Nerve Growth Factor Stimulates Proliferation and Survival of Human Breast Cancer Cells through Two Distinct Signaling Pathways*

Received for publication, November 20, 2000, and in revised form, February 14, 2001

Published, JBC Papers in Press, February 28, 2001, DOI 10.1074/jbc.M010499200

Simon Descamps‡‡, Robert-Alain Toillon‡, Eric Adriaenssens‡, Valérie Pawlowski‡‡, Simon M. Cool*‡, Victor Nurcombe**, Xuefen Le Bourhis‡, Bénôni Boilly‡, Jean-Philippe Peyrat‡, and Hubert Hondermarck‡‡ ‡‡

From the §Équipe Facteurs de Croissance, UPR EA-1033 Biologie du Développement, Université des Sciences et Technologies de Lille, 59655 Villeneuve d’Ascq France, the ¶Immunopathologie Cellulaire des Maladies Infectieuses, CNRS, UMR 8527, Institut de Biologie de Lille, 59000 France, the **Department of Anatomical Sciences, University of Queensland, St. Lucia, Queensland 4072, Australia, and the †Laboratoire d’Oncoologie Moléculaire Humaine, Centre Oscar Lambret, 59020 Lille, France

We show here that the neurotrophin nerve growth factor (NGF), which has been shown to be a mitogen for breast cancer cells, also stimulates cell survival through a distinct signaling pathway. Breast cancer cell lines (MCF-7, T47-D, BT-20, and MDA-MB-231) were found to express both types of NGF receptors: p140trkA and p75NTR. The two other tyrosine kinase receptors for neurotrophins, TrkB and TrkC, were not expressed. The mitogenic effect of NGF on breast cancer cells required the tyrosine kinase activity of p140trkA as well as the mitogen-activated protein kinase (MAPK) cascade, but was independent of p75NTR. In contrast, the anti-apoptotic effect of NGF (studied using the ceramide analogue C2) required p75NTR as well as the activation of the transcription factor NF-kB, but neither p140trkA nor MAPK was necessary. Other neurotrophins (BDNF, NT-3, NT-4/5) also induced cell survival, although not proliferation, emphasizing the importance of p75NTR in NGF-mediated survival. Both the pharmacological NF-κB inhibitor SN50, and cell transfection with IkB, resulted in a diminution of NGF anti-apoptotic effect. These data show that two distinct signaling pathways are required for NGF activity and confirm the roles played by p75NTR and NF-κB in the activation of the survival pathway in breast cancer cells.

Nerve growth factor (NGF) is the archetypal member of the neurotrophin superfamily, which also includes brain-derived neurotrophic factor (BDNF), neurotrophin-3 (NT-3), NT-4/5, and NT-6. NGF interacts with two classes of membrane receptor: the TrkA proto-oncogene product p140trkA, which possesses intrinsic tyrosine kinase activity, and a secondary receptor, p75NTR, that belongs to the tumor necrosis factor (TNF) receptor family. The stimulation of cell survival and cell differentiation by NGF and other neurotrophins have been described primarily in neuronal cell systems. Although the neurotrophic effect through p140trkA is known to involve the MAPK cascade, the role of p75NTR is still controversial; there is evidence that it can both positively and negatively regulate neuronal cell death and differentiation, depending on the cell type examined. In some cases, p75NTR is an inducer of apoptosis, even without NGF stimulation, whereas in other cases the activation of p75NTR by NGF results in a protection from cell death. In addition to its neurotrophic function, other activities of NGF have been described. For example, NGF can modulate gene expression in monocytes, it is chemotactic for melanocytes, and its inhibition on p75NTR can block the migration of Schwann cells. NGF also stimulates the proliferation of chromaffin cells, lymphocytes, and keratinocytes. We have previously shown that NGF is mitogenic for cancerous but not normal human breast cells, and these data, as well as others showing a role for NGF in the stimulation of prostatic cancer cells, implicates NGF in non-neuronal carcinogenesis.

Both cellular proliferation as well as tumor cell survival are crucial for malignant progression. The effect of NGF on the survival of cancer cells through the p75NTR receptor has been shown for neuroblastoma (18) and schwannoma (6). In prostate cancer, p75NTR has been shown to be a mediator of NGF’s effects during critical phases of developmental cell death and carcinogenic progression (19). To date only the mitogenic effect of NGF for breast cancer cells has been described (13), with its roles in the control of breast cancer cell survival unknown.

