Expression of Alternatively Spliced src Messenger RNAs Related to Neuronal Differentiation in Human Neuroblastomas

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ABSTRACT

Neuroblastoma, the most common malignant solid cancer of children, has an ability to differentiate in vitro and in vivo. This biological property has a significant influence upon the prognosis of patients with neuroblastomas. Neuronal cells express three alternatively spliced forms of c-src mRNA (nonneuronal c-src, neuronal c-srcN1, and neuronal c-srcN2), which are found at different levels in adult and fetal human brain tissue. In this study, the transcriptional levels of the three c-src mRNAs were examined in relation to the neural differentiation in eight human neuroblastoma cell lines and two clonal sublines and in seven primary neuroblastoma tissues by S1 nuclease protection assays. Neuronal c-srcN1 mRNA was expressed at high levels in neuroblastoma cell lines with the ability to differentiate but not in the cell lines lacking the capacity to mature in response to chemical inducers irrespective of N-myc gene amplification and overexpression. In terminally differentiated neuroblastoma cells, the expression of neuronal c-srcN2 mRNA, which was barely detectable at a steady-state level in the uninduced cells, increased to significant levels. Infantile neuroblastomas identified by mass screening tests expressed both neuronal c-srcN1 and c-srcN2 mRNAs at levels almost identical to that found in human brain tissue, but terminally differentiated neuroblastomas from older children identified based on clinical symptoms, did not. These results suggest that neuronal c-src gene expression and the ability of neuroblastomas to differentiate in vitro and in vivo may be correlated.

INTRODUCTION

NB,1 the most common solid cancer found in children, arises from immature neuroblastic cells in the adrenal and sympathetic ganglion chain. It seems likely that NB cells develop because of an alteration in neural differentiation, because most NBs continue to express the N-myc protooncogene. The N-myc protooncogene is normally expressed in fetal neuronal tissues at an early developmental stage (1–3) and acts as a regulator for gene expression (4). Infantile NBs often mature in response to chemotherapy or spontaneously, but advanced NBs diagnosed after 1 yr of age, in which the N-myc gene is often amplified, do not have a promising prognosis despite recent advances in multimodal treatments. Although decreased expression of N-myc precedes morphological changes during the induced differentiation of human NB cell lines (5), the exact genes responsible for NB cell differentiation after diminishing N-myc expression have not been elucidated. Recent studies have shown that enhanced levels of Ha-ras and trk protooncogenes, which are involved in the signal transduction pathway of neural differentiation, strongly correlate with the favorable prognosis of patients with NB (6, 7). We previously observed that infantile NBs with favorable prognosis also express higher levels of c-src mRNA (8). However, the relationship of the expression of neuron-specific c-src mRNAs generated by alternative splicing to neural differentiation of NBs remains to be clarified.

The src protooncogene, the normal cellular counterpart of the retroviral transforming gene v-src, encodes a membrane-bound tyrosine-specific protein kinase. High levels of the src gene product and its specific kinase activity, notably those of neuronal form of the src product, are observed in immature neural cells and tissues at the onset of differentiation (3, 9–14). In mammalian brain tissue, src mRNA is expressed in three distinct forms by alternative splicing of exons NI and NII located between exons 3 and 4 (15). Nonneuronal c-src mRNA does not contain neuronal exons, whereas neuronal c-srcN1 and c-srcN2 mRNAs contain exon N1 and both exons N1 and NII, respectively. Since neuronal c-srcN2 mRNAs are expressed at a higher ratio relative to c-src and c-srcN1 in the adult brain than in the fetal brain (15), the three c-src isoforms encoded by these mRNAs may provide different functions during the development of the human brain.

In analyses of src protein expression in NBs (16–23), neuron-specific src protein was reported to be expressed in NB cells and tissues, and its expression could be related to neural differentiation of NBs. However, immunoblotting or immunoprecipitation techniques used in these studies could not distinguish molecular weight differences of the three c-src proteins, although the differences in nonneuronal and neuronal isoforms could be. In this report, we quantitatively analyzed the levels of the three alternatively spliced c-src mRNAs in eight NB cell lines and two clonal sublines of neuronal and epithelial-like cells and those during RA-induced differentiation of the NB cell lines. To clarify the significance of neuronal c-src mRNA expression in clinically observed NBs, we also examined the three c-src mRNAs levels in seven NB tissues which were found by clinical symptoms or NB mass screening tests.

