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Cancer Res 1992;52:4221-4226.

[CANCER RESEARCH 52, 4221–4226, August 1, 1992]

Inhibition of Proliferation by c-*myb* Antisense RNA and Oligodeoxynucleotides in Transformed Neuroectodermal Cell Lines[1]

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ABSTRACT

Transfection of a neuroblastoma cell line with expression vectors containing two different segments of human c-*myb* complementary DNA in antisense orientation yielded far fewer transfectant clones than did the transfection with the identical segments in sense orientation. In cell clones expressing c-*myb* antisense RNA, levels of the c-*myb* protein were down-regulated and the proliferation rate was slower than that of cells transfected with sense constructs or the untransfected parental cell line. Treatment of neuroblastoma and neuroepithelioma cell lines with a c-*myb* antisense oligodeoxynucleotide strongly inhibited cell growth. These data indicate a definite involvement of c-*myb* in the proliferation of neuroectodermal tumor cells extending the role of this protooncogene beyond the hematopoietic system. The availability of cell clones that transcribe c-*myb* antisense RNA provides a useful tool to study the involvement of other genes in the proliferation and differentiation of neuroblastoma cells.

INTRODUCTION

The c-*myb* protooncogene is the cellular homologue of the viral v-*myb* carried by avian myeloblastosis virus and E26 (1, 2), both of which transform hematopoietic cells with a distinct myeloid phenotype (1–3). The protein encoded by v-*myb* is localized in the nucleus (4, 5) and binds to DNA *in vitro* (6). The DNA-binding domain of the v-myb protein is composed of two imperfectly conserved 52-amino acid direct repeats located near the amino terminus (7) and corresponds to a truncated version of that found in chicken and mammalian c-myb proteins (8–10). The v-myb protein synthesized in bacteria binds specifically to the nucleotide sequence pyAACG/TG (11), and concatemers of this consensus sequence can confer v-*myb*-dependent inducibility to otherwise unresponsive promoters, suggesting that the v-myb protein acts as sequence-specific DNA binding transcription factor (12). Also, v-*myb* directly regulates the expression of a cellular gene, *MIM-1*, in chicken myeloblasts infected with an avian myeloblastosis virus temperature-sensitive mutant (13). Nuclear localization and DNA binding activity are necessary but not sufficient for the oncogenic potential of *myb* (14). The c-*myb* protooncogene and v-*myb* share several biochemical and functional properties, including nuclear localization, DNA binding, and transcriptional regulator activity (15). These properties appear to be important in transformation of chicken myeloid cells (16).

It has long been suggested that c-*myb* expression is linked to proliferative and differentiative processes in the hematopoietic system (17, 18). Antisense c-*myb* oligodeoxynucleotides block normal hematopoiesis *in vitro* (19). More recently, the need for c-*myb* expression on fetal liver hematopoiesis has been demonstrated in transgenic animals in which the c-*myb* gene has been inactivated by homologous recombination (20).

Elevated c-*myb* expression has been demonstrated in human leukemia cells (21) the *in vitro* proliferation of which has been shown to be *myb*-dependent (22). Several solid tumors of different embryonic origin such as colon carcinoma (23), small cell lung carcinoma (24), teratocarcinoma (25), and neuroblastoma (26) also demonstrate c-*myb* expression.

NB[4] is a malignant childhood tumor thought to arise in migratory cells of the embryonal neural crest (26). NB is histopathologically indistinguishable from NE and the two malignancies are often considered as one entity. Nevertheless, Thiele *et al.* (26) have reported that the pattern of protooncogene expression differs in these tumors. In fact, N-*myc* expression is generally high in NB and its amplification correlates with tumor progression and aggressiveness (27), whereas NE generally does not express N-*myc* (27). On the contrary, c-*myc* expression is high in NE but low in NB. However, both NE and NB express c-*myb*. NB cell lines induced to differentiate by retinoic acid demonstrate a rapid and sharp decrease in c-*myb* expression due to a decreased transcription rate rather than instability of c-*myb* mRNA (28). The temporal relationship between the levels of c-*myb* mRNA and the differentiative and proliferative processes occurring in NB makes c-*myb* a possible candidate for a key role in the proliferation and/or differentiation of neuroectodermal tumors.

In the present study we evaluated the effects of c-*myb* down-regulation in NB and NE cell lines by transfection of expression vectors carrying different domains of the c-*myb* cDNA in antisense orientation and by exposing cell cultures to antisense oligodeoxynucleotides.

MATERIALS AND METHODS

Cloning of Antisense and Sense c-*myb* Vectors. *Sst*II-*Eco*RI (DNA-binding domain) and *Bam*HI-*Bam*HI (3′ untranslated region) fragments were obtained from clone pMbm I dihydrofolate reductase (18) containing a full-length human c-*myb* cDNA. After end-repair with Klenow enzyme (Promega, Madison, WI), the fragments were cloned in the polylinker region of pRc/CMV vector (Invitrogen, San Diego, CA) as described (29). Sense and antisense orientation of the cloned fragments was determined by restriction analysis.

Cell Lines and Transfection. Neuroblastoma cell line LAN-5 (30) was grown in RPMI 1640 (Sigma, St. Louis, MO) supplemented with fetal bovine serum (Sigma). Cell lines SK-N-SH and SK-N-MC (31) were grown in minimal essential medium (GIBCO, Grand Island, NY) with 10% fetal bovine serum.

DNA transfections in LAN-5 cells were performed by the calcium phosphate precipitation technique according to standard procedures (29). Briefly, cells were seeded at a density of 10^6/dish and 48 h later were exposed to plasmid DNA (pRc/CMV) at 20 μg/plate for 6 h. After

Received 12/13/91; accepted 5/27/92.

