Tumor Escape Mutants Develop within an Immune-Privileged Environment in the Absence of T Cell Selection

Peter W. Chen, Toshihiko Uno and Bruce R. Ksander

*J Immunol* 2006; 177:162-168; doi: 10.4049/jimmunol.177.1.162
http://www.jimmunol.org/content/177/1/162

**References**
This article cites 33 articles, 15 of which you can access for free at:
http://www.jimmunol.org/content/177/1/162.full#ref-list-1

**Subscription**
Information about subscribing to *The Journal of Immunology* is online at:
http://jimmunol.org/subscription

**Permissions**
Submit copyright permission requests at:
http://www.aai.org/About/Publications/JI/copyright.html

**Email Alerts**
Receive free email-alerts when new articles cite this article. Sign up at:
http://jimmunol.org/alerts
Tumor Escape Mutants Develop within an Immune-Privileged Environment in the Absence of T Cell Selection

Peter W. Chen,* Toshihiko Uno,† and Bruce R. Ksander1‡§

The establishment of tumor escape mutants, which can be driven by innate and/or adaptive immune effector cells, presents a significant obstacle in the development of successful tumor immunotherapies. Our study documents that tumors growing within an immune-privileged site within the eye develop a tumor escape phenotype in the absence of selective T cell pressure. P815 tumor cells that are recovered from progressively growing tumors within the anterior chamber of the eye escape elimination when injected into the flanks of a second group of syngeneic DBA/2 mice that were previously immunized against P815 tumor cells. The escape phenotype of eye-derived P815 tumors was stable and permanent when the tumor cells were cultured in vitro. Eye-derived tumor cells recovered from the anterior chamber of CB-17 SCID mice also escaped elimination when injected into the flanks of immunized mice, demonstrating that selective pressure by tumor Ag-specific T cells did not contribute to the development of the escape phenotype. In vitro studies demonstrated that eye-derived tumor cells were not lysed by specific CTL and were unable to restimulate primed Ag-specific T cells. Immune escape of eye-derived tumor cells was not due to down-regulation of either MHC class I or ICAM-1. Our data demonstrate that the immune-privileged environment within the eye induces a tumor escape phenotype that is not driven by selective T cell pressure. We predict that immune escape within the eye is driven by the unique ocular environment that permanently alters gene expression in eye-derived tumor cells. The Journal of Immunology, 2006, 177: 162–168.

Genetic instability plays a major role in the ability of tumor cells to develop escape mutants that evade immune elimination. This fundamental characteristic of tumor cells is a major reason why many promising immunotherapies designed to elicit potent tumor Ag-specific T cell immunity ultimately fail, and it poses a considerable challenge in the development of successful cancer vaccine strategies (1, 2). Thus, immunotherapies designed to establish Ag-specific T cell immunity against tumors present an interesting paradox; tumor-specific immunity that effectively eliminates the tumor also applies selective pressure that promotes the development of tumor escape mutants that are resistant to T cell elimination. Numerous reports indicate that tumors escape immune elimination by the selective growth of tumor cells expressing random mutations that either initiate or silence genes through point mutations, frameshift mutations, genomic translocations, insertions, or deletions (3, 4). Tumor cells that escape immunity due to selective T cell pressure typically display mutations induced through genomic modifications. T cells, B cells, and NK cells have been reported to apply selective pressure that results in the development of tumor escape mutants (5–8). This report examines whether the immune privileged environment induces the development of a tumor escape phenotype and whether it is due to selective T cell pressure.

Abbreviation used in this paper: MLTC, mixed lymphocyte-tumor cell.

Materials and Methods

Cell lines

P815 murine mastocytoma cells were obtained from American Type Culture Collection. Cultured P815 tumor cells were periodically passaged in vivo to ensure a consistent phenotype. Cells were cultured in complete RPMI 1640 medium containing 10% FBS (HyClone), 100 U of penicillin, 50 μg of streptomycin, 0.1% Fungizone (BioWhittaker), 2.0 mM glutamine (BioWhittaker), 0.01 M HEPES buffer (BioWhittaker), and 0.5% 2-ME (Sigma-Aldrich). L1210 is a DBA/2-derived leukemia cell line that does not express any of the characterized P815 tumor Ags (9). P815 CD80−IL-12 tumor cells were obtained by coelectroporation of the pBMG-luc plasmid containing the cDNA for the IL-12 p35 chain and the pBMG-his plasmid containing the cDNA for the IL-12 p40 chain into P815 CD80−transfected tumor cells (12). Transfected cells were cultured in complete RPMI 1640 medium containing 10% FBS, 800 μg/ml neomycin, and 0.05 mM histidinol. Eye-derived P815 cells were infected cells were cultured in complete RPMI 1640 medium containing 10% FBS, 18 U.S.C. Section 1734 solely to indicate this fact.