MATERIALS AND METHODS

Cell Culture. The human NB cell lines SK-N-SH (24), IMR32 (25), GOTO (26), and NB-1 (27) were obtained from the Japanese Cancer Research Resources Bank. The human NB cell line LA-N-5 (28) was kindly provided by Dr. Robert C. Seeger (Children’s Hospital of Los Angeles, Los Angeles, CA). RT-BM-1 (29) was kindly provided by Dr. Tohru Sugimoto (Kyoto Prefectural University of Medicine, Kyoto, Japan), and NB69 (30) was kindly provided by Dr. Yoshisuke Nishi (Life Science Research Laboratory, Japan Tobacco, Inc., Kanagawa, Japan). The human NB cell line cNB1 was established in our laboratory and has been cultured for over 9 yr. NB69N and NB69S are clonal sublines of NB69, isolated in our laboratory. All NB cell lines, except for NB69 and its sublines, were cultured in RPMI 1640 medium supplemented with 10% heat-inactivated FCS. NB69 and its sublines were cultured in RPMI 1640 medium containing 15% FCS. The HeLa S3 and FL cell lines were obtained from American Type Culture Collection and grown in Eagle’s basal medium supplemented with 10% FCS. Neural differentiation of NB cells (3 × 106) cultured for 2 days in 90-mm tissue culture plates was induced by incubation in culture medium containing 5 μM trans-RA (Sigma), 1 mM dibutyryl cyclic AMP (FCS), fetal calf serum; m, nucleotide.

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1 Supported in part by a grant-in-aid from the Institute of Whole Body Metabolism (Chiba, Japan).

2 To whom requests for reprints should be addressed.

3 The abbreviations used are: NB, neuroblastoma; cDNA, complementary DNA; PCR, polymerase chain reaction; RA, retinoic acid; dbcAMP, dibutyryl cyclic AMP; FCS, fetal calf serum; m, nucleotide.

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gen prior to testing. Specimens were confirmed to consist of tumor cells by pathological examination prior to analyses. The clinical profiles of the patients from which the tumor tissues were obtained are summarized in Table 2.

**RNA Preparation.** Total RNA was isolated from cultured cells by the method of Chomczynski and Sacchi (31). Extracted RNAs were precipitated with isopropanol in a microfuge tube, washed with 80% ethanol, and dissolved in 400 µl of distilled water. The RNA concentration was determined by measuring absorbance at 260 nm and 280 nm. The RNA was then precipitated with ethanol and redissolved in distilled water to a concentration of 1 µg/µl. After confirmation of the final RNA concentration by absorbance, 40 µg of RNA were subjected to an S1 nuclease protection assay, and 10 µg were subjected to Northern blot analysis. Two µg of RNA were electrophoresed through a formalin gel prior to Northern blot analysis in order to confirm its integrity and abundance. RNAs from normal human and neuroblastoma tissues were isolated by grinding frozen tissues to a fine powder in liquid nitrogen and lysis in a heated guanidinium solution as described previously (32). Total RNA was extracted as described above.

**RNA PCR and cDNA Cloning.** The oligonucleotide primers used for first-strand cDNA synthesis and PCR were synthesized by a DNA synthesizer (380B, ABI). The sequences of the primers are as follows: TAGGATC-CCTAAGCTTGGAGGCTTCA (exon 2, sense) and AAAAAAGTGAGGAGGCCCCACTGA (exon 4, antisense) (33). The primers contained BamHI and HindIII sites, respectively, flanked by two bases to facilitate restriction endonuclease digestion. To synthesize the first-strand cDNA, 5 µg of total RNA from brain tissue were denatured by addition of CH3HgOH annealed with 30 pmol of the antisense primer at 65°C for 5 min, and chilled on ice. The annealed RNA template was transcribed at 42°C for 1 h in a reaction containing 50 mM Tris hydrochloride (pH 8.0), 70 mM KCl, 10 mM MgCl2, 2 mM deoxynucleoside triphosphates, and 14 units of Rous associated virus 2 reverse transcriptase (Takara) in a volume of 20 µl. One fifth of the reaction mixture was subjected to PCR in a reaction containing 50 mM KCl, 10 mM MgCl2, 2 mM Tris hydrochloride (pH 8.3), 1.5 mM MgCl2, 0.01% gelatin, 1.25 mM deoxynucleoside triphosphates, 20 pmol of each primer, and 2.5 units of Taq DNA polymerase (Perkin Elmer Cetus). PCR was performed by 35 cycles of denaturing step at 94°C, a 2-min annealing step at 60°C, and a 2-min extension step at 72°C. PCR products were cloned into the BamHI-HindIII site of the phagemid pTV119N (Takara). Nucleotide sequences of the three c-src cDNAs were determined by the dideoxy chain-termination method (34).

