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Diversity of Cellular Molecules in Human Cells Detected by Monoclonal Antibodies Reactive with c-*myc* Proteins Produced in *Escherichia coli*

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Six clones of monoclonal antibodies, MYC-1 to -6, were prepared by using two kinds of truncated c-*myc* proteins, p23 and p42, produced in *Escherichia coli* as immunogens. Analysis with enzyme-linked immunosorbent assays and immunoblotting assays with peptides produced in *Escherichia coli* showed that 5 clones of monoclonal antibodies, MYC-1 to -4 and -6, were reactive with c-*myc* protein encoded by exon 2. The remaining one clone, MYC-5, was reactive with the portion of c-*myc* protein encoded by exon 3. All monoclonal antibodies were also reactive with phosphorylated c-*myc* protein produced by insect cells infected by the baculovirus expression vector with the human c-*myc* gene. With immunoblotting assays using cellular lysates, MYC-1 and -3 detected bands at the levels of 58 kDa and 60 kDa, MYC-5 detected a band at 56 kDa and MYC-6 detected bands at 68 kDa and 75 kDa. All of these bands were detectable in nuclear extracts of HL-60 and Colo320, both of which have amplified c-*myc* genes, and also the extract of RmycYl which is the c-*myc* gene transfectant into 3Yl rat cells. None of them was detectable in peripheral blood mononuclear cells and 3Yl, both of which lacked activated c-*myc* genes. This indicates that these nuclear proteins are either c-*myc* gene products or molecules closely related to the c-*myc* gene. The remaining two clones, MYC-2 and -4, detected a band at the level of 85 kDa in cytoplasmic extracts of all the above-mentioned cells independent of the presence of the c-*myc* gene. This suggests that 85 kDa protein might be irrelevant to the c-*myc* gene. The 56 kDa protein was detectable by MYC-5 in phytohemagglutinin-stimulated peripheral blood mononuclear cells as well as leukemic cells of some patients.

Key words: c-*myc* — Monoclonal antibody

We have recently produced two kinds of truncated human c-*myc* proteins, p23 and p42, in *Escherichia coli* (*E. coli*).[1] The p23 protein corresponds to 155 amino acids (aa) from the 98th gln to the 252 ser, plus five aa (gly-gly-thr-arg-arg) at the C-terminus, plus 21 aa from the Ha-*ras* gene at the N-terminus. The p42 protein corresponds to 342 aa from the 98th gln to the C-terminus, plus 21 aa derived from the Ha-*ras* gene at the N-terminus. DNA-cellulose column chromatography showed that p42 protein binds to DNA, but that p23 does not, indicating that the DNA-binding activity of c-*myc* protein is localized in the peptide encoded by exon 3 of the c-*myc* gene.[1]

For further structural and functional analyses of c-*myc* protein, we prepared several clones of monoclonal antibodies (MoAb)[6] by immunizing mice with p23 and p42. We found that these antibodies reacted specifically with the c-*myc* protein produced in *E. coli*. They, however, detected several different protein molecules either in the nucleus or cytoplasm of human cells. The relevance of these proteins to the c-*myc* gene is analyzed and reported in this paper.

MATERIALS AND METHODS

Construction and expression of pTRmyc10 and pTRmyc 22 The construction and expression of pTRmyc10 and pTRmyc22 have been previously described.[2] Briefly, *E. coli* transfected with pTRmyc10 and pTRmyc22 produced p23 and p42, respectively. The primary structure of the p23 protein deduced from the nucleotide sequence of the constructed plasmid was as follows: an N-terminal 21 aa derived from the Ha-*ras* gene, and 155 aa encoded by a part of the c-*myc* gene from the *Pvu*II site to the 3′ end of exon 2 (residues 98–252), plus a 5 aa sequence (gly-gly-thr-arg-arg). DNA fragments coding the c-*myc*

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[6] Abbreviations: aa, amino acid(s); *E. coli*, *Escherichia coli*; MoAb, monoclonal antibody; SDS, sodium dodecyl sulfate; PAGE, polyacrylamide gel electrophoresis; ELISA, enzyme-linked immunosorbent assay; ABC, avidin-biotin complex; PBS, phosphate-buffered saline; PBMC, peripheral blood mononuclear cells.

in pTRmyc10 were replaced by the c-*myc* gene for 342 aa (residues 98–439) in pTRmyc22.