1 Address correspondence and reprint requests to Dr. Bruce R. Ksander, Schepens Eye Research Institute, 20 Staniford Street, Boston, MA 02114. E-mail address: ksander@vision.eri.harvard.edu
2 Abbreviation used in this paper: MLTC, mixed lymphocyte-tumor cell.

Received for publication December 20, 2005. Accepted for publication April 17, 2006.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

*Department of Ophthalmology, University of Texas Southwestern Medical Center, Dallas, TX 75390; †Ehime University School of Medicine, Ehime, Japan; ‡Schepens Eye Research Institute, Boston, MA 02114; and §Department of Ophthalmology, Harvard Medical School, Boston, MA 02114
were recovered from enucleated P815 tumor-containing eyes 10 days after injection of 1 x 10^5 P815 tumor cells into the anterior chamber as described below. Tumor cells and eye tissue were separated, and a single cell suspension was prepared by mechanical manipulation. In a single series of experiments in which we recovered eye-derived tumor cells 3, 5, 7, or 10 days postinjection, the eye-derived tumor cells were isolated and cultured for 3 days in vitro.

**Materials and Methods**

**Mice**

Female DBA/2 mice (H-2^d^) and CB-17 SCID mice were obtained from Taconic Laboratories and housed in pathogen-free conditions in animal facilities of the Schepens Eye Research Institute (Boston, MA). Experiments were performed on 8-wk-old mice. All experimental protocols in this study were approved by the Schepens Eye Research Institute’s animal care and use committee and conform to resolution of the Association for Research in Vision and Ophthalmology on the use of animals in research.

**Isolation of P815 tumor cells**

P815 cells or eye-derived P815 cells were either untreated or cultured in vitro for 72 h with IFN-γ (10 U/ml). Cells were washed three times in HBSS and labeled with FITC-conjugated anti-H-2K^d^, anti-H-2D^d^, or anti-H-2L^d^ (BD Pharmingen) or ICAM-1 (Pharmingen) Abs for 30 min at 4°C. Labeled cells were washed three times and analyzed by flow cytometry on a Coulter EPICS instrument. Fifty thousand events were detected and recorded for each sample, and results were analyzed using Coulter EPICS FACS analysis software.

**RESULTS**

**Eye-derived tumors develop an immune escape phenotype**

To test whether tumor cells growing within an immune-privileged environment develop an escape phenotype, naive DBA/2 mice received an anterior chamber injection of 1 x 10^5 P815 tumor cells. Ten days later tumor-bearing eyes were enucleated, and freshly isolated eye-derived P815 cells were recovered as described in Materials and Methods. Eye-derived tumor cells (1 x 10^5 P815 cells/mouse) were then injected into the shaved flank of a second group of DBA/2 mice that were previously immunized with P815 CD80^+^ IL-12 tumor cells. Tumor growth was measured as the mean tumor diameter on subsequent days (Fig. 1). Eye-derived tumor cells injected into the flank of immunized mice grew progressively at a rate that was not significantly different from that of the negative control group. Moreover, the doubling time of eye-derived tumor cells in vitro was not significantly different from that of wild-type P815 cells, and the growth rate of eye-derived tumor cells in vivo in naive DBA/2 mice was also not significantly different from that of wild-type mice (data not shown). As a negative control, naive DBA/2 mice received a flank injection of P815 cells that grew progressively. As a positive control, immunized mice received a flank injection of P815 cells that were rejected completely by day 16. To rule out the possibility that we were transferring normal eye-derived cells mixed with eye-derived P815 cells, we obtained cells from normal eyes and mixed them at an equal ratio with wild-type P815 cells. This mixture of cells was injected into the flank of immunized mice. The transfer of normal eye-derived cells and tumor cells was not sufficient to induce the immune escape phenotype. We conclude from these data that eye-derived P815 tumor cells acquire an immune escape phenotype when they grow within the immune-privileged anterior chamber of the eye.