In this study, we have shown that, in addition to its mitogenic effect, NGF is also an anti-apoptotic factor for breast cancer cells. These cells express mRNA for both p140trkA and p75NTR receptors. Our results indicate that the mitogenic effect of NGF requires p140trkA and the MAPK cascade, but not the p75NTR receptor, whereas the promotion of cell survival strictly requires p75NTR as well as NF-κB, but not p140trkA and MAPK. Thus the mitogenic and anti-apoptotic effects of NGF on breast cancer cells are mediated through two distinct signaling pathways.
Intracellular Signaling Pathways of NGF in Breast Cancer Cells

17865

cancer cells are mediated through two different signaling pathways.

EXPERIMENTAL PROCEDURES

Materials—Cell culture reagents were purchased from BioWhittaker (France) except insulin, which was obtained from Organon (France). Recombinant human nerve growth factor, brain derived growth factor (BDNF), and neurotrophins 3 (NT-3) and 4 (NT-4) were from R & D Systems (UK). K-252a (inhibitor of trk-tyrosine kinase activity) and PD98059 (inhibitor of MAPK cascade) were from Calbiochem (France). The mouse monoclonal anti-NF receptor (p75<sup>TR</sup>) antibody was from Eurodemed (France) and was previously described for its ability to block the interaction between p75<sup>TR</sup> and NGF (20). The anti-lamin B (C-20), goat polyclonal IgG, and the polyclonal anti-p140<sup>Src</sup>-kinase (trk763) were from Santa Cruz Biotechnology. C2 cereamide analogue (N-acetyl-d-sphingosine), Hoechst 33258, and electrophoresis reagents were from Sigma (St. Louis, MO, USA). Mouse monoclonal anti-NGF receptor (p75NTR) antibody was from Santa Cruz Biotechnology. NT-2 (Ntera/D1) human neural precursor cells in minimal essential medium (Earle’s salts) supplemented with 20 mM L-glutamine were purchased (13). 35-mm diameter dishes were inoculated with 2 × 10<sup>5</sup> cells/dish in 2 ml of medium containing 10% FCS. After 24 h, cells were washed twice with serum-free medium. Next day, the medium was replaced with 2 ml of serum-free medium containing 100 ng/ml NGF or various concentrations of other neurotrophins (BDNF, NT-3, NT-4/5). To study the effect of pharmacological inhibitors or blocking antibodies, various concentrations were added simultaneously with NGF (100 ng/ml). After 2 days of NGF exposure, cells were harvested by trypsinization and counted using a hemocytometer.

Determination of the Percentage of Apoptotic Cell Nuclear—Apoptosis of breast cancer cells was induced by the ceramide analogue C2, which has been described as a pro-apoptotic agent for human breast cancer cells (21, 22). Apoptosis was obtained by treatment with 2 μM C2 for 24 h. To quantify the percentage of apoptotic cells, the TdT-medi-ated dUTP nick end labeling (TUNEL) assay was performed as described by Herrmann et al. (23). Cells were then incubated with monoclonal anti-NGF receptor (p75NTR) antibody (10 μg/ml), and then pelleted by centrifugation (10,000 × g, 2 min). The pellet was then resuspended in ice-cold hypotonic buffer (10 mM Hepes, pH 7.5, 0.1% SDS, 100 μM diithiobis (2-mercaptoethanol) and boyled 5 min. The reaction was stopped by the addition of an equal volume of 2.5 M sodium carbonate, 2 mM EDTA, 0.1% SDS, and 0.1% Triton X-100. After 30 min on ice with frequent agitation, the insoluble fraction was pelleted by centrifugation at 10,000 × g for 10 min. The supernatant was replaced with 2 ml of 10% sucrose containing 100 ng/ml NGF or various concentrations of other neurotrophins (BDNF, NT-3, NT-4/5). The reaction mixture was then added to the pellet for 24 h. After centrifugation at 10,000 × g for 10 min, the supernatant was replaced with 2 ml of serum-free medium containing 100 ng/ml NGF or various concentrations of other neurotrophins (BDNF, NT-3, NT-4/5). To study the effect of pharmacological inhibitors or blocking antibodies, various concentrations were added simultaneously with NGF (100 ng/ml). After 2 days of NGF exposure, cells were harvested by trypsinization and counted using a hemocytometer.

