Prognostic Value of TrkA Protein Detection by Monoclonal Antibody 5C3 in Neuroblastoma

Kim Kramer, William Gerald, Lynne LeSauteur, H. Uri Saragovi, and Nai-Kong V. Cheung

Departments of Pediatrics [K. K., N.-K. V. C.] and Pathology [W. G.], Memorial Sloan-Kettering Cancer Center, New York, New York 10021, and Department of Pharmacology and Therapeutics [L. L., H. U. S.], and McGill Cancer Center [H. U. S.], McGill University, Montreal, Quebec H3G 1Y6, Canada

ABSTRACT

The effects of nerve growth factor, a neurotrophin mediating growth and differentiation of neural crest-derived cells, are mediated by the receptor TrkA. TrkA mRNA expression has been associated with a good prognosis in human neuroblastoma (NB). We describe the use of monoclonal antibody 5C3 in detecting TrkA expression by immunochemistry in NB and other malignant tumors.

A murine anti-TrkA IgG1 monoclonal antibody, 5C3, was generated against the extracellular domain of human p140 TrkA. 5C3 detected a 140-kDa band on Western blots. 5C3 was optimized for immunostaining and used to detect p140 TrkA in 113 frozen NB samples and 42 samples from nine other malignancies. MOPC21 IgG1 antibody was used as a control. Results by immunohistochemistry were compared to TrkA expression assessed by reverse transcription-PCR and Western analysis. The prognostic value of TrkA expression by these methods was evaluated and compared to other known prognostic variables, including stage, age, and MYCN copy number.

TrkA expression was detected by immunohistochemistry in 73 of the 113 NB tumor specimens and strongly correlated with nonmetastatic disease. TrkA expression was specific for NB among small round blue cell tumors. Both TrkA expression by immunohistochemistry and localized/4s disease correlated with survival. Tumors from 55 of 60 patients with localized/4s NB exhibited homogeneous or a mixed pattern of TrkA immunohistochemistry, whereas only 18 of 53 patients with stage 4 NB were immunoreactive. Detection of TrkA by reverse transcription-PCR and Western analysis was much more sensitive and no longer correlated with survival.

5C3 enables rapid detection of p140 TrkA by immunohistochemistry and identifies patients more likely to have localized NB with a favorable clinical outcome. Lack of TrkA expression is correlated with metastatic, malignant NB. A subset of patients with NB, however, died of aggressive metastatic disease despite TrkA expression. As a mimic of nerve growth factor, 5C3 may be useful in the study of TrkA-expressing tumors.

INTRODUCTION

Human NGF, a member of the neurotrophin family, is responsible for the growth, differentiation, maturation, and survival of sympathetic and neural crest-derived neurons (1, 2). The effects of NGF are mediated by the cellular expression of the transmembrane glycoprotein tyrosine kinase receptor, TrkA, which initiates signal transduction and differentiation (3, 4). The NGF-TrkA signaling pathway plays a crucial role in the development of both the peripheral and central nervous systems (5).

NB, a common childhood tumor of neural crest origin, exists in defined stages with distinct behavior. Whereas localized/regional neuroblastoma is curable with minimal therapy, metastatic neuroblastoma is highly aggressive, infiltrating local and distant sites.

TrkA is expressed in many primary neuroblastomas, and TrkA mRNA expression has been associated with a favorable outcome (6, 7). Although the precise role of the TrkA receptor in the pathogenesis of NB is unclear, evidence does support its role in tumor cell differentiation in vitro and in vivo (8). Its potential role in regulating growth, differentiation, and regression of NB has been postulated.