Cells RmycY1 which is the human c-*myc* gene transfectant to rat 3Y1 cells was prepared and kindly provided by Dr. K. Shiroki, Institute of Medical Science, University of Tokyo, Tokyo.[2] Northern blot hybridization showed that RmycY1 contains an enhanced amount of transcripts of the c-*myc* gene.[2]

Production of MoAbs Female (BALB/c×C57BL/6)F_1 mice were immunized more than 4 times with purified p23 or p42 at 2-week intervals. For the first immunization, 50 μg of c-*myc* protein in complete Freund's adjuvant was injected subcutaneously, and for subsequent immunizations 100 μg of c-*myc* protein was injected intraperitoneally.

Spleen cells of immunized mice were fused with NS-1 myeloma cells as described previously.[3,4] The anti p23 or p42 antibodies produced by the hybridomas were detected in enzyme-linked immunosorbent assays (ELISA) and immunoblotting assays as described below. Hybridomas producing antibodies were subcloned 2 to 3 times by limiting dilution until their monoclonality was confirmed.

Preparation of nuclear and cytoplasmic fractions of human cell lines Nuclear and cytoplasmic fractions of HL-60 and Colo 320 cells were prepared according to the published method.[5] The cells were washed twice with phosphate-buffered saline (PBS). The pellets were washed once with the isolation buffer: 3.75 m*M* Tris-HCl (pH 7.5), 0.05 m*M* spermin, 0.125 m*M* spermine, 1% thiodiglycerol and 20 m*M* KCl. Then 5 volumes of the isolation buffer with 0.1% digitonin (Wako Pure Chemical Industries, Tokyo) and 0.1 m*M* PMSF were added to one volume of the washed pellets. They were immediately broken in a Dounce-type tissue homogenizer with about 15 strokes. The supernatant was collected as the cytoplasmic fraction after centrifugation (900*g*, 10 min). The pellets were washed with the isolation buffer containing 0.1 m*M* PMSF and the final pellet was used as the nuclear fraction.

Immunoblotting assay Bacterial or cellular lysates separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) were electroblotted onto Immobilon PVDF membranes (Millipore, Bedford, MA) for 150 Vh in the blotting buffer: 0.25 *M* Tris, 0.192 *M* glycine and 10% methanol. The protein blots were incubated in PBS with 3% non-fat dry milk overnight at room temperature. The membranes were washed with TPBS (PBS containing 0.05% Tween 20), and then incubated with MoAb for 2 h at room temperature. The membranes were washed with TPBS and incubated with biotinylated horse anti-mouse IgG antibodies (Vector Lab., Burlingame, CA) for 1 h at room temperature, and washed with TPBS. They were then incubated with peroxidase-labeled avidin-biotin complexes (ABC) (Vector Lab.) for 30 min. After washing with TPBS, membranes were treated with 4-chloro-1-naphthol containing 0.01% H_2O_2 as a substrate.

PHA-stimulated peripheral blood mononuclear cells (PBMC) PBMC were isolated from heparinized blood of healthy volunteers by the Ficoll-Conray method. PBMC were suspended at a concentration of 5×10^6/ml in RPMI1640 supplemented with 10% fetal calf serum and cultured in a 5% CO_2 incubator at 37°C. One μg/ml of PHA-P (GIBCO, Grand Island, NY) was added to the culture. PBMC were sequentially harvested and their lysates were analyzed by immunoblotting assays.

RESULTS

Production of MoAbs and their reactivities with proteins produced in *E. coli* Four clones, MYC-1 to -4, were obtained by fusing spleen cells of a mouse immunized with p23. Two other clones, MYC-5 and -6, were obtained from spleen cells immune to p42. Supernatants of five clones, MYC-1 to -4 and -6, were reactive with both p23 and p42 in ELISA and immunoblotting assays as shown in Table I and also in Fig. 1. Supernatant of MYC-5, however, was exclusively reactive with p42. None of them was reactive with *ras* p21[4,6] or lysates of *E. coli* not transfected with the c-*myc* gene. These results indicate that all six MoAbs, MYC-1 to -6, were reactive with peptides encoded by the c-*myc* gene and produced in *E. coli*.