**Northern (RNA) Blot Hybridization.** RNA (7.5 µg) was separated on a 1% agarose-6% formaldehyde gel and transferred to a nitrocellulose filter. The RNA blots were hybridized with a [α-32P]dCTP-labeled probe, the BglII-EcoRI fragment of the N-myc third exon (35, 36), under stringent conditions as described previously (32). Rehybridization with mouse β-actin cDNA (37) was extended further by addition of 1 pmol each of dATP, dGTP, dCTP, and dTTP and incubation at 37°C for 30 min. The DNA was then digested at the EcoRI site of the polylinker and separated on a 5% acrylamide gel. The 400-nt-long probe, including a 93-nt sequence derived from the vector, was eluted from the gel and hybridized with 40 µg of total RNA in 80% formamide, 40 mM piperazine-N,N'-bis(2-ethanesulfonic acid) (pH 6.4), 400 mM NaCl, and 1 mM EDTA at 55°C for 12 h. The protected fragments from the digestion with 1000 units/ml of S1 nuclease (Boehringer Mannheim) at 30°C for 45 min were analyzed on a 6% acrylamide/7 M urea gel (38).

**Quantitation of mRNA Expression.** All mRNA signals obtained by S1 analyses and Northern blot hybridizations were measured using a bioimaging analyzer (LAS 2000, Fujix) by exposure to the imaging plate for 16 (S1 analyses) or 4 h (Northern blot hybridization). This system can calculate signal intensities more precisely than densitometric scanning to autoradiograms, independent of the exposure time of the radioactive bands. Expression levels were determined by subtracting the background signal. The autoradiograms were performed for 3 to 5 days (S1 analyses) or 24 to 48 h (Northern blot hybridization) at ~80°C after quantitation by the imaging analyzer.

**RESULTS**

**Isolation of c-src cDNA.** Initially, an attempt was made to isolate cDNAs of the three c-src mRNAs from human brain tissue by RNA PCR. The PCR primers were designed to amplify c-srcN2 cDNA, so that the three c-src mRNA species could be distinguished simultaneously when the c-srcN2 cDNA was used as a uniformly labeled probe for S1 analyses. To this end, the 5' primer was positioned at exon 2, and the 3' primer was positioned at exon 4 as indicated in Fig. 1. RNA PCR using these primers yielded three PCR products of the expected size on a 3% agarose gel, which were then cloned into the pTV119N vector. Sequencing analyses confirmed that the products were cDNAs of nonneuronal c-src, neuronal c-srcN1, and neuronal c-srcN2 (data not shown).

**S1 Analysis of c-src mRNA Splicing in NB Cells.** To detect the three c-src mRNAs quantitatively, single-stranded DNA complemen-
ter to c-srcN2 mRNA was synthesized and used as a uniformly labeled probe for S1 nuclease protection assays. As shown in Fig. 1, three distinct bands corresponding to c-src (168-nt band), c-srcN1 (186-nt band), and c-srcN2 (307-nt band) were observed in brain tissue. The NB cell lines, LA-N-5 and SK-N-SH, with and without N-myc amplification, respectively, expressed mRNAs of both nonneuronal c-src and neuronal c-srcN1, whereas only nonneuronal c-src mRNA was expressed in spleen, liver, and FL cells. Transcripts containing only exon NII and exon 4, which would result in a 121-nt band if expressed, were not observed (data not shown).

