Proc. Natl. Acad. Sci. USA
Vol. 91, pp. 4499–4503, May 1994
Medical Sciences

Biologic and therapeutic significance of *MYB* expression in human melanoma

NOBUKO HIJIYA*, JIN ZHANG*, MARIUSZ Z. RATAJCZAK*, JEFFREY A. KANT*, KIM DERIEL†, MEENHARD HERLYN‡, GERALD ZON¶, AND ALAN M. GEWIRTZ*§‖

Departments of *Pathology and §Internal Medicine, University of Pennsylvania School of Medicine, †Temple University School of Medicine, and ‡Wistar Institute for Anatomy and Biology, Philadelphia, PA 19104; and ¶Lynx Therapeutics, Inc., Hayward, CA 94545

Communicated by Peter C. Nowell, November 25, 1993

ABSTRACT **We investigated the therapeutic potential of employing antisense oligodeoxynucleotides to target the disruption of *MYB*, a gene which has been postulated to play a pathogenetic role in cutaneous melanoma. We found that *MYB* was expressed at low levels in several human melanoma cell lines. Also, growth of representative lines *in vitro* was inhibited in a dose- and sequence-dependent manner by targeting the *MYB* gene with unmodified or phosphorothioate-modified antisense oligodeoxynucleotides. Inhibition of cell growth correlated with specific decrease of *MYB* mRNA. In SCID mice bearing human melanoma tumors, infusion of *MYB* antisense transiently suppressed *MYB* gene expression but effected long-term growth suppression of transplanted tumor cells. Toxicity of the oligodeoxynucleotides was minimal in mice, even when targeted to the murine *Myb* gene. These results suggest that the *MYB* gene may play an important, though undefined, role in the growth of at least some human melanomas. Inhibition of *MYB* expression might be of use in the treatment of this disease.**

Cutaneous melanoma is a highly malignant and increasingly common neoplasm (1). Because metastatic melanoma remains incurable, new treatment approaches are needed. The ability to selectively disrupt the function of genes involved in malignant cell growth is an increasingly attractive therapeutic strategy. Technologies relevant to this purpose include homologous recombination, which actually destroys the targeted gene (2), and use of reverse complementary (antisense) RNA (3) or DNA (4–6) to interfere with utilization of the target gene's mRNA. The latter may be particularly well suited to therapeutic purposes since antisense oligodeoxynucleotides (ODNs) can be chemically synthesized and introduced directly into cells without the need for viral vectors. Problems attendent to the use and manufacture of viral vectors are thereby avoided. Further, the transient effect of antisense DNA on gene expression, as opposed to permanent alteration of the genome, may be desirable under these circumstances.

We previously reported that phosphorothioate-modified antisense ODNs (antisense [S]ODNs) targeted to the *MYB* protooncogene controlled the growth of a human leukemia in a SCID mouse model (7). *MYB* encodes a transcriptionally active nuclear binding protein, MYB, which plays an important regulatory role in cell proliferation (8) and differentiation (9). *MYB* is located on chromosome 6q22–23 in humans (10), where some human melanomas also have structural aberrations. Altered *MYB* expression has been implicated in the pathogenesis of melanoma (11–14). Accordingly, we targeted the *MYB* gene in human melanoma cells with antisense ODNs to learn more about the biologic importance of its expression in this disease and the therapeutic potential of disrupting its function.

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

MATERIALS AND METHODS

ODNs. Unmodified phosphodiester ODNs and [S]ODNs (Lynx Therapeutics, Hayward, CA) complementary to the *MYB* gene were synthesized as reported (8, 15).

Cell Culture and *in Vitro* ODN Exposure. Melanoma cell lines were obtained from the American Type Culture Collection (Hs294T, A375, C32) or from Dupont Guerry (University of Pennsylvania School of Medicine) (SK-MEL-37, WM39). Cells were cultured in 96-well plates (10^3 cells/200 μl per well) to which ODNs (10–100 μg/ml) were added once or on two or five consecutive days. Effects on cell proliferation were determined by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium (MTT) assay (CellTiter 96 nonradioactive cell-proliferation assay, Promega) (16).

Mice. Seven- to 8-week-old female CB-17/ACRTAC/*scid*/SDS mice were obtained from Taconic Farms. Animals with elevated IgM levels, as measured by ELISA (17), were excluded from the study.

