Expression of Brain-derived Neurotrophic Factor and p145TrkB Affects Survival, Differentiation, and Invasiveness of Human Neuroblastoma Cells

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ABSTRACT

A large number of poor prognosis neuroblastoma (NB) tumors constitutively express brain-derived neurotrophic factor (BDNF) and variably express the gene for its tyrosine kinase (Trk) receptor TrkB. Good prognosis NB tumors typically express high levels of TrkA mRNA, which encodes the signal transducing receptor for nerve growth factor, p140TrkA. These neurotrophins are necessary for neural cell survival and differentiation. This study evaluates the effects of activation of the BDNF-TrkB signal transduction pathway on the growth, survival, morphology, and invasive capacity of NB cells. We find that the addition of BDNF to SY5Y cells induced to express p145TrkB by retinoic acid treatment does not significantly affect cell proliferation yet will support cell survival. Activation of the BDNF-TrkB signal transduction pathway stimulates disaggregation of cells and extension of neuritic processes which can be blocked by a BDNF-neutralizing antibody. Treatment of cells with K252a, an inhibitor of Trk, reverses the cellular disaggregation. An evaluation of the effects of BDNF and nerve growth factor on the ability of NB cells to penetrate basement membrane proteins indicated that BDNF stimulated a 2-fold increase while nerve growth factor inhibited RA-SY5Y cell invasion. Thus, activation of the p145TrkB signal transduction pathway stimulates NB cell survival, disaggregation, and invasion; all characteristics of metastatic cells. Furthermore, these studies indicate that activation of different Trk signal transduction pathways in NB cells results in distinct differences in tumor cell biology and these may be relevant to the clinical course of the patients.

INTRODUCTION

Human NB tumors are derived from pluripotent embryonal neural crest cells. The neural crest is a transient embryonal structure that arises during the closure of the neural tube, at which time cells migrate ventrally and laterally to give rise to the sympathetic and sensory ganglia of the peripheral nervous system, the chromaffin cells of the adrenal gland, melanocytes, and a variety of other tissues. Furthermore, neural crest cells have high proliferative and migratory potential characteristics also found in aggressive tumors (1). Several clinical observations suggest that NB tumors may arise from genetic alterations affecting processes controlling differentiation: (a) NB in situ occurs at a much higher rate than the frequency of NB in the population (2). (b) NB tumors exhibit a high degree of cellular heterogeneity reminiscent of different stages of neural cell development ranging from immature blasts to ganglion cells (3). (c) Cases of spontaneous as well as therapy-induced maturation of tumors to benign ganglioneuromas have been documented (4, 5).

The survival, maturation, and function of neural cells is influenced by NTs encountered in the extracellular milieu during development. NGF is the prototypic NT in a family that includes BDNF, NT-3, and NT-4/5 (6-8). These polypeptides are structurally and functionally related and are expressed in tissues, both in discreet yet often overlapping patterns during development (6-8). The effects of NTs are mediated by the Trk family of tyrosine kinase receptors (TrkA, TrkB, and TrkC) that encode both full-length (p130–145 kDa proteins) as well as truncated variants that lack the intracellular Trk domain (9–12). Signals transduced by NGF are mediated primarily by TrkA, BDNF, NT-3, NT-4/5 by TrkB, and NT-3 predominantly by TrkC (13).

Analysis of NT receptors in NB tumors has shown that good prognosis NB tumors express high levels of TrkA (14–17), while many poor prognosis NB tumors and cell lines express BDNF (18, 19) and tumors (18), but not typically cell lines, express either full-length or truncated TrkB (18, 19). High levels of BDNF have been detected in NB tumors and cell lines containing amplified N-myc genes, a marker of poor prognosis in patients with NB tumors (20). NB cell lines variably express TrkA and p75NTR (21, 22) and in many of these cell lines signal transduction via endogenous TrkA is impaired (23). However, NGF signal transduction may be intact since NGF treatment of a primary culture from an NB tumor stimulated cell survival (14). Furthermore, NGF treatment of NB cells expressing high levels of TrkA by transfection cause early response gene activation, neurite extension, and cell growth arrest in a NB cell line (23). Typically NB cell lines have been derived from poor prognosis NB tumors. While these tumors express TrkB and BDNF, few NB cell lines express readily detectable levels of TrkB mRNA (18). Recently, we determined that many NB cell lines constitutively express BDNF, while their ability to express high levels of TrkB is regulated by RA (18). The cell line, SY5Y (24), does not express high levels of BDNF; however, RA induces TrkB and neurite extension occurs with the addition of exogenous BDNF (18).