Reactivities of MoAbs with human c-*myc* protein in insect cells with the baculovirus expression vector Reactivities of MoAbs with phosphorylated c-*myc* protein produced in insect cells with the baculovirus expression vector were examined. Lysates of Ac373/hc-myc-infected cells and control AcNPV-infected cells were provided by Dr. C. Miyamoto (Nippon Roche Research Center, Kamakura). Ac 373/hc-myc cells are derived from *Spodoptera frugiperda* with baculovirus expression vector which produced a significant amount of c-*myc* protein.[7] Figure 2 shows an example of immunoblotting assays of MoAbs using their lysates. All of MYC-1 to -6 and control anti c-*myc* protein MoAb CT-14 (Cambridge Research Biochemicals, Cambridge, UK) detected bands at the level of 56–58 kDa, though the bands with MYC-2, -4 and -6 were faint. No MoAb was reactive with AcNPV cells without the human c-*myc* gene.

Reactivities of MoAbs with cellular proteins of human cells Reactivities of MoAbs with human cellular proteins were analyzed. Two human cell lines, HL-60 and Colo 320, were used as targets and their nuclear and cytoplasmic proteins were prepared according to the procedures described in "Materials and Methods." It is known that HL-60 and Colo 320 have amplified c-*myc*

Table I. Profiles of the Six MoAbs (MYC-1 to -6) Reactive with c-*myc* Proteins: A Summary of Immunoblotting Assay Results

	MoAb						
	MYC-1	MYC-2	MYC-3	MYC-4	MYC-5	MYC-6	CT-14[a]
Proteins used for immunization	p23	p23	p23	p23	p42	p42	31 amino acids in C-terminus
Reactivity with:							
truncated c-*myc* proteins							
p23	+	+	+	+	−	+	−
p42	+	+	+	+	+	+	+
c-*myc* protein with the baculovirus vector	+	±	+	±	++	±	++
HL-60 and Colo 320							
nuclear lysates	+ (58K, 60K)[b]	± (85K)	+ (58K, 60K)	± (85K)	++ (56K)	+ (68K, 75K)	+ (56K)
cytoplasmic lysates	−	++ (85K)	−	++ (85K)	−	−	++ (56K)
PBMC[c] lysates	−	+ (85K)	−	+ (85K)	−	± (68K, 75K)	−
RmycY1 lysates (c-*myc* gene transfectant)	±～+ (56K)	++ (85K)	±～+ (56K)	++ (85K)	++ (56K)	+ (68K, 75K)	++ (56K)
3Y1 lysates	−	++ (85K)	−	++ (85K)	−	−	−

a) Control anti c-*myc* protein MoAb (see reference 8).
b) Size of bands detected.
c) Peripheral blood mononuclear cells.

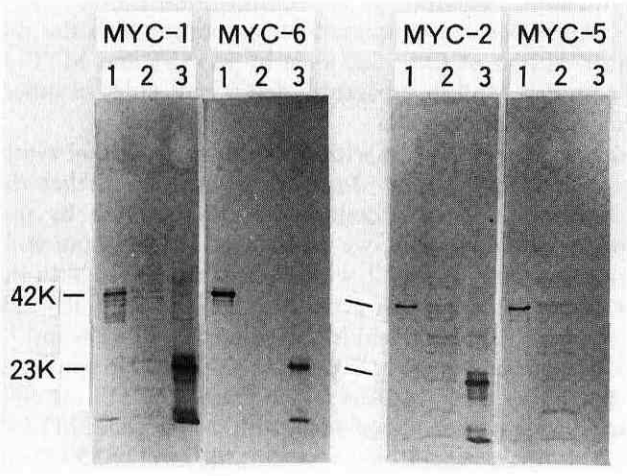

Fig. 1. Immunoblotting assays with anti c-*myc* protein MoAbs utilizing proteins produced in *E. coli*. Reactivities of MYC-1, MYC-2, MYC-5 and MYC-6 with truncated c-*myc* proteins p23 and p42, and also with Ki-*ras* p21 were analyzed. All proteins were produced in *E. coli*. Undiluted culture supernatant of hybridomas was used in the ABC assay; lane 1, c-*myc* p42; lane 2, Ki-*ras* p21; lane 3, c-*myc* p23.