Melanoma Transplantation into SCID Mice and Administration of [S]ODNs. Single-cell suspensions of Hs294T human melanoma cells (2×10^6 in 0.2 ml of Dulbecco's modified Eagle's medium) were injected subcutaneously in the right lower dorsal region of mice. [S]ODNs were administered by subcutaneously implanted Alzet constant-infusion pumps (Alza) (7).

Evaluation of Tumor Growth in Mice. Tumor weights *in vivo* were estimated from three separate diagonal measurements (18). Actual tumor weights were obtained at the time of sacrifice. Statistical significance of tumor weight differences was assessed by the Mann–Whitney nonparametric method.

RNA Extraction and Reverse Transcription–Polymerase Chain Reaction (RT–PCR). *MYB* and β-actin mRNA transcripts were detected by RT–PCR as described save that RT was primed with random hexamers (2 ng/μl) (19). The *MYB* amplification primers corresponded to mRNA nt 195–215 (5′ primer) and nt 444–464 (3′ primer) (10). The PCR product was detected with a probe corresponding to nt 355–375. β-Actin expression was detected with 5′ and 3′ primers corresponding to nt 600–621 and 905–885, respectively. The β-actin PCR product was detected with a ^{32}P-labeled probe corresponding to nt 795–815 (20).

Detection of Antisense [S]ODN in Tumor Tissue. Excised tumors were minced and multiply washed. DNA was extracted by standard methods, and 50 μg was electrophoresed in a 4% low-melting agarose gel (FMC) and then transferred to a nylon membrane (Pall) in 3 M NaCl/0.3 M sodium citrate, pH 7. DNA was probed with a ^{32}P-end-labeled *MYB* sense oligomer complementary to the antisense DNA sequence.

Abbreviations: ODN, oligodeoxynucleotide; [S]ODN, phosphorothioate ODN; RT, reverse transcription.

‖To whom reprint requests should be addressed at: Room 230–John Morgan Building, University of Pennsylvania School of Medicine, 36th Street and Hamilton Walk, Philadelphia, PA 19104.

RESULTS

***MYB* Expression in Human Melanoma Cell Lines.** Though the integrity of the *MYB* locus on chromosome 6 has been scrutinized (11, 13, 14, 21), data concerning *MYB* expression in melanomas are sparse (22, 23). We therefore screened five human melanoma cell lines (Hs294T, SK-MEL-37, A375, C32, and WM39) for *MYB* mRNA by Northern analysis (19). Total RNA from each cell line (20 μg) was blotted to nitrocellulose and then probed with a ^{32}P-labeled human *MYB* cDNA (24). None of the lines gave a positive signal with this technique. However, when a sensitive RT–PCR was employed, *MYB* mRNA was unambiguously detected in all five cell lines (data not shown). Accordingly, *MYB* is most likely expressed in some melanoma cell lines, albeit at a low level. In accord with this finding, MYB protein levels were below detection limits in Western blot analysis of protein extracted from 7×10^3 SK-MEL-37 and Hs294T cells (data not shown).

Effect of Disrupting *MYB* Expression in Human Melanoma Cells. To determine the biological significance of low-level *MYB* expression in melanoma cells, we targeted the *MYB* message in SK-MEL-37 and Hs294T cells with unmodified or phosphorothioate antisense ODNs. Control DNA sequences were evaluated simultaneously to ensure that any effects observed were sequence specific. In Hs294T cells, for example, exposure to *MYB* sense sequences had no statistically significant effect on cell proliferation in comparison to untreated controls. In contrast, the *MYB* antisense DNA inhibited growth in a dose-responsive manner up to ≈60% ($P < 0.001$) of control cell values (data not shown). Growth inhibition was accompanied by loss of RT–PCR-detectable *MYB* mRNA, but not β-actin mRNA, suggesting that growth inhibition was secondary to pertubation of *MYB* expression.

Visual examination of the cultures revealed some clue regarding the mechanism of inhibition, which appeared to vary with the cell line. For example, after exposure to *MYB* antisense, Hs294T cells appeared to undergo cytolysis, suggesting that *MYB* perturbation could be a lethal event in these cells (Fig. 1*A*), whereas SK-MEL-37 cells appeared to undergo growth arrest with or without what appeared morphologically to represent differentiation toward a more mature phenotype (Fig. 1*B*).

