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Functional Knock-Out of c-myb by an Intracellular Anti-c-Myb Single-Chain Antibody

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Aberrant expression of the c-myb proto-oncogene is a key factor in the development of the neoplastic phenotype in a variety of contexts. On this basis, it has been proposed that ablation of c-myb function might be an effective approach for therapy. To this end, we have employed an intracellular single-chain antibody (sFv) approach to achieve the functional knock-out of the c-Myb onco-protein. We derived an anti-c-Myb sFv, which was configured into eukaryotic expression plasmids. We confirmed the expression of the cytoplasmic and nuclear forms of the sFvs in the correct subcellular compartments by immunofluorescent staining. Importantly, the anti-c-Myb sFvs strongly inhibited the transactivation activity of c-Myb. Furthermore, cytotoxic effect of the sFv was observed only in the c-Myb positive cell line K562. These results suggest that anti-c-Myb sFv is a valuable tool for understanding the molecular mechanisms of c-myb induced transformation. In addition, this approach may have potential utility in the gene therapy for c-myb-dependent malignant diseases.

Key Words: **single-chain antibody; c-myb; gene therapy; leukemia; transactivation.**

The c-myb proto-oncogene is the cellular homologue of the viral oncogene, v-myb found in avian myeloblastosis virus (AMV) and E26 retroviruses (1,2). The product of the c-myb gene, c-Myb, is a transcriptional activator which plays an important regulatory role in cell proliferation and differentiation of hematopoietic cells (3–5). In this regard, high levels c-myb expression are detected in immature hematopoietic cells; during differentiation, the expression of c-myb is down-regulated in the context of normal hematopoietic development (4–7).

In addition to its role in normal processes of differentiation, c-myb has been linked to the pathobiology of various neoplasms. Specifically, c-myb over-expression has been reported in human myelogenous leukemia (8–10) and lymphoreticular malignancies (8,11). In addition, it has recently been shown that c-myb overexpression is observed in malignant cells of nonhematopoietic origin, including colon cancer, melanoma, breast cancer, neuroblastoma and neuroepithelioma (8, 12–15).

Whereas c-myb overexpression has been linked to the neoplastic phenotype, its precise role in this context has not been determined. In this regard, we have developed a strategy for knockout of this oncoprotein by the intracellular sngle-chain antibody (sFv) approach. We have previously achieved down regulation and functional knock-out of oncoproteins such as erbB-2, Bcl-2, cyclin D1, Human papilloma virus 16 E7 (HPV 16 E7) and Epstein-Barr virus (EBV) latent membrane protein 1 (Lmp1) using the sFv technology (16–21). In this report, we demonstrate the functional knock-out of c-myb proto-oncogene product by an intracellularly expressed anti-c-Myb sFv. This technique may thus allow precise definition of the role of c-myb in neoplastic transformation. In addition, this approach may allow the development of the gene therapy strategies for c-myb-dependent malignancies.

MATERIALS AND METHODS

Cell lines. K562 human chronic myelogenous leukemia cells and COS-1 african green monkey kidney cells were obtained from American Type Culture Collection (Rockville, MD). They were maintained in DMEM/F12 (COS-1) or RPMI1640 (K562) tissue culture media (Mediatech, Inc, Herndon, VA) supplemented with L-glutamine (2 mM), penicillin (100 IU/ml), streptomycin (25 μg/ml), and 10% heat-inactivated fetal calf serum (FCS) at 37°C in a humidified 5% CO_2 atmosphere.

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Bacterial expression and purification of human c-Myb recombinant protein. To express the amino-terminal half of c-Myb protein, the c-myb gene coding for amino acids 1-358 in the described plasmid, pET-8c/p42 (22) was cloned into BamHI and NotI site of PGEX4T-1, a GST-protein fusion system (Pharmacia Biotech Inc., Piscataway, NJ). According to the standard procedure, bacteria carrying the c-myb expression vector were grown and induced by isopropyl-B-D-thiogalactopyranoside (IPTG, Fisher Scientific, Fair Lawn, NJ). Resuspended bacteria were sonificated in the buffer (100 mM Tris-HCl pH 7.9 containing 300 mM NaCl and 1mg/ml of lysozyme). The GST-c-Myb fusion protein in the soluble fraction was then collected by glutathione sepharose 4B according to the manufacturer's instructions (Pharmacia Biotech Inc.). After cleavage of the GST from the fusion protein by thrombin, reduced glutathione was added to release c-Myb protein from glutathione sepharose 4B. The purified c-Myb protein was then dialyzed using a Slide-A-Lyzer (pore size 10,000 MW, PIERCE, Rockford, IL) against 1.0 L of phosphate buffered saline (PBS) pH 7.4 with three buffer changes. The purified human c-Mybp42 was detected by immunobloting using conditioned supernatant of hybridoma 4/14 secreting anti-c-Myb monoclonal antibody (MAb) (23).

