

Ocular Tumors React with Anti-neuroblastoma Monoclonal Antibodies

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The reactivities of mouse monoclonal antibodies directed against human neuroblastoma and peripheral melanoma associated antigens, with human retinoblastoma and choroidal melanoma cell lines were tested. Segregation of antigenic determinants according to each tumor class and cell line were observed. Three retinoblastoma cell lines and one fresh tumor explant showed determinants detected by two human neuroblastoma antisera, while the choroidal melanoma showed one determinant present on a peripheral melanoma but not neuroblastomas nor retinoblastomas, suggesting certain potential distinctive tumor related determinants. *Invest Ophthalmol Vis Sci* 24:1150-1152, 1983

The application of monoclonal antibody technology permits the analysis of the phenotypic heterogeneity of both normal and tumor tissues. This is based upon the observation that hybridoma-derived monoclonal antibodies can be used to detect specific antigenic determinants in cells. Although the function and importance of these determinants are generally unknown, knowledge of their distribution among tumors may have utility as a complementary technique to light and electron microscopy for characterizing different tumor cell phenotypes and possibly for resolving the complex interrelationships between tumor cells of even the same type. We decided to use monoclonal antibodies to define more clearly the relationship of eye tumors and other tumors commonly considered to be analogs eg, retinoblastoma: neuroblastoma and choroidal: peripheral melanoma. We screened the reactivities of three retinoblastoma and one ocular melanoma cell lines with eight monoclonal antibodies raised against antigens of different tissues. The latter include fetal brain, neuroblastoma cells, melanoma cells, and primitive neuroectodermal tumor.

Materials and Methods. Six monoclonal antibodies were obtained from J. T. Kemshead.¹ An additional antineuroblastoma monoclonal antibody BRL-7982 AS was obtained from a commercial source (Bethesda Research Laboratories, Gaithersburg, MD). This antibody is widely reactive with neuroectodermal tumor cells and appears to recognize a cell surface glycoprotein of $M_r = 2000$ on the human neuroblastoma cell line IMR-5. This cell surface determinant is present on human fetal brain cells, pre-B cells, mature B cells, and their corresponding leukemias.² The antibody does not recognize foreign fibroblasts, or two of eight human neuroblastoma cell lines in our laboratory, indicating that the antigenic determinant it does recognize is not of a major histocom-

patibility complex, or a class I molecule unique to melanoma. We also tested the reactivity of a monoclonal antibody (DW-1) directed against a primitive neuroectodermal tumor cell line established from one of the patients in our institution^{3,4} with these ocular tumors. The monoclonal antibodies Q14 and R24 were obtained from A. H. Houghton of the Memorial Sloan-Kettering Cancer Center.⁵

The tumor cell line SK-RB-DU was derived from a previously untreated patient with bilateral retinoblastoma.⁶ The patient's tumor was initially passaged in nude mice and then later transferred to cell culture. The cell line growing as a monolayer was passaged continuously for over 50 subtransfers during one year. The SK-RB-MI and SK-RB-SP lines were also derived from patients with bilateral retinoblastoma. These were initially cultured in the presence of a fibroblast feeder cell line derived from the conjunctiva of another retinoblastoma patient. These cells tend to remain as clumps of 30 or 40 cells loosely attached to the fibroblast monolayer and are readily harvested by simple agitation and collection. The tumor cells grow poorly or not at all in the absence of these feeder fibroblasts, and attempts at culture with normal skin fibroblasts or autologous tumor associated fibroblasts were consistently unsuccessful. These cell lines were tested for reactivity with monoclonal antibodies prior to and after short-term culture. Retinoblastoma tumor tissue, SK-RB-Ne, was obtained with 1/2 hr after enucleation of a tumored eye from an untreated patient with bilateral retinoblastoma. Tumor imprints on polylysine-treated glass slides were made, air dried, and fixed and stained as described below. This same tumor is currently in short-term culture and it is too early to consider it an established cell line. The ocular melanoma cell line SK-OM-FO was obtained from an untreated patient and maintained in vitro for 8 months prior to antibody testing. The peripheral melanoma cell line RPMI 7931, originally established by G. Moore in Rosewell Park Memorial Institute, New York, was obtained from Jorgen Fogh of Memorial Sloan-Kettering Cancer Center and maintained in our laboratory since 1975. The human neuroblastoma cell line SK-N-MC was included in this study for comparison. It is a well-established cholinergic neuroblastoma cell line and is one of the two neuroblastoma cell lines of eight tested that did not react with the UJ13A monoclonal antibody. It is also distinctive in that it is only one of eight human neuroblastoma cell lines that contained a determinant