Relationship of ODN Dose, Frequency of Exposure, and Inhibition of Cell Growth. We also examined cell growth inhibition as a function of ODN concentration and frequency of exposure. When SK-MEL-37 cells were exposed to ODNs, the most important factor for achieving growth inhibition was initial exposure to high concentrations of ODN (Fig. 2). For example, no effect on cell growth was observed when the ODNs were added to cultures in divided doses of 20 μg/ml per day for 2 days, or 10 μg/ml per day for 5 days. In contrast, when cells were exposed to a single bolus of 50 μg/ml, cell growth was inhibited ≈25% in comparison to untreated controls. This relationship was even more apparent at higher doses. A single total ODN dose of 100 μg/ml inhibited growth much more significantly than the same total dose delivered in divided doses of 20 μg/ml per day for 5 days (Fig. 2*A*). Even at doses up to 250 μg/ml, 50 μg/ml per day for 5 days was not as effective as a total of 200 μg/ml given as 100 μg/ml per day for 2 days (50% vs. 70% inhibition, respectively).

To determine whether these results were influenced by possible degradation of unmodified ODN, we carried out similar experiments with Hs294T cells exposed to the more stable [S]ODN. A similar but less strict relationship between extracellular concentration and inhibition of cell growth was again observed (Fig. 2*B*). That is, initial high concentrations were more effective than equivalent final concentrations built up by cumulative lower doses. Accordingly, it appears that for either type of compound, sufficient cellular uptake to inhibit the target gene is achieved only by initial exposure to some critical "high" concentration of compound. Why cumulative lower doses of a stable compound are less effective is uncertain, but examination of this phenomenon may provide valuable information on oligomer uptake mechanisms, intracellular trafficking, and interactions with target mRNA.

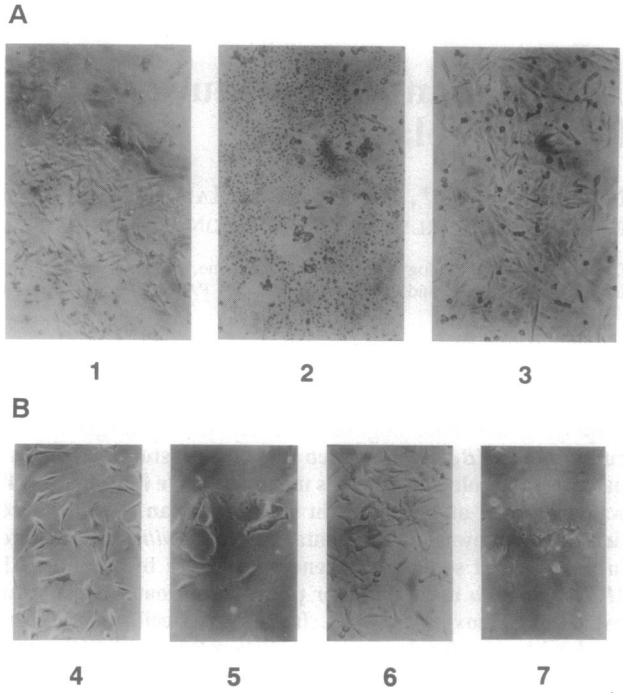

FIG. 1. Photomicrographs of Hs294T and SK-MEL-37 human melanoma cells incubated with *MYB* ODNs (100 μg/ml per day for 5 days). (*A*) Hs294T cells incubated with sense (*1*) or antisense (*2*) [S]ODNs or without ODNs (*3*). (*B*) SK-MEL-37 cells incubated with *MYB* sense (*4*) or antisense (*5*) ODNs without treatment (*6*), or with the differentiation-inducing agent mitomycin C (50 ng/ml per day for 5 days) (*7*). Note the morphologic similarity of cells in *5* and *7*. Under each culture condition cells display a flattened, stellate appearance, multiple dendrites, and increased numbers of pigment granule, all characteristics which suggest that the cells have undergone differentiation (25).