NGF Receptors and PARP Immunoblotting—Subconfluent cell cultures were harvested by scraping in serum-free medium. After centrifugation (1000 × g, 5 min), the pellet was treated with lysis buffer (0.3% SDS, 1% Triton X-100, 0.1% SDS, 100 μM diithiobis (2-mercaptoethanol) and boyled 5 min. The reaction was stopped by the addition of an equal volume of 2.5 M sodium carbonate, 2 mM EDTA, 0.1% SDS, and 0.1% Triton X-100. After 30 min on ice with frequent agitation, the insoluble fraction was pelleted by centrifugation at 10,000 × g for 10 min. The pellet was then resuspended in ice-cold hypotonic buffer (10 mM Hepes, pH 7.5, 0.1% SDS, 100 μM diithiobis (2-mercaptoethanol) and boyled 5 min. The reaction was stopped by the addition of an equal volume of 2.5 M sodium carbonate, 2 mM EDTA, 0.1% SDS, and 0.1% Triton X-100. After 30 min on ice, the supernatant was replaced with 2 ml of serum-free medium containing 100 ng/ml NGF or various concentrations of other neurotrophins (BDNF, NT-3, NT-4/5). To study the effect of pharmacological inhibitors or blocking antibodies, various concentrations were added simultaneously with NGF (100 ng/ml). After 2 days of NGF exposure, cells were harvested by trypsinization and counted using a hemocytometer.

Determination of the Percentage of Apoptotic Cell Nuclear—Apoptosis of breast cancer cells was induced by the ceramide analogue C2, which has been described as a pro-apoptotic agent for human breast cancer cells (21, 22). Apoptosis was obtained by treatment with 2 μM C2 for 24 h. To quantify the percentage of apoptotic cells, the TdT-mediated dUTP nick end labeling (TUNEL) assay was performed as described by Herrmann et al. (23). Cells were then incubated with monoclonal anti-NGF receptor (p75NTR) antibody (10 μg/ml), and then pelleted by centrifugation (10,000 × g, 2 min). The pellet was then resuspended in ice-cold hypotonic buffer (10 mM Hepes, pH 7.5, 0.1% SDS, 100 μM diithiobis (2-mercaptoethanol) and boyled 5 min. The reaction was stopped by the addition of an equal volume of 2.5 M sodium carbonate, 2 mM EDTA, 0.1% SDS, and 0.1% Triton X-100. After 30 min on ice, the supernatant was replaced with 2 ml of serum-free medium containing 100 ng/ml NGF or various concentrations of other neurotrophins (BDNF, NT-3, NT-4/5). To study the effect of pharmacological inhibitors or blocking antibodies, various concentrations were added simultaneously with NGF (100 ng/ml). After 2 days of NGF exposure, cells were harvested by trypsinization and counted using a hemocytometer.

NGF Receptors and PARP Immunoblotting—Subconfluent cell cultures were harvested by scraping in serum-free medium. After centrifugation (1000 × g, 5 min), the pellet was treated with lysis buffer (0.3% SDS, 1% Triton X-100, 0.1% SDS, 100 μM diithiobis (2-mercaptoethanol) and boyled 5 min. The reaction was stopped by the addition of an equal volume of 2.5 M sodium carbonate, 2 mM EDTA, 0.1% SDS, and 0.1% Triton X-100. After 30 min on ice, the supernatant was replaced with 2 ml of serum-free medium containing 100 ng/ml NGF or various concentrations of other neurotrophins (BDNF, NT-3, NT-4/5). To study the effect of pharmacological inhibitors or blocking antibodies, various concentrations were added simultaneously with NGF (100 ng/ml). After 2 days of NGF exposure, cells were harvested by trypsinization and counted using a hemocytometer.

Determination of the Percentage of Apoptotic Cell Nuclear—Apoptosis of breast cancer cells was induced by the ceramide analogue C2, which has been described as a pro-apoptotic agent for human breast cancer cells (21, 22). Apoptosis was obtained by treatment with 2 μM C2 for 24 h. To quantify the percentage of apoptotic cells, the TdT-mediated dUTP nick end labeling (TUNEL) assay was performed as described by Herrmann et al. (23). Cells were then incubated with monoclonal anti-NGF receptor (p75NTR) antibody (10 μg/ml), and then pelleted by centrifugation (10,000 × g, 2 min). The pellet was then resuspended in ice-cold hypotonic buffer (10 mM Hepes, pH 7.5, 0.1% SDS, 100 μM diithiobis (2-mercaptoethanol) and boyled 5 min. The reaction was stopped by the addition of an equal volume of 2.5 M sodium carbonate, 2 mM EDTA, 0.1% SDS, and 0.1% Triton X-100. After 30 min on ice, the supernatant was replaced with 2 ml of serum-free medium containing 100 ng/ml NGF or various concentrations of other neurotrophins (BDNF, NT-3, NT-4/5). To study the effect of pharmacological inhibitors or blocking antibodies, various concentrations were added simultaneously with NGF (100 ng/ml). After 2 days of NGF exposure, cells were harvested by trypsinization and counted using a hemocytometer.