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[1] This work was supported in part by NIH Grant CA46782, by American Cancer Society Grant CH-455A, and by a grant from the Associazione Italiana Ricerca sul Cancro (AIRC).

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[4] The abbreviations used are: NB, neuroblastoma; NE, neuroepithelioma; cDNA, complementary DNA; PBS, phosphate-buffered saline; CMV, cytomegalovirus; RT, reverse transcriptase; PCR, polymerase chain reaction.

2 days cells were placed in medium containing the antibiotic G418 (Sigma; 400 μg/ml) and transfectant clones were isolated 3 weeks later.

Oligodeoxynucleotides. Unmodified deoxynucleotides were synthesized on an Applied Biosystem 380B DNA synthesizer by means of β-cyanoethylphosphoramidite chemistry. Oligodeoxynucleotides were purified by ethanol precipitation and multiple washes in 70% ethanol. Nucleotide (nt) numbers and codon positions for each c-*myb* oligodeoxynucleotide refer to the published human c-*myb* cDNA (10).

The primers for the PCR to control integration of the antisense inserts in the 5′ and 3′ antisense clones and to detect antisense RNA transcription are

CMV1: 5′ AATGGGAGTTTGTTTTGGCACCAA3′ nt 699–722 of hRc/CMV-*myb*-1: 5′ TGCCAAGCACTTAAAGGGGAGAAT3′ nt 467–490
myb-2: 5′ AACTTGTTTGGGAGACTCTGCATT3′ nt 2959–2983
myb-3: 5′ GCTGGCACTGCACATCTGTT3′ complementary to nt 333–352
myb-4: 5′ GCTGGCACTGCACATCTGTT3′ nt 128–147
myb-5: 5′ CTGAAGAAGCTGGTGGAACAGAATG3′ nt 264–289
myb-6: 5′ CTAGCAGCATGTCTACAGGC3′ complementary to nt 2708–2729
myb-7: 5′ CCATGTGACATTTAATCCAG3′ nt 2496–2515
myb-8: 5′ GCTCATTTATGGTTAATGAC3′ nt 2525–2544

The c-*myb* sense oligodeoxynucleotide used in cell cultures was 5′GCCCGAAGACCCCGGCAC3′ corresponding to codons 2–7. The c-*myb* antisense was 5′GTGCCGGGGTCTTCGGGC3′, complementary to the codons 2–7.

RNA Extraction and RT-PCR Analyses. Total RNA was prepared by acid guanidinium thiocyanate-phenol-chloroform extraction (32). RNA for RT-PCR analysis to detect c-*myb* antisense RNA in transfected clones was treated with RNase-free DNase I (Promega). Reverse transcription, PCR analysis, and hybridization to the specific probes were carried out as described (33). Plasmid integration was determined by PCR after isolating genomic DNA as described (29).

Immunocytochemical Analysis. LAN-5 cells were seeded in Labtek chamber slides (NUNC, Naperville, IL) at a density of 5×10^3 cells/cm^2. After 48 h, cells were rinsed in PBS, fixed in 4% paraformaldehyde in PBS for 10 min at 4°C, permeabilized in PBS containing 0.01% Triton X-100 for 5 min at 4°C, and treated with 1% bovine serum albumin (Sigma) for 30 min at room temperature. Incubation with sheep polyclonal antibody to c-myb protein (Cambridge Research Biochemicals, Valley Stream, NY) (34) at 1:100 dilution and immune sheep serum as control was carried out for 18 h at 4°C. After extensive washings in PBS, slides were treated with peroxidase-labeled rabbit anti-sheep IgG antibody (KPL, Gaithersburg, MD) and stained with an immunocytochemical staining kit (KPL).

RESULTS

Cloning Efficiency and Proliferation of a Human Neuroblastoma Cell Line Expressing c-*myb* Antisense Transcripts. Two different regions of the human c-*myb* cDNA were cloned in antisense orientation in the expression vector pRc/CMV in which the CMV promoter-enhancer (35) drives the transcription of the cloned genes and the SV40 promoter drives transcription of the gene encoding the G-418 resistance used to select transfected cells. Transient transfection assays to compare the efficiency of the Rous sarcoma virus, SV40, and CMV promoters in LAN-5 neuroblastoma cells showed that the CMV had highest activity in driving the transcription of a reporter gene.[5]

Neuroblastoma cell line LAN-5 which expresses c-*myb* mRNA constitutively (26, 28) was transfected with plasmids expressing antisense and sense c-*myb* transcripts. Both size and number of G418 resistant clones were reduced in the transfections with constructs expressing the 5 or 3′ antisense *myb* sequences as compared to those expressing the sense transcripts (Fig. 1). On average, the cloning efficiency of 5′ antisense-*myb* transfectants was reduced 66% (from 62 to 70%) as compared