RNAs derived from the eight NB cell lines were subjected to S1 analyses to examine expression levels of the three c-src mRNAs and Northern blot analyses to detect N-myc mRNA (Figs. 2 and 3). Six of the NB cell lines, IMR32, GOTO, NB-1, LA-N-5, cNBI, and RT-BM-1, contained from 25 to 150 copies of the amplified N-myc gene (data not shown) which expressed enhanced N-myc mRNA (Fig. 2). The SK-N-SH and NB69 cell lines contained a single copy of the N-myc gene and expressed very low levels of N-myc mRNA, which were detected only after extended exposure (7 days) of the autoradiograms (8; data not shown). These NB cell lines expressed various levels of the three c-src mRNAs. The SK-N-SH, IMR32, GOTO, NB-1, and cNBI cell lines predominantly expressed nonneuronal c-src mRNA and very low levels of c-srcN1 mRNA (Fig. 2). The LA-N-5, RT-BM-1, and NB69 cell lines expressed both nonneuronal c-src and c-srcN1 mRNAs at high levels (Figs. 2 and 3) and very low levels of c-srcN2 mRNA which were detected only after extended exposure (10 days) of the autoradiograms of the S1 analyses (data not shown). As reported for other NB cell lines (39), NB69 cells consist of neuroblastoid cells, whereas the NB69N and NB69S consist of neuroblastic small round cells and substrate-adherent flat-epithelial cells. The morphologically distinct sublines of NB69, NB69N, and NB69S consist of neuroblastic small round cells and flat-epithelial-like cells, respectively (Fig. 4). Whereas the NB69S expressed only nonneuronal c-src mRNA, NB69N expressed high levels of both nonneuronal c-src mRNA and c-srcN1 mRNA (Fig. 3) and very low levels of c-srcN2 mRNA (data not shown). The NB69 cells expressed the three c-src mRNAs in a pattern that was characteristic of both NB69N and NB69S. As shown in Table 1, the expression levels and splicing pattern of c-src mRNA did not correspond with the morphological appearance of NB cells or N-myc oncogene amplification and expression levels.

Expression of Neuronal c-src mRNAs in Relation to NB Cell Differentiation. To study expression of the three c-src mRNAs in relation to neuronal maturation of NBs, the eight NB cell lines and the two clonal sublines were treated with RA or dbcAMP or both. The extent of neural differentiation was determined by the formation of ganglion-like clusters which were interconnected by extended neurite processes (28) and downregulation of N-myc or c-myc mRNA expression (5, 40, 41). Terminal differentiation was induced in LA-N-5, RT-BM-1, and NB69N, which expressed c-srcN1 mRNA at high levels (Fig. 4). The other cell lines showed no apparent neuronal change following treatment with RA or dbcAMP or both, although IMR32 cells exhibited neurite processes which extended slightly, and the neuroblastic cells in NB69 had extended long neurite processes.
Fig. 4. Morphological changes of LA-N-5, RT-BM-1, NB69N, and NB69S cells treated with 5 μM RA plus 1 mM dbcAMP (LA-N-5) or 5 μM RA (RT-BM-1, NB69N, and NB69S cells) for 12 days. After the cells (3 × 10⁶) were grown in a 90-mm dish for 2 days, the medium was removed, and the differentiation medium was added. The differentiation medium was changed every 3 days.

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<th>Table 1 Characteristics and c-src mRNA splicing in NB cell lines</th>
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- Morphological appearance of NB cells: N, neuroblastic small round cells with short neurites; S, substrate adherent, flat epithelial-like cells.
- For N-myc mRNA expression levels, the signal intensity of N-myc mRNA in the SK-N-SH is assigned as a value of 1.
- Levels of c-src and c-srcN1 mRNA expression: C, nonneuronal c-src; N1, c-srcN1.

Total RNA was isolated 12 days after neural induction from terminally differentiated LA-N-5 (10 μM RA and 1 mM dbcAMP), RT-BM-1 (5 μM RA), and NB69N (5 μM RA) and from nondifferentiated IMR32 and NB69S (Fig. 4), and S1 analyses were performed (Figs. 3 and 5). In the terminally differentiated LA-N-5 and RT-BM-1 cells, expression of c-srcN2 mRNA was clearly detected, and c-srcN1 mRNA increased slightly, whereas nonneuronal c-src mRNA expression showed no quantitative change (Fig. 5). An increase in c-srcN2 mRNA expression was also detected in the differentiated NB69N cells, although this change was not as clear as that observed in the LA-N-5 and RT-BM-1 cells, and downregulation of c-myc expression was faint (Fig. 3). In nondifferentiated IMR32 and NB69S cells, in which N-myc or c-myc mRNA did not decrease significantly, induction of the c-srcN2 mRNA was not observed (Figs. 3 and 5).

