Surface and cytoplasmic antigens in retinoblastoma NORMAN T. FELBERG AND LARRY A. DONOSO.

Surface and cytoplasmic antigens in retinoblastoma were examined by antisera prepared against two tissue-cultured retinoblastoma cell lines. In addition, several other antisera, including anti-rhodopsin, anti-rod outer segment, anti-large-molecular-weight protein, anti-S, and anti-P antigen, were also utilized in a complement-dependent cytotoxicity assay in order to explore the tumor cell surface. Antisera to tissue-cultured retinoblastoma cell lines were cross-reactive to both cell lines as was rod outer segment antisera.

Many lines of evidence support the concept that antigenic changes in the cell surface or cytoplasmic makeup are associated with malignant transformation.1 2 The establishment of retinoblastoma tissue culture cell lines,3 4 the production of antisera to specific photoreceptor cell proteins,5 and the description of sugar-specific lectins6 have made new techniques available, all of which are applicable to the study of the nature of the cell surface of the malignant cell.

We have undertaken an investigation of the surface and cytoplasmic antigens in retinoblastoma tumors, utilizing both paraffin-embedded tissue sections as well as two tissue-cultured retinoblastoma cell lines. This report describes our findings using a variety of antisera prepared against retinoblastoma cells and a variety of photoreceptor cell proteins.

Materials and methods

Tissue. Retinoblastoma tumor specimens were obtained within 5 min after enucleation. The tissue was fixed in cold ethanol and processed according to the procedure of Sainte-Marie.7

Both WERI-Rbl and Y79 tissue-cultured retinoblastoma cell lines have been maintained in our laboratory for over 2 years. The characteristics of each of these cell lines have been well described.3 4 In(207,1354),(856,1396)

Reagents. Poly-D-lysine, trypan blue, and diaminobenzidine were obtained from Sigma Chemical Co. Horseradish peroxidase–conjugated goat anti-rabbit IgG (heavy chain–specific) was from Cappel Laboratories, as was guinea pig complement. All other reagents were ‘reagent grade’ unless specified.

Antisera. Anti-retinoblastoma sera were prepared in 2.5 kg New Zealand white rabbits by intradermal injection of 0.5 ml of 5 × 10^6 retinoblastoma cells/ml mixed with complete Freund’s adjuvant followed by weekly intravenous injections of 0.5 ml of 5 × 10^6 tumor cells/ml resuspended in Hanks’ balanced salt solution. Injections were repeated until complement-dependent cytotoxicity titers reached a plateau.

Antiserum was prepared against human rod outer segments (ROSs) obtained by a single discontinuous 1M sucrose density gradient centrifugation.8 ROSs from one globe were suspended in 0.5 ml of phosphate-buffered saline (PBS), emulsified with an equal volume of complete Freund’s adjuvant, and injected intradermally. A second challenge was given 2 weeks later with an equal amount of ROSs in incomplete Freund’s adjuvant. The rabbit was bled 2 weeks after the second challenge.

Guinea pig anti-S, guinea pig anti-P, fluorescein isothiocyanate–labeled rabbit anti–guinea pig immunoglobulins, and normal guinea pig serum were the gifts of Waldon B. Wacker, Ph.D., Louisville. Goat anti-frog rhodopsin and rabbit anti-bovine large-molecular-weight protein were kindly supplied by David Papermaster, Ph.D., New Haven.

Cell attachment. Both Y79 and WERI-Rbl grow in suspension and do not adhere to culture flasks as monolayers. Cell attachment to Teflon-coated slides (Shandon Scientific Co.) was made possible by the use of 10 μg/ml poly-D-lysine HBr (MW 70,000) in PBS, a technique previously described for poly-D-ornithine.9

Immunofluorescence or immunoperoxidase assay. Rabbit anti-retinoblastoma sera were diluted 1:10 or 1:20 in PBS and incubated for 30 min at 37° in a moist chamber. Horseradish peroxidase–conjugated goat anti-rabbit IgG was used at a dilution of 1:100 or 1:200. Antibody binding was detected with Karnovsky’s reagent.10