Determination of the Percentage of Apoptotic Cell Nuclear—Apoptosis of breast cancer cells was induced by the ceramide analogue C2, which has been described as a pro-apoptotic agent for human breast cancer cells (21, 22). Apoptosis was obtained by treatment with 2 μM C2 for 24 h. To quantify the percentage of apoptotic cells, the TdT-mediated dUTP nick end labeling (TUNEL) assay was performed as described by Herrmann et al. (23). Cells were then incubated with monoclonal anti-NGF receptor (p75NTR) antibody (10 μg/ml), and then pelleted by centrifugation (10,000 × g, 2 min). The pellet was then resuspended in ice-cold hypotonic buffer (10 mM Hepes, pH 7.5, 0.1% SDS, 100 μM diithiobis (2-mercaptoethanol) and boyled 5 min. The reaction was stopped by the addition of an equal volume of 2.5 M sodium carbonate, 2 mM EDTA, 0.1% SDS, and 0.1% Triton X-100. After 30 min on ice, the supernatant was replaced with 2 ml of serum-free medium containing 100 ng/ml NGF or various concentrations of other neurotrophins (BDNF, NT-3, NT-4/5). To study the effect of pharmacological inhibitors or blocking antibodies, various concentrations were added simultaneously with NGF (100 ng/ml). After 2 days of NGF exposure, cells were harvested by trypsinization and counted using a hemocytometer.
facturer. Briefly, MCF-7 cells were incubated for 48 h in Opti-MEM transfection medium containing 8 μl of Lipofectin reagent, 0.8 μg of green fluorescence protein (GFP)-carrying vector and 0.2 μg of empty vector PCDNA3 or 0.2 μg of IkBm. In the case of c-rel or rel-A, cells were cotransfected with 0.8 μg of GFP-carrying vector and 0.6 μg of PSVK3 (empty plasmid), c-rel, or rel-A. Cells were then grown for 24 h with 10% FCS minimal essential medium and rinsed for 2 h in serum-free medium before incubation in serum-free medium in the presence or absence of 100 ng/ml NGF and/or 2 μM C2 for another 24 h. Cells were then fixed with paraformaldehyde 4% (4 °C) for 30 min, and the percentage of apoptotic cell nuclei in GFP-stained cells was determined as described above.

RESULTS

NGF Mitogenic and Anti-apoptotic Activity for Breast Cancer Cells—The effects of 100 ng/ml NGF on cell proliferation and C2-induced apoptosis were evaluated by cell counting and Hoechst staining, respectively. The results show that NGF induces an increase in cell number for all breast cancer cell lines tested (Fig. 1A). We have previously demonstrated that NGF has a direct mitogenic effect on breast cancer cells by recruiting cells in G0 phase and by shortening the G1 length (Descamps et al., 1998). In addition, NGF rescued breast cancer cells undergoing C2-induced apoptosis; the maximum survival was observed at 200 ng/ml NGF (Fig. 1B). The morphology of cells undergoing this NGF-induced anti-apoptotic rescue was quite distinct (Fig. 2A). The induction of apoptosis by C2 was found to involve cleavage of poly(A)DP-ribose polymerase (PARP); this cleavage was reversed by NGF (Fig. 2B).

TrkA and p75NTR Expression—RT-PCR was used to show the expression of mRNA for both high and low affinity NGF recep-

Fig. 1. Effect of NGF on the growth and survival of breast cancer cells. A, breast cancer cells were serum-deprived in minimum essential medium, and after 24 h the NGF (100 ng/ml) was added. After 48 h, cells were harvested and counted. B, cells were serum-deprived in minimum essential medium and treated with 2 μM C2 with or without 100 ng/ml NGF. After 24 h, cells were counted and the proportion of apoptotic nuclei was determined after Hoechst staining under an Olympus-BH2 fluorescence microscope. For measurement of both cell number and apoptosis, results are expressed as the means ± S.D. of five separate experiments. Significance was determined using the Tukey’s test (*, p < 0.01).