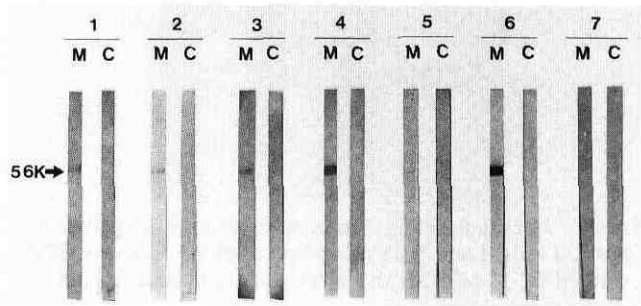

Fig. 2. Reactivities of anti c-*myc* protein MoAbs with lysates of insect cells with the human c-*myc* gene. Each MoAb was analyzed with lysates of Ac373/hc-myc cells with the human c-*myc* gene (M) and control AcNPV cells without human c-*myc* gene (C) in immunoblotting assays. Lane 1, MYC-1; lane 2, MYC-2; lane 3, MYC-3; lane 4, MYC-5; lane 5, MYC-6; lane 6, CT-14 (control anti c-*myc* MoAb; see reference 8); lane 7, control culture supernatant. Undiluted culture supernatant of hybridomas was used in the ABC assay. In this assay, all MoAbs detected 56–58 kDa c-*myc* protein in Ac373/hc-myc cells (M) except MYC-6 in lane 5 whose reactivity with c-*myc* protein of Ac373/hc-myc cells was usually weak and occasionally negative. None of the MoAbs was reactive with control AcNPV cells (C).

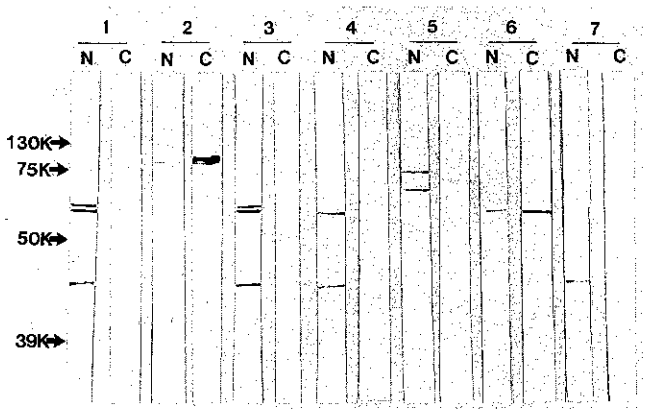

Fig. 3. Immunoblotting assays with nuclear preparations (N) and cytoplasmic preparations (C) of HL 60 cells. Lane numbers are as described for Fig. 2. A band of 45 kDa in nuclear preparations (N) in lanes 1, 3 and 4 is non-specific since it is also observed in lane 7 with negative control supernatant.

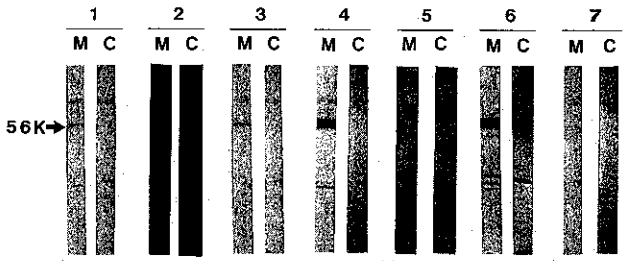

Fig. 5. Immunoblotting assays with lysates of c-*myc* gene transfected rat cells. Reactivities of MoAbs with lysates of RmycYl cells (M), transfectants of human c-*myc* gene, and lysates of 3Yl rat cells (C) without transfection of the human c-*myc* gene. Lane numbers are as described for Fig. 2. MYC-1 (lane 1), MYC-3 (lane 3), MYC-5 (lane 4) and CT-14 (lane 6) detected a single 56 kDa band only in RmycYl (M). MYC-6 (lane 5) detected 68 kDa and 75 kDa bands only in RmycYl. MYC-2 (lane 2), however, detected a single 85 kDa band in both RmycYl and 3Yl cells. Though not shown here, MYC-4 also detected an identical 85 kDa band.