Table 1. Cell line

Monoclonal antibody	Neuroblastoma SK-N-MC	Retinoblastoma				Choroidal melanoma SK-OM-FO	Peripheral melanoma RPMJ 7931
		SK-RB-DU	SK-RB-MI	SK-RB-SP	SK-RB-NE		
Antineuroblastoma							
BRL-7920 AS	+	+	+	-	-	+	+
UJ13A	-	+	+	+	+	+	-
UJ181.4	+	+	+	+	+	-	-
UJ127:11	-	-	-	-	-	-	-
UJ308	-	-	-	-	-	-	-
UJ223.8	-	-	-	-	-	-	-
UJ167	-	-	-	-	-	-	-
DW-1	-	-	-	-	-	+	+
Antimelanoma							
Q14	-	-	-	nd	nd	+	+
R24	-	-	-	nd	nd	+	-

recognized by UJ181.4. These latter studies were done using conditions identical to those described in this study for these tumor lines.

The cells were assayed for reactivity to antibody using an indirect fluorescent method. The tumor cells were permitted to attach and grow as a monolayer on a LAB-TEK® plate. (Miles Laboratories, Naperville, IL). They were then fixed with ice cold methyl alcohol and then washed with cold phosphate buffered saline pH 7.4 (PBS). They were then exposed for 45 min at 4 C to antihuman neural tissue antibodies. After two additional washes with PBS, fluorescinated goat antimouse IgG or IgM (Meloy Labs., Gaithersburg, VA) was applied for 1 hr at 4 C. After thorough washing with PBS, the cells were mounted in cold 1% glycine in water at a pH 7.2 and fluorescence visually scored using a halogen epifluorescent light source on a Nikon microscope. Visual scoring was registered as positive (+) when bright green fluorescence was observed at the lowest antibody dilution and this same dilution of antibody stained cells using the vectastain™ ABC Kit, with biotinylated antimouse IgG (Vector Labs., Burlingame, CA).⁷

In each experiment, the fluorescinated or biotinylated antimouse antibody added to replicate cells without monoclonal antibody served as controls. In all cases the absence of fluorescence in controls was obligatory to exclude nonspecific absorption.

Results. The BRL-7920 AS antibody was reactive with determinants on both melanoma, 3/5 retinoblastoma and 6/8 neuroblastoma cell lines tested. The cells of ocular origin in this study contained an antigen recognized by UJ13A, while the UJ181.4 may be recognizing an antigen peculiar to this one unusual neuroblastoma, and 4/4 retinoblastoma cells. This notion of segregation of determinants was reinforced by the selective reactivity of antimelanoma antibody Q14, R24, and the antiprimitive neuroectodermal

tumor antibody DW-1. Within this group of tumors, the ocular melanoma is distinguishable from the peripheral melanoma by the reactivity with the R24 antibody (Table 1).

Discussion. The BRL-7920 AS recognized a product present in most of these cells tested. In additional studies with this antibody, it did not recognize two out of eight neuroblastoma cell lines. In view of the information known about this antibody, it does not seem probable that it is recognizing HLA-D/DR determinants or a molecule specific to these tumors. Since the product being recognized appears to be distributed among a variety of cell classes including lymphocytes, the use of this antibody for classification of these ocular tumors is probably of little value.

UJ13A is an antibody raised against human fetal brain tissue. It recognizes a surface glycoprotein in many tumors of neural origin. These include neuroblastomas at a variety of levels of differentiation. This determinant found in the choroidal melanoma and the four retinoblastomas suggest these tumors to be less differentiated than the peripheral melanoma, or the cholinergic neuroblastoma, both of which were derived from older patients. The SK-N-MC neuroblastoma not only did not exhibit the particular antigenic determinant recognized by UJ13A but was unique among neuroblastomas because it also expressed an antigen recognized by UJ181.4. In a personal communication, J. T. Kemshead reported that the UJ181.4 was restricted to fetal brain, neuroblastoma, medulloblastoma, and retinoblastoma. This antibody did detect determinants in four retinoblastoma cell lines, suggesting that in addition to the pan-neuroectodermal antigen seen by UJ13A, the retinoblastoma exhibit fetal antigens that may be also found in neuroblastomas or other neuroectodermal tissues.

The DW-1 antibody, raised against a primitive

neuroectodermal tumor antigen was reactive with both the choroidal and the peripheral melanoma, suggesting the presence of an additional antigen in these cells which is separate from the "fetal" product recognized by UJ13A.