Fig. 2. Anti-apoptotic effect of NGF. A, Hoechst staining of apoptotic cell nuclei in control, C2 and C2+NGF-treated MCF-7 cells. Cells were serum-deprived in minimum essential medium and treated with C2. NGF was added at 100 ng/ml. After 24 h, cells were counted and the proportion of apoptotic nuclei was determined after Hoechst staining. B, immunoblot detection of PARP cleavage. C2-induced PARP cleavage was reversed by p75NTR activation mediated by NGF. MCF-7 cells were serum-deprived in minimum essential medium for 24 h and were then treated with 100 ng/ml NGF in the presence or absence of 2 μM C2, 10 nM K-252a, 10 μM PD98059, or 10 μg/ml anti-p75NTR-blocking antibody (Euromedex) for another 24-h period. Proteins were detected after SDS-PAGE of cell preparations from MCF-7 breast cancer cells, electroblotting onto nitrocellulose, and immunodetection with anti-PARP antibodies.
it has been shown before that the level of a given cellular protein cannot be simply deduced from mRNA transcript level (24). One could hypothesize that the stability of mRNA and/or protein for NGF receptors, differs between breast cancer cells and neuroblastoma cells, leading to the observed disproportionality between mRNA and protein levels.

**Involvement of p140<sub>TrkA</sub> and p75<sub>NTR</sub> in Mitogenic and Survival Activities of NGF**—We used a combination of specific antibodies and pharmacological inhibitors to study the putative functions of p140<sub>TrkA</sub> and p75<sub>NTR</sub> in the stimulation of proliferation and cell survival induced by NGF. The Trk tyrosine kinase inhibitor K-252a, and the MEK inhibitor PD98059, both strongly inhibited the growth-stimulatory effect of NGF on MCF-7 cells, but had no effect on its anti-apoptotic effects (Fig. 4). Conversely, neither the anti-p75<sub>NTR</sub> blocking antibody nor the NF-κB inhibitor SN50 affected NGF-stimulated proliferation, although both strongly reduced the anti-apoptotic effects (Fig. 4). The tyrosine kinase activity of p140<sub>TrkA</sub> was inhibited by K-252a but not by the anti-p75<sub>NTR</sub> or PD98059 (Fig. 5). On the other hand, the activity of the MAPKs was inhibited by K-252a and PD98059 but not by the anti-p75<sub>NTR</sub> (Fig. 5). It should be noted that the SN50 peptidic inhibitor of NF-κB, similarly to the anti-p75<sub>NTR</sub> inhibited the anti-apoptotic effect of NGF but neither its proliferative effect nor its activation of p140<sub>TrkA</sub> and MAPKs. The effect of other neurotrophins on MCF-7 cell growth and survival was also evaluated (Fig. 6A). In contrast to NGF, no proliferative effect was provided by BDNF, NT-3, or NT-4/5. However, all neurotrophins tested exhibited a rescue effect on C2-treated cells that was not altered in the presence of the trk inhibitor K-252a (Fig. 6B). These data suggest that trk receptors are not involved in NGF survival activity. Moreover, the participation of trkB and trkC in these events can be ruled out, because they are not expressed in these breast cancer cells (Fig. 6C).

**NF-κB Involvement in the Anti-apoptotic Effect of NGF**—The inhibitory effect of SN50 on the NGF anti-apoptotic activity indicated the potential involvement of NF-κB in the signaling leading to the protective activity of this growth factor. To further investigate this phenomenon, we studied the effect of NGF on the nuclear translocation of NF-κB, as well as the consequence of transfection by IkBm (an inhibitor of NF-κB) or by c-rel and rel-A (constitutively active subunits of NF-κB) on the NGF-mediated anti-apoptotic activity in MCF-7 cells. Western blotting revealed no change in the nuclear levels of NF-κB (p65) during apoptosis induced by C2 (Fig. 7). In contrast, the addition of NGF on C2-treated cells induced a translocation of NF-κB from cytoplasm to nucleus. Computerized quantification revealed a doubling p65 band intensity normalized to the total intensity of the lane (data not shown). Moreover, this NF-κB nuclear translocation was inhibited by the presence of p75<sub>NTR</sub>-blocking antibody or SN50, but was not affected by K-252a and PD98059. Interestingly, in the absence of C2-induced apoptosis NGF was not able to induce the nuclear translocation of NF-κB, confirming previous observations that p75<sub>NTR</sub>-mediated NF-κB activation requires cell stress (25). Transfection of MCF-7 cells with IkBm, an inhibitor of NF-κB, reversed the anti-apoptotic effect of NGF (Fig. 8A). As a control, we transfected MCF-7 cells with an empty vector; no effect was observed. In addition, transfection with activators of the NF-κB pathway, c-rel or rel-A (Fig. 8B), resulted in an inhibition of C2-induced apoptosis of MCF-7 cells, even in absence of NGF,
confirming the involvement of NF-κB family members in human breast cancer cell survival.