The effects of BDNF-TrkB signal transduction on NB cell growth, survival, and changes in cell morphology have been evaluated. Since a number of poor prognosis NB tumors constitutively express BDNF and TrkB mRNA (19), we have used the SY5Y NB cell line because it does not constitutively express BDNF, thus enabling the biological effects of activation of the TrkB signal transduction pathway by BDNF to be evaluated. In this study, we have found that BDNF increases NB cell survival, induces neurite extension, cellular disaggregation, and stimulates the invasiveness of NB cells.

MATERIALS AND METHODS

Cell Culture. The NB cell line SH-SY5Y (SY5Y) (24) was cultured in RPMI 1640 (Media Tech, Herndon, VA) supplemented with 10% FCS (Biofluid, Rockville, MD), glutamine (2 mm), penicillin (50 units/ml), and streptomycin (50 μg/ml) at 37°C with 5% CO₂.

Received 10/18/94; accepted 2/15/95.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

1 This research was sponsored in part by the National Cancer Institute, NIH, Department of Health and Human Services, under Contract NO1-CO-74101 with ABL (D. R. K.), an NIH First Award (R. K. W.), and a grant from the Concern II Foundation (R. K. W.).

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3 The abbreviations used are: NB, neuroblastoma; NGF, nerve growth factor; BDNF, brain-derived neurotrophic factor; RA, retinoic acid; NT, neurotrophin; Trk, tyrosine kinase.
Institute-Frederick Cancer Research and Development Center). Anti-TrkB antibody was used to estimate relative changes in gene or protein expression.

**Morphological Studies.** SY5Y cells were plated in 24-well dishes at a density of 2.5 x 10⁴ cells/well in duplicate. After 24 h, cells were treated with various concentrations of RA in combination with 1 μM all-trans RA (RA; Sigma Chemical Company, St. Louis, MO) or solvent control for 6 days. Antibody studies, a 1:100 dilution of anti-BDNF, anti-NGF, or preimmune serum was added in the absence or presence of 1 μM RA or control solvent for 6 days. For antibody experiments, using K252a (29, 30), SY5Y cells were plated at a density of 5 x 10⁴/well and after 24 h were treated with 5 μM RA and 10 ng/ml BDNF. After 5 days, cells were washed and recultured in media containing 5 μM RA and 10 ng/ml BDNF in the presence of indicated concentrations of K252a for 24 h. The photographs were taken with a Nikon Diaphot inverted microscope using Hoffman modulation lens. Cell counts were performed using a hemocytometer and viability was assessed by trypan blue exclusion.

**[3H]dThd Incorporation.** SY5Y cells (5 x 10⁵/well) were plated in quintuplicate in 96-well plates. After 24 h, cells were treated with 1 μM RA and 10 ng/ml BDNF. After 5 days, cultures were washed and medium was changed to serum free supplemented with 10 ng/ml BDNF or control media for 6 days. Twenty h prior to harvest, cells were labeled with 1 μCi/well [3H]dThd (ICN, Costa Mesa, CA). For RA dose-response experiments, 5 x 10⁵ cells/well were plated in 96-well plates, then treated with 1 μM RA, 1 μM RA, 1 μM RA, 5 μM RA, or solvent control for varying times. For neutrophin experiments, cells were treated with 0, 10, and 100 ng/ml each of BDNF, NGF, or NT-3 for 6 days. For RA and BDNF concentration experiments, SY5Y cells were plated as described above and treated with a combination of 0, 10, or 100 ng/ml BDNF with 1 μM RA, 1 μM RA, or solvent control. After 6 days, cells were incubated with [3H]dThd for 20 h and harvested using an Inotech harvester (Inotech, Lansi, MI) and counted in a Beckman LS1801 scintillation counter (Beckman Instruments Inc., Fullton, CA).