In comparing the reactivity of the anti-melanoma antibodies with other antibodies, we were not surprised to find a degree of specificity since these former antibodies may be recognizing class I molecules; distinguishing yet another set of antigens.

Similar reactivity among these tumors of rather disparate origin, ie, infants with neuroblastoma and adults with neuroblastoma or melanoma, is not unexpected. In fact, reactivity of neuroblastoma, retinoblastoma, and melanoma cells with a single monoclonal antibody has already been reported.⁸ What may eventually have more clinical significance is the distribution among cells and the biochemical identification of the specific determinants being recognized by these monoclonal antibodies.

The data suggest that there may be fundamental antigens common to most neuroectodermal tissues or tumors that are independent of the state of differentiation such as that recognized by BRL-7920 AS. There may also be a variety of differentiation antigens exhibited potentially by all neuroectodermal tissues but whose expression is variable.

If the UJ13A and the UJ181.4 antibodies recognize "fetal" antigenic substances then this suggests the presence of lesser differentiated status for the retinoblastoma and choroidal melanoma tumor types than the cholinergic neuroblastoma and peripheral melanoma.

The perspective of the primitive neuroectodermal tumor as a nosologic entity is based upon histologic criteria that assumes it to be a preneuroblastoma. The reactivities observed with DW-1 suggest that there are additional antigens being exhibited in the melanomas that are of a less differentiated nature, but distinct from those found in fetal central nervous system tissues.

In conclusion, ocular tumors may not be very unique compared to other neuroectodermal tumors in terms of expression of antigenic determinants. The retinoblastomas appear to retain what may be two "fetal"-type antigenic products that can be detected by two different monoclonal antibodies. The retinoblastomas may be distinguished from the melanomas in that the latter appear to express both fetal (UJ13A, DW-1) and antigens associated with more differentiated tissue and recognized by Q14 and R24. The antigenic substances defined by the UJ181.4 monoclonal antibody in human retinoblastoma cells appear to have rather a unique pattern of distribution. This is based only on its reactivity in five tumors from

five patients with bilateral disease and may not be expressed by unilateral retinoblastomas. The antigens are probably not tumor specific but may represent different stages of differentiation. By analyzing antigenic determinants using a battery of monoclonal antibodies of more cell lines and tumors from a wider variety of patients, a more comprehensive perspective of these tumors may be possible.

Key words. ocular tumors, monoclonal antibodies

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References

1. Kemshead JT, Fritschy JG, Asser U, Brown S, and Greaves MF: Characterisation of an antigen present on neuroblastoma and melanoma cells recognized by monoclonal antibody UJ 127:11. *In* Pediatric Oncology. Proceedings of the XIII Meeting of the International Society of Pediatric Oncology, Marseilles, 1981, Raybaud C, Clement R, Libreuil G, and Bernard JL, editors. Amsterdam, Excerpta Medica, 1982, pp. 85-89.
2. Kennet RH, Jonak Z., and Bechtol KB: Characterization of antigens with monoclonal antibodies. *In* Advances in neuroblastoma Research. Evans AE, editor. New York, Raven Press, 1980, pp. 205-219.
3. Helson L, Helson C, Lieberman P, and Schneider B: Primitive neuroectodermal tumors (PNET). Proc. XVII Annual Meeting of Am Soc Clin Oncol April 30-May 2, 1981. Abstract #C-14, p. 336.
4. Kohler G, and Milstein C: Derivation of specific antibody-producing tissue culture and tumor lines by cell fusion. *Eur J Immunol* 6:511, 1976.
5. Dippold WG, Lloyd KO, Li LTC, Ikeda H, Oettgen HF, and Old LJ. Cell surface antigens of human malignant melanoma; definition of six antigenic systems with mouse monoclonal antibodies. *Proc Natl Acad Sci USA* 77:6114, 1980.
6. Helson L, Kitchin FD, Abramson D, and Ellsworth RM: Metastatic retinoblastoma and L-phenylalanine mustard-dianhydrogalactitol. *Anticancer Res* 2:235, 1982.
7. Hsu SM, Raine L, and Fanger H: A comparative study of the peroxidase-antiperoxidase method and an avidin-biotin complex method for studying polypeptide hormones with radioimmunoassay antibodies. *Am J Clin Pathol* 75:734, 1981.
8. Kemshead JT, Fritschy J, Asser U, Sutherland R, and Greaves MF: Monoclonal antibodies defining markers with apparent selectivity for particular haemopoietic cell types may also detect antigens on cells of neural crest origin. *Hybridoma* 1:109, 1982.