The eye-derived escape phenotype developed in tumors that grew for 10 days within the anterior chamber of the eye. To determine the minimum time that tumors needed to grow within the anterior chamber to develop the immune escape phenotype, P815 cells were injected into the anterior chamber of naive DBA/2 mice.
and then recovered on day 3, 5, 7, or 10 postinjection. Eye-derived tumor cells were then injected into the flank of previously immunized DBA/2 mice, and the percentage of mice with progressively growing flank tumors, as well as the growth rate, was determined (Fig. 2). Eye-derived P815 cells recovered on either day 7 or 10 postinjection grew progressively in the flank of 100% of the immunized mice at a rate that was not significantly different from that of the negative control. By contrast, eye-derived P815 cells recovered 5 days postinjection grew progressively in the flank of only 60% of the immunized mice and at a rate that was considerably slower than that of the negative control. None of the eye-derived P815 cells recovered 3 days postinjection grew progressively in the flanks of immunized mice. Wild-type P815 cells were either injected into the flanks of naive DBA/2 mice and grew progressively (negative control) or into the flanks of immunized DBA/2 mice and were rejected (positive control). These data indicate that the eye-derived immune escape phenotype begins to appear in tumors that grow for 5 days within the anterior chamber of the eye but is not complete until after 7 days.

Eye-derived tumors recovered from immunodeficient mice

To determine whether the immune escape phenotype we observed in eye-derived tumors required selective T cell pressure, we recovered eye-derived tumors from the anterior chamber of immunodeficient mice. CB-17 SCID mice received an anterior chamber injection of 2 × 10^5 P815 cells and, 10 days later, the tumor-containing eyes were removed and eye-derived tumor cells were prepared. A single cell suspension of eye-derived P815 cells was injected into the flanks of previously immunized DBA/2 mice (2 × 10^5 eye-derived P815 tumor cells per mouse). Growth of flank tumors was recorded and displayed in Fig. 3. Tumor cells harvested from the anterior chamber of CB-17 SCID mice also grew progressively, and their growth rate was not significantly different from that of eye-derived tumor cells recovered from immunocompetent mice. Wild-type P815 cells were either injected into the flanks of naive DBA/2 mice and grew progressively (negative control) or into the flanks of immunized DBA/2 mice and were rejected (positive control). As in the previous experiment, eye-derived P815 cells (recovered from naive DBA/2 mice) grew progressively in the flanks of immunized mice. We conclude that neither T cells nor B cells are required for the generation of the immune escape phenotype in tumors growing in the anterior chamber of the eye.

The immune escape phenotype is stable in vitro

In the preceding experiments, tumor cells were freshly isolated from the anterior chamber of the eye and then immediately injected into the flanks of immunized mice. To determine whether eye-derived P815 cells maintained their immune escape phenotype even when they were cultured in vitro, we serially passaged eye-derived P815 cells in vitro for varying lengths of time before injecting them into the flanks of immunized mice (Fig. 4). If eye-derived P815 cells were cultured for either 10 or 20 serial passages in vitro before injection into the flank of immunized mice, these eye-derived tumors still grew progressively in the flank and there was no significant difference between their growth rate and the growth rate of freshly isolated tumor cells. As a negative control, wild-type P815 cells were rejected from the flanks of immunized mice. As a positive control for the eye-derived escape phenotype, P815 cells that were freshly isolated from the anterior chamber and not cultured in vitro grew progressively in the flanks of immunized mice. We conclude that the eye-derived immune escape phenotype is stable and permanent when the tumor cells are cultured in vitro in the absence of the local environment within the anterior chamber.