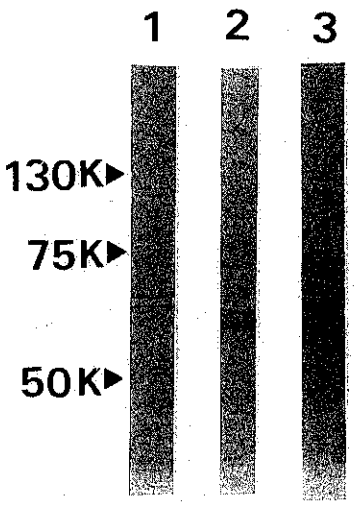

Fig. 4. Immunoblotting assays with nuclear preparation of Colo 320 cells. Lane 1, MYC-1; lane 2, MYC-5; lane 3, MYC-1 and MYC-5. MYC-1 and MYC-5 detect bands of different molecular weight.

genes. With cellular extracts of HL-60, MYC-1 and -3 detected two bands at the levels of 58 kDa and 60 kDa in the nuclear fraction (Fig. 3 and Table I). MYC-2 and -4 detected bands at the level of 85 kDa in the cytoplasmic protein extracts. MYC-5 detected a band at the level of 56 kDa. CT-14 used as a control anti c-*myc* protein MoAb[8] also detected a band at the same level. Our MYC-5, however, detected a band only in the nuclear extract whereas CT-14 detected it more dominantly in the cytolasmic preparation. MYC-6 detected two bands at the levels of 68 kDa and 75 kDa in the nuclear extract. These bands were also detected by each MoAb in the protein extract of Colo 320 (data not shown). No difference of the size and intracellular location of proteins detected by MoAbs was observed between the two cell lines. Though MYC-1, -3, -5, -6 and CT-14 failed to detect any band in the protein preparation of PBMC, MYC-2 and -4, detected a 85 kDa band in their cytoplasmic protein.

Figure 4 shows immunoblotting assays with the nuclear extract of Colo 320 cells by MYC-1 and MYC-5. These two MoAbs apparently detect molecules of different molecular weights.

Reactivities of MoAbs with the cellular protein of c-*myc* gene transfected cells In order to analyze further the relevance of these protein molecules detected by our MoAb to c-*myc* genes, we utilized rat 3Yl cells and their transformants, RmycYl, which were transfected with the activated human c-*myc* gene. MYC-1, -3 and -5 detected a single 56 kDa band and MYC-6 detected 68 kDa and 75 kDa bands in RmycYl (Fig. 5 and Table I). No band was detected by these MoAbs in the extract of 3Yl rat cells that had not been transfected with c-*myc* gene. MYC-2 and -4, however, detected a band at the level of 85 kDa in the extract of both Rmyc Yl and 3Yl cell lines.

Reactivities of MYC-5 with PHA-stimulated PBMC and non-cultured leukemic cells Four MoAbs, MYC-1, -3, -5 and -6, seemed to be immediately relevant to c-*myc* genes since they consistently reacted with protein preparations of cells with activated c-*myc* genes. In particular, MYC-5 always detected a band identical to that of CT-14

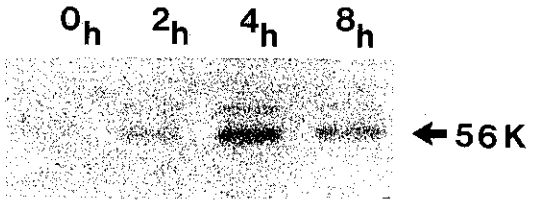

Fig. 6. Reactivity of MYC-5 with cellular lysates of PHA-stimulated PBMC. A 56 kDa band appeared within 2 h of incubation of PBMC with PHA and became maximal at 4 h. An additional 58 kDa band was also observed at 4 h.