Effect of *MYB* Antisense [S]ODN on Melanoma Tumor Growth *in Vivo*. We examined the effect of the *MYB* antisense

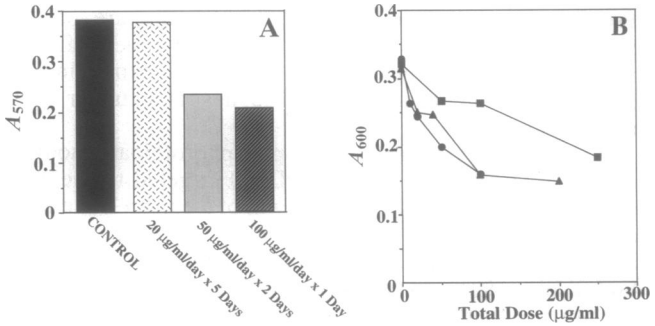

FIG. 2. Effects of concentration and frequency of ODN exposure on melanoma cell growth. (*A*) Effect of a total ODN dose of 100 μg/ml on SK-MEL-37 cell growth. Cells were exposed to *MYB* antisense ODNs for five consecutive days (20 μg/ml per day), two consecutive days (50 μg/ml per day), or once (100 μg/ml). Cell growth was quantitated by MTT assay (A_{570} or A_{600}) 10 days after the start of culture. (*B*) A similar experiment carried out with [S]ODNs on Hs294T cells. Total [S]ODN dose to which the cells were exposed is shown on the abscissa. The total dose was delivered either as a single dose (●) or in equal divided daily doses given over 2 days (▲) or 5 days (■).

DNA on human melanoma cell growth in a SCID mouse model. In the first of three experiments to assess this question, 41 mice were inoculated with Hs294T cells. When tumor nodules became palpable, animals were randomly assigned to receive no treatment (13 animals) or 7-day infusions (500 μg/day; 25 μg/g of body weight) of *MYB* sense (14 animals) or antisense (14 animals) [S]ODN. Animals were examined daily for 40 days to determine the effects of the [S]ODN on survival and tumor growth. The antisense [S]ODN treatment significantly inhibited local tumor growth in comparison to the untreated and sense [S]ODN-treated groups. In fact, until ≈day 35, calculated tumor weights in the antisense group were ≈50% lower than the other groups. After this time, growth in the antisense-treated group recovered and essentially paralleled that seen in the untreated and sense-treated animals. Nevertheless, when the mice were sacrificed on day 40, tumors removed from the antisense-treated animals were significantly smaller ($P < 0.05$), 2.5 ± 0.5 g (mean ± SD), than those from the untreated and sense-treated groups, 3.5 ± 1.7 g and 3.3 ± 1.2 g, respectively.

We then examined the growth-inhibitory effects of the *MYB* antisense [S]ODN against a subclinical tumor burden. In this experiment, mice were subcutaneously inoculated with 2×10^6 Hs294T tumor cells. Three days later animals were randomized to receive no treatment (9 mice) or a 7-day infusion (500 μg/day) of *MYB* sense (8 mice) or antisense (10 mice) [S]ODN. Tumor growth was evaluated over a 65-day period (Fig. 3*A*). While no untreated mice were lost during this period, 3 sense- and 4 antisense-treated mice died of uncertain causes. In the remaining animals, inhibition of tumor growth in the antisense-treated group was again noted throughout the observation period and appeared to be greater than that observed in the first experiment. When the mice were sacrificed at 60 days after the pumps were implanted, mean tumor weights of untreated, sense, and antisense groups were 4.5 ± 1.7 g, 4.0 ± 1.5 g, and 2.1 ± 1.2 g, respectively. The differences between these groups were statistically significant ($P < 0.05$). Fig. 4 illustrates typical tumors observed in these mice.

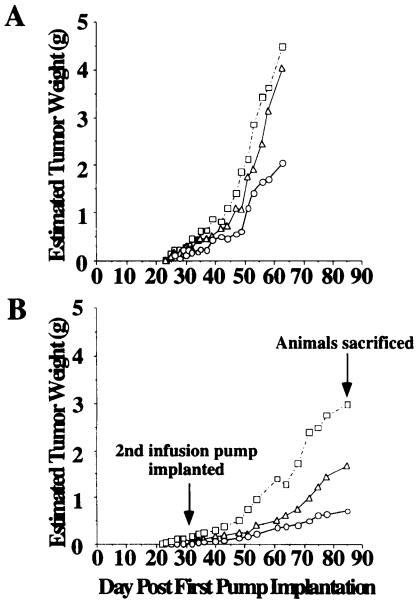

FIG. 3. Effect of *MYB* [S]ODN infusion on human melanoma tumor growth in SCID mice. Doses were 500 μg/day for 7 days initiated 3 days after melanoma cell inoculation (*A*) or 500 μg/day for 14 days administered 3 days after inoculation and then again 16 days after the first infusion ended. ○, Antisense-treated; △, sense-treated; □, untreated.