A mouse mAb, 5C3, was generated against the extracellular domain of human p140 TrkA. It immunoprecipitates a 140-kDa tyrosine kinase activated during NGF treatment. Cerebrosides complementarity-determining regions of mAb 5C3 are homologous to specific turn regions of NGF. Ligand binding assays revealed that 5C3 inhibited 125I-labeled NGF binding to human TrkA-expressing E25 cells. In reciprocal experiments, NGF blocked the binding of mAb 5C3 in a dose-dependent manner (9). 5C3 mimics NGF function in receptor phosphorylation, induction of receptor internalization, protection of apoptotic death in serum-free media, and increased transformation of TrkA-expressing cells (9). 5C3 is specific for human p140 TrkA and has demonstrated no cross-reactivity with human TrkB or TrkC (9).

5C3 may be useful in the clinical diagnosis and therapy of certain human tumors. We used mAb 5C3 to detect TrkA expression in frozen human tumors, to investigate the heterogeneity of TrkA expression in human NB, and to correlate TrkA protein expression with stage and clinical outcome.

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2 To whom requests for reprints should be addressed, at Department of Pediatrics, Memorial Sloan-Kettering Cancer Center, 1275 York Avenue, New York, NY 10021. Phone: (212) 639-8401; Fax: (212) 744-2245.

3 The abbreviations used are: NGF, nerve growth factor; NB, neuroblastoma; mAb, monoclonal antibody; RT-PCR, reverse transcription-PCR.
PATIENTS AND METHODS

Frozen tumors from 124 patients with NB and 42 tumors from patients with other malignant tumors (medulloblastoma, malignant glioma, rhabdomyosarcoma, Ewing's sarcoma, Wilms' tumor, primitive neuroectodermal tumor, osteosarcoma, melanoma, breast carcinoma, and lung carcinoma) were analyzed. Tumors were randomly selected from a tumor bank repository; selection was based on the availability of the tumor tissue and the known clinical staging of the tumor sample. All diagnoses of tumor samples were confirmed by histological assessment of tumor specimens. Eleven NB tumor samples were excluded because of poor neuroblast content, tumor necrosis, or inadequate tissue sections for histological assessment. Patients with NB were stratified by stage according to the International Neuroblastoma Staging System. Sixty patients had localized/ stage 4s disease (group 1: stage 1, n = 19; stage 2, n = 22; stage 3, n = 11; stage 4s, n = 8) and 53 had metastatic stage 4 disease (group 2). Of the 113 cases of NB, 42 occurred in patients less than 1 year of age at the time of diagnosis (32 in group 1 and 10 in group 2). Of the 60 patients in group 1, 54 samples were taken at the time of the initial diagnosis, and 6 samples were analyzed at the time of a second look surgical procedure or recurrence. Of the group 2 patients, 26 were analyzed at initial diagnosis, 22 were analyzed at a second surgical procedure, 4 were taken at a time of a recurrence, and 1 was taken at the time of autopsy.

Immunohistochemical Studies. Eight-μm cryostat frozen tumor sections were fixed in acetone and washed in PBS. Endogenous peroxidases were blocked in 0.3% H₂O₂ in PBS. Sections were incubated in 10% normal horse serum (Life Technologies, Inc., Gaithersburg, MD) and incubated with murine anti-TrkA IgG mAb 5C3 for 1 hr. MOPC21 IgG1 myeloma (Sigma Chemical Co., St. Louis, MO) was used as a control. Sections were incubated with a secondary antimouse biotinylated antibody (Vector Laboratories, Burlingame, CA); they were then incubated with avidin-biotin complex (Vector Laboratories) and stained with the Vector VIP substrate (Vector Laboratories). A 10% hematoxylin counterstain for 4 min was used. Staining was graded as positive (homogeneous) if >90% of cells were immunoreactive, mixed (heterogeneous) if 10–90% of cells were immunoreactive, or negative if <10% of tumor cells were reactive.