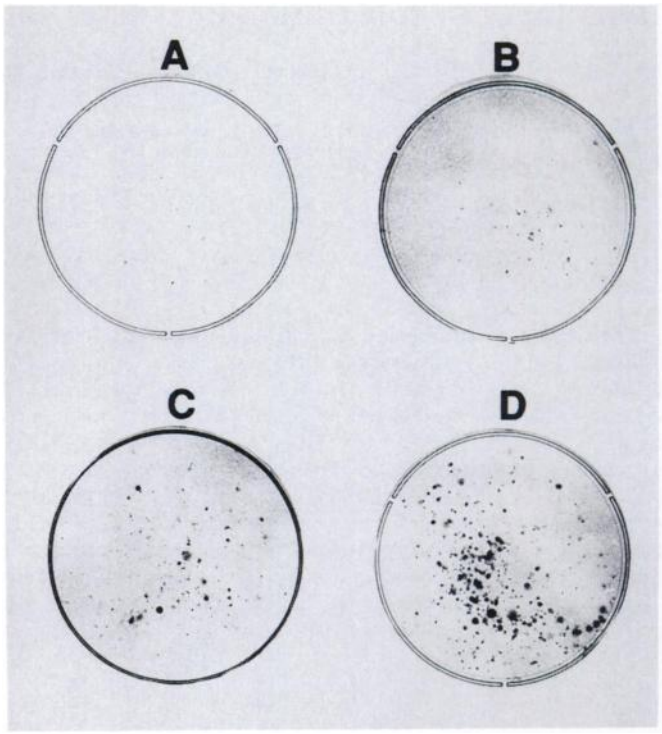

Fig. 1. Cloning efficiency of LAN-5 cells transfected with plasmids transcribing c-*myb* cDNA in the sense and antisense orientation. *A*, 5′ antisense *myb*; *B*, 3′ antisense *myb*; *C*, 5′ sense *myb*; and *D*, 3′ sense *myb*. Results are representative of 3 separate experiments.

to 5′ sense-*myb*; for the 3′ antisense-*myb* transfections, the average reduction was 91% (from 84 to 97%) as compared to 3′ sense-*myb* (mean of 3 separate experiments).

Twelve single clones were isolated from the 5′ and 3′ antisense *myb* transfectants and the integration of each construct was determined by PCR. Primers for PCR were chosen in order to amplify 267 base pairs upstream of the *Not*I cloning site in addition to 208 base pairs of 5′ antisense-*myb* and 219 bp of 3′ antisense *myb*. Fig. 2 shows a diagram illustrating PCR strategies with primers and probes used for the analysis of the clones. All 3′ antisense *myb* clones showed the expected amplified fragment (486 base pairs) (Fig. 3*A*), whereas several 5′ antisense *myb* clones (Fig. 3*B*, *Lanes 2*, *4*, *7*, and *8*) did not,

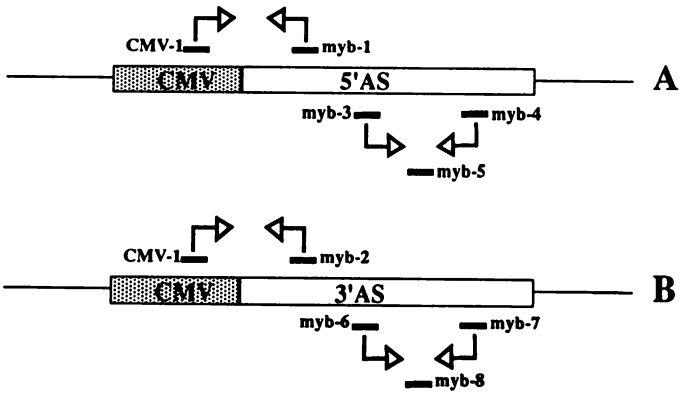

Fig. 2. PCR strategies to detect vector integration and transcription of antisense RNA in antisense transfectants. In *A*, CMV-1 and *myb*-1 are the PCR primers to determine the integration of the 5′ antisense (*AS*) clones; *myb*-3 and *myb*-4 are the primers for RT-PCR; and *myb*-5 is the probe to detect antisense RNA transcripts in 5′ antisense clones. In *B*, CMV-1 and *myb*-2 are the PCR primers to control the integration of the 3′ antisense clones; *myb*-6 and *myb*-7 are the RT-PCR primers; and *myb*-8 is the probe for antisense RNA detection in 3′ antisense clones.

[5] G. Raschellà et al., unpublished observations.

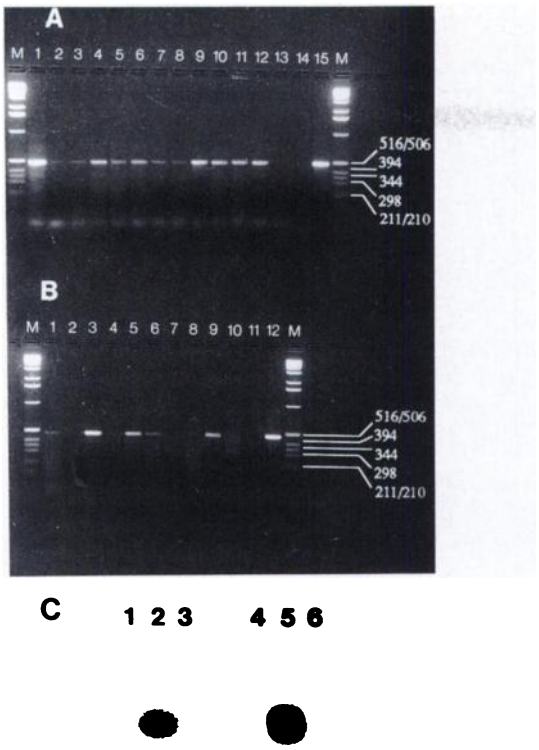

Fig. 3. Detection of construct integration and antisense transcripts by PCR analysis. *A*, 3′ antisense *myb* clones. *M*, size marker. *Lane 1*, pooled transfectant clones; *Lanes 2–12*, individual clones; *Lane 13*, transfectant clone containing the pRc/CMV vector (negative control); *Lane 14*, no DNA; *Lane 15*, DNA from vector 3′ antisense *myb* (positive control). *B*, 5′ antisense *myb* clones. *M*, size marker. *Lane 1*, pooled transfectant clones; *Lanes 2–9*, individual clones; *Lane 10*, transfectant clone containing the pRc/CMV vector (negative control); *Lane 11*, no DNA; *Lane 12*, DNA from vector 5′ antisense *myb* (positive control). *C*, hybridization analysis of antisense RNA transcripts in transfectant clones. *Lane 1*, RT-PCR without RNA; *Lane 2*, RT-PCR of 5′ antisense clone in *B*, *lane 9*; *Lane 3*, PCR from RNA of the same clone; *Lane 4*, RT-PCR without RNA; *Lane 5*, RT-PCR of 3′ antisense *myb* clone, in *A*, *Lane 7*; *Lane 6*, PCR from RNA of the same clone. Primers and probes are as described "Materials and Methods."

possibly because of rearrangement or deletion involving the antisense insert preventing the transcription of the antisense RNA. Accordingly, the inhibition of LAN-5 cell proliferation resulting from transfection with the 5′ antisense *myb* construct might be underestimated. Clones positive for integration of the 5′ antisense construct showed the expected amplified 475-base pair fragment.