Construction of anti-c-Myb sFvs. The 4/14 hybridoma cells, producing an anti-c-Myb MAb, were developed by H. Bading et. al. (23). The epitope recognized by the MAb is in the transactivating domain of human c-Myb, especially the sequence of amino acids from 123 to 134 (24). Messenger RNA was isolated from 4/14 mouse hybridoma cells using Poly (A) pure mRNA isolation kit (Ambion, Austin, TX). Anti-c-Myb sFvs were constructed using Recombinant Phage Antibody System Mouse sFv Module according to the manufacturer's instructions (Pharmacia Biotech). Briefly, the cDNAs of the variable heavy chain (VH) and light chain (VL) regions of anti-c-Myb IgG transcripts were synthesized using reverse transcriptase polymerase chain reaction (RT-PCR), following amplification of VH and VL using their respective primers. The sFvs were assembled by adding the linker $(Gly_4Ser)_3$. The sFv fragments were then digested with restriction endonucleases, SfiI and NotI, and cloned into the bacterial expression vector pCANTAB5E (Pharmacia Biotech), which contains an E-tag sequence (GAPVPYPDPLEPR) at the C-terminus. The cloned sFvs were then directly transfected in *E.coli* HB2151 by the electroporation method. Screening of recombinant sFv clones was accomplished by the colony lift assay as described previously (25).

Expression and functional assay of the sFv in a prokaryotic system. Positive sFv clones from the colony lift assay were isolated, and expression of the sFv protein was induced by IPTG in 8 ml of 2 × YT media. To collect the periplasmic extract, the culture pellets were resuspended in 1 ml of cold PBS pH 7.4 containing 1 mM EDTA, and incubated on ice for 30 minutes with occasional vortexing. The mixture was centrifuged at 14,000 × g at 4°C, and the supernatant containing the soluble sFv protein was used for immunobloting or the assay of binding ability of sFv by ELISA.

Expression of anti-c-Myb sFv and c-Myb protein in eukaryotic cells. The eukaryotic expression vector pcDNA3 (Invitrogen, Carisbad, CA) was used to construct a series of plasmids to achieve expression of the sFv or c-Myb protein in eukaryotic cells. Figure 1 shows the plasmids containing sFvs targeted to different subcellular compartments. The cytoplasmic sFv vector (pCyMybsFv) contains the anti-c-Myb sFv gene fused with c-Myc tag (EQKLISEEDLN). The nuclear sFv vector (pNuMybsFv) contains the SV40 nuclear localization signal sequences (TMPPKKKRKVGGAQPA) (26) plus the c-Myc tag at the c-terminal of the sFv. To achieve exogenous expression of the human c-Myb protein, the c-myb cDNA fragment isolated from pET-8c/p42, was cloned into pcDNA3 between BamHI and NotI site (pc-mybp42). Cells were transfected with the described plasmids by the adenovirus poly-L-lysine vector system (AdpL method), as previously described (16,27). At 48 h or the described time points, expression of the sFv or the c-Myb protein was determined by immunobloting using anti-c-Myc tag polyclonal antibodies (A-14, Santa Cruz Biotechnology, CA) or conditioned medium from 4/14 hybridoma cells containing anti-c-Myb MAb, respectively.

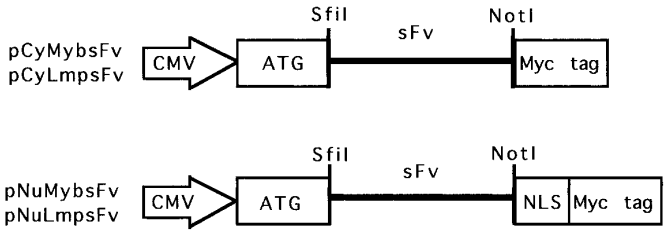

FIG. 1. Schema of the eukaryotic expression vectors of sFvs. All sFvs were cloned into pcDNA3 backbone plasmid. NLS: Nuclear localization signal.

Immunoblot analysis. For the detection of anti-c-Myb sFv protein, 30 μg of the cell lysate was separated on a 10% SDS-PAGE gel. After electrophoresis, protein was transferred onto a nitrocellulose membrane (BIO-RAD). An anti-E tag MAb (Pharmacia Biotech), at 1:1,000 for the prokaryotic system, or anti-c-Myc tag, at 1:3,000 for eukaryotic system, was added to the membrane as the primary antibody. After incubation, a HRP-conjugated goat anti-mouse, or anti-rabbit IgG (Jackson laboratories, Bar Harbor, ME), was added to the membrane and incubation was continued for an additional one hour. The immunoblot was developed by the Renaissance reagent system (Dupont, Boston, MA) according to the manufacturer's instructions.