DISCUSSION

This study shows that, in addition to its mitogenic activity, NGF is anti-apoptotic for breast cancer cells, and that these two biological effects are differentially mediated by the p140<sup>TrkA</sup> and p75<sup>NTR</sup> receptors, respectively. The growth of breast cancer results from a balance between cell proliferation and apoptosis, both of which can be modulated by various regulatory peptides. For example, epidermal growth factor, fibroblast growth factors, and insulin-like growth factor-1 can all stimulate the proliferation and survival of breast cancer cells (26). On the other hand, agents such as transforming growth factor-β or tumor necrosis factor-α can inhibit growth and induce apoptosis in these cells (27). Recently we have shown that NGF, which was primarily described for its neurotrophic properties, is a strong mitogen for cancerous but not for normal human breast epithelial cells, suggesting a crucial function for this factor in the initiation and progression of human breast tumors (13). In the present study, we have shown that the breast cancer cells express transcripts for both TrkA and p75<sup>NTR</sup> receptors. In contrast, no expression of TrkB and TrkC was found in any of the breast cancer cells tested, in accordance with the fact that BDNF, NT-3, or NT-4/5 have no mitogenic effect for these cells. The presence of NGF receptors has been detected previously in breast cancer cells (28), and low levels of NGF receptor expression have recently been reported in other breast cancer cell lines (29), leading to the hypothesis of a recruitment and cooperation between p140<sup>TrkA</sup> and p185<sup>Her-2</sup> for the induction of mitogenesis by NGF. Our results indicate a stimulation of p140<sup>TrkA</sup> tyrosine kinase activity and of the MAPK cascade by NGF, and the use of the pharmacological inhibitors K-252a and PD98059 demonstrate the requirement for these signals in NGF-induced MCF-7 cell proliferation. The induction of MAPK activity required p140<sup>TrkA</sup> activation, but p75<sup>NTR</sup> did not appear to be involved, because p75<sup>NTR</sup>-blocking antibodies did not have any effect on NGF-induced MAPK.
activation and cell proliferation. In contrast, p75NTR-blocking antibodies exhibited an inhibition of NGF-induced survival, attesting to the functionality of these blocking antibodies. Thus, the mitogenic activity of NGF requires the p140ptk2 MAPK cascade independently of the p75NTR receptor. This signaling pathway for the NGF-proliferative effect appears to be similar to that which is described for the neurotrophic activity of NGF in neurons in both in vitro and in vivo (32). However, the intracellular signaling involved in the anti-apoptotic activity of NGF in neurons remains controversial. The p140ptk2/MAPK cascade is generally described as protective for neuronal cell death, although there has been a recent report of a novel apoptotic pathway mediated by p140ptk2/MAPK in medulloblastoma cells (33). Unlike the p140ptk2 receptors, the definition of the precise physiological role of p75NTR has proven difficult (4). The p75NTR receptor belongs to the TNF-receptor family, including among others, types I and II of the TNF receptor, the Fas antigen, and CD40 (34). The common cellular responses to activation of this family of receptors are the activation of gene transcription via nuclear factor-kB (NF-kB) and the regulation of cell survival/apoptosis. In some cases apoptosis was shown to develop following NGF binding to p75NTR, although in other cases it appeared to occur in the absence of ligand (spontaneous apoptosis) and was reversed by NGF (35). The C2 reagent used here is known to induce apoptosis in breast cancer cells such as MCF-7 (21, 22). Morphological analysis after Hoechst staining and the inhibition of PARP cleavage demonstrated that NGF rescues breast cancer cells from C2-induced cell death. Interestingly, K-252a and PD98059 did not affect the anti-apoptotic activity of NGF, indicating that p140ptk2 tyrosine kinase and MAPK activities are not necessary for the protective effect. Previous reports have noted that NGF is able to elicit its biological effects through p75NTR receptors and independently of p140ptk2 in neurons (36, 37) and Schwann cells (38). In our experiments, a specific role for p75NTR in the cell survival effect was first suggested by the fact that other neurotrophins (interacting with p75NTR or with p75NTR) are also able to protect cells from death while having no impact on cell proliferation. The crucial role of p75NTR was further demonstrated by the use of p75NTR-blocking antibodies, which completely reversed the protective effect of NGF from C2-induced apoptosis. Moreover, BDNF, NT-3, and NT-4/5, all of which can bind p75NTR, can also stimulate breast cancer cell survival. Because TrkB and TrkC are not expressed in breast cancer cells, these data emphasize the role played by p75NTR in the anti-apoptotic effect of NGF. Activation of p75NTR specifically induces NF-kB independent of p140ptk2 in several cell types, including Schwann cells (38). To explore the involvement of NF-kB in the NGF survival effect, we first tested SN50, which inhibits the nuclear translocation of this transcription factor (39). We found that it blocked the anti-apoptotic effect of NGF without affecting the p140ptk2/MAPK cascade or cellular proliferation. The involvement of
NF-κB was further demonstrated by transfection with a mutated form of IkBa, which blocked NF-κB translocation to the nucleus. MCF-7 cells transfected by mutated IkBa were not rescued from C2-induced apoptosis by NGF, confirming the involvement of NF-κB in the anti-apoptotic activity mediated by p75
tn
. Similar observations have been made in PC12 cells in which the blocking of p75
tn
-mediated activation of NF-κB resulted in an enhancement of apoptosis (40). In addition, transfections by c-rel or rel-A, which are constitutively activated forms of NF-κB, had a protective effect on MCF-7 cells treated by C2 in absence of NGF stimulation. c-rel and rel-A belong to the NF-κB family of transcription factors. The protection from apoptosis observed after transfection with this factor emphasizes the role played by NF-κB molecules in the control of breast cancer cell survival.