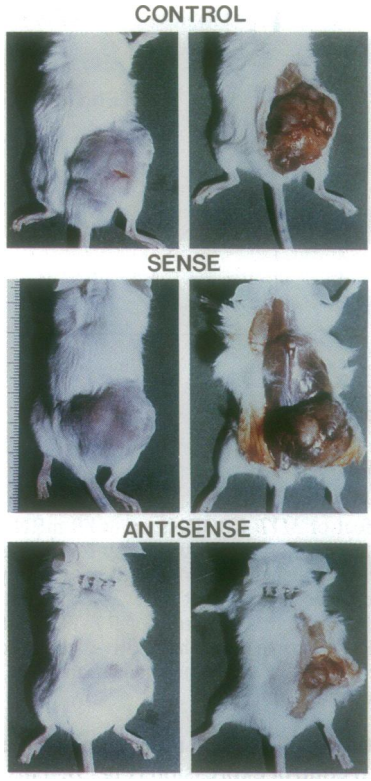

FIG. 4. Representative photomicrographs of melanomas *in situ* in animals which received *MYB* ODN infusions (500 μg/day for 7 days) beginning 3 days after tumor cell inoculation. Infusion pumps can be seen in the sense- and antisense-treated mice.

We lastly examined the effect of a repeat infusion on tumor growth. Mice (10 per group) were again inoculated with 2×10^6 tumor cells. Three days later they were randomized to receive no treatment or an infusion of sense or antisense [S]ODN (500 μg/day for 14 days). Sixteen days after the first infusion ended, a repeat infusion of identical dose and duration was begun. In this experiment, 3 control animals died tumor-related deaths during the observation period. In the antisense-treated animals tumor growth inhibition was more dramatic than in the previous experiments and persisted throughout the observation period (Fig. 3*B*). When animals were sacrificed 85 days after the first pump was implanted, mean tumor weights of control ($n = 7$), sense ($n = 9$), and antisense ($n = 10$) groups were 3.0 ± 2.0 g, 1.7 ± 1.5 g, and 0.7 ± 0.5 g. The difference in tumor weights between the untreated and antisense-treated groups was highly significant ($P < 0.01$), as was the difference between the sense-treated and antisense-treated groups ($P < 0.05$). Though it appeared that tumor sizes differed between the untreated and sense-treated groups, the differences were not of statistical significance ($P > 0.05$).

In contrast to the experiments carried out with a lower total dose of [S]ODN, none of the animals in the high dose, repeat-infusion sense- or antisense-treated groups died before the experiment was terminated. These results suggest that [S]ODN toxicity was not a cause of animal deaths.

[S]ODN Uptake in Tumor Tissue and Correlation of Growth Inhibition with *MYB* mRNA Levels. To determine the extent of [S]ODN uptake into tumor tissue and to correlate effects on *MYB* expression with tumor growth, five mice with established tumors (≈0.5 g) were infused with *MYB* antisense [S]ODN (500 μg/day) for 7 days. On days 7, 9, and 11 after the infusion was begun, an animal was sacrificed and its tumor was excised to determine *MYB* mRNA levels in the tissue. *MYB* mRNA was measurably decreased (as normal-

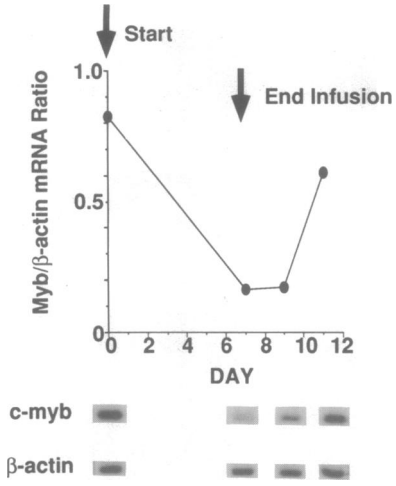

FIG. 5. Effect of *MYB* [S]ODNs on *MYB* mRNA expression in tumor tissue. *MYB* [S]ODNs (500 μg/day for 7 days) were infused into mice with established tumors (≈1.0 g). On days 7, 9, and 11 tumors were excised and total RNA was extracted from approximately equivalent amounts of tissue for RT–PCR detection of *MYB* and β-actin mRNA expression. The relative amount of *MYB* mRNA in each sample was estimated by normalization to the β-actin mRNA detected in the same sample.

ized to β-actin mRNA), but not completely eliminated, in comparison to control expression (Fig. 5). This decrement in *MYB* expression persisted for ≈2 days after the infusion finished but returned toward baseline thereafter.