**RNA Analysis.** RNA isolation and hybridization were performed as described previously (31, 32). Total RNA was extracted from subconfluent 150-cm² plates of indicated cell lines. Total RNA (25 μg) from each sample was electrophoresed on 1% agarose-2.2 M formaldehyde gels and transferred to Nitran membranes (Schleicher and Schuell, Keene, NH) by capillary transfer. Blots were hybridized with 32P-labeled DNA. DNA inserts were isolated from plasmids containing rat TrkB (33, 34), human TrkB (35), BDNF (36), and glyceraldehyde-3-phosphate dehydrogenase. Blots were hybridized, washed, and exposed to X-ray films after rehybridization of the membrane was performed after treating Nitran membranes for 1 h in 50% formamide and 1X SSC at 75°C.

**Protein Analysis.** Cells (2 x 10⁶) were plated in 150-cm² dishes for 24 h and treated with solvent control, 1 μM RA, 1 μM RA, 1 μM RA, or 5 μM RA for 6 days. cultures were fed every 2 days with fresh RA media and were treated with 100 ng/ml BDNF for 5 min with rocking at 37°C. To detect TrkB protein, cells were lysed, and the lysates were immunoprecipitated as described previously (18, 37, 38). The lysates were immunoprecipitated with anti-pan Trk 203 (39). Immunoprecipitations (performed for 2-4 h at 4°C) were collected with protein A-Sepharose and washed three times with NP40 lysis buffer and once with water. The immunoprecipitates were boiled in sample buffer (2% SDS, 100 mM DTT, 10% glycerol, and 0.25% bromophenol blue) for 5 min and electrophoresed on 7.5% SDS-polyacrylamide gels (PAGE) before transfer to nitrocellulose. Protein blots were probed overnight at 4°C with antiphosphotyrosine antibody 4G10, and TrkB antibody was diluted in Tris-buffered saline (pH 8.0) and 0.2% Tween 20. Blots were analyzed using an ECL chemiluminescence system (Amersham Corp., Arlington Heights, IL). Protein blots were then stripped of antibody in 62.5 mM Tris (pH 6), 2% SDS, and 100 mM 2-mercaptoethanol at 70°C for 1 h and reprobed.

**Invasion Assay.** SY5Y cells were pretreated with 1 μM RA for 3 days. A single-cell suspension containing 5 x 10⁵ cells in 0.2 ml of serum-free Iscove's modified Dulbecco's medium was placed in the upper compartment
of a modified Boyden chamber (Falcon, Oxnard, CA) that had been coated with 40 μg Matrigel, an artificial basement membrane preparation (Collaborative Research, Bedford, MA). The bottom chamber held 0.5 ml medium and both chambers contained 1 nm RA and varying amounts (0, 5, 10, or 50 ng/ml) of either BDNF or NGF. The cells were incubated for 24 h at 37°C. Under these conditions, cells are unable to penetrate to the lower surface of the filter without elaborating proteolytic enzymes. After incubation, noninvasive cells and Matrigel on the upper surface of the filter were removed using a moist cotton swab. Adherent invasive cells on the under surface of the filter were fixed with 2% paraformaldehyde, stained in 0.1% crystal violet, and counted under high-power magnification. Nonadherent invasive cells found in the bottom chamber were collected by centrifugation and quantified by hemocytometer. The values reported for each point represent the mean and SE of quadruplicate experiments as a percentage of the control mean and SE values.