In conclusion, our results demonstrate that NGF is an anti-apoptotic factor for human breast cancer cells and that the signaling pathway leading to this survival activity is distinct from the signaling pathway, which leads to mitogenic stimulation. Although p140trkA and the MAPKs mediate the mitogenic activity of NGF, its anti-apoptotic activity required p75
tn
 and NF-κB alone. NGF is present in the mammary gland (41, 42) as well as its transcripts, 2 and our present finding therefore emphasizes that NGF is a crucial regulator of mammary tumor growth. The inhibition of breast cancer progression through the targeting p140trkA and p75
tn
 should be considered as a potential perspective for the treatment of this pathology.

Acknowledgments—We thank Rachel Connor (Imperial College, London) and David G. Fernig (University of Liverpool) for critical reading of this manuscript.

REFERENCES

1. Barbacid, M. (1995) Curr. Opin. Cell Biol. 7, 148–155
2. Friedman, W. J., and Greene, L. A. (1999) Exp. Cell Res. 253, 131–142
3. Lewin, G. R., and Barde, Y.-A. (1996) Ann. Rev. Neurosci. 19, 289–317
4. Barker, P. A. (1998) Cell Death Differ. 5, 346–356
5. Barbacid, S., Oh, J., Zhang, L.-T., Yang, J., Bitler, C. M., Butcher, L. L., and Bredesen, D. E. (1993) Science 261, 345–348
6. Gentry, J. J., Casaccia-Bonnefil, P., and Carter, B. D. (2000) J. Biol. Chem. 275, 7558–7565
7. Ehrhard, P. B., Ganter, U., Stalder, A., Bauer, J., and Otten, U. (1993) Proc. Natl. Acad. Sci. U. S. A. 90, 5425–5427
8. Yaar, M., Grossman, K., Eiller, M., and Gilchrest, B. A. (1991) J. Cell Biol. 115, 821–828
9. Anton, E. S., Weskamp, G., Reichardt, L. F., and Matthew, W. (1994) Proc. Natl. Acad. Sci. U. S. A. 91, 2795–2799
10. Lillien, L. E., and Claude, P. (1985) Nature 317, 632–634
11. Otten, U., Ehrhard, P., and Peck, R. (1989) Proc. Natl. Acad. Sci. U. S. A. 86, 10059–10063
12. Di Marco, E., Mathur, M., Bondanza, S., Cutuli, N., Marchisio, P. C., Cancenda, R., and De Luca, M. (1993) J. Biol. Chem. 268, 22838–22846
13. Descamps, S., Lebourhis, X., Delehedde, M., Boilly, B., and Hondermarck, H. (1998) J. Biol. Chem. 273, 16659–16662
14. Djakiew, D., Delsite, R., Pflug, B., Wrathall, J., Lynch, J. H., and Onoda, M. (1991) Cancer Res. 51, 3304–3310
15. Djakiew, D., Pflug, B., Delsite, R., Onoda, M., Lynch, J. H., Arand, G., and Thompson, E. W. (1993) Cancer Res. 53, 1416–1420
16. Pflug, B., and Djakiew, D. (1998) Mol. Carcinog. 23, 106–114
17. Sortino, M. A., Condorelli, F., Vancheri, C., Chiarenza, A., Bernardini, R., Consoli, U., Canonico, P. L. (2000) Mol. Endocrinol. 14, 124–136
