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Monoclonal Antibodies Against the Viral and Human Cellular *myb* Gene Product

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Retroviruses code for oncogenes which cause tumors in animals. The viral oncogenes have evolved from normal cellular proto-oncogenes, to which they are closely related. The viral and cellular oncogenes differ in point mutations and size, the viral genes often being truncated and, in some cases, fused to unrelated cellular genes. These differences may be responsible for the transforming function of the viral oncogenes.

In human tumor cells, activated cellular oncogenes resembling the viral oncogenes have been identified. They also carry mutations and/or deletions, and in many cases are overexpressed from amplified genes. It is of interest to determine whether these activated cellular oncogenes are characteristic of certain tumors and whether their gene products can serve as tumor markers.

To date, nearly two dozen viral oncogenes have been identified. Some of them are closely related to each other, and on the basis of their sequence homology, cellular location, and associated enzymatic activities, they can be roughly classified into three groups. The largest group is the *src* gene family, which consists of tyrosine-specific protein kinases that are predominantly located at the inner side of the plasma membrane. Another group consists of protein kinases which are not tyrosine- but serine-/threonine-specific and are located in the cytoplasm, such as *mil/raf*. The third group comprises oncogene proteins located in the nucleus, such as *myc, myb*, and *fos*. They presumably play a role in the regulation of gene expression in tumor cells.

The c-*myb* oncogene, the homolog to the transforming gene (v-*myb*) of avian myeloblastosis virus (AMV), is specifically expressed in hematopoietic cells and appears to be tightly regulated during cell differentiation and proliferation [1, 5]. A five- to tenfold amplification of the c-*myb* gene was found in cultured cells of a patient with acute myelogenous leukemia (AML) [3]. Tumor cell lines have been established from AMV-transformed bone marrow cells and from a human immature T-cell line, designated BM-2 and MOLT4, respectively.

To analyze viral and cellular oncogene proteins, antisera are required. They were obtained against bacterially expressed oncogenes. An EcoRI/XbaI DNA fragment which comprises almost the complete viral oncogene *myb* (804 out of 1145 nucleotides) was cloned into pPLc24 expression vector [2]. The bacterially expressed proteins served not only for immunization of rabbits and mice but also for the identification of monoclonal antibodies in enzyme-linked immunoabsorbent assay (ELISA) tests. Monoclonal antibodies against the viral *myb* protein were obtained by standard hybridoma technology. Twenty-one myb-reacting monoclonal antibodies were obtained and subcloned until they were stable and characterized for their reactivities in various assays such as ELISA, Western blots, and indirect radioimmunoprecipitation. For some of the clones,

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Table 1. Summary of monoclonal antibody clones isolated against bacterially expressed viral *myb* protein

Clone no.	Clone	ELISA	Titer	Ig class	Blot		RIP	
					BM-2	MOLT4	BM-2	MOLT4
1/10	Lost	+++	10^{0}		–	–	(+)	–
1/12	+	++	10^{-2}		–	–	(+)	–
1/21	+	+++	10^{-2}		+	–	++	–
2/22	Lost							
3/12	+	+++	10^{-2}		–	–	++	–
3/20	+	+++	10^{-3}	IgG_{2a}	+++	–	+++	–
4/10	+	+++	10^{-1}		–	–	+	–
4/14	+	+++	10^{-1}	IgG_{2b}	+	+	+++	++
5/18	+	+++	10^{-2}		–	–	++	–
9/ 1	+	++	10^{0}		–	–	+	–
9/12	+	+++	10^{-1}	IgM	+	–	+	–
9/14	+	+++	10^{-3}	IgG_{1}	++	–	++	–
9/19	+	+++	10^{-3}	IgG_{2b}	+++	–	+++	–
10/ 7	Lost						–	–
10/22	Lost						(+)	–
14/14	+	+++	10^{-1}		(+)	–	+	–
15/23	+	+++	10^{0}		+	–	++	–
18/ 3	+	+++	10^{-1}		+	–	++	–
18/ 9	Lost						–	–
18/24	Lost						–	–
19/16	+	+++	10^{-2}	IgG_{2a}	(+)	–	+	–
M 46					++	++	+++	++

The reactivities in various tests were graded by +++, ++, +, and (+), according to the intensities of the signals obtained.
The serum titer was about 1000-fold higher than that of the hybridoma culture supernatants. Isolation of monoclonal antibodies followed standard techniques.
ELISA, enzyme-linked immunoabsorbent assay; RIP, indirect radioimmunoprecipitation; M46, mouse 46 used for fusion.

their immunoglobulin subgroups were determined and ascites fluid produced. These properties are summarized in Table 1.

Clone 4/14 precipitates the viral and human cellular *myb* gene products from ^{35}S-methionine metabolically labeled BM-2 and MOLT4 cells (Fig. 1). The p48$^{v\text{-}myb}$ and p75$^{hu\text{-}c\text{-}myb}$ proteins are predominantly precipitated (Fig. 1, slot 4, left and right). The specificities of the two precipitations are proven by use of excess bacterial *myb* protein for competition of the antigen-antibody reactions (Fig. 1, slot 5, left and right).