Western Blot Analysis. Total protein, DNA, and RNA were extracted from tumors using the Tri-Reagent method (Molecular Research Center, Inc., Cincinnati, OH). Ten μg of total protein were electrophoretically separated on a 7.5% polyacrylamide gel system according to Laemmli (10) and transferred to a polyvinylidene difluoride membrane. Membrane extracts of NIH 3T3 mouse cells transfected with human TrkA (100,000 cells/50 μl/lane, 1 × 10⁶ receptors/cell) were used as positive controls. MOPC21 IgG was used as a control antibody. The membrane was incubated in hybridoma supernatant diluted 1:1 in blocking buffer for 30 min at room temperature and washed in Tris-buffered saline-0.5% Tween 20. Bound antibody was detected by HRP labeled goat anti-mouse IgG (Sigma, St. Louis, MO) diluted at 1:1000 in blocking buffer. Bands were detected by chemiluminescence (ECL, Amersham, United Kingdom).

RT-PCR. Total cellular RNA was extracted form 44 selected human neuroblastoma tumors (20 from group 1 patients and 24 from group 2 patients), four cell lines (NMB7, IMR32, LAN 1, and LAN 5), and normal human tissues (liver, lung, heart, thyroid, frontal cortex, and cerebellum) using TRI-Reagent (Molecular Research Center, Inc., Cincinnati, OH). cDNA was synthesized by reacting 1 μg of RNA, 1 μl of 50 μM random hexamer, and an 8.5-μl solution of reverse transcriptase, RNase inhibitor (Promega, Madison, WI), chelating buffer, diethylpyrocarbonate-treated water, and dNTP (Life Technologies, Inc., Gaithersburg, MD). Samples were incubated at 68° to reduce RNA secondary structure and then at 37°C for 1 h. The reactions were inactivated by incubation at 90°C for 5 min. RNA integrity was verified by RT-PCR amplification of B₂ microglobulin. The sequences of the biotin-labeled primers for the TrkA molecule were: 5’ AAC CAG CCC ACC CAC GTC AAC AAC 3’ and 5’ AAG GAG CÔCOT AGA AAG GAA GAG 3’. The product generated from these primers is 225 bp, corresponding to position 1165–1390 of the published TrkA cDNA sequence. PCR amplifications were performed by adding 0.5 μl of cDNA to a 49.5-μl mixture of Tris-HCL buffer (pH 8.3), 3 mM MgCl₂, 25 mM dNTP, 50 μM primer, 0.2 units AmpliTaq DNA polymerase (Perkin-Elmer Cetus, Norwalk, CT), and 0.5 units Perfect Match (Stratagene, La Jolla, California). Optimization of PCR was accomplished by use of the PCR optimization kit (Stratagene, La Jolla, California). The amplification was performed for 25 cycles of 94°C for 1 min, 62°C for 1 min, and 72°C for 1 min and a final extension of 72°C for 8 min. The PCR product was separated by 1.5% agarose gel electrophoresis and transblotted onto a nylon membrane (Bio-Rad, Munich, Germany). The membrane was blocked in 0.2% 1-Block (Tropix, Bedford, MA) in PBS with 0.5% SDS for 20 min and incubated in the streptavidin-alkaline phosphatase conjugate (1:5000 in blocking buffer; Tropix, Bedford MA) for 30 min. The membrane was washed in PBS with 0.1% SDS and incubated in assay buffer [0.1 M diethanolamine and 1 mM MgCl₂ (pH 10.0)] for 4 min. The chemiluminescent substrate CSPD (0.25 mM disodium-3-phenyl phosphate in assay buffer; Tropix, Bedford, MA) was applied at 0.25 mm in assay buffer for 1 h and exposed to film.

Sequence Analysis. The 225-bp TrkA RT-PCR product was excised from the agarose gel and purified by using the GeneClean procedure (Bio 101, Inc., Vista, CA). Fifty ng of pT7 Blue T-vector was ligated with 0.2 pmol of amplified product in a volume of 10 μl and set at 16°C overnight. One μl of ligation reaction was added to NovaBlue competent cells (Novagen, Madison, WI) for transformation. Colonies were incubated and purified using the Wizard Miniprep DNA purification system (Promega, Madison, WI). PRISM Ready Reaction Dideoxy Terminator Premix (Applied Biosystems, Foster City, CA) and T7 and U19 primers (Novagen, Madison, WI) were used for cycle sequencing. The sequence was analyzed using the Applied Biosystems Automated DNA sequencer (ABI, Foster City, CA) and the Seq Ed software program (ABI).