CTL fail to lyse eye-derived tumor cells

Boon and coworkers (11, 13–20) reported that P815 cells express multiple tumor Ags that are recognized by tumor Ag-specific
CTLS. In the next series of experiments, we examined whether the failure of mice to reject eye-derived P815 cells was due to the failure of CTL to lyse these tumor cells. DBA/2 mice were immunized with a s.c. injection of P815 CD80− IL-12-transfected tumor cells. Three weeks later, lymphocytes from the draining lymph nodes were isolated and restimulated with irradiated untransfected P815 cells in a 5-day MLTC culture. Restimulated effector cells were tested for their ability to lyse 51Cr-labeled wild-type P815 cells or eye-derived P815 cells (Fig. 5). CTLs were unable to lyse eye-derived P815 cells. As a negative and a positive control, respectively, naive effector cells were unable to lyse wild-type P815 target cells, whereas primed effector cells lysed wild-type P815 target cells. As a control for specificity, primed effector cells were unable to lyse DBA/2-derived L1210 target cells that do not express any of the tumor Ags found on P815 cells. We conclude that tumor cells that grow within the immune-privileged anterior chamber of the eye developed the ability to escape lysis by fully differentiated effector CTLs.

Eye-derived P815 cells fail to activate primed T cells

To produce fully lytic CTLs, precursor CTLs must be restimulated with tumor cells in vitro in the MLTC cultures. In the next series of experiments we examined whether eye-derived P815 cells were capable of triggering precursor T cells to proliferate and differentiate into fully lytic CTLs. DBA/2 mice were subcutaneously immunized with P815 CD80− IL-12-transfected tumor cells. Three weeks later, primed lymphocytes from the draining lymph nodes were isolated and restimulated in a 5-day MLTC culture with either irradiated P815 cells or irradiated eye-derived P815 cells. Three weeks later, draining lymph node cells from either immunized or naive mice were restimulated for 5 days with irradiated P815 cells or irradiated syngeneic spleen cells. CTLs were harvested and tested for their ability to lyse target P815 cells (●), eye-derived P815 (P815e) cells (○), L1210 cells (△), or naive P815 cells (□). The data are displayed as the percentage of specific lysis.

![Graph](Image)

**FIGURE 4.** The immune escape phenotype is stable in vitro. Naive DBA/2 mice were s.c. immunized with P815 CD80− IL-12 tumor cells and, 3 wk later, were challenged with P815 cells (△), freshly isolated eye-derived P815 cells (●), eye-derived P815 cells serially passaged 10 (p10) times in vitro (○), or eye-derived P815 cells serially passaged 20 times (p20) in vitro (■). Tumor growth was recorded three times per week for 3 wk; n = 10 per group.

![Graph](Image)

**FIGURE 5.** CTLs fail to lyse eye-derived P815 cells. Naive DBA/2 mice were s.c. immunized with P815 CD80− IL-12 tumor cells. Three weeks later, draining lymph node cells from either immunized or naive mice were restimulated for 5 days with irradiated P815 cells or irradiated syngeneic spleen cells. CTLs were harvested and tested for their ability to lyse target P815 cells (●), eye-derived P815 (P815e) cells (○), L1210 cells (△), or naive P815 cells (□). The data are displayed as the percentage of specific lysis.

To determine whether eye-derived P815 cells were capable of triggering primed lymphocytes to proliferate in vitro, DBA/2 mice were subcutaneously immunized with P815 CD80− IL-12-transfected tumor cells. Three weeks later, primed lymphocytes from the draining lymph nodes were isolated and restimulated in a MLTC culture with either irradiated P815 cells or irradiated eye-derived P815 cells. On day 4 of the MLTC culture, lymphocytes were pulsed with 5 μCi of [3H]thymidine and incubated for an additional 18 h. Pulsed cells were harvested, and proliferation was determined by incorporation of [3H]thymidine (Fig. 7). To determine the background level of proliferation, two negative controls were used; naive lymphocytes were restimulated with irradiated P815 cells, and primed lymphocytes were restimulated with irradiated DBA/2 splenocytes. As a positive control, primed lymphocytes were restimulated with irradiated P815 cells, resulting in significant proliferation. By contrast, when similarly primed lymphocytes were restimulated with irradiated eye-derived tumor cells, there was significantly less lymphocyte proliferation. We conclude that eye-derived P815 cells have a reduced capacity to induce primed lymphocytes to proliferate in vitro.