Transcription of antisense c-*myb* RNA was confirmed in RT-PCR experiments (Fig. 3*C*). Total RNA from transfectant clones was extracted and treated with RNase-free DNase I before the RT-PCR reaction. Primers were designed to amplify a 224-base pair product for 5′ antisense *myb* clones and a 231-base pair product for 3′ antisense *myb* clones (Fig. 2). After size fractionation on an agarose gel and transfer to a nylon membrane, the amplified products were hybridized to specific probes for unambiguous identification; antisense c-*myb* RNA transcripts were clearly detected in both 5′ and 3′ antisense-*myb* transfectants (Fig. 3*C*). Most of the antisense transfectants do not show evident morphological alterations. All tested antisense clones retain the capability to differentiate toward a neural phenotype under the effect of retinoic acid (not shown). A more detailed phenotypic characterization of the transfectant clones is now in progress.

Comparison of growth curves for 3′ and 5′ antisense-*myb* clones which were found positive for integration of the constructs (Fig. 4) revealed consistently slower proliferation rates and generally lower growth plateaus than those of the LAN-5 parental cells and sense controls.

Immunocytochemical analysis using an anti-*myb* polyclonal antibody indicated the clear presence of myb protein in the nucleus of the LAN-5 parental cells and the 5′ and 3′ sense controls (Fig. 5, *A–C*), whereas myb protein was barely detectable in the 5′ and 3′ antisense transfectants (Fig. 5, *D* and *E*).

Effect of c-*myb* Antisense Oligodeoxynucleotides on Growth of Neuroblastoma and Neuroepithelioma Cell Lines. To further examine the role of c-*myb* in the proliferation of neuroectodermal tumors, we inhibited c-*myb* expression using antisense oligodeoxynucleotides and analyzed the effects of this

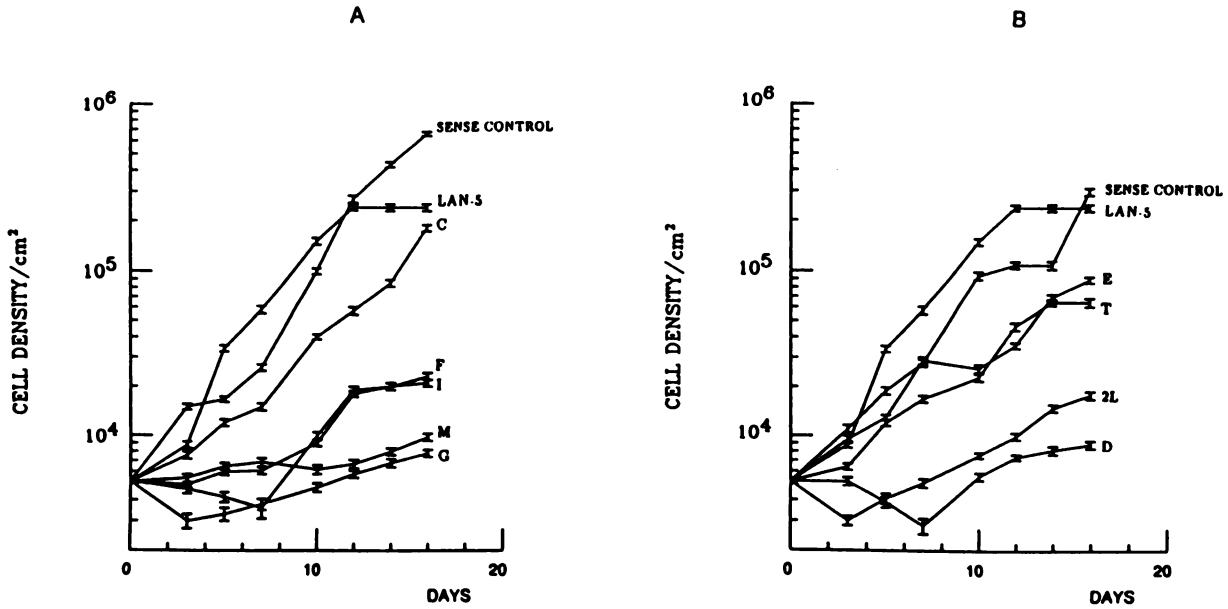

Fig. 4. Growth curves of antisense-*myb* transfectants. *A*, 3′ antisense *myb*; *B*, 5′ antisense *myb*. Values are the mean of two independent experiments. The sense-*myb* control curve in each panel is derived from the mean values using three different 5′ (*A*) and 3′ (*B*) sense clones. Each sense clone had a comparable growth rate. Initial seeding density was 5×10^3 cells/cm^2. *Letters*, individual clones. *Bars*, SE.