MYCN PCR Analysis. MYCN copy number was obtained by Southern blotting as previously described by Seeger et al. (11) for 51 samples. For all other samples, the PCR was used to detect amplification of the MYCN oncogene using genomic DNA extracted from cryosections of tumor samples (12). DNA extracted from peripheral blood lymphocytes of healthy volun-

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teers served as single-copy MYCN controls. DNA extracted from cell lines IMR32, Lan 1, and Lan 5 were used as MYCN-amplified controls. Two primer pairs were used to coamplify a 428-bp fragment of the MYCN oncogene, along with a 268-bp fragment of the single copy β-globin gene. PCR amplification was performed according to Crabbe et al. (12). Products were separated on a 5% polyacrylamide gel, transblotted onto a nylon membrane, and detected by chemiluminescence as described above. MYCN gene amplification was detected by visual examination and densitometric (PDI Images, Huntington Station, NY) calculation of the relative intensity of the MYCN and β-globin gene bands.

Statistical Analysis. Statistical analyses of the subgroups were compared using Fisher’s exact test. Survival probabilities were calculated according to the method of Kaplan and Meier (13), and survival of the subgroups was compared by log-rank tests. Univariate and multivariate analyses were performed using the Cox proportional model (14).

RESULTS

Immunohistochemistry

NB. We used mAb 5C3 to detect TrkA expression by immunohistochemistry. In general, the small round blue cells typical of undifferentiated NB had minimal or no staining for TrkA. In most NBs showing varying degrees of differentiation, a mixed heterogeneous pattern was noted, with minimal or no staining in some areas and focal staining most prominent in the mature ganglion cells and their cellular processes.

TrkA expression was detected in 73 of 113 NB tumors and was strongly correlated with localized/4s disease ($P < 0.0001$). A high level of homogeneous expression was found in 38 of 60 tumors from patients with localized NB but only 13 of 53 tumors from group 2 patients (Fig. 1A). Seventeen of 59 tumors from group 1 patients and 5 of 53 from group 2 patients displayed a mixed pattern of TrkA expression (Fig. 1B). Many of these samples having this mixed pattern of expression were faintly positive and showed no expression in certain areas of the tumor. No staining was observed in 5 of 60 group I tumors (one stage 2, two stage 3, and two stage 4s) or in 35 of 53 group 2 tumors (Fig. 1C). Group 1-negative tumors tested positive when examined for the expression of other tumor markers (GD2, CD44) to eliminate the possibility of tissue degradation. By groups, infants less than 1 year of age demonstrated a near-identical pattern of TrkA expression, as did older patients (Table 1). It is recognized that tumor histology can change dramatically with treatment as a result of tumor cell differentiation, necrosis, and so forth. For this reason, TrkA expression was reexamined after treatment or at recurrence in 15 additional samples. 5C3...
Table 1  TrkA expression in NB

<table>
<thead>
<tr>
<th>No.</th>
<th>Group 1</th>
<th>Group 2</th>
<th>Infants &lt;1 year of age</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. positive (%)</td>
<td>59</td>
<td>38 (64)</td>
<td>17 (29)</td>
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<tr>
<td>No. mixed (%)</td>
<td></td>
<td>13 (25)</td>
<td>5 (9)</td>
</tr>
<tr>
<td>No. negative (%)</td>
<td></td>
<td>5 (19)</td>
<td>4 (16)</td>
</tr>
<tr>
<td>No.</td>
<td>53</td>
<td>13 (25)</td>
<td>5 (9)</td>
</tr>
<tr>
<td>No. positive (%)</td>
<td></td>
<td>5 (19)</td>
<td>4 (16)</td>
</tr>
</tbody>
</table>

a Fifteen samples were repeated after chemotherapy, at the time of second surgery or recurrence: 5C3 staining patterns remained unchanged in 14 tumors; 1 negative tumor subsequently positive postchemotherapy in regions of maturing elements.