The anti-*myb* monoclonal antibody 3/20 proved useful for the purification of *myb*-specific protein from BM-2 cells. An immunoaffinity column was prepared from ascites fluid, and the immunoglobulin fraction was isolated by protein A chromatography and subsequently coupled to carrier beads. Details of this procedure have been published [2]. The purification of the p48$^{v\text{-}myb}$ protein from ^{35}S-methionine metabolically labeled BM-2 cells is about 3000-fold (Fig. 2). The p75$^{hu\text{-}c\text{-}myb}$ protein can be purified from MOLT4 cells, using an identical approach with clone 4/14 (data not shown). The analysis of human tumor cell lines is hampered by the fact that the level of human *myb* protein expression is about 10-fold lower than that of the avian viral system.

It is of interest to determine the reactivity of the *myb* monoclonal antibody 4/14 with human and avian tumor cells. In order first to establish its specificity in immunoperoxidase staining, clone 4/14 was tested with

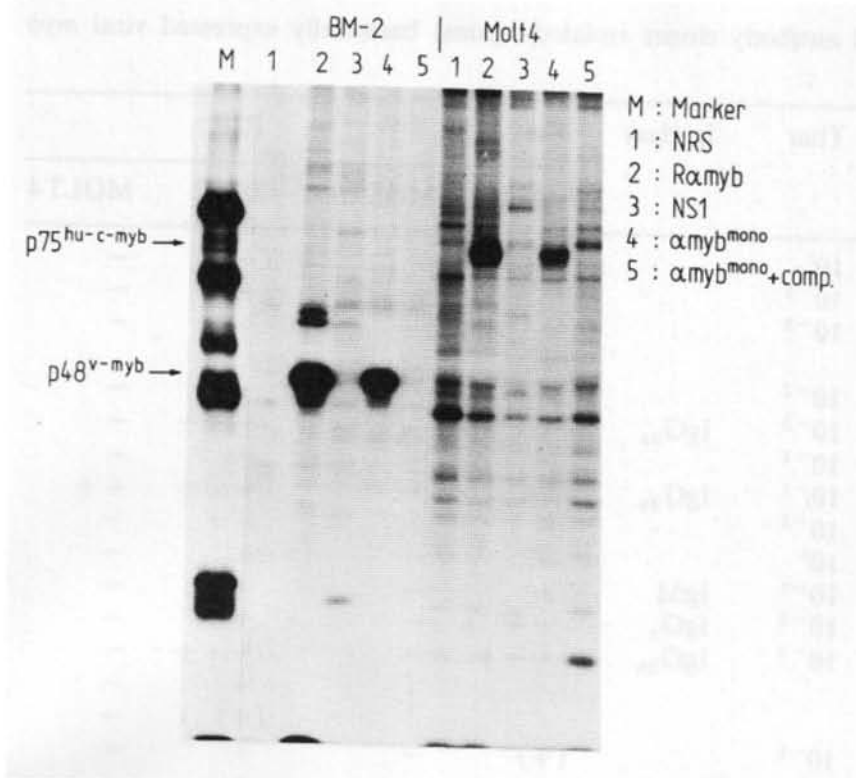


Fig. 1. Indirect immunoprecipitation of *myb* protein from ^{35}S-methionine metabolically labeled avian BM-2 and human MOLT4 cell lines (2×10^6 cells per precipitation labeled with 500 μCi/ml ^{35}S-methionine for 90 min and processed as described in [2]). For precipitation, 5 μl of normal rabbit serum (*slot 1*) and rabbit antibacterial *myb* serum (*slot 2*) were used. 1 ml of supernatant from NS-1 myeloma cells (*slot 3*) and from hybridoma clone 4/14 (*slot 4*) were used. Competition of the precipitation reaction was performed using 5 μg of purified *myb* antigen (*slot 5*). *M* indicates marker proteins, from *top* to *bottom:* 92K, 68K, 54K, 45K, and 32K. Exposure time: 1 week