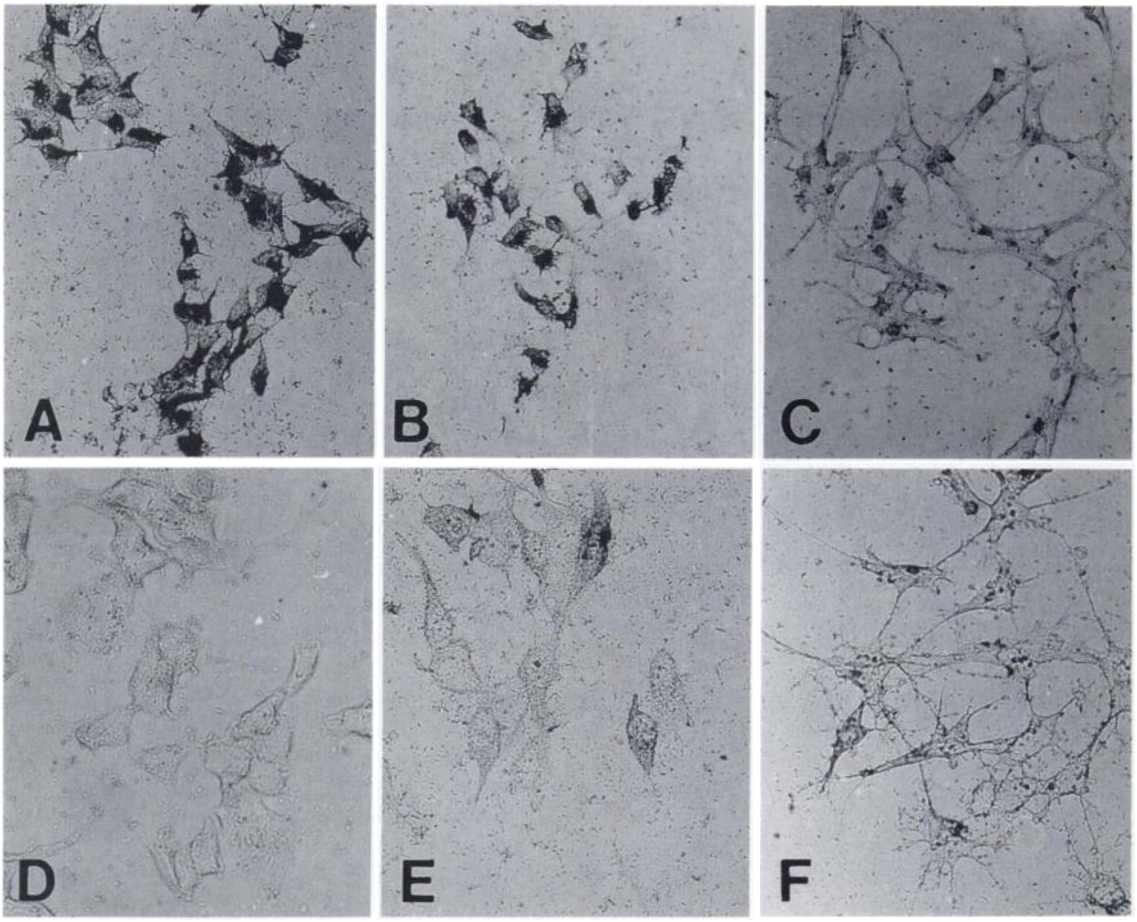

Fig. 5. Expression of c-*myb* protein in antisense clones. Cells were treated with an anti-*myb* specific polyclonal antibody (*A–E*) or nonspecific preimmune serum (*F*) as described in "Materials and Methods." *A*, LAN-5; *B*, 5′ sense *myb*; *C*, 3′ sense*myb*; *D*, 5′ antisense *myb*; *E*, 3′ antisense *myb*; and *F*, 3′ sense *myb* treated with immune serum. × 200.

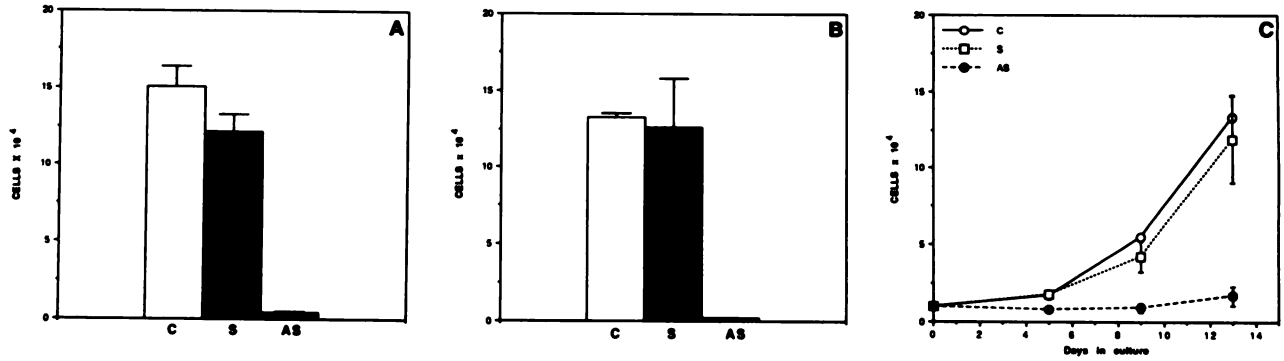

Fig. 6. Effect of c-*myb* antisense oligodeoxynucleotide on cell growth of neuroblastoma and neuroepithelioma cell lines. SK-N-SH (*A*), SK-N-MC (*B*), and LAN-5 (*C*) cells were untreated (*C*), sense-treated (*S*) and antisense-treated (*AS*). Cells were counted after 7 days for SK-N-SH (*A*) and after 9 days for SK-N-MC (*B*). LAN-5 (*C*) cells were counted at days 0, 5, 9, and 13 of culture. Cell count values, mean ± SD (*bars*) of experiment performed in triplicate.

inhibition on the growth of neuroblastoma cell lines LAN-5 and SK-N-SH and neuroepithelioma cell line SK-N-MC (31). In a typical experiment, 1×10^4 cells were seeded in the presence of antisense or sense oligodeoxynucleotides (80 μg/ml at 0 h, 40 μg/ml after 18 h, and 40 μg/ml after 36 h). Cells were counted after 7 or 9 days. As shown in Fig. 6, antisense c-*myb* oligodeoxynucleotide treatment resulted in almost complete growth inhibition in all three cell lines. To determine whether this inhibition correlated with c-*myb* transcript levels, total RNA was extracted from each tumor cell line 24 h after exposure to 120 μg of c-*myb* oligodeoxynucleotides and c-*myb* expression was measured by RT-PCR; c-*myb* mRNA was barely detectable in antisense-treated cells, but abundantly expressed in sense-treated and untreated cells (Fig. 7).