staining patterns remained unchanged in 14 tumors. One originally negative tumor subsequently stained faintly positive in areas with maturing elements postchemotherapy. Of the 26 stage 4 tumors examined at diagnosis, 5 (19%) had a homogeneous TrkA expression, 4 (16%) were heterogeneous, and 17 (65%) had no expression. This is unchanged from the overall analysis of the 53 group 2 patients (24%, 10%, and 66%, respectively). Results are presented in Table 1.

Other Malignancies. No 5C3 reactivity was found in 41 of 42 tumor samples representing nine other types of human malignancies (rhabdomyosarcoma, primitive neuroectodermal tumor, Ewing’s sarcoma, osteosarcoma, Wilms’ tumor, melanoma, breast carcinoma, lung carcinoma, and central nervous system tumors). Of the five Wilms’ tumor specimens, four were negative. One tissue sample, from a 19-year-old female with a diagnosis of metastatic Wilms’ tumor, was faintly and heterogeneously positive.

Western Blot Analysis

Tumor protein extracts and 5C3 were used to detect TrkA protein expression by Western analysis. Thirty-three tumors (13 group 1 and 20 group 2) and the cell line Lan 5 were analyzed for TrkA by the Western blot technique. Five of the 20 group 2 tumors were MYCN amplified. 5C3 detected a 140-kDa band on Western blot analysis of proteins extracted from frozen tumor specimens. No protein was detected using the control antibody. The 140-kDa TrkA protein was detected in all 13 group 1 tumors and 16 of 20 group 2 tumors. Lan 5 was positive for the TrkA band (Fig. 2). Of the 5 MYCN amplified tumors, 3 were found positive by Western analysis (Table 2).

RT-PCR Analysis

RT-PCR was performed to detect TrkA mRNA expression in NB tumor samples. Twenty group 1 and 24 group 2 patients were analyzed for TrkA expression by RT-PCR. All tumor specimens with 5C3 immunoreactivity were found to have the 225-bp TrkA product by RT-PCR with 25 cycles of amplification (Fig. 3). Of five group 1 patients with no detectable 5C3 reactivity, RT-PCR identified faint TrkA mRNA expression in all five. Of 11 group 2 5C3-negative samples, 4 were positive by RT-PCR. Samples that were negative by Western analysis were also negative by RT-PCR under these conditions. When PCR conditions were increased to 35 cycles using 2 μg cDNA, all neuroblastoma cell lines, including Lan 1, Lan 5, IMR 32, and NMB7, as well as all tumor samples from both group 1 and group 2 samples, demonstrated TrkA mRNA expression. Normal tissues, including breast, thyroid, cardiac, ileum, frontal cortex, and cerebellum, failed to demonstrate TrkA mRNA expression at 25 or 35 cycles. These results are summarized in Table 3. The sequence of the 225-bp PCR-amplified product corresponded to that of the known TrkA cDNA sequence position 1165–1390.

MYCN Amplification

PCR was performed using genomic DNA from NB tumor samples to correlate TrkA expression with the presence or absence of MYCN amplification. Of the group 1 tumors, all were found to have single copy of MYCN. Of the group 2 tumors, eight had evidence of MYCN amplification.
IrkA detection by immunocytochemistry and RT-PCR

<table>
<thead>
<tr>
<th>Immunochemistry</th>
<th>RT-PCR positive</th>
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<tr>
<td>Group 1</td>
<td></td>
</tr>
<tr>
<td>SC3 positive</td>
<td>15</td>
</tr>
<tr>
<td>SC3 negative</td>
<td>4</td>
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<td>SC3 positive</td>
<td>11</td>
</tr>
<tr>
<td>SC3 negative</td>
<td>11</td>
</tr>
<tr>
<td>SC3 mixed</td>
<td>2</td>
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</tbody>
</table>

eight MYCN-amplified tumors were IrkA negative by immunohistochemistry, with one demonstrating homogeneous IrkA expression.