Normalization of *MYB* expression may have been related to [S]ODN concentration in tissue falling below a critical level. In support of this hypothesis, [S]ODN levels in the tumor tissue decreased rapidly from an estimated 500 ng (per 50 μg of extracted DNA) during the infusion to 10–50 ng (per 50 μg of extracted DNA) on day 8, 1 day after the infusion finished (Fig. 6). Nevertheless, while human *MYB* expression will be selectively suppressed by the antisense DNA, the PCR primers employed for detection will amplify both human *MYB* mRNA and murine Myb mRNA. Since murine blood and stromal elements gradually infiltrate the tumor, some of the *MYB* mRNA detected could be contributed from this source.

Toxicity of Murine *MYB* Antisense Oligomers in Mice. Mice receiving human *MYB* ODN behaved and fed normally and lost no weight. In addition, histopathologic examination of tissue fixed at the time of sacrifice revealed no organ damage (data not shown). As mentioned above, however, a small number of animals died of unexplained causes during the infusion studies. Such deaths occurred approximately equally in each treatment group and were therefore unrelated to the ODN sequence infused. Further, since there were no unexplained deaths in the animals that received the highest

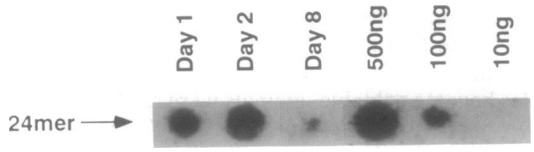

FIG. 6. Detection of *MYB* antisense ODN in tumor tissue. Mice with established tumors were infused with *MYB* antisense ODN (500 μg/day for 7 days). The tumors were excised on days 1, 2, and 8 after start of the infusion. Tumor DNA was extracted, electrophoresed in 4% agarose, and blotted to nylon membranes as noted above. Tumor [S]ODN content was estimated by probing the blotted material with a ^{32}P-end-labeled *MYB* sense oligomer. Relative band intensity was then compared with known DNA standards of 500, 100, and 10 ng (shown in the three lanes at right).

ODN doses (i.e., two infusions), it appears that while [S]ODN toxicity cannot be ruled out as a cause of death in these cases, this explanation seems unlikely.

To model potential toxicity in a human host, mice were also infused with murine *MYB* sense and antisense sequences, up to 1 mg/day for 14 days. No clinically significant untoward effects were observed in these mice (Table 1). The mice behaved normally and their body weights did not change. Of interest, however, mice did manifest thrombocytopenia which appeared to be neither sequence nor dose related. The level of thrombocytopenia was mild to moderate and caused no detectable bleeding abnormality. Animals receiving the highest dose of antisense DNA also manifested splenomegaly. Histopathology of hypertrophied spleens demonstrated lymphoid and myeloid hyperplasia with increased megakaryocytes. We therefore posit that the spleen, an important hematopoietic organ in the mouse, may have been compensating for hematopoietic suppressive effects of the *MYB* antisense [S]ODN.

DISCUSSION

Though the *MYB* gene has been postulated to play a role in the pathogenesis of malignant melanoma, data supporting this hypothesis have been scant and largely inferential (11–14). Using antisense DNA, we now provide some direct evidence that *MYB* gene expression is important for the growth and maintenance of several human melanoma cell lines. Why MYB deprivation causes cytolysis in some cells (Hs294T) and apparent differentiation in others (SK-MEL-37) is unclear. Also enigmatic is the prolonged growth suppression (>2 months) associated with the transient suppression of *MYB*. In the first *in vivo* experiment this might have been more apparent than real, since after an initial delay, growth of the tumor mass in antisense-treated animals appeared to parallel that seen in the control groups. However, this did not seem to be the case in the second (Fig. 4*A*) and third (Fig. 4*B*) *in vivo* experiments, where tumor growth was considerably suppressed. Here one could postulate that