RESULTS

Effect of NTs on NB Cell Growth and Survival. Since a number of tumors derived from patients with an unfavorable prognosis express TrkB and BDNF, we evaluated the effects of BDNF on NB tumor cell proliferation and survival. The SY5Y NB cell line constitutively expresses TrkA yet does not express readily detectable TrkB and BDNF by Northern blot and Western blot analyses (18). Using the SY5Y NB cell model system, it is possible to study the effects of the addition of exogenous BDNF on NB cells that lack p145TrkB or can be induced by RA to express p145TrkB. Although RA is known to inhibit NB cell growth (32, 41), an analysis of the effects of different concentrations of RA on SY5Y cell proliferation indicated that 1 nm RA had no significant effect on [3H]dThd uptake in SY5Y NB cells while 1 μM RA resulted in a 50% reduction at 6 days. Concentrations as high as 5 μM RA are required to completely arrest SY5Y cell growth (Fig. 1A). Our previous experiments showed an increase in TrkB protein in NB cells treated with 1 nm RA (9-fold), and Trk protein increased at 5 μM RA (28-fold; Ref. 18). Northern blot analysis indicated that concentrations of RA as low as 1 nm induced both the 8.4- and 9.5-kilobase TrkB mRNA species (8.5-fold) with increasing levels of TrkB mRNA (28-fold) detected at 5 μM RA (Fig. 1B). In addition, a 5-min BDNF treatment of 1 nm RA-treated SY5Y cells caused a dramatic increase in p145TrkB autophosphorylation (94-fold), a level similar to that detected in 1 μM (106-fold) or 5 μM RA (102-fold)-treated cells (Fig. 1C). Autophosphorylation of p145TrkB is an early step in the BDNF-TrkB signal transduction pathway.

To evaluate the effects of NTs on NB cells, SY5Y cells were treated with 0, 10, or 100 ng/ml BDNF, NGF, or NT-3. After 4 days, cells were labeled with 1 μCi/well [3H]dThd, incubated for an additional 20 h, and harvested at day 5. Results indicated that the addition of concentrations of NT as high as 100 ng/ml to SY5Y cells did not alter cell proliferation (Fig. 2A). To evaluate the effects of BDNF on TrkB-expressing SY5Y cells, cells were treated with either 1 nm RA or 1 μM RA to induce p145TrkB and cultured in the presence of 0, 10, or 100 ng/ml BDNF for 5 days. The results indicate that BDNF did not significantly alter the growth of TrkB-expressing SY5Y cells (Fig. 2B). Thus, in contrast to the growth-inhibiting effects of NGF on PC12 cells (42), neither BDNF, NT-3, nor NGF significantly affect the growth of the SY5Y NB cells.

One of the properties of NTs is to promote neuronal cell survival. To determine if BDNF affected the survival of TrkB-expressing NB cells, SY5Y cells were treated with 1 μM RA or 5 μM RA and 10 ng/ml BDNF. After 5 days, cultures were washed and reincubated in serum-free media in the absence or presence of 10 ng/ml BDNF and incubated for an additional 7 days. At various times, [3H]dThd uptake was monitored and viability was assessed by trypan blue exclusion. A representative experiment (of four performed) depicted in Fig. 2C indicates that there was a significant decrease in [3H]dThd incorporation in cells cultured in the absence of BDNF. Cell viability was assessed at day 7. Ten percent of the cells were nonviable in cultures lacking BDNF compared to only 4% nonviable cells in cultures treated with BDNF. In similar experiments in which the cell number was assessed using a colorimetric assay for protein, a 20% decrease in
cells was found in cultures incubated in the absence of BDNF (data not shown). These studies are consistent with those of Nakagawara et al. (19) and indicate that while BDNF does not significantly affect the growth state of TrkB-expressing NB cells, it does enhance their survival when cultured in serum-free conditions.