### Statistical Analysis

Using Fisher’s exact test, IrkA positive and mixed immunostaining strongly correlated with localized and 4s disease ($P < 0.0001; n = 113$). Expression no longer correlated with disease stage when assessed by RT-PCR/chemiluminescence ($P = 0.46; n = 44$). Lack of TrkA expression by immunohistochemistry strongly correlated with MYCN amplification ($P < 0.002$). In a univariate analysis, both TrkA expression by immunohistochemistry ($P < 0.0001$), and localized/4s disease ($P < 0.0001$) correlated with survival, with localized/4s disease stage being the more significant of these two variables (Fig. 4). Both TrkA expression by immunostaining and localized/4s disease status were more significant in predicting survival outcome than was TrkA analysis by RT-PCR, age, or MYCN copy number ($P = 0.10, 0.04,$ and $0.08$, respectively.) Among stage 4 patients, the only variable shown to be significant in terms of survival was age ($P < 0.0001$). No survival advantage was seen among stage 4 TrkA immunostaining-positive patients ($P > 0.5$) or among patients with a single copy MYCN ($P > 0.5$).

### DISCUSSION

The neurotrophic hypothesis suggests that all or most nerve cells require neurotrophic factors during embryogenesis and during postnatal life for their survival and/or for maintenance of their phenotypic characteristics (15). NGF is required for neural crest-derived sensory and sympathetic neuronal development. The interaction of NGF with TrkA appears to play a role in human NB cell growth, differentiation, and cell death. The TrkA receptor is known to have signal-transducing tyrosine kinase activity similar to that exhibited by other members of the neurotrophin family. Recent data support the role of other neurotrophins, such as pleiotrophin, in the developmental regulation of NB tumorigenesis (16). Of particular importance is the role of TrkB and brain-derived neurotrophic factor, recently identified as factors potentiating the survival, differentiation, and invasiveness of human NB cells (17, 18).

We used mAb 5C3 to detect TrkA expression in NB and other human malignancies, to investigate the heterogeneity of TrkA expression in human NB, and to determine whether the pattern of TrkA expression in NB correlated with stage and clinical outcome. Expression correlated with localized and stage 4s NB, a clinical group characterized by a favorable outcome. Among patients in this group, 55 of 60 biopsies exhibited TrkA expression. Among patients with stage 4 NB, TrkA expression was demonstrated in 18 of 53 patients, with 13 of 18 having a homogeneous pattern of expression. Lack of TrkA protein expression was correlated with metastatic, malignant NB. When analyzed by groups, a similar TrkA protein expression was demonstrated in infants less than 1 year of age. A mixed heterogeneous pattern of TrkA protein expression was found in
a subset of both localized and advanced NBs but was significantly more common among localized tumors demonstrating various degrees of tumor cell differentiation. Among small round blue cell tumors of childhood, TrkA protein expression was specific for human NB.

Seven of eight MYCN-amplified tumors were found to be negative by 5C3 staining, confirming the correlation between MYCN gene amplification and loss of TrkA gene expression (6, 7). This is consistent with the findings that constitutive expression of MYCN inhibits exit from the cell cycle and blocks neuronal differentiation (19). Two of eight patients with MYCN-amplified tumors are alive, and both stained negative for TrkA. The one patient with TrkA-positive MYCN-amplified stage 4 disease suffered disease recurrence 4 years after diagnosis and died of treatment-related complications following reinduction chemotherapy.