DISCUSSION

We have shown that down-regulation of c-*myb* expression exerts a strong inhibitory effect on the proliferation of neuroectodermal tumor cells. Two different strategies were used in our work. Neuroblastoma cell line LAN-5 was transfected with vectors carrying two different segments of the human c-*myb* cDNA in the antisense orientation and transfection efficiency was assayed; the yield of transfectants was dramatically reduced

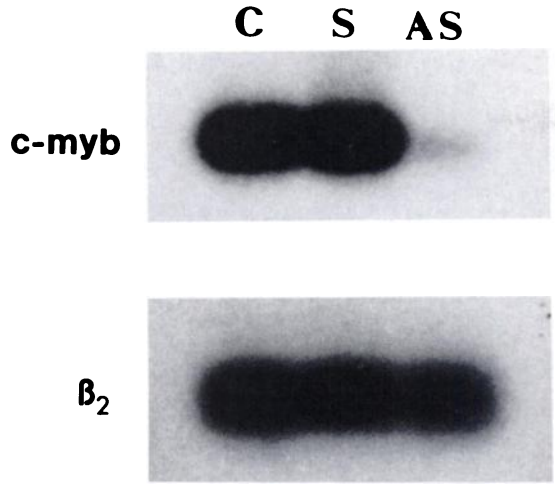

Fig. 7. Expression of c-*myb* mRNA in LAN-5 cells exposed to c-*myb* oligodeoxynucleotides. LAN-5 cells (10^5/ml) were untreated (*C*) or exposed to ~80 μg/ml of c-*myb* sense (*S*) or antisense (*AS*) oligodeoxynucleotides at time 0. After 12 h a second dose (40 μg/ml) was added. Cells were harvested 12 h later. Total RNA was isolated and divided into two aliquots that were separately amplified by RT-PCR with c-*myb*- and β_2-microglobulin-specific primers as described (44). The resulting cDNAs were hybridized to specific ^{32}P-end-labeled probes as described (29). Results are from a representative experiment.

when either antisense vector was used as compared to their sense controls. Although the isolated antisense transfectants are heterogeneous in their growth rate, they show consistently a slower proliferation as compared to sense controls. Furthermore, they transcribe antisense c-*myb* mRNA and have a marked reduction of myb protein synthesis as indicated by immunocytochemistry. Together these data indicate that the slower proliferation of the isolated antisense clones is due to down-regulation of c-*myb* expression caused by antisense RNA production.

The second strategy involved exposure of neuroblastoma and neuroepithelioma cell cultures to a c-*myb* antisense oligodeoxynucleotide in order to suppress c-*myb* mRNA expression. Again, the same inhibitory effect on the proliferation rate of these cells was observed. The rescue of antisense stable transfectants is in apparent contrast with the nearly complete growth inhibition resulting from the exposure of neuroectodermal tumor lines to c-*myb* antisense oligodeoxynucleotides. Most likely these findings rest in the low level of antisense c-*myb* RNA transcribed by the transfectant clones and detectable by the sensitive RT-PCR technique but insufficient to completely block the function of c-*myb* mRNA. In this regard, Cotten *et al.* (36) have demonstrated the requirement for an antisense RNA-:target mRNA ratio of 6:1 to completely abolish the function of protein U7. Nevertheless, the slow proliferation rate of the c-*myb* antisense transfectants indicates that even incomplete down-modulation of c-*myb* has a readily detectable effect on cell growth.

In neuroblastoma several structural abnormalities such as the deletion of the short arm of chromosome 1 (del1p32-pter), double-minute chromosomes and homogeneously staining regions are frequent findings (37–39) and have been associated with the development and the progression of this neoplasia (40). Furthermore, N-*myc* gene amplification has been correlated with advanced clinical stages and poor clinical outcome (27, 41). However, the proliferative activity of neuroblastoma has not been clearly associated with a distinct pattern of altered gene expression.

Two recent reports describe the block of N-*myc* expression by means of antisense RNA and oligodeoxynucleotides (42, 43). In both cases neuroblastoma proliferation was only partially affected by N-*myc* down-regulation and was probably secondary to the induction of differentiative processes. In addition, a large percentage of terminal neuroblastomas do not show amplification or detectable expression of N-*myc*. Together, those data suggested that other gene activities besides N-*myc* are involved in the proliferation of neuroblastoma cells. The inhibitory effect on cell growth obtained by abolishing the expression of c-*myb* strongly suggests the involvement of this protooncogene in the regulation of neuroblastoma cell proliferation. Moreover the findings reported here, provide direct evidence for the essential role of c-*myb* in nonhematopoietic tissues, perhaps through its effects on the expression of genes directly involved in DNA synthesis and cell cycle progression. The stable transfectant cell lines that express antisense c-*myb* RNA should prove useful in evaluating the possible cooperation of c-*myb* and other genes in regulating proliferative and differentiative processes in neuroblastoma, which, in turn, may lead to the development of an antisense-based therapy of these neoplastic disorders.