18. Lièvrement, J. P., Sciorati, C., Morandi, E., Paolucci, C., Bunone, G., Della Valle, G., Moldolesi, J., and Clementi, E. (1999) J. Biol. Chem. 274, 15466–15472
19. Rabizadeh, S., Rabizadeh, S., Ye, X., Wong, J. J., and Bredesen, D. E. (1999) Cell Death Differ. 6, 1222–1227
20. Ross, A. H., Groh, P., Bothwell, M., Elder, D. E., Ernst, C. S., Marano, N., Ghrist, B. F., Siempp, C. C., Herlyn, M., Atkinson, B., and Koprowski, H. Proc. Natl. Acad. Sci. U. S. A. 81, 6681–6685
21. Gill, Z. P., Pecks, C. M., Newcomb, P. V., and Holly, J. M. P. (1997) J. Biol. Chem. 272, 25692–25697
22. Pirianov, G., Danielsson, C., Carlberg, C., James, S. Y., and Colston, K. W. (1999) Cell Death Differ. 6, 890–901
23. Herrmann, J. L., Icham, A. W., Sarkiss, M., Chiao, P. J., Rands, M. T., Bruckheimer, E. M., Brisbay, S., and McDonnell, T. J. (1997) Exp. Cell Res. 237, 101–109
24. Gysy, S. P., Roohan, Y., Franza B. R., and Aebi, S. R. (1999) Mol. Cell Biol. 19, 1720–1730
25. Bhakar, A. L., Roux, P. P., Lachance, C., Kryl, D., Zeindler, C., Barker, P. A. (1999) J. Biol. Chem. 274, 21443–21449
26. Ethier, S. P. (1995) J. Natl. Cancer Inst. 87, 964–973
27. Le Bourhis, X., Toillon, R. A., Boilly, B., and Hondermarck, H. (2000 Breast Cancer Res. Treat. 60, 251–258
28. Rakowicz-Szulczynska, E. M. (1993) J. Cell. Physiol. 154, 64–70
29. Tagliabue, E., Castigliani, F., Giarelli, C., Modugno, M., Assnghi, L., Someni, G., Melani, C., and Menard, S. (2000) J. Biol. Chem. 275, 5388–5394
30. Barker, P. A., and Shooter, E. M. (1994) Neuron 13, 203–215
31. Klesse, L. J., Meyers, K. A., Marshall, C. J., and Parada, L. F. (1999) Oncogene 18, 2055–2068
32. Casacca-Bonnefil, P., Kong, H., and Chao, M. V. (1998) Cell Death Differ. 5, 357–364
33. Chou, T. T., Trojanowski, J. Q., and Lee, V. M. (2000) J. Biol. Chem. 275, 565–570
34. Baker, S. J., and Reddy, E. P. (1998) Oncogene 17, 3261–3270
35. Carter, B. D., and Lewin, G. R. (1997) Neuron 18, 167–190
36. Prade, J. M., Rodriguez-Tebah, A., and Barde, Y. A. (1996) Nature 383, 166–168
37. Longo, F. M., Manthorpe, M., Xie, Y. M., and Varon, S. (1997) J. Neurosci. Res. 48, 1–17
38. Carter, B. D., Kaltschmidt, C., Kaltschmidt, B., Offenhauser, N., Bohm-Matthei, R., Baeuerle, P. A., and Barde, Y. A. (1996) Science 272, 542–545
39. Yao-Zhong, L., Yao, S. Y., Veach, R. A., Torgerson, T. R., and Hawiger, J. (1995) J. Biol. Chem. 270, 14235–14248
40. Foehr, E. D., Lin, X., O’Maloney, A., Gerezuinias, R., Bradshaw R. A., Greene, W. C. (2000) J. Neurosci. 20, 7556–7563
41. Gruber, A., Lakshmanan, B., Tarrant, R., Alm, J., and Fisher, D. A. (1985) Pediatr. Res. 19, 934–937
42. Lossing, C., and Hannson, H.-A. (1993) Plast. Reconstr. Surg. 91, 1277–1286