**Effects of NTs on NB Cell Differentiation.** To determine the effects of NTs on neuronal differentiation, SY5Y cells were treated with 100 ng/ml BDNF, NGF, or NT-3 alone or in combination with 1 μM RA for 6 days (Fig. 3). SY5Y NB cells grow as loosely substrate-adherent aggregates of cells with an occasional cell extending neurite-like projections that typically do not exceed the soma length (Fig. 3, IA). Treatment of SY5Y with 1 μM RA caused the cells to become more substrate adherent and there was a small increase in neurite-bearing cells (Fig. 3, 2A). In the presence of NTs, SY5Y cells were more substrate adherent, with an increase in the number of neurite-bearing cells. SY5Y express TrkA and modestly extend pro-
Fig. 4. BDNF-induced differentiation of RA-treated SYSY cells is dose dependent. Cells were treated with media (A), 1.0 ng/ml (B), 3 ng/ml (C), or 10 ng/ml (D) BDNF in culture containing either 95% ethanol (1A–D) or 1 µM RA (2A–D) for 6 days. Photographs were taken using a Nikon microscope with Hoffman modulation.
Fig. 5. Activation of TrkB is BDNF dose dependent. After $2 \times 10^6$ SY5Y cells were treated with 1 $\mu$m RA for 6 days to induce TrkB, cells were incubated with indicated concentrations of BDNF for 5 min and lysates were evaluated by Western blot analysis with anti-PTyr and TrkB antibodies.

Fig. 6. Anti-BDNF blocks differentiation of SY5Y cells. SY5Y cells were plated in 24-well plates at a concentration of $2.5 \times 10^4$ cells/well in duplicate. Cells were treated with 1 $\mu$m RA and 10 ng/ml BDNF in the presence or absence of 1:100 dilution of anti-BDNF (B), anti-NGF (D), or preimmune serum (A and C) for 6 days. Photographs were taken using a Nikon Diaphot microscope with Hoffman modulation.

Fig. 7. Effect of BDNF and RA on maintenance of differentiated phenotype. SY5Y cells ($5 \times 10^4$/well) were plated in 96-well plates and treated with 5 $\mu$m RA and 10 ng/ml BDNF for 5 days. After 5 days, cells were washed and new media added containing 5 $\mu$m RA and 10 ng/ml BDNF (A), 5 $\mu$m RA alone (B), 10 ng/ml BDNF alone (C), or solvent control (D). Photographs were taken at day 4 using Hoffman modulation on a Nikon Diaphot microscope.
seen in Fig. 6A. After 5 days of treatment, cultures were washed three times in complete media and cultured in media, RA, or BDNF alone, or in the combination of RA and BDNF. After 6 days, cultures were evaluated for evidence of morphological differentiation. Analysis of over 200 cells in three independent experiments indicate that the continuous presence of BDNF in the media was required to maintain the number of neurite-bearing cells (Fig. 7, A and C). Cultures lacking BDNF (Fig. 7, B and D) show a decrease in neurite-bearing cells accompanied by an increase in cellular aggregation. Addition of BDNF to cultures such as depicted in Fig. 7D was sufficient to stimulate cellular disaggregation and neurite extension to the extent seen in Fig. 7A after 24 h (data not shown).

To determine if signals transduced via TrkB are important for the BDNF stimulated changes in cellular aggregation and maintenance of the differentiated phenotype, SY5Y cells were treated with K252a, an inhibitor of Trk (29). SY5Y cells were treated with 1 μM RA and 10 ng/ml BDNF and after 5 days media were changed and cells incubated in media containing 1 μM RA, 10 ng/ml BDNF, and increasing concentrations of K252a (Fig. 8, A–D). Within 24 h of the addition of K252a there was a dramatic change in cellular morphology. Changes in the morphology were detected as early as 6 h after the addition of K252a (data not shown). Treatment with 300 nM K252a was needed to cause a change in cellular aggregation or adhesion and a decrease in the number of neurite-bearing cells (Fig. 8C). Although phenotypic changes are apparent at 300 nM K252a, a decrease in BDNF-induced p145TrkB autophosphorylation is only detected at 1 μM K252a (Fig. 8E). It may be possible that K252a induces a qualitative rather than quantitative alteration in TrkB autophosphorylation sites at 300 nM. It should also be noted that cells cultured in the presence of BDNF had reduced BDNF-stimulated TrkB autophosphorylation (Lane 3) compared to cells cultured in the absence of BDNF (Lane 2). A similar observation has been previously reported in NGF-stimulated PC12 cells (37) and may be due to a reduced membrane receptor as a consequence of ligand-induced receptor cycling.