Time-dependent alteration of the expression levels of the three c-src and N-myc mRNAs during neural differentiation of RT-BM-1 was investigated after addition of RA (Fig. 6). No apparent morphological changes were observed within the first 24 h. After 3 to 5 days of induction, cell aggregates resolved into a monolayer with neurite processes extended. After 7 days of RA treatment, the cells began to assemble into ganglion-like clusters, which by Day 12 interconnected into an extensive neurite network. N-myc expression decreased to 35% by Day 1 after RA treatment, preceding the morphological changes. Between Days 3 and 14, N-myc expression decreased to less than 25%. The expression levels of the three c-src mRNAs increased at different times and in different patterns. Nonneuronal c-src mRNA increased quickly and transiently, reached a maximum of 2-fold induction at Day 3, and returned to the original level by Day 7. c-srcN1 mRNA also increased rapidly, reached a maximum of 3-fold induction between Days 3 and 5, and decreased to lower levels (2-fold) between Days 7 and 14. c-srcN2 mRNA expression increased gradually and reached a maximum of 4-fold induction by Day 14. The increase in c-srcN2 mRNA was more obvious after prolonged exposure (10 days) of the autoradiograms of the S1 analyses (data not shown). N-myc and c-src transcription in NB cells was not affected by serum starvation.
Expression of Neuronal c-src mRNAs in Primary NBs. To clarify the significance of neuronal c-src mRNA expression to neuronal maturation in clinically observed NBs, RNAs from seven primary NB specimens were subjected to S1 analysis. Four of the seven cases were found by NB mass screening tests, and no N-myc amplification was observed in these tumors (data not shown). The remaining three cases were identified based on clinical symptoms after 1 yr of age, and all showed N-myc gene amplification (data not shown). The clinical profiles of these cases are summarized in Table 2. The S1 assay revealed a distinct alteration in the c-src mRNA splicing patterns between infantile NBs and aggressive NBs diagnosed after 1 yr of age (Fig. 7). All four infantile NBs expressed significant levels of c-srcN2 mRNA and high levels of c-srcN1 mRNA. No or low levels of the c-srcN2 mRNA were observed in aggressive NBs, which expressed either c-src mRNA singly or both c-src and c-srcN1 mRNAs. The patient in Case 7, who had a tumor expressing low levels of c-srcN2 mRNA, showed no evidence of disease after autologous bone marrow transplantation, although the N-myc gene was amplified and overexpressed at high levels in her tumor.

DISCUSSION

In this study, we first examined the transcriptional levels and splicing patterns of c-src mRNAs in seven NB cell lines (SK-N-SH, IMR32, GOTO, NB-1, LA-N-5, cNBI, RT-BM-1). We found that the splicing pattern of the c-src mRNAs does not correlate with steady-state levels of c-myc mRNA, which caused growth arrest of the cells but did not induce morphological changes (data not shown).

Expression of Neuronal c-src mRNAs in Primary NBs. To clarify the significance of neuronal c-src mRNA expression to neuronal maturation in clinically observed NBs, RNAs from seven primary NB specimens were subjected to S1 analysis. Four of the seven cases were found by NB mass screening tests, and no N-myc amplification was observed in these tumors (data not shown). The remaining three cases were identified based on clinical symptoms after 1 yr of age, and all showed N-myc gene amplification (data not shown). The clinical profiles of these cases are summarized in Table 2. The S1 assay revealed a distinct alteration in the c-src mRNA splicing patterns between infantile NBs and aggressive NBs diagnosed after 1 yr of age (Fig. 7). All four infantile NBs expressed significant levels of c-srcN2 mRNA and high levels of c-srcN1 mRNA. No or low levels of the c-srcN2 mRNA were observed in aggressive NBs, which expressed either c-src mRNA singly or both c-src and c-srcN1 mRNAs. The patient in Case 7, who had a tumor expressing low levels of c-srcN2 mRNA, showed no evidence of disease after autologous bone marrow transplantation, although the N-myc gene was amplified and overexpressed at high levels in her tumor.

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* Origin of the tumor: Ret, retroperitoneum; Adr, adrenal gland; Med, mediastinum.

* Histology of the tumor: 3a, rosette fibrillary type.

* For N-myc mRNA expression levels, the signal intensity of N-myc mRNA in the SK-N-SH is assigned as a value of 1.

* For c-srcN2 mRNA expression levels, the signal intensity of c-srcN1 mRNA in the RT-BM-1 is assigned as a value of 100. ND, not detectable.

* Clinical course: NED, no evidence of disease; REC, recurrence; DOD, death of disease.