Immunofluorescence staining. To detect the localization of the anti-c-Myb sFv *in situ,* COS-1 cells were plated on glass coverslips, and transfected with plasmids using the AdpL method. After 48 h, the cells were washed and fixed by PBS containing 4% paraformaldehyde. Then the cell membranes were permeabilized with PBS containing 0.2% TritonX100 (Sigma). An anti-c-Myc polyclonal antibody at 1:400 was added and incubated for 1 h at room temperature, followed by the incubation with fluorescein isothiocyanate (FITC)-conjugated polyclonal goat anti-rabbit IgG antibody at 1:400 dilution for an additional 1 h. The stained cells were then evaluated by immunofluorescence microscopy.

ELISA analysis. Supernatant (100 μl) from the periplasmic extract of bacteria was added to the antigen-coated wells and incubated for 2 hour at room temperature. After treatment with wash buffer (PBS pH7.4 containing 0.1% Tween 20), 100 μl of anti-E tag antibody at 1:1000 was added to the plate, followed by a further 1 h incubation. After washing, HRP-cojugated anti-mouse IgG at 1:3000 was added to each wells. After the final washing, the color was developed with ABTS chromogen reagent (Sigma), and OD wad read at 405 nm.

Transcriptional assays. To observe the inhibitory effect of anti-c-Myb sFv on the transcriptional activity of c-Myb, we employed a human c-myc promotor-luciferase fusion gene, pMyc-Luc (28) as a target of c-Myb. In this regard, it has been reported that c-Myb stimulates the human c-myc promotor, P1 and P2 (29). Co-transfection of plasmids, pMyc-Luc and pc-mybp42 into COS-1 cells showed stimulatory effect of c-Myb on the c-myc promotor. After establishing the transcriptional assay system, we also co-transfected pNuMybsFv or pCyMybsFv as a third plasmid. COS-1 cells were transfected with these plasmids by the AdpL method. Effector (pc-mybp42), reporter (pMyc-Luc) and inhibitor (pNuMybsFv or pCyMybsFv) plasmids were transfected in relative amounts of 34 : 1 : 67. Forty eight hours post-transfection, the cells were lysed in 100 μl of lysis buffer (Promega). 10 μl of each sample were subsequently mixed with 50 μl of luciferase assay reagent (Promega) according to the manufacturer's instructions, and duplicate determination of triplicate samples were assayed in a Berthhold luminometer. The luciferase activity was standardized by the amount of total protein.

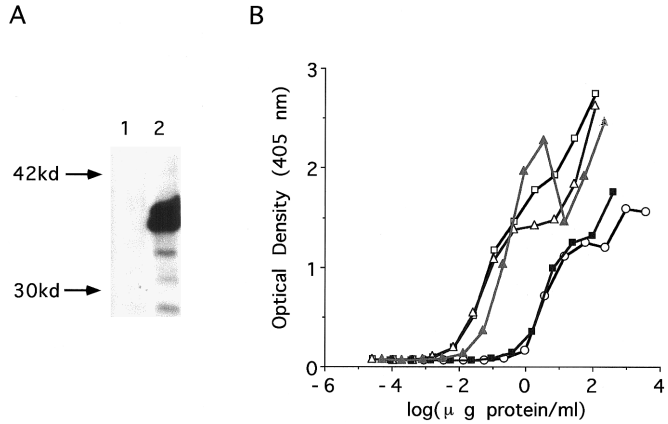

FIG. 2. Expression and binding ability of an anti-c-Myb sFv expressed in *E.coli*. (A) Immunoblot analysis of the anti-c-Myb sFv expressed in *E.coli*. 10 l of periplasmic extract was separated by SDS-PAGE (12%) and sFv expression was determined by anti-E-tag antibody. HB2151 is the control periplasmic extract from non-transfectected bacteria. (B) Binding ability of bacterial expressed sFv clones. ELISA was used to measure the binding activity of the periplasmic expressed anti-c-Myb sFv clones. Described concentrations of protein (100 μl) from periplasmic extracts or supernatant of hybridoma 4/14 (○) were added to a 96-well plate coated with purified recombinant human c-Mybp42 (10g/ml). Clone 3 (□), clone 12 (△), clone 23 (■) and clone 33 (▲) were positive clones from colony lift assay.

Stable transfections. For analysis of phenotypic effect of the sFv on leukemia cells, 4×10^6 K562 cells were transfected with 20 μg of the linealized plasmids by electroporation. As a control, either linealized pcDNA3 or anti-Lmp1 sFv-containing vector was also employed for transfection. After 48 h of incubation in non-selective medium, the transfected cells were plated in 96 well plates at different concentrations, and the number of G418 resistant colonies were counted after 21 days of selection. For COS-1 cells, the cells (25,000 per well) were plated in 6 cm dishes and allowed to recover for 24 h before transfection. Cells were transfected using Lipofectamine (GibcoBRL) according to the manufacturer's recommendations. Three weeks later, the number of G418-resistant colonies were counted by crystal violet staining.

Statistics. Comparison of individual conditions were assessed using the Students' t test for equal means using StatView for Macintosh (SAS Institute Inc, San Francisco, CA).