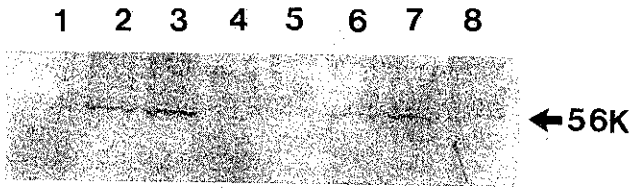

Fig. 7. Detection of 56 kDa band by MYC-5 in lysates of leukemic cells. Lysates were prepared from leukemic cells in the peripheral blood of seven patients with acute myelogenous leukemia (lanes 1 to 7) and also from normal bone marrow (lane 8). A 56 kDa band was detected in lysates of 5 patients (lanes 2, 3, 5, 6 and 7) and of normal bone marrow (lane 8).

which was previously reported to detect c-*myc* protein.[8] We therefore examined cellular protein molecules detected by MYC-5 in PHA-stimulated PBMC and also in leukemic cells derived from several patients.

PBMC were prepared and stimulated by PHA as described in "Materials and Methods." Lysates were prepared from cells sequentially collected after initiation of PHA stimulation. Immunoblotting analysis with MYC-5 on these cell lysates is shown in Fig. 6. A 56 kDa band appeared in 2 h and became the most dominant in 4 h after PHA stimulation. The band then became fainter within 8 h. Though not shown in Fig. 6, the faint band remained detectable for 24 h after the initiation of the culture. In addition to a band of 56 kDa, an additional band of 58 kDa, though much fainter, was also observed at 4 h.

We also examined whether the cellular molecules in non-cultured leukemic cells were detectable by MYC-5. Lysates were prepared from cryopreserved leukemic cells obtained from patients' peripheral blood. As shown in Fig. 7, in 5 out of 7 cases of acute myelogenous leukemia, a band of 56 kDa was detectable, though in one case it was extremely faint. In two cases no band was detectable. In normal bone marrow cells, a 56 kDa band was consistently detectable.

DISCUSSION

Table I summarizes the analyses of the 6 MoAbs, MYC-1 to -6. MYC-1 to -4 and -6 were reactive with both p23 and p42 in immunoblotting assays and in ELISA. MYC-5 exclusively reacted with p42. Though both p23 and p42 consist of 21 N-terminal aa of *ras* proteins, none of these MoAbs reacted with *ras* p21, indicating that they were not reactive with *ras* gene-coded peptides of p23 and p42. The reactivities of MYC-1 to -4 and -6 with p42 of pTRmyc22 clearly showed that they are not directed against the additional 5 aa sequence at the C-terminus of p23 since p42 does not have this sequence. The possibility that MYC-1 to -4 and -6 reacted with unique junctional conformations between *ras* and *myc* gene products must also be considered. However, all of these antibodies reacted with c-*myc* protein produced in insect cells with the baculovirus vector carrying the human c-*myc* gene, though the binding affinities of antibodies vary. Collectively, these results indicate that MYC-1 to -4 and -6 react with c-*myc* gene products coded by exon 2 and that MYC-5 was reactive with c-*myc* gene products coded by exon 3.

Proteins in mammalian cells detected by these antibodies are more diverse. All of the 58 kDa and 60 kDa molecules (p58/60) detected by MYC-1 and MYC-3, 56 kDa molecules (p56) by MYC-5 and 68 kDa and 75 kDa molecules (p68/75) by MYC-6 were primarily detectable in nuclear extracts rather than cytoplasmic extracts. These molecules were detectable in cells with activated c-*myc* genes such as HL-60, Colo 320 and RmycYl, though MYC-1 and -3 detected only a single 56 kDa band in RmycYl cells. None of them was detectable in non-stimulated PBMC and rat 3Yl cells, both of which lacked activated c-*myc* gene. Taken together, this evidence seems to indicate strongly that p58/60, p56, and p68/75 are immediate c-*myc* gene products or proteins closely related to c-*myc* genes.