BDNF-TrkB Stimulates NB Cell Invasion. Since BDNF altered cellular aggregation and, seemingly, the motility of the NB cells and since one of the characteristics of poor prognosis NB tumors is their metastatic capability, an evaluation of the ability of the BDNF-TrkB signal trans-
The expression of TrkA is expressed by good prognosis NB tumors (14–17) has stimulated interest in determining the role and biological consequences of the expression of the Trk family of receptors and their ligands in NB tumors. Recently we found that a number of cell lines constitutively expressed BDNF but failed to express p145TrkB unless stimulated with RA (18). In a study of poor prognosis NB tumors and cell lines, it was found that poor prognosis NB tumors constitutively express BDNF and variably express p145TrkB or p95TrkB (19).

We have utilized RA to induce TrkB in the SY5Y cell line that does not constitutively produce readily detectable levels of BDNF or TrkB. Clearly, RA stimulates a number of changes in NB cells which may affect BDNF-TRkB signal transduction; however, the ability to add exogenous BDNF to SY5Y cell-expressing TrkB enables biological and biochemical studies of the consequences of the activation of the TrkB signal transduction pathway by BDNF to be evaluated. Our study did not reveal any evidence that BDNF (Fig. 4B) or the other TrkB ligands, NT-3 and NT-4/5 (data not shown), significantly altered cell proliferation in NB cells. The data indicated that under serum-free conditions, the survival of TrkB-expressing SY5Y cells was maintained by treatment with BDNF (Fig. 5) and cell numbers decreased in cultures lacking BDNF. This supports a previous finding that TrkB mediated a survival response under conditions of low serum in one NB cell line that was found to constitutively express p145TrkB and BDNF (19). The survival capability of NTs and their receptors is well documented in normal neural cells and constitutive TrkB expression during tumorigenesis could impart a survival advantage to an immortal or tumorigenic cell. Furthermore, the biological effects of BDNF-TRkB signal transduction in NB cells are distinct from those noted in two other model systems in which TrkB-mediated signal transduction was analyzed. In PC12 cells transfected with TrkB, treatment with BDNF arrests cell growth and induces differentiation (43), while in NIH-3T3 cells transfected with TrkB, transformed foci form (44). These findings support the concept that the intracellular environment contributes to the potential diversity of biological responses that may occur upon activation of a signal transduction pathway. Furthermore, they underscore the necessity to study the biological effects of activation of endogenous receptors in their natural milieu.

Aside from mediating neuronal survival, NTs stimulate morphological and biochemical differentiation of neural cells. In NB cells, the effects of NTs alone are modest; however, treatment of cells with NTs in combination with RA results in dramatic changes. BDNF stimulated cellular disaggregation and neurite extension to a degree not apparent in cultures treated with the other NTs. Cellular disaggregation and neurite extension occurred in cells treated with 1 nm and 1 μM RA, indicating that the expression of a differentiated phenotype can occur in proliferating cells. Since most NB cell lines and tumors constitutively produce BDNF, we focused our attention on the analysis of the effects of this NT. We found that concentrations as low as 1 ng/ml stimulated autophosphorylation of p145TrkB, while 3 ng/ml consistently induced cellular disaggregation and neurite extension. Experiments utilizing the anti-BDNF blocking antibody and the wash-out experiments indicated the effects on cell morphology were specific and reversible. Cellular disaggregation and the proportion of neurite-bearing cells was altered when the BDNF-TRkB signal transduction pathway was inhibited with Trk-specific tyrosine kinase inhibitor K252a.