Table 1. Toxicity of murine *MYB* antisense [S]ODNs for BALB/c mice

[S]ODN	Dose, μg/day	Leukocytes, no. × 10^{-3} per mm^3			Hematocrit, %			Platelets, no. × 10^{-3} per mm^3			Spleen weight, mg
		Day 0	Day 14	Day 21	Day 0	Day 14	Day 21	Day 0	Day 14	Day 21	Day 21
Sense	100	3.2	7.7	3.2	53	52	52	554	635	215	140
	300	3.4	6.7	2.9	52	51	51	642	619	218	123
	1000	2.7	3.5	3.6	52	49	51	491	421	346	111
Antisense	100	2.6	4.5	2.6	53	47	48	740	412	188	165
	300	2.1	5.1	2.7	52	44	48	595	397	183	181
	1000	2.7	3.3	4.8	52	44	49	503	230	279	273

[S]ODNs corresponding to murine *Myb* mRNA codons 2–9 were administered to BALB/c mice at various doses for 14 days by Alzet pumps. Blood cells were counted in a hemocytometer and hematocrit was determined by centrifugation of heparinized blood on days 0, 14, and 21. Spleens were weighed immediately after death.

deprivation of MYB led to a persistent, though apparently nonlethal, growth-deprived state. Alternatively, MYB deprivation may have led to cell death in a sensitive subset of cells and to cytostasis in another which eventually recovered. Therefore, the protein probably exerts different functions within and between cells of a given type, and these functions most likely depend on the state of differentiation and cell cycle status. Unmasking these functions through inhibition studies of this type may provide useful clues to the function of MYB in nonhematopoietic cells and to the identity of potential protein partners in these effects.

That we obtained growth inhibition by targeting a gene expressed at such low levels may be viewed as surprising. However, recent reports suggest that low-level gene expression can have significant biological import (26, 27). For example, Burk *et al.* (27) reported that MYB protein cooperatively interacted with C/EBP transcription factor proteins, but only when *MYB* was expressed at a low level. High-level *MYB* expression abrogated this synergy. Accordingly, it is reasonable to hypothesize from all these studies that low-level *MYB* expression is of biological significance in melanoma cells.

The experiments reported herein serve as a paradigm of ODN-based therapeutics for human malignancies. Nevertheless, while it is clear that *MYB* is an effective target in human melanoma, it is not necessarily the best target for this strategy. It is equally straightforward that further development of the antisense strategy will be needed before the successful application of this technique in the clinic can be anticipated. Knowledge concerning DNA uptake mechanisms, intracellular ODN trafficking, mRNA disruption mechanisms, and, of equal importance, how apparent resistance develops will all contribute significantly to the effective pharmaceutical use of these compounds. Accordingly, while this area remains in its scientific infancy, these *in vivo* studies and those of our colleagues (28–30) convince us that modulation of gene expression with antisense DNA is a therapeutic strategy worth pursuing.

The editorial assistance of E. R. Bien is gratefully acknowledged. This work was supported in part by U.S. Public Health Service Grants CA54384 and CA51083 (A.M.G.) and CA25874 (M.H.). A.M.G. is the recipient of a Research Career Development Award from the National Cancer Institute.

