-transfected with NF-κB-luciferase, β-galactosidase reporters, and 0.5 μg of expression plasmids encoding either wild type TRADD or the mutant TRADDΔDD lacking the death domain, the dominant negative p75^{NTR} (*m p75^{NTR}*), or the corresponding empty vector. A control luciferase vector (pLuc-MCS) lacking the NF-κB binding site was also used. After 24 h, cells were serum-starved and treated with or without 200 ng/ml NGF, in the presence of 10 μM ceramide C2, for 24 h. The effects of ectopically expressed proteins are assessed as induced NF-κB-luciferase normalized for β-galactosidase activity. The induction of NF-κB was calculated by assessing the luciferase expression in cells expressing NF-κB promoter element construct or control vector (pLuc-MCS) lacking the NF-κB binding sequences. NF-κB activity was normalized with the pCMVβ-galactosidase control vector. Results are expressed as the means ± S.D. of five separate experiments.

TRADD Is Required for p75^{NTR}-mediated Antiapoptotic Effect of NGF—We have previously shown that p75^{NTR}-mediated activation of NF-κB results in the survival of breast cancer cells (12). We show here that transfection with TRADDΔDD prevents the antiapoptotic effect of NGF in MCF-7 breast cancer cells (Fig. 4*A*). However, the antiapoptotic activity of NGF was not totally inhibited with TRADDΔDD, since transfection with a vector coding a mutated form of IκB suggests that TRADD is

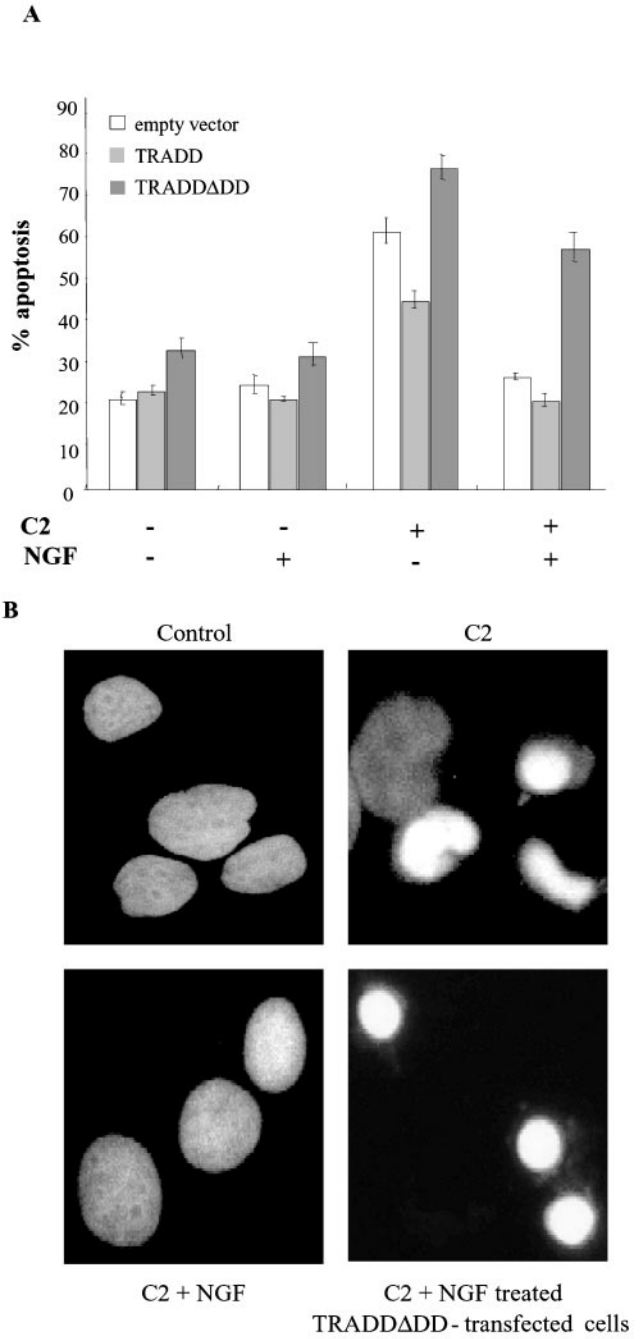

FIG. 4. **TRADD is required for p75^{NTR}-mediated antiapoptotic effect of NGF.** *A*, apoptosis was assessed in MCF-7 cells transiently cotransfected with green fluorescent protein-carrying vector along with either expression plasmid for wild type TRADD or dominant negative TRADΔDD. 24 h after transfection, cells were serum-starved and treated with 10 μM of C2 with or without 200 ng/ml NGF for another 24-h period. Control consists of MCF-7 cells transfected with PRK5 control vector. *B*, Hoechst staining of cell nuclei in control, C2, C2 + NGF-treated MCF-7, and C2 + NGF-treated TRADΔDD-transfected cells. Cells were serum-starved and treated with C2 + NGF as described above. Apoptosis was monitored 24 h after transfection, using the Hoechst 33258 staining as described under "Experimental Procedures." Results are expressed as the means ± S.D. of five separate experiments.

not the only intermediate associating with p75^{NTR} to activate NF-κB. In contrast, transfection with TRADD enhances the antiapoptotic effect of NGF, confirming the involvement of TRADD in p75^{NTR}-mediated survival activity. The morphology of cell nuclei observed after Hoescht staining is shown in Fig. 4*B*.