Evan *et al.* raised 6 clones of MoAb by utilizing synthetic peptides as immunogens.[8] All of these MoAbs detected 62 kDa molecules in human cells by immunoprecipitation, occasionally accompanied with 64 kDa molecules. CT-14, one of the MoAbs that Evan *et al.* raised against 31 aa at the C-terminus of c-*myc* gene, was used as a control in our analysis. CT-14 consistently detected a 56 kDa band at exactly the same level as that detected by MYC-5 directed to c-*myc* gene products of exon 3. A band by MYC-5, however, was apparently dominant in nuclear extracts whereas CT-14 detected it in cytoplasmic as well as nuclear preparations. MYC-5 and CT-14 may therefore detect similar molecular groups or different epitopes of the same molecule. In this regard it has to be mentioned that MYC-5 failed to react with

the synthetic peptide of 31 aa at the C-terminus of the c-*myc* gene.

In immunoblotting assays, MYC-5 detected 56 kDa molecules in PHA-stimulated PBMC as well as in the acute myelogenous leukemic cells of several patients. In PHA-stimulated PBMC, an additional 58 kDa molecule was also detected when products of c-*myc* were maximized after 4 h of incubation. The relevance of this 58 kDa molecule to the c-*myc* gene also has to be examined together with that of the molecules detected by the other MoAbs.

The presence of heterogeneous molecules related to c-*myc* genes has been suggested for quite some time. Products of c-*myc* genes have been identified in cellular extracts by various polyclonal antibodies raised against synthetic peptides or c-*myc* proteins.[9-12] There have been discrepancies in the reported sizes of c-*myc* molecules that have been identified by SDS-PAGE either in immunoprecipitation assays or in immunoblotting assays, with values ranging between 62 kDa and 68 kDa. Some antibodies also identified more than one protein molecule. Differences in precise molecular weights determined by various investigators can possibly be attributed to the presence of different peptides either encoded by the c-*myc* gene or by other genes, or may be attributable to post translational modifications such as phosphorylation, or variations in experimental conditions such as cellular preparations and SDS-PAGE. In fact, in several experimental systems modification of molecular weight due to phosphorylation has been suggested.[7, 12] In addition to post translational modification of molecules, a recent analysis by Hann *et al.* showed the presence of two different peptides encoded by c-*myc* genes; one with a smaller molecular weight (p64) encoded by exons 2 and 3 and another with a slightly higher molecular weight (p67) owing to the presence of 14 or 15 N-terminal aa.[13] These additional aa were coded by a non-AUG translational initiation of exon 1. The MoAbs that we prepared detected 56–58 kDa molecules in the lysates of Ac 373/hc-myc cells with the baculovirus vector carrying the c-*myc* gene, and 56 kDa molecules in those of RmycYl cells transfected with the c-*myc* gene. Since c-*myc* genes used for these experiments were lacking exon 1, these cells might have produced peptides coded only by exons 2 and 3 though this is still to be determined.

The MoAbs we prepared may identify a series of these diverse c-*myc* proteins composed of different peptides as well as having different phosphorylation status. The results of simultaneous use of MoAbs in the current study strongly argue against the possibility that the molecular variations are due to experimental conditions such as cellular preparations and immunoblotting assays (examples are shown in Fig. 4). It is also important to stress that the size and number of molecules detected by a certain MoAb may also vary depending on the cells used as targets. Examples are MYC-1 and -3, which recognized 58 kDa and 60 kDa molecules in HL-60 and Colo 320 and detected a single 56 kDa molecule in Rmycl cells. Another example was MYC-5, which consistently detected a single 56 kDa molecules in HL-60, Colo 320 and RmycYl despite detecting an additional 58 kDa band in PHA-stimulated PBMC. Detailed analyses of molecules defined by MYC-1, -3, -5, and -6 might therefore be particularly important in clarifying the diverse molecular profiles of c-*myc* gene products.