The metastatic process has been shown to be a multistep process marked by changes in cellular adhesion, attachment to basement membrane, elaboration of proteolytic enzymes, locomotion, and additional changes in cell adhesion to form a metastatic nodule (45, 46). The changes in morphology noted in BDNF-treated SY5Y cells, the ability of α-BDNF antibodies to block this effect, and the ability of the Trk inhibitor K252a to reverse BDNF-TRkB-induced morphological changes support the proposal that the BDNF-TRkB signal transduction pathway stimulates the changes in cellular aggregation that are a prerequisite for metastasis. The finding that BDNF stimulates a 2-fold increase in NB cell invasion while another NT, NGF, actually inhibits cell invasion supports the hypothesis that the BDNF-TRkB signal transduction pathway contributes to the metastatic behavior of poor prognosis NB cells. Although the low-affinity NT receptor p75NTR has been shown to stimulate melanoma cell invasion (47), it is unlikely that this receptor mediates these effects in NB since NGF, which also binds p75NTR, inhibits cell invasion. We used 1 nm RA to induce TrkB because this concentration does not affect the proliferation potential of SY5Y cells although it remains to be determined whether growth inhibition affects the invasion of NB cells. The biochemical mechanisms mediating the BDNF-TRkB-induced changes in cellular adhesion and invasion are currently under investigation.

The expression of BDNF and TrkB by NB tumor cells may be due to their neural crest cell origin. The processes involved in dispersion of migratory neural crest cells and the subsequent aggregation of these cells into tissues such as sympathetic ganglia or the adrenal medulla are analogous to those occurring in the metastatic process. A study by Kalcheim and Gendreau (48), showed that BDNF and laminin, but not NGF, were necessary for the formation of sympathetic ganglia by migrating neural crest cells, indicating that the BDNF-TRkB signal transduction pathway may play a role in normal neural crest migration.

Our findings that the activation of the BDNF-TRkB signal transduction pathway in NB cells stimulates cell survival and invasiveness is in sharp contrast to the biological effects stimulated by NGF. In this study we show cellular invasion is stimulated by BDNF-induced signal transduction, but inhibited by NGF. Studies by Nakagawara et al. (19) and confirmed in this report indicate that BDNF does not inhibit NB cell growth, yet supports NB cell survival. These results show a contrasting role for the BDNF compared to another member of the NT family NGF, which induces cell growth arrest in NB cells expressing high levels of transfected TrkB (23) and survival of primary cultures of NB tumor cells (14). Thus, in NB cells in vitro, the expression of TrkA or TrkB results in distinct biological responses when their respective signal transduction pathways are activated.

One of the hallmarks of poor prognosis NB tumors is that tumors are metastatic and survive despite intensive chemotherapeutic regimens. Our observation that in NB cell lines activation of the BDNF-
TrkB signal transduction pathway increases cell survival and invasive-ness suggests that this signal transduction pathway plays a role in these processes and affects the biological behavior of these tumors which may contribute to the poor outcome in these patients. Furthermore, the differential Trk gene expression noted in NB tumors supports the hypothesis that good and poor prognosis NB tumors correspond to distinct lineages of neural crest cells or cells at a different stage of development. This raises the possibility that differences in the clinical course of patients with these tumors may reflect differences in the basic biology of the neural crest cells at the time at which the genetic alterations leading to tumorigenesis occurred. These findings suggest that the biological behavior of these tumors is affected by their expression of BDNF-TrkB. Strategies aimed at interfering with this signal transduction pathway may be useful in the treatment of poor prognosis NB tumors.

ACKNOWLEDGMENTS

We thank J. Carnahan for making available the BDNF-neutralizing antibody before it was published. Additionally, we are grateful to J. Winslow, K. Nikolics, G. Burton, R. Scott, and J. Vaugh for NT-3 and BDNF; A. Rosenthal for NT-4/5; D. Morrison for anti-PTyr; D. Martin-Zanca for the human Trk cDNA; D. Middlemiss and T. Hunter for probes and cDNA to TrkB; L. Reichardt for the BDNF cDNA; and M. Glickman for the K252a. We also thank J. Hughes for manuscript preparation and editorial assistance.

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