Table I. Complement-dependent cytotoxicity

<table>
<thead>
<tr>
<th>Antiserum toward</th>
<th>Y79*</th>
<th>WERI-Rbl*</th>
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</thead>
<tbody>
<tr>
<td>Human Y79 retinoblastoma</td>
<td>1000</td>
<td>200</td>
</tr>
<tr>
<td>Human WERI-Rbl retinoblastoma</td>
<td>50</td>
<td>500</td>
</tr>
<tr>
<td>Human ROSs</td>
<td>500</td>
<td>10</td>
</tr>
<tr>
<td>Bovine rhodopsin</td>
<td>&lt;2</td>
<td>&lt;2</td>
</tr>
<tr>
<td>Bovine large-molecular-weight protein</td>
<td>&lt;2</td>
<td>8</td>
</tr>
<tr>
<td>Guinea pig S antigen</td>
<td>&lt;2</td>
<td>&lt;2</td>
</tr>
<tr>
<td>Guinea pig P antigen</td>
<td>&lt;2</td>
<td>&lt;2</td>
</tr>
</tbody>
</table>

*Retinoblastoma cell lines.

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Fig. 1. Staining pattern associated with the cell surface obtained after incubation of viable tissue cultured Y79 retinoblastoma cells with rabbit anti-Y79 serum, followed by peroxidase-conjugated goat anti-rabbit immunoglobulin G (heavy chain-specific). Surface-bound antibody was observed as a dark membrane or "halo" appearance. Occasional cells with black cytoplasm were not viable during the incubation with rabbit anti-Y79 serum, and the antibody penetrated into the cytoplasm. (x250.)

Guinea pig anti-S and anti-P reactions were measured as described by Wacker et al.  

Complement-dependent cytotoxicity. Various dilutions of antisera were mixed with viable tissue culture Y79 or WERI-Rbl cells attached to a Teflon-coated glass slide treated with poly-D-lysine (see above). After 30 min at 37° in a humidified 5% CO₂ incubator, the serum was replaced with 1:5 dilution of guinea pig complement for an additional 30 min incubation. Excess reagent was removed, and the viable cells were determined by trypan blue exclusion. End point was taken as 50% viability.

Results. Rabbit anti-Y79 sera reacted against both paraffin-embedded retinoblastoma tissue sections as well as viable tissue-cultured tumor cells, indicating the presence of antibodies against cytoplasmic and cell surface antigens, respectively. When viable tissue-cultured cells were incubated with the antisera, a cell surface "rimming" pattern appeared to be the predominant staining pattern observed (Fig. 1). No gross qualitative staining pattern differences could be discerned between the two tissue culture cell lines. The specificity of the anti-Y79 serum was examined with human fetal lung fibroblasts (IMR-90) and cutaneous malignant melanoma cells (MLA-14). No reactions were seen when viable or fixed cells were studied at dilutions as low as 1:2, suggesting the antisera did not contain antibodies reactive toward nonspecific human cell surface or cytoplasmic antigens.

When histologic tissue sections from enucleated globes containing both retinoblastoma tumors and areas of normal retina were incubated with either anti-S or anti-P serum, only an occasional tumor cell reacted, whereas the photoreceptor cell layer in areas of normal retina reacted strongly with the antisera. Some sections contained well-defined rosettes or fleurettes, and special emphasis was directed at these areas of the tumor; however, S or P antigen did not appear to be associated with
these structures. When tissue-cultured Y79 or WERI-Rbl retinoblastoma cells were incubated with the antisera, no fluorescence was associated with the tumor cells.

Shared antigens between the two tissue-cultured tumor cell lines were found by complement-dependent cytotoxicity. Rabbit anti-Y79 serum had a complement-dependent cytotoxicity titer of 1:1000 toward Y79 cells (Table I). Anti-Y79 serum was also cytotoxic to WERI-Rbl cells at a dilution of 1:200, suggesting common surface antigens between the two cell lines. Conversely, rabbit anti–WERI-Rbl serum showed cross-reactivity to Y79 (Table I). In addition, rabbit anti-human ROS serum was cytotoxic to both cell lines, suggesting that antigens common with photoreceptor cells persisted in both tumor cell lines. In contrast, no significant cytotoxic effects to either tumor cell line could be elicited with the use of anti-frog rhodopsin, guinea pig anti-S or anti-F, or anti-bovine large-molecular-weight protein, suggesting the absence of these antigens on the tumor cell lines. The possible exception seen in Table I is the moderate cytotoxicity of anti-bovine large-molecular-weight protein serum against WERI-Rbl.