DISCUSSION

We have shown here for the first time that nerve growth factor stimulation of the common neurotrophin receptor p75^{NTR} results in the recruitment of TRADD. It has previously been shown that p75^{NTR} interacts with TRAF protein family members (15, 21) and that this interaction modulates p75^{NTR}-induced cell death and NF-κB activation with contrasting effects (15). TRAF4 inhibited the NF-κB response, whereas TRAF2 and TRAF6 enhanced p75^{NTR}-induced NF-κB activation. However, these experiments were carried out in immortalized human epithelial kidney HEK 293T cells in which p75^{NTR} stimulation by NGF resulted in cell death induction. In contrast, for breast cancer cells, p75^{NTR} stimulation by NGF has been shown to be antiapoptotic and mediated by the nuclear translocation of NF-κB (12). TRADD is a multifunctional intracellular signaling adaptor protein that is recruited by TNFR1, leading to IKK activation and so to NF-κB nuclear translocation (22). Our results indicate that, in breast cancer cells, TRADD is also recruited by p75^{NTR}, resulting in an activation of NF-κB, similar to the signaling initiated by TNFR1. Cell survival induced by NGF was observed from 50 ng/ml, and the maximum effect was obtained at 200 ng/ml, providing a coherence between the p75^{NTR}/TRADD interaction and the biological effect of NGF in breast cancer cells. In addition, it should be emphasized that our experiments were performed without transfection of p75^{NTR} or TRADD, strengthening the physiological relevance of the p75^{NTR}/TRADD interaction in breast cancer cells.

Two intracellular domains have been identified within p75^{NTR}. The first one is homologous to the binding domain for TRAF proteins, and the second is homologous but distinct from the death domain of TNFR1 (23). TRADD death domain has been reported to bind to the death domain of TNFR1 (17). In our experiments, the death domain of TRADD was required for interaction with p75^{NTR}, suggesting that the death domain of p75^{NTR} is involved in TRADD binding. Therefore, the activation of p75^{NTR} and TNFR1 appears to involve a similar mechanism of interaction with TRADD, further suggesting similar downstream signaling pathways. Interestingly, it has been shown by NMR spectroscopy and crystal structure determination that TRAF2 has a higher affinity for TRADD than it has for TNFR1 itself (24, 25). TRAF2 has been shown to interact with p75^{NTR}, an interaction we have confirmed in breast cancer cells (data not shown); these data suggest that TRADD linked to p75^{NTR} might interact with TRAF2. However, further structural analyses are required to define the precise molecular mechanism involved in TRADD interaction with p75^{NTR}.

The role of NF-κB activation is well established for the survival of breast cancer cells, and its involvement in the antiapoptotic effect of NGF is clearly related to p75^{NTR} signaling (12). TRADD is a potent activator of the IκB kinase, IKK, which leads to the ubiquitination/degradation of IκB and nuclear translocation of the free NF-κB (17). We have shown that transfection of breast cancer cells with a dominant negative form of TRADD resulted in an inhibition of the NGF-mediated antiapoptotic effect, demonstrating the involvement of TRADD in the NF-κB-mediated survival pathway. The trophic function of NGF for breast cancer cells, involving both mitogenic and antiapoptotic activities, has recently been shown (8, 12). The prognostic value of both p75^{NTR} and TrkA receptors has also secondarily been reported (26), as has the possibility of targeting p140^{TrkA}-mediated mitogenic signaling with tamoxifen, a drug commonly used in breast cancer therapy (27). Our present data also suggest that targeting the p75^{NTR}/TRADD/NF-κB signaling pathway may be a promising avenue for future treatment of breast cancer.

In conclusion, the findings reported here provide new insights into the proximal elements of the p75^{NTR} signaling pathway by demonstrating the involvement of TRADD as a critical intermediary for NF-κB activation. In immortalized striatal neurons, p75^{NTR} was found to induce apoptosis without the participation of TRADD as an adaptor protein (28). Whether or not the interaction of TRADD with p75^{NTR} provides a more specific pathway for NGF-induced antiapoptotic activity remains to be determined, but TRADD should now be considered as a significant influence in the balance between proapoptotic and antiapoptotic signaling pathways initiated by p75^{NTR}.

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