REFERENCES

1. Bister, K., Nunn, M., Moscovici, G., Perbal, B., Baluda, M. A., and Duesberg, P. H. Acute leukaemia viruses E26 and avian myeloblastosis virus have related transformation-specific RNA sequences but different genetic structures. Proc. Natl. Acad. Sci. USA, *72:* 3677–3681, 1982.
2. Roussel, M., Saule, S., Langrow, C., Rommens, C., Beug, H., Graf, T., and Stehelin, D. Three types of viral oncogenes for haematopoietic transformation. Nature (Lond.), *281:* 452–455, 1979.
3. Moscovici, C., and Gazzolo, L. Transformation of hemopoietic cells and avian leukemia viruses. *In:* Advances Viral Oncology, Vol. 1, New York: Raven Press, pp. 83–106, 1982.
4. Klempnauer, K. H., Symonds, G., Evan, G. I., and Bishop, J. M. Subcellular localization of proteins encoded by oncogenes of avian myeloblastosis virus and avian leukemia virus E26 and by the chicken c-*myb* gene. Cell, *37:* 537–547, 1984.
5. Boyle, W. J., Lampert, M. A., Lipsick, J. S., and Baluda, M. A. Avian myeloblastosis virus and E26 virus oncogene products are nuclear proteins. Proc. Natl. Acad. Sci. USA, *81:* 4265–4269, 1984.
6. Moelling, K., Pfoff, E., Beng, H., Beimling, P., Bunte, T., Scheller, H. E., and Graf, T. DNA-binding activity is associated with purified Myb proteins from AMV and E26 viruses and is temperature-sensitive for E26 *ts* mutants. Cell, *40:* 983–990, 1985.
7. Klempnauer, K. H., and Sippel, A. E. The highly conserved amino-terminal region of the protein encoded by the v-*myb* oncogene functions as a DNA-binding domain. EMBO J., *6:* 2719–2723, 1987.
8. Rosson, D., and Reddy, P. Nucleotide sequence of chicken c-*myb* complementary DNA: implications for *myb* oncogene activation. Nature (Lond.), *319:* 604–606, 1986.
9. Gonda, T. J., Gough, N. M., Dunn, A. R., and De Blaquere, J. Nucleotide sequence of cDNA clones of the murine *myb* protooncogene. EMBO J., *4:* 2003–2007, 1985.
10. Majello, B., Kenyon, L. C., and Dalla Favera, R. Human c-*myb* protooncogene: nucleotide sequence of cDNA and organization of the genomic locus. Proc. Natl. Acad. Sci. USA, *83:* 9636–9640, 1986.
11. Biedenkapp, H., Borgmeyer, U., Sippel, A. E., and Klempnauer, K. H. Viral *myb* oncogene encodes a sequence-specific DNA-binding activity. Nature (Lond), *335:* 835–837, 1988.
12. Weston, K., and Bishop, J. M. Transcriptional activation by the V-*myb* oncogene and its cellular progenitor c-*myb*. Cell, *58:* 85–94, 1986.
13. Ness, S. A., Marknell, A., and Graf, T. The v-*myb* oncogene product binds to and activates the promyelocyte-specific *MIM-1* gene. Cell, *59:* 1115–1124, 1989.
14. Ibanez, C. E., and Lipsick, J. S. Structural and functional domains of the c-*myb* oncogene: requirements for nuclear transport, myeloid transformation, and colony formation. J. Virol., *62:* 1981–1988, 1988.
15. Sakura, H., Kanei-Ishii, C., Nagase, T., Nakagoshi, H., Gonda, T. J., and Ishii, S. Delineation of three functional domains of the transcriptional activator encoded by c-*myb* protooncogene. Proc. Natl. Acad. Sci. USA, *86:* 5758–5762, 1989.
16. Grasser, F. A., Graf, T., and Lipsick, J. E. Protein truncation is required for the activation of the c-*myb* proto-oncogene. Mol. Cell. Biol., *11:* 3987–3996, 1991.
17. Westin, E. H., Gallo, R. C., Arya, S. K., Eva, A., Souza, L. M., Baluda, M. A., Aaronson, S. A., and Wong-Staal, F. Differential expression of the AMV gene in human hematopoietic cells. Proc. Natl. Acad. Sci. USA, *79:* 2194–2198, 1982.
18. Clarke, M. F., Kukowska-Latallo, J. F., Westin, E., Smith, M., and Prochownik, E. V. Constitutive expression of a c-*myb* cDNA blocks Friend