* Numbers in parentheses, months after diagnosis.
state levels of N-myc expression and the morphological appearance of the NB cells. However, they did correlate with the ability of NB cells to differentiate in response to RA and dbcAMP. Enhanced expression of c-srcN1 mRNAs at steady state was observed in LA-N-5 and RT-BM-1 cells, which underwent neuronal differentiation when treated by RA and dbcAMP. Our preliminary studies demonstrated that treatment of NB69 cells with RA resulted in terminal differentiation of neuroblastic small round cells, but morphological change was not observed in substrate-adherent flat-epithelial-like cells. We isolated two sublines, NB69N and NB69S, from NB69 and found that NB69N cells which were able to differentiate expressed c-srcN1 mRNA at high levels. Interestingly, also, the splicing pattern of the c-src mRNAs clearly differed in NB69S and NB69N. NB69S expressed only c-src mRNA and showed no morphological change by the chemical modulator. This finding sustains our hypothesis that expression of neuronal c-srcN1 mRNA is associated with the ability of NBs to mature in vitro irrespective of N-myc amplification and expression and phenotypic appearance.

Our observation that the expression of c-srcN2 mRNA was enhanced in the brain and nearly undetectable in NB cell lines is consistent with those reported by Pyper and Bolen (15). They documented that nonneuronal c-src and c-srcN1 mRNAs are expressed at higher levels in fetal brain tissue than in adult brain tissue and that c-srcN2 mRNA cannot be detected in two retinoblastoma cell lines, which are of neuroectodermal origin. These findings suggest the possibility that c-srcN2 mRNA expression is confined to neuronal cells with a differentiated phenotype, while expression of nonneuronal c-src mRNA and c-srcN1 mRNA is associated with undifferentiated cell phenotypes. In fact, terminally differentiated cells of LA-N-5, RT-BM-1, and NB69N expressed significant levels of c-srcN2 mRNA. However, changes in the transcriptional levels of the three c-src mRNAs during differentiation of RT-BM-1 cells were more complicated than anticipated. Increased expression of nonneuronal c-src and c-srcN1 mRNAs preceded the morphological changes and reached maximal levels when the cells began to differentiate. During the later stages of differentiation, c-src and c-srcN1 mRNAs were downregulated, and c-srcN2 mRNA increased at significant levels. These observations suggest the possibility that the three c-src isoforms have different functional roles during different stages of neural differentiation.

Analyses of the seven primary NB tissues showed differential splicing patterns of the three c-src mRNAs between the cases younger and older than 1 year of age. All four infantile NBs found by mass screening expressed significant levels of c-srcN2 mRNA in addition to high levels of c-srcN1 mRNAs. Advanced NBs diagnosed after 1 year of age expressed c-src mRNA alone or both c-src and c-srcN1 mRNAs, but no low levels of c-srcN2 mRNA. The splicing patterns found in these advanced NBs are almost identical with those found in the established NB cell lines that were analyzed. This observation raises the possibility that NBs which lack c-srcN2 mRNA expression have a selective advantage for establishment of a continuous cell line. It seems likely that expression of c-srcN1 mRNA alone is not sufficient for good prognosis in patients with NBs lacking c-srcN2 mRNA expression, even though we found that these tumors are able to mature. Although all infantile NBs analyzed here have a histologically undifferentiated appearance, these tumors expressed c-srcN2 mRNA at nearly equivalent levels as those found in human brain tissue and terminally differentiated NB cells induced by a chemical modulator. These infantile NBs might have a functionally mature potential or might already be progressing toward neural differentiation.

Genomic amplification of the N-myc protooncogene is found in 30 to 40% of NB tumors with an aggressive phenotype (42, 43) and results in poor patient prognosis. The expression levels of N-myc mRNA, however, do not always correlate with the extent of gene amplification (44). In our studies, two of three cases with N-myc amplification expressed enhanced levels of the mRNA compared with those found in established cell lines, whereas one of three expressed very low levels of the N-myc mRNA. This indicates that the lack of c-srcN2 mRNA expression observed in aggressive NBs does not result from the enhanced levels of N-myc mRNA.

In this study, we demonstrated that neuronal c-srcN1 mRNA expression was related to the ability of NBs to mature in vitro. Furthermore, we found that neuronal c-srcN2 mRNA expression was enhanced in infantile NBs with favorable prognosis. It may be possible that the pattern and level of expression of the three c-src mRNAs’ expression are a biological marker in the ability of NBs to differentiate in vitro.

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Expression of Alternatively Spliced src Messenger RNAs Related to Neuronal Differentiation in Human Neuroblastomas

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