1. American Cancer Society (1992) *American Cancer Society: Cancer Facts and Figures-1992* (ACS, Atlanta).
2. Mansour, S. L., Thomas, K. R. & Capecchi, M. R. (1988) *Nature (London)* **336**, 348–352.
3. Prochownik, E. V., Kukowska, J. & Rodgers, C. (1988) *Mol. Cell. Biol.* **8**, 3683–3695.
4. Zamecnik, P. C. & Stephenson, M. L. (1978) *Proc. Natl. Acad. Sci. USA* **75**, 280–284.
5. Gewirtz, A. M. & Calabretta, B. (1991) in *Prospects for Antisense Nucleic Acid Therapy of Cancer and AIDS*, ed. Wickstrom, E. (Liss, New York), pp. 53–61.
6. Ratajczak, M. Z., Hijiya, N., Catani, L., DeRiel, K., Luger, S. M., MacGrave, P. & Gerwirtz, A. M. (1992) *Blood* **79**, 1956–1961.
7. Ratajczak, M. Z., Kant, J. A., Luger, S. M., Hijiya, N., Zhang, J., Zon, G. & Gewirtz, A. M. (1992) *Proc. Natl. Acad. Sci. USA* **89**, 11823–11827.
8. Gewirtz, A. M. & Calabretta, B. (1988) *Science* **242**, 1303–1306.
9. Todokoro, K., Watson, R. J., Higo, H., Amanuma, H., Kuramochi, S., Yanagisawa, H. & Ikawa, Y. (1988) *Proc. Natl. Acad. Sci. USA* **85**, 8900–8904.
10. Majello, B., Kenyon, L. C. & Dalla-Favera, R. (1986) *Proc. Natl. Acad. Sci. USA* **83**, 9636–9640.
11. Linnenbach, A. J., Huebner, K., Reddy, E. P., Herlyn, M., Parmiter, A. H., Nowell, P. & Koprowski, H. (1988) *Proc. Natl. Acad. Sci. USA* **85**, 74–78.
12. Trent, J. M., Thompson, F. H. & Meyskens, F. L. J. (1989) *Cancer Res.* **49**, 420–423.
13. Meese, E., Meltzer, P. S., Witkowski, C. M. & Trent, J. (1989) *Genes Chromosomes Cancer* **1**, 88–94.
14. Dasgupta, P., Linnenbach, A. J., Giaccia, A. J., Stamato, T. D. & Reddy, E. P. (1989) *Oncogene* **4**, 1201–1205.
15. Zon, G. & Stec, W. J. (1991) in *Oligonucleotides and Analogues: A Practical Approach*, ed. Eckstein, F. (Oxford Univ. Press, Oxford), pp. 87–108.
16. Promega Corporation (1991) in *Technical Bulletin #112* (Promega, Madison, WI).
17. Coligan, J. E., Kruisbeek, A. M., Margulies, D. H., Shevach, E. M. & Strober, W. (1993) *Current Protocols in Immunology* (Greene Associates, New York), pp. 2.1.2–2.1.22.
18. Morrison, S. D. (1983) *J. Natl. Cancer Inst.* **71**, 407–408.
19. Sambrook, J., Fritsch, E. F. & Maniatis, T. (1989) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Lab. Press, Plainview, NY).
20. Nakajima-Iijima, S., Hamada, H., Reddy, P. & Kakunaga, T. (1985) *Proc. Natl. Acad. Sci. USA* **82**, 6133–6137.
21. Kacker, R. K., Giovanella, B. C. & Pathak, S. (1990) *Anticancer Res.* **10**, 859–871.
22. Fumagalli, S., Doneda, L., Nomura, N. & Larizza, L. (1993) *Melanoma Res.* **3**, 23–27.
23. Husein, Z., FitzGerald, G. B. & Wick, M. M. (1990) *J. Invest. Dermatol.* **95**, 571–575.
24. Leprince, D., Saule, S., de Taisne, C., Gegonne, A., Begue, A., Righi, M. & Stehelin, D. (1983) *EMBO J.* **2**, 1073–1078.
25. Trent, J. M., Stanbridge, E. J., McBride, H. L., Meese, E. U., Cassey, G., Araujo, D. E., Witkowski, C. M. & Nagel, R. B. (1990) *Science* **247**, 568–571.
26. Sims, J. E., Gayle, M. A., Slack, J. L., Alderson, M. R., Bird, T. A., Giri, J. G., Colotta, F., Re, F., Mantovani, A., Shanebeck, K., Grabstein, K. H. & Dower, S. K. (1993) *Proc. Natl. Acad. Sci. USA* **90**, 6155–6159.
27. Burk, O., Mink, S., Ringwald, M. & Klempnauer, K.-H. (1993) *EMBO J.* **12**, 2027–2038.
28. Agrawal, S., Goodchild, J., Civeira, M. P., Thornton, A. H., Sarin, P. S. & Zamecnik, P. C. (1988) *Proc. Natl. Acad. Sci. USA* **85**, 7079–7083.
29. Bayever, E., Iversen, P., Smith, L., Spinolo, J. & Zon, G. (1992) *Antisense Res. Dev.* **2**, 109–110.
30. Kitajima, I., Shinohara, T., Bilakovics, J., Brown, D. A., Xu, X. & Nerenberg, M. (1992) *Science* **258**, 1792–1795.