RESULTS

Derivation of an anti-c-Myb sFv. Several positive colonies were isolated by colony lift assay and expression of the anti-c-Myb sFv protein was confirmed by immunobloting. The sFv protein is predicted to be composed of approximately 270 amino acids with an estimated molecular weight of 30 Kd. However, the position of the protein on the SDS-PAGE gel is slightly higher than the calculated molecular weight (Fig. 2A). Of note, this has also been observed in the context of other studies observing expression of sFvs in *E.coli* or mammalian cells (18,21). The binding activities of the engineered anti-c-Myb sFvs to the recombinant c-Myb protein were determined by ELISA assay (Fig. 2B). Four of these sFv clones showed a dose-dependent binding affinity. Periplasmic extract of bacteria includes many types of proteins. Therefore, absolute binding affinities of these sFvs were not able to be calculated in this experiment. However, the optical densities achieved by sFv clones exceeded that exhibited by supernatant of original hybridoma cells secreting anti-c-Myb MAb. After repeating this experiments several times, we found that clone 23 had the lowest nonspecific binding affinity to the control protein, bovine serum albumin (BSA). For this reason, sFv clone 23 was used in subsequent experiments.

Expression of anti-c-Myb sFvs in eukaryotic cells. After validating the binding affinity of the derived anti-c-Myb sFvs expressed in bacteria, we constructed a series of plasmids to achieve the expression of the sFv in the eukaryotic cells. These plasmids were designed to localize the sFvs to specific subcellular compartments. COS-1 cells were transfected with pCyMybsFv or pNuMybsFv by the AdpL method, and stained with an anti-c-Myc antibody at 48 h post-transfection. Cytoplasmic sFv (pCyMybsFv) was expressed mainly in the cytosol. There was also some nuclear staining of intracellular sFv with this plasmid as well (Fig. 3A). The expression of the nuclear-targeted sFv (pNuMybsFv) was restricted to the nucleus (Fig. 3B). Thus, these studies confirmed that the anti-c-Myb sFvs were expressed in correct subcellular localizations in COS-1 cells.

Time course of the expression of anti-c-Myb sFvs. We also observed the time course of the expression of anti-c-Myb sFv. COS-1 cells were transfected with pCyMybsFv or pNuMybsFv by the AdpL method. After transfection, cell lysates were collected at various time points. The expression of the sFv protein was determined by immunoblot analysis. The expression of a cytosolic form of sFv was observed from 15 h to 160 h post-transfection (Fig. 4). The expression of a nuclear form of sFv was also detected from 15 h post-transfection. Although the expression level of a nuclear-targeted sFv was lower than that of cytoplasm-targeted sFv, peak of the expressions of both type of sFvs were observed around 90 to 120 h after transfection.

Functional knock-out of c-Myb by anti-c-Myb sFvs. We next determined the ability of the sFv to ablate the transactivation activity of c-Myb. To this end, we employed a human c-myc promotor-luciferase fusion gene. Previous studies showed that human c-myc promotor, P1 and P2 include c-Myb binding sites, and are strongly stimulated by human c-Mybp42 (29). In our experiments, we used the pMyc-Luc plasmid including P1 and P2 regions of human c-myc promotor fused with the luciferase gene (28). The c-Myb expression plasmid stimulated the luciferase activity several fold (Fig. 5, bar 2). Co-transfection of the COS-1 cells with