Discussion. This study demonstrated the presence of cell surface and cytoplasmic antigens in paraffin-embedded retinoblastoma tumors as well as in two viable retinoblastoma cell lines with the use of heterologous antisera prepared against tissue-cultured Y79 and WERI-Rbl retinoblastoma cell lines. Belehradek et al.11 demonstrated the presence of organ-specific antigen(s) in three retinoblastoma cell lines by immunofluorescence assay with guinea pig autoantibody to normal guinea pig retina, indicating the presence of shared antigens between the two tissues. The specificity of their antisera was shown by the failure to react with two cell culture lines derived from endometrial and oral epidermoid carcinoma. Similarly, our Y79 antisera did not cross-react with cell lines derived from human fetal lung fibroblasts or malignant melanoma, suggesting that the antisera did not contain nonspecific cross-reacting antibodies toward these human-derived cell lines. Several other lines of evidence, however, suggest that there may be common antigens between the retinoblastoma tumor cell and photoreceptor cells.

Saari et al.12 examined cellular extracts of a variety of tumors for the presence of cellular binding proteins commonly found on the normal retina and specific for retinoids. They were able to show that both Y79 and WERI-Rbl contained cellular-binding proteins specific for retinoic acid in high concentration. Russell et al.13 have recently separated and purified receptor proteins for [3H]retinol and [3H]retinoic acid from cultured Y79 retinoblastoma cells.

Common antigens between the Y79 and WERI-Rbl cell lines were shown by complement-dependent cytotoxicity assay. Rabbit anti-Y79 serum was cytotoxic to Y79 cells (titer 1:1000, Table I) as well as WERI-Rbl cells (titer 1:200, Table I). Conversely, rabbit anti–WERI-Rbl serum showed cross-reactivity to Y79 (Table I).

Antiserum prepared against human ROSs was cytotoxic to both tumor cell lines, suggesting that these antigens have been retained during transformation. Other proteins, however, do not appear to be conserved. Antiserum to rhodopsin, guinea pig S antigen (a 50,000 dalton retinal surface protein responsible for the production of experimental autoimmune uveitis), guinea pig P antigen (rhodopsin), and bovine large-molecular-weight protein (the second most abundant protein of ROSs) did not appear to react against the tumor cell lines. To our knowledge, rhodopsin has never been demonstrated as a product of retinoblastoma tumors. Cell lines from retinoblastoma tumors may be too undifferentiated to retain these antigens. We have not been able to demonstrate S antigen in freshly obtained well-differentiated retinoblastomas containing rosettes or fleurettes. In the same sections, S antigen is readily apparent in the nontumorous parts of the retina, perhaps suggesting that the rosette is still primitive in comparison to the fully differentiated retina. In addition, patients with uveitis show similar cellular immune responsiveness to either bovine- or human-derived retinal extracts of S antigen,14 indicating no significant species specificity differences for this particular antigen.

Cell-mediated immunity with a cultured retinoblastoma cell line was shown by Char et al.15 in patients with a variety of pigmentary retinal degenerations. These studies suggest the presence of antigens common not only to the retinoblastoma and the photoreceptor but also to the retinal pigment epithelium as well. Surface receptors for immunoglobulin and the third component of complement also appear to be present on pigment epithelial cells16; however, recent studies in our laboratory have failed to reveal these same receptors in the Y79 or WERI-Rbl cell lines. A detailed analysis of a variety of surface Y79 and WERI-Rbl receptors will be the subject of a future report.
Waldon B. Wacker, Ph.D., and Carolyn Kalsow, Ph.D., supplied the reagents for the anti-S and anti-P studies. David Papernest, M.D., supplied antisera to rhodopsin and large-molecular-weight protein. Barbara Hankinson and Susan Piperata provided technical assistance.


Key words: retinoblastoma, surface antigens, cytoplasmic antigens, cancer

References


Studies on the angioarchitecture of the posterior choroid in rat and role of posterior ciliary vein. Hiroshi Yoshimoto, Mikio Murata, Kiyoshi Yamagami, and Shuichi Matsuyama.

Vascular casts were made by injection of low-viscosity plastic in eight Wistar-Kyoto rats, and the posterior half of the ocular wall was observed using the semilunar section method. Veins which connect with the capillaries of the posterior choroid include a venous system independent of the vortex vein and running parallel to the long and short posterior ciliary arteries. The veins flow into a venous ring which is located in the region at which the arterial circle of Zinn is said to be. There was no structure in this region which could be described as an arterial circle. From the above findings, it is concluded that these venous systems are part of the posterior ciliary vein and that they play an important role as a pathway for irrigation of blood from the posterior choroid.

Despite the clinical importance of the choroidal circulatory system, its fine structure has not been as fully clarified as that of the retinal circulatory system because of the anatomic difficulty of direct, in vivo, detailed observation. Nevertheless, due to the development of low-viscosity plastics suitable for preparation of vascular casts, the circulatory...