murine erythroleukemia cell differentiation. Mol. Cell. Biol., *8:* 884–892, 1988.
19. Gewirtz, A. M., and Calabretta, B. A c-*myb* antisense oligodeoxynucleotide inhibits normal human hematopoiesis *in vitro*. Science (Washington DC), *242:* 1303–1306, 1988.
20. Mucenski, M. L., McLain, K., Kier, A. B., Swerdlow, S. H., Schreiner, C. M., Miller, T. A., Pietryga, D. W., Scott, Jr., W. J., and Potter, S. S. A functional c-*myb* gene is required for normal murine fetal hepatic hematopoiesis. Cell, *65:* 677–689, 1991.
21. Slamon, D. S., deKernion, J. B., Verma, I. M., and Cline, M. S. Expression of cellular oncogenes in human malignancies. Science (Washington DC), *224:* 256–261, 1984.
22. Calabretta, B., Sims, R. B., Valtieri, M., Caracciolo, D., Szczylik, C., Venturelli, D., Beran, M., and Gewirtz, A. M. Normal and leukemic hematopoietic cells manifest differential sensitivity to inhibitory effects of c-*myb* antisense oligodeoxynucleotides: an *in vitro* study with relevance to bone marrow purging. Proc. Natl. Acad. Sci. USA, *88:* 2351–2355, 1991.
23. Alitalo, K., Winquist, R., Linn, C. C., de La Chapelle, A., Schwab, M., and Bishop, J. M. Aberrant expression of an amplified c-*myb* oncogene in two cell lines from a colon carcinoma. Proc. Natl. Acad. Sci. USA, *81:* 4534–4538, 1984.
24. Griffin, C. A., and Baylin, S. B. Expression of the c-*myb* oncogene in human small cell lung carcinoma. Cancer Res., *45:* 272–275, 1985.
25. Janssen, J. W. G., Vernole, P., de Boer, P. A. J., Oosterhuis, J. W., and Collard, J. G. Sublocalization of c-*myb* to 6q21-q23 by *in situ* hybridization and c-*myb* expression in human teratocarcinoma with 6q rearrangements. Cytogenet. Cell Genet., *41:* 129–135, 1986.
26. Thiele, C. J., McKeon, C., Triche, T. J., Ross, R. A., Reynolds, C. P., and Israel, M. A. Differential protooncogene expression characterizes histopathologically indistinguishable tumors of the peripheral nervous system. J. Clin. Invest., *80:* 804–811, 1987.
27. Seeger, R. C., Brodeur, G. M., Sather, H., Dalton, A., Siegel, S., Wong, K. Y., and Hammond, D. Association of multiple copies of the N-*myc* oncogene with rapid progression of neuroblastoma. N. Engl. J. Med., *313:* 1111–1116, 1985.
28. Thiele, C. J., Cohen, P. S., and Israel, M. A. Regulation of c-*myb* expression in human neuroblastoma cells during retinoic acid-induced differentiation. Mol. Cell. Biol., *8:* 1677–1688, 1988.
29. Sambrook, J., Fritsch, E. F., and Maniatis, T. *In*: Molecular Cloning, A Laboratory Manual, Ed. 2., Cold Spring Harbor, NY: Cold Spring Harbor Laboratory, 1989.
30. Seeger, R. C., Danon, Y. L., Rayner, S. A., and Hoover, F. Definition of a THY-1 determinant on human neuroblastoma, glioma, sarcoma, and teratoma cells with a monoclonal antibody. J. Immunol., *128:* 983–989, 1982.
31. Biedler, J. L., Helson, L., and Spengler, B. A. Morphology and growth, tumorigenicity and cytogenetics of human neuroblastoma cells in continuous culture. Cancer Res., *33:* 2643–2649, 1973.
32. Chomczynski, P., and Sacchi, N. Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. Anal. Biochem., *162:* 156–159, 1987.
33. Szczylik, C., Skorski, T., Nicolaides, N. C., Manzella, L., Malaguarnera, L., Venturelli, D., Gewirtz, A. M., and Calabretta, B. Selective inhibition of leukemia cell proliferation by BCR-ABL antisense oligodeoxynucleotides. Science (Washington DC), *253:* 562–565, 1991.
34. Maly, A., and Krchnak, V. Identification of c-myb (chicken), c-myb (mouse) and v-myb (AMV) protein products by immunoprecipitation with antibodies directed against a synthetic peptide. FEBS Lett., *205:* 104–108, 1986.
35. Foecking, M. K., and Hofstetter, H. Powerful and versatile enhancer promoter unit for mammalian expression vectors. Gene, *45:* 101–105, 1986.
36. Cotten, M., Schaffner, G., and Birnstiel, M. L. Ribozyme, antisense RNA, and antisense DNA inhibition of U7 small nuclear ribonucleoprotein-mediated histone pre-mRNA processing *in vitro*. Mol. Cell. Biol., *9:* 4479–4487, 1989.
37. Brodeur, G. M., Green, A. A., Hayes, F. A., Williams, K. J., Williams, D. L., and Tsiatis, A. A. Cytogenetic features of human neuroblastomas and cell lines. Cancer Res., *41:* 4678–4686, 1981.
38. Franke, F., Rudolph, B., Christiansen, H., Harbott, J., and Lampert, T. Tumor karyotype may be important in the prognosis of human neuroblastoma. J. Cancer Res. Clin. Oncol., *111:* 266–272, 1986.
39. Gilbert, F., Feder, M., Balaban, G., Brangman, D., Lurie, D. K., Podolski, R., Rinaldt, V., Vinikoor, N., and Weinsband, J. Human neuroblastomas and abnormalities of chromosomes 1 and 17. Cancer Res., *44:* 5444–5449, 1984.
40. Gilbert, F. Chromosome abnormalities, gene amplification, and tumor progression. *In:* A. E. Evans, G. J. D'Angio, and R. C. Seeger, (eds.), Advances in Neuroblastoma Research, pp. 151–159. New York: Alan R. Liss, 1985.
41. Dominici, C., Negroni, A., Romeo, A., Castello, M. A., Clerico, A., Scopinaro, M., Mauro, F., and Raschellà, G. Association of near-diploid DNA content and N-*myc* amplification in neuroblastomas. Clin. Exp. Metastasis, *7:* 201–211, 1989.
42. Whitesell, L., Rosolen, A., and Neckers, L. M. Episome-generated n-myc antisense RNA restricts the differentiation potential of primitive neuroectodermal cell lines. Mol. Cell. Biol., *11:* 1360–1371, 1991.
43. Negroni, A., Scarpa, S., Romeo, A., Ferrari, S., Modesti, A., and Raschellà, G. Decrease of proliferation rate and induction of differentiation by a MYCN antisense DNA oligomer in a human neuroblastoma cell line. Cell Growth Differ., *2:* 511–518, 1991.
44. Venturelli, D., Mariano, M. T., Valtieri, M., Lange, B., Crist, W., Link, M., and Calabretta, B. Down-regulated c-*myb* expression inhibits DNA synthesis of T-leukemia cells in most patients. Cancer Res., *50:* 7371–7375, 1990.