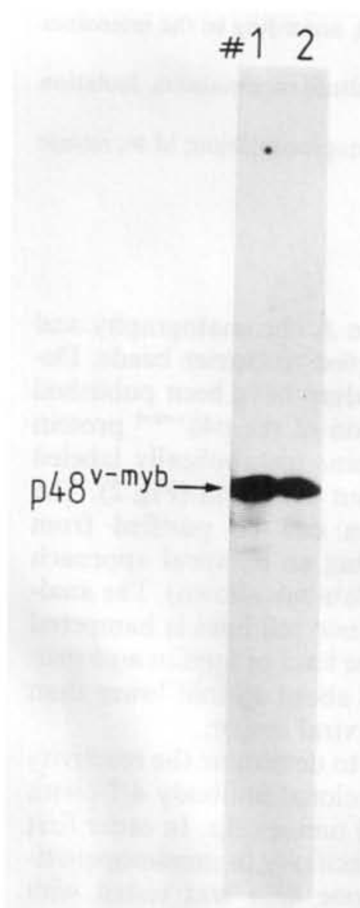
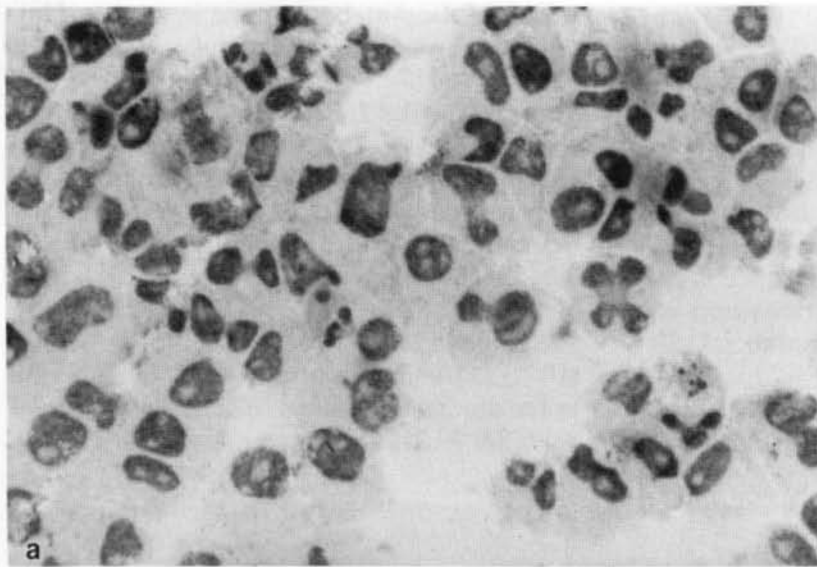


Fig. 2. Immunoaffinity column purification of p48$^{v\text{-}myb}$ protein from ^{35}S-methionine metabolically labeled BM-2 cells, using the monoclonal clone 3/20. The column was prepared according to previously published procedures [2]. 5×10^7 cells labeled with 500 μCi/ml for 90 min were used. An aliquot (5%) of the eluted fractions (2 ml each) was analyzed on sodium dodecyl sulfate polyacrylamide gel and exposed for autoradiography (time: 1 week)



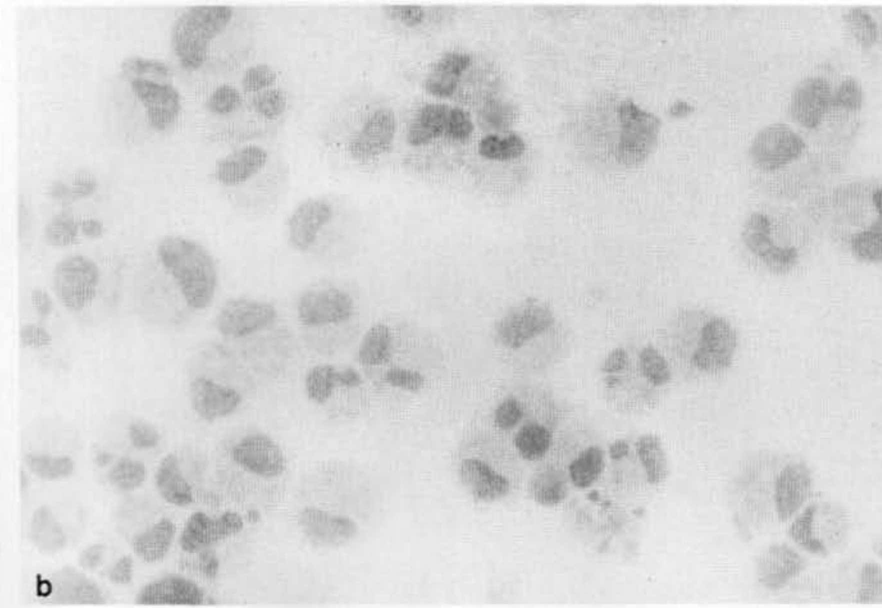


Fig. 3 a, b. Immunoperoxidase staining of BM-2 cells with monoclonal antibody, clone 4/14. Ascites fluid was used at a dilution of 1 : 600 (**a**). Competition by excess of antigen (5 μg) (**b**). Immunoperoxidase staining was performed as described in [4]

BM-2 cells. As is shown in Fig. 3, the monoclonal antibody gives rise to nuclear staining in BM-2 cells. The specificity of the reaction is proven by competition of the antibody binding with excess of antigen, which is shown in Fig. 3 b. Preliminary evidence indicates that the human cellular *myb* gene product can be detected in the human tumor cell line MOLT4 by an identical technique, in spite of the fact that it is expressed at about 10-fold lower levels in those cells (data bot shown).

In summary, of the monoclonal antibodies isolated against v-*myb*, 1 out of 21 recognizes the human cellular *myb* gene product in several experimental approaches. The differences between the viral and cellular *myb* genes are extensive, since besides several point mutations the viral *myb* gene is truncated to about two third the size of the cellular *myb* gene. In spite of this, the cross-reactive monoclonal clone 4/14 suggests the existence of a conserved antigenic site located on an EcoRI/XbaI fragment of v-*myb*. Since the expression of c-*myb* protein may be related to different malignancies, antibody clone 4/14 is a useful tool for investigating whether or not c-*myb* protein can be used as a tumor marker. Such investigations are now under way.

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