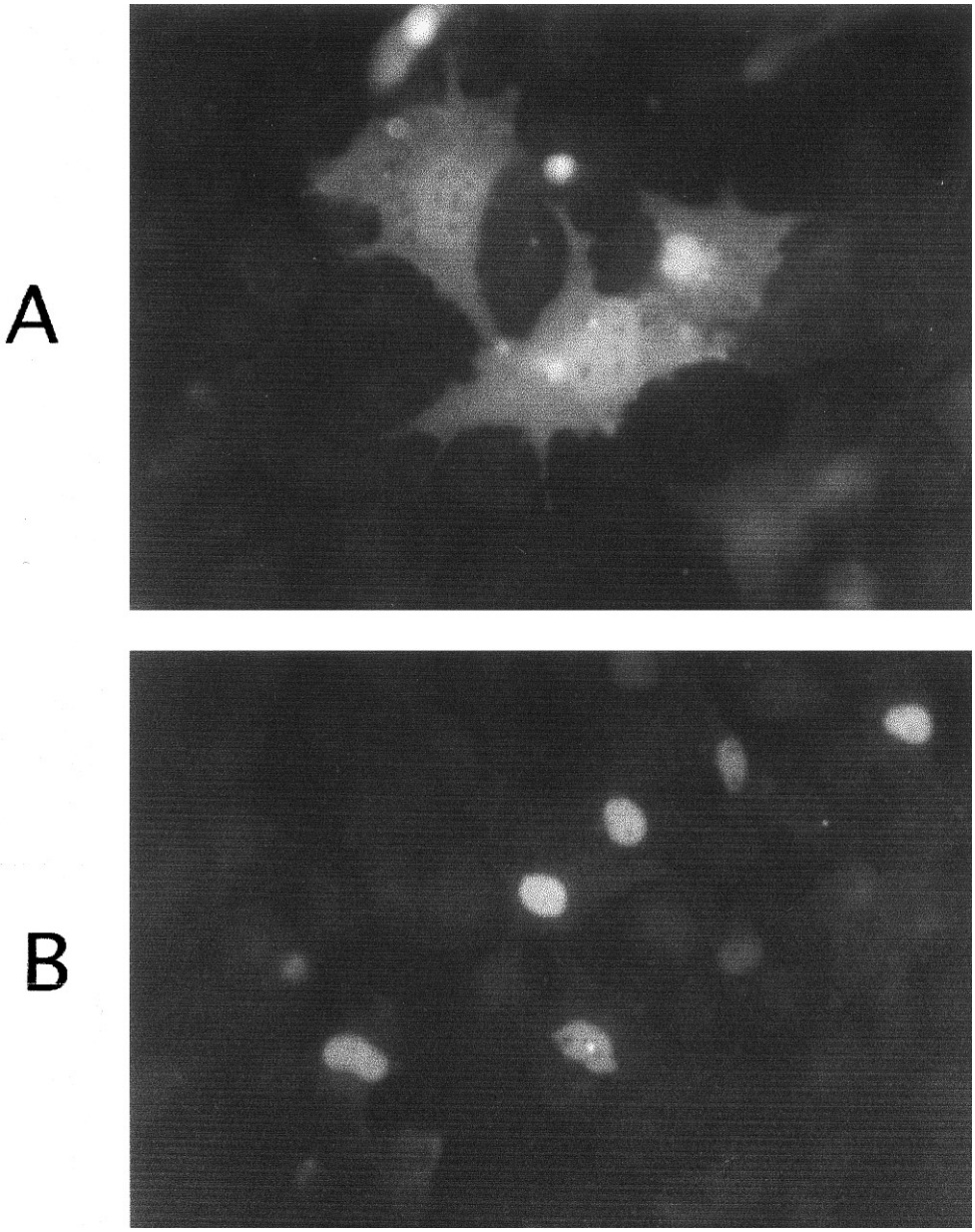

FIG. 3. Immunofluorescent staining of cytoplasmic- and nuclear-targeted anti-c-Myb sFvs. (A) COS-1 cells were transfected with cytoplasmic form of anti-c-Myb sFv, pCyMybsFv by the AdpL method. After 48h, the sFv was detected by anti-c-Myc-tag antibody and FITC-labeled anti-rabbit IgG. (B) COS-1 cells were transfected with nuclear form of anti-c-Myb sFV, pNuMybsFv.

pNuMybsFv, pc-mybp42 and pMyc-Luc showed that the nuclear form of the anti-c-Myb sFv inhibited the transactivating activity of c-Myb to the basal level (Fig. 5, bar 4). The cytoplasmic form of the anti-c-Myb sFv has the same effect on the function of c-Myb (Fig. 5, bar 6). Importantly the control plasmid pNuLmpsFv and pCyLmpsFv showed no significant inhibitory effect on the activity of c-Myb (Fig. 5, bar 3 and 5). Control sFvs, pNuLmpsFv and pCyLmpsFv did not show the inhibitory effect on the activity of c-Myb (Fig. 5, bar 3 and 5). These results suggest that nuclear and cytoplasmic forms of anti-c-Myb sFv specifically blocked the function of c-Myb.

Effect of anti-c-Myb sFv on derivation of stable clone in c-myb-positive and -negative cell lines. To evaluate the effects of the anti-c-Myb sFv, we attempted to derive stable clones from several cell lines. A differential in stable clone derivation is a common assay used to indicate specific functional effects of the sFv in cell lines. The plasmid pNuMybsFv, encoding nuclear-targeted anti-c-Myb sFv, and control plasmids pcDNA3 or pNuLmpsFv were used in this experiments. Trans-

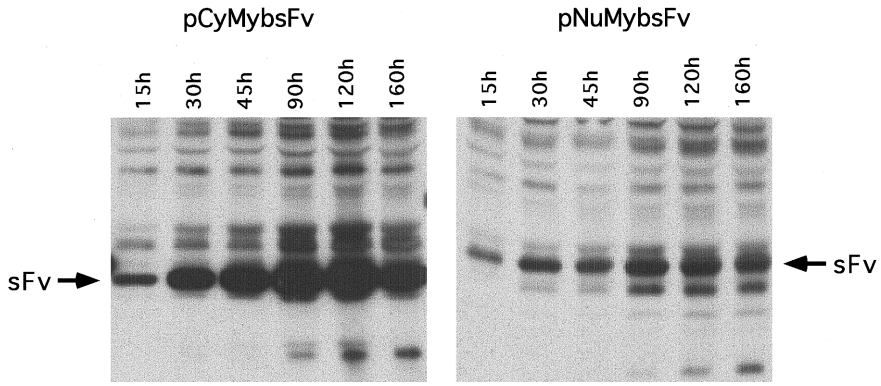

FIG. 4. Time course of the expression of anti-c-Myb sFvs. COS-1 cells were transfected with pCyMybsFv (left) or pNuMybsFv (right) by the AdpL method. At the described time points, cell lysates were separated on a 10% SDS-PAGE gel.