As a prognostic tool in patients with NB, 5C3 enables rapid identification of TrkA expression by immunohistochemistry and Western blot technique. Interestingly, TrkA expression, as determined by immunohistochemistry, correlated better with stage and clinical outcome than did expression determined by Western analysis or RT-PCR. This is attributed to the increased sensitivity of the latter two methods. It is also recognized that nonlinear relationships exist between the concentration of TrkA mRNA and the concentration of the p140TrkA protein. When more PCR cycles and the more sensitive detection by chemiluminescence are used, all NB samples demonstrate some degree of TrkA expression. When Western analysis was performed, tumors having rare positive ganglion cells by immunohistochemistry were also TrkA positive. Recently, another mAb detecting p140TrkA was reported, describing its sensitivity when compared to that of Northern blot (20). Although the sensitivity of the immunohistochemistry was lower than that of Northern analysis, a concordance rate of 79% was obtained. The prognostic significance of these findings was not reported. In our analysis, detection of p140TrkA with 5C3 by immunohistochemistry provides the most valuable prognostic information in patients with NB.

Our results confirm the findings of other investigators that TrkA expression in NB is associated with a favorable outcome (6, 7, 21–24). This finding is expected when a variable, such as TrkA, is so closely correlated with the stage of disease. A subset of state 4 patients, however, still demonstrates TrkA immunohistochemical expression; 18 of 53 patients with lethal disease have TrkA expression in this series. For this subset of patients, it appears that biological parameters other than the expression of the TrkA receptor dictate the clinical behavior of NB. Despite the presence of a receptor known to induce differentiation in developing sympathetic neuroblasts, aggressive metastatic behavior is demonstrated in some patients. No survival advantage is seen in TrkA-positive stage 4 NB. In our series of stage 4 patients, only age at the time of diagnosis is predictive of an improved survival, regardless of TrkA status. This indicates that TrkA expression by itself may not be sufficient to confer a benign phenotype and suggests the importance of other prognostic variables, including those that interact with other putative neurotrophic proteins. In the setting of TrkA-positive stage 4 NB, failure to differentiate may be caused by interference with the signal transduction pathway, the absence of appropriate levels of NGF, or the presence of substances that interact or compete with NGF to induce signal transduction (15). In these cases, tumor progression occurs despite TrkA expression. Rare localized tumors maintain the ability to differentiate with low or absent TrkA receptor expression.

5C3 has potential as a diagnostic and therapeutic device in experimental and human malignancies involving TrkA-expressing tumors. As a mimic of NGF function, 5C3 directly binds to the extracellular domain of TrkA; mediates receptor phosphorylation, dimerization, and internalization; and inhibits NGF binding to several receptor-expressing cells (9). In this regard, it may used to further study the biological actions of NGF in the context of neurological disorders, as well as TrkA-expressing malignancies. Dose-response relationships, quantification of cell survival, and growth characteristics may be studied on primary cell culture systems and serve as a model for the developmental processes occurring in vivo. Ultimately, 5C3 or receptor-binding small peptide antagonists (25) may be useful for tumor imaging in patients with NB and as a potential therapeutic agent to promote NB cell differentiation and tumor regression. Recent experiments have shown that 99mTc-5C3 is useful for imaging TrkA-expressing tumors in nude mice.

We conclude that immunohistochemical detection of TrkA protein expression is specific for human NB; as such, it may be useful for differential diagnosis of small round blue cell tumors of childhood. TrkA protein expression is correlated with localized and stage 4s NB, whereas lack of TrkA protein expression is correlated with metastatic, malignant NB. Despite the presence of the TrkA receptor, a subset of patients with NB will exhibit an aggressive clinical course. RT-PCR analysis of the TrkA gene confirms the association of TrkA protein expression and localized NB and, in addition, will detect TrkA mRNA expression not apparent at the protein level. TrkA expression is a powerful prognostic marker that complements other known markers such as age at diagnosis (26, 27), MYCN copy number (12, 28), deletion of the short arm of chromosome 1 (29), CD44 expression (30, 31), cellular DNA content (32, 33), and tumor histology (34). A larger study is needed to define the independent prognostic impact of 5C3 when these other important factors are considered. As a mimic of NGF, 5C3 or its structural analogs may be useful for radioimaging or for the treatment of TrkA-expressing tumors.

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REFERENCES


* H. U. Saragovi, manuscript in preparation.


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