The last molecule, p85, detected by MYC-2 and -4 seems to be widely distributed in a way independent of the presence of the activated c-*myc* gene. Evidence so far obtained raises a question as to whether this cellular molecule is directly encoded by the c-*myc* gene.

The availability of these six MoAbs will enable us to define the diversity of c-*myc* gene products precisely at the molecular level. Since our preliminary analysis indicates that some of these reagents can directly detect c-*myc* gene products in either immuno-cytostainings or immuno-histostainings, they may also be useful for detecting c-*myc* gene activation at the cellular level.

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REFERENCES

1) Naoe, T., Shiku, H., Yamada, K., Okazaki, T. and Kurosawa, Y. Production of a truncated human c-*myc* protein which binds to DNA. *FEBS Lett.*, **40**, 49–54 (1988).

2) Shiroki, K., Segawa, K., Koita, Y. and Shibuya, M. Neoplastic transformation of rat 3Yl cells by a transcriptionally activated human c-*myc* gene and stabilization of p53 cellular tumor antigen in the transformed cells. *Mol. Cell.*

Biol., **6**, 4379–4386 (1986).
3) Kato, K., Naoe, T., Hiraiwa, A., Namikawa, R., Suzuki, S., Yamada, K. and Shiku, H. Production and analysis of HH 10 monoclonal antibodies reactive to immature hematopoietic cells and their use for monitoring acute leukemia cells. *Jpn. J. Cancer Res.*, **76**, 524–531 (1985).
4) Yoshida, K., Hamatani, K., Koide, H., Ikeda, H., Nakamura, N., Akiyama, M., Tsuchiyama, H., Nakayama, E. and Shiku, H. Preparation of anti-*ras* Mr 21,000 protein monoclonal antibodies and immunohistochemical analyses on expression of *ras* genes in human stomach and thyroid cancers. *Cancer Res.*, **48**, 5503–5509 (1988).
5) Mirkovitch, J., Mirault, M. and Laemmli, U. K. Organization of higher-order chromatin loop: specific DNA attachment site on nuclear scaffold. Cell, **39**, 223–232 (1984).
6) Matsui, T., Hirano, M., Naoe, T., Yamada, K. and Kurosawa, Y. Production of chimeric protein coded by the fused viral H-*ras* and human N-*ras* gene in *Escherichia coli*. *Gene*, **52**, 215–223 (1987).
7) Miyamoto, C., Smith, G. E., Farrell-Towt, J., Chizzonite, R., Summers, M. D. and Ju, G. Production of human c-*myc* protein in insect cells infected with a baculovirus expression vector. *Mol. Cell. Biol.*, **5**, 2860–2865 (1985).
8) Evan, G. I., Lewis, G. K., Ramsay, G. and Bishop, J. M. Isolation of monoclonal antibodies specific for human c-*myc* proto-oncogene product. *Mol. Cell. Biol.*, **5**, 3610–3616 (1985).
9) Ramsay, G., Evan, G. I. and Bishop, J. M. The protein encoded by the human proto-oncogene c-*myc*. *Proc. Natl. Acad. Sci. USA*, **81**, 7742–7746 (1984).
10) Hann, S. R. and Eisenman, R. N. Proteins encoded by the human c-*myc* oncogene: differential expression in neoplastic cells. *Mol. Cell. Biol.*, **4**, 2486–2497 (1984).
11) Watt, R. A., Shatzman, A. R. and Rosenberg, M. Expression and characterization of the human c-*myc* DNA-binding protein. *Mol. Cell. Biol.*, **5**, 448–456 (1985).
12) Persson, H., Gray, H. E., Godeau, F., Braunhut, S. and Bellve, A. R. Multiple growth-associated nuclear proteins immunoprecipitated by antisera raised against human c-*myc* peptide antigens. *Mol. Cell. Biol.*, **6**, 942–949 (1986).
13) Hann, S. R., King, M. W., Bentley, D. L., Anderson, C. W. and Eisenman, R. N. A non-AUG translational initiation in c-*myc* exon 1 generates an N-terminally distinct protein whose synthesis is disrupted in Burkitt's lymphomas. *Cell*, **52**, 185–195 (1988).