fection of the anti-c-Myb sFv did not elicit suppression of colony formation in the c-myb-negative cell line, COS-1 (Table 1). In contrast, there was a 60% reduction of colony formation in the c-myb-positive K562 cells transfected with anti-c-Myb sFv. Thus, the effects of the anti-c-Myb sFv appeared to be dependent upon the expression of c-myb in the target cells. Nonetheless, the control sFv plasmid, pNuLmpsFv, also caused a level of suppression of colony formation of the cell lines. The basis for this effect may reflect the antibody cross-reactivity with alternate cellular target, or may represent a nonspecific effect of the intracellular antibody on normal cellular physiology.

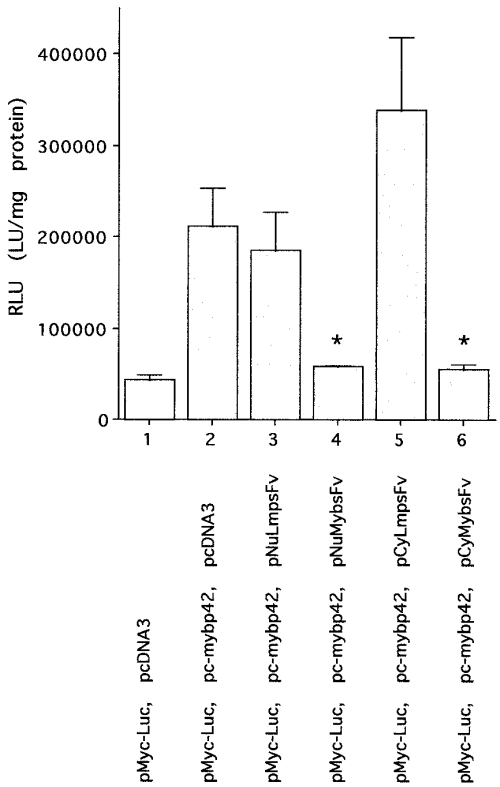

FIG. 5. Transcriptional assay of the c-myc promotor. Lane 1 showed the basal c-myc promotor activity. Subconfluent COS-1 cells in a 12-well plate were transfected with myc promotor-luciferase fusion plasmid, pMyc-Luc (0.002μg) and mock plasmid, pcDNA3 (0.2μg). After 48 h, luciferase activity was measured. COS-1 cells were transfected with pMyc-Luc (0.002μg), c-Myb expression plasmid, pc-mybp42 (0.067μg) and mock plasmid (0.133μg) (bar 2). COS-1 cells were transfected with pMycLuc, pc-mybp42, and pNuLmpsFv (bar 3), pNuMybsFv (bar 4), pCyLmpsFv (bar 5) or pCyMybsFv (bar 6). * $P < 0.01$, bar 2 vs bar 4 or bar 6.

DISCUSSION

In this report, we demonstrate the functional knockout of c-Myb proto-oncoprotein by an intracellular anti-c-Myb single-chain antibody. We observed the anti-c-Myb sFvs were expressed in mammalian cells and could be targeted to subcellular compartments. Without any signal sequence, the sFv proteins were translated in cytosol and distributed in a diffuse pattern. In our experiments, cytoplasm-targeted sFv was detected both in the cytosol and nucleus. This is probably because the small sized proteins easily enter the nucleus through the pores in the nuclear membrane without nuclear leader signal peptide. For example, green flu-

TABLE I

Effect of Anti-c-Myb sFv on Derivation of Stable Clones

	Number of G418-resistant colonies		
Cell line	pcDNA3	pNuLmpsFv	pNuMybsFv
COS-1	347 ± 51	305 ± 20	369 ± 48
K562	35 ± 3.8	23 ± 2.2	15 ± 3.2*

Note. Cell lines were transfected with either the pcDNA3, pNuLmpsFv or pNuMybsFv using Lipofectamine (COS-1) or electroporation (K562). At 48 h, G418 was added to the media. At 21 days after transfection, colony numbers were evaluated. Results are from four separate experiments and are expressed as mean ± SD. *$P <$ 0.01, pcDNA3 vs PNuMybsFv.

orescent protein which has similar molecular weight as sFv doesn't include a nuclear leader signal, but it can be detected both in the cytosol and the nucleus (data not shown). We observed the expression of the nuclear form of the sFv was restricted to the nucleus. SV-40 nuclear leader peptide has strong activity to target the protein to the nucleus (26). Compared to the cytoplasm-tageted sFv, the expression levels of nucleus-targeted sFv were low. However, the expression levels of the nuclear oncoproteins in malignant cells are also very low. In some malignant cells, only Northern blot analysis could detect the expression of c-myb (30). Importantly, the biological effects of antisense oligodeoxynucleotides on c-myb mRNA positive cells suggest that low level of c-Myb can have a strong effect on the phenotypic change of the cells (30, 31). Therefore, a small amount of expressed sFv may be sufficient to ablate the function of the nuclear oncoprotein. In our experiments, anti-c-Myb sFvs, pNuMybsFv and pCyMybsFv didn't decrease the expression level of c-Myb protein (data not shown). Usually, ER-targeted sFvs, anti-ErbB2, anti-Lmp1 and anti-Bcl-2 sFvs in our laboratory inhibit the expression of target protein in the cells (18,21). However, the binding of cytoplasmic form of sFv to the target protein was not necessary for down regulation of the expression of the target protein (32). Therefore, the cytosol- and nuclear-targeted anti-c-Myb sFv specifically blocked the function of c-Myb. In our experiment, both proteins, sFv and c-Myb were controlled under the CMV promotor. It required double amount of the pNuMybsFv or pCyMybsFv to totally block the effect of pc-mybp42 expressing c-Myb.

To detect the phenotypic effect of the sFv on malignant cells, we assayed the colony formation of cells transfected by the sFv. Reduced number of colonies derived from cells transfected by anti-c-Myb sFv compared to control suggests a cytotoxic effect of the sFv on the leukemia cells expressing c-myb. Consistent with this observation, c-myb negative cells did not show any reduction of colony formation by anti-c-Myb sFv. The results suggested that anti-c-Myb sFv has specific cytotoxic effect on leukemia cells expressing c-myb. In our experiment, anti-Lmp1 sFv also slighly reduced the number of clones especially in K562 cells. The parental Mab of anti-Lmp1 sFv, S12, recognizes the cytosolic domain of EBV derived TNF receptor family protein, Lmp1 (33,34). Previous study have shown that S12 MAb reacted with some EBV-negative normal tissues (35). In addition, the expression level of anti-Lmp1 sFv was much higher than that of anti-c-Myb sFv (data not shown). Therefore, it is possible that the control sFv has some cross reactivity with other target protein or proteins. Or the effects of the control sFv may reflect a nonspecific effect of the intracellular sFv on normal cellular physiology.

On the basis of our achievement, inhibition of the function of c-Myb will enable a more direct method of investigation of c-myb function. Until now, we could only use the over expression of exogenous c-Myb to approach the functional analysis of the oncoprotein. Although c-myb antisense oligodeoxynucleotides showed an inhibitory effect on the growth of malignant cells in previous reports (36,37), there is no direct evidence of functional knockout of c-Myb by the antisense oligodeoxynucleotides. We are currently evaluating the effect of the anti-c-Myb sFv on malignant cells expressing c-myb which may help a strategy for the gene therapy of leukemia and other c-myb-dependent malignancies.

ACKNOWLEDGMENTS

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REFERENCES

1. Klempnauer, K. H., Gonda, T. J., and Bishop, J. M. (1982) *Cell* **31,** 453–463.
2. Radke, K., Beug, H., Kornfeld, S., and Graf, T. (1982) *Cell* **31,** 643–653.
3. Duprey, S. P., and Boettiger, D. (1985) *Proc. Natl. Acad. Sci. USA* **82,** 6937–6941.
4. Gonda, T. J., and Metcalf, D. (1984) *Nature,* **310,** 249–251.
5. Sheiness, D., and Gardinier, M. (1984) *Mol. Cell. Biol.* **4,** 1206–1212.
6. Westin, E. H., Wong-Staal, F., Gelmann, E. P., Dalla Favera, R., Papas, T. S., Lautenberger, J. A., Eva, A., Reddy, E. P., Tronick, S. R., Aaronson, S. A., and Gallo, R. C. (1982) *Proc. Natl. Acad. Sci. USA* **79,** 2490–2494.
7. Craig, R. W., and Bloch, A. (1984) *Cancer Res.* **44,** 442–446.
8. Slamon, D. J., DeKernion, J. B., Verma, I. M. and Cline, M. J. (1984) *Science* **224,** 256–262.
9. Slamon, D. J., Boone, T. C., Murdock, D. C., Keith, D. E., Press, M. F., Larson, R. A., and Souza, L. M. (1986) *Science* **233,** 347–354.
10. Mavilio, F., Sposi, N. M., Petrini, M., Bottero, L., Marinucci, M., De Rossi, G., Amadori, S., Mandelli, F., and Peshle, C. (1986) *Proc. Natl. Acad. Sci. USA* **83,** 4394–4398.
11. Bading, H., Gerdes, J., Schwarting, R., Stein, H., and Moelling, K. (1988) *Oncogene* **3,** 257–265.
12. Thiele, C. J., McKeon, C., Triche, T. J., Ross, R. A., Reynolds, C. P., and Israel M. A. (1987) *J. Clin. Invest.* **80,** 804–811.
13. Thiele, C. J., Cohen, P. S., and Israel, M. A. (1988) *Mol. Cell. Biol.* **8,** 1677–1683.
14. Griffin, C. A., and Baylin, S. B. (1985) *Cancer Res.* **45,** 272–275.
15. Alitalo, K., Winrvist, R., Lin, C. C., De La Chapelle, A., Schwab, M., and Bishop, J. M. (1984) *Proc. Natl. Acad. Sci. USA* **81:** 4534-–538.
16. Deshane, J., Loechel, F., Conry, R. M., Siegal, G. P., King, C. R., and Curiel, D. T. (1994) *Gene Ther.* **1,** 332–337.

17. Grim, J., Deshane, J., Feng, M., Lieber, A., Kay, M., and Curiel, D. T. (1996) *Am. J. Respir. Cell Mol. Biol.* **15,** 348–354.
18. Piché, A., Grim, J., Rancourt, C., Gómez-Navarro, J., Reed, J. C. and Curiel, D. T. (1998) *Cancer Res.* in press.
19. Guadalupe B., Bartek, J., Rodenburg, C., Kasono, K., Grim, J., Khazaeli, M. B., Siegal, G. P., Strong, T. V. And Curiel, D. T. (1998) *Cancer Res.* submitted.
20. Johanning, F. W., Gillespie G. Y., Grim, J., Rancourt, C., Alvarez, R.D., Siegal, G. P., and Curiel, D. T. (1998) *Cancer Res.* in press.
21. Piché, A., Kasono, K., Johanning, F., Curiel, T. J. and Curiel, D. T. (1998) *Gene Ther.* in press.
22. Guehmann, S., Vorbrueggen, G., Kalkbrenner, F., and Moelling, K. (1992) *Nucleic Acids Res.* **20,** 2279–2286.
23. Bading, H., Beutler, C., Beimling, J., Gerdes, J., Stein, H., and Moelling, K. (1987) *Haematol. Blood Transfus.* **31,** 488–492.
24. Bading, H., Hansen, J., and Moelling, K. (1987) *Oncogene* **1,** 395–401.
25. Pharmacia Biotech. (1996) Simultaneous screening of large numbers of recombinant antibody clones using a colony lift assay. *Science Tools from Pharmacia Biotech* Vol. 1, pp. 10–11.
26. Kalderon, D., Roberts, B. L., Richardson, W. D. and Smith, A. E. (1984) *Cell* **39,** 499–509.
27. Curiel, D. T., Wanger, E., Cotten, M., Birnstiel, M. L., Agarwal, S., Li, C. M., Loechel, S., and Hu, P. C. (1992) *Human Gene Ther.* **3,** 147–154.
28. Kim, H. G., and Miller, D. M. (1995) *Biochemistry* **34,** 8165–8171.
29. Zobel, A., Kalkbrenner, F., Vorbrueggen, G., and Moelling, K. (1992) *Biochem. Biophys. Res. Com.* **186,** 715–722.
30. Hijiya, N., Zhang, J., Ratajczak, M. Z., Kant, J. A., DeRiel, K., Herlyn, M., Zon, G. and Gewirtz, A. M. (1994) *Proc. Natl. Acad. Sci. USA* **91,** 4499–4503.
31. Melani, C., Rivoltini, L., Parmiani, G., Calabretta, B. and Colombo, M. P. (1991) *Cancer Res.* **51,** 2897–2901.
32. Duan, L., Bagarsa, O., Laughlin, M. A., Oakes, J. W. and Pomerantz, R. J. (1994) *Proc. Natl. Acad. Sci. USA* **91,** 5075–5079.
33. Mann, K. P., Staunton, D. and Thorley-Lowson, D. A. (1988) *J. Virol.* **55,** 710–720.
34. Baker, S. J. and Reddy, E. P. (1996) *Oncogene* **12,** 1–9.
35. Jiwa, N. M., Oudejans, J. J., Dukers, D. F., Vos, W., Horstman, A., van der Valk, P., Middledorp, J. M., Walboomers, J. M. M. and Meijer, C. J. L. M. (1995) *J. Clin. Pathol.* **48,** 438–442.
36. Anfossi, G., Gewirtz, A.M., and Calabretta, B. (1989) *Proc. Natl. Acad. Sci. USA* **86,** 3379–3383.
37. Ratajczak M. Z., Kant, J. A., Luger, S. M., Hijita, N., Zhang, J., Zon, G., and Gewirtz, A. M. (1992) *Proc. Natl. Acad. Sci. USA* **89,** 11823–11827.