**MHC class I and ICAM-1 on eye-derived tumor cells**

A common mechanism tumors use to escape immune elimination is to down-regulate either MHC class I or T cell adhesion molecules (21, 22). Therefore, we examined whether the expression of MHC class I or ICAM-1 on eye-derived P815 cells was either reduced or not regulated by treatment with IFN-γ. Wild-type and eye-derived P815 cells were cultured for 72 h in either the presence or absence IFN-γ and then stained with Abs specific for MHC H-2Kd, H-2Dd, H-2Ld, or ICAM-1. There was no significant difference in the level of MHC class I expressed on eye-derived P815
cells as compared with wild-type P815 cells (Fig. 8). In addition, there was no significant difference in the ability of wild-type and eye-derived P815 cells to up-regulate MHC class I when treated with IFN-γ (data not shown). ICAM-1 expression on eye-derived and wild-type P815 cells was not significantly different in either the absence or presence of IFN-γ (data not show). We conclude that eye-derived P815 cells do not escape immune elimination via the failure to express either MHC class I or ICAM-1.

Immunization with eye-derived tumor cells

We wanted to determine whether immunization with eye-derived P815 cells transfected with CD80+ IL-12 genes protected mice against a subsequent challenge with eye-derived P815 cells that did not express either CD80 or IL-12. To establish a vaccine against eye-derived P815 tumor cells, we inoculated P815 CD80+ IL-12 tumor cells into the anterior chamber of DBA/2 mice and 10 days later recovered eye-derived P815 CD80+ IL-12 tumor cells. A second group of naive DBA/2 mice were immunized with the eye-derived P815 CD80+ IL-12 cells via a s.c. flank injection. These eye-derived immunized mice were then subcutaneously challenged with either wild-type or eye-derived P815 cells, and the percentage of mice with progressively growing tumors, as well as the growth rate, was determined (Fig. 9). In the preceding experiments, mice immunized against wild-type P815 cells always rejected P815 cells (100% tumor rejection) and were unable to reject eye-derived P815 cells (0% tumor rejection). By contrast, mice immunized with the eye-derived vaccine were less effective at eliminating P815 cells (40% tumor rejection) but more effective at eliminating eye-derived P815 cells (60% tumor rejection). As negative controls, naive mice were challenged in the flank with either P815 cells or eye-derived P815 cells, resulting in progressive tumors in all mice. We conclude that eye-derived tumors are weakly immunogenic and can provide partial protection from eye-derived and wild-type tumors.

Discussion

Immune evasion by tumor cells has recently become one of the most important barriers to successful cancer immunotherapy (23–25). Over the past decade, tumor immunologists have become more and more successful at developing novel strategies to immunize cancer patients against tumor-specific and tumor-associated Ags, only to find that immunized patients develop recurrences displaying a variety of immune escape mutations that allow the tumor cells to grow progressively and avoid elimination (4). Primed tumor-specific T cells (either CD4+ T cells, or CD8+ T cells) that eliminate tumor cells are paradoxically also able to induce escape mutant tumor cells under certain conditions. It is believed that specific T cells provide selective pressure for the survival of mutations that lead to immune escape. This selective T cell pressure is, therefore, an example of Darwinian natural selection at the cellular level (2). This mechanism of tumor escape has been shown to occur in vitro and in vivo in patients that were immunized against specific tumor Ags (26 - 28).

Our previous research was directed at developing a tumor cell vaccine using a murine model that would allow us to eliminate progressively growing ocular tumors from the immune-privileged anterior chamber of the eye. To our surprise, mice immunized with this vaccine were only able to eliminate a subsequent tumor challenge administered into a nonimmune-privileged site (s.c. tissues in the flank) and were not able to eliminate tumors from the immune-privileged eye (9). In other words, systemically immunized
mice with primed T cells were unable to terminate immune-privileged and eliminate ocular tumors. In our efforts to understand how these tumors escaped elimination, we discovered in the current series of experiments that eye-derived tumor cells acquired an immune “escape phenotype.” Eye-derived tumor cells developed their own ability to avoid elimination, regardless of whether the eye-derived tumors were placed into a privileged or nonprivileged site. These experiments revealed a previously unrecognized aspect of the ocular environment, i.e. that tumor cells growing within the anterior chamber acquired an immune escape phenotype.

Two pieces of data from our studies indicate that the eye-derived immune escape phenotype was not due to selective T cell pressure. First, the escape phenotype developed rapidly within the anterior chamber of naive mice (within 7–10 days postinoculation), well before a selective T cell response could develop and too quickly for the development and selection of random mutations among the P815 cells. Second, the eye-derived escape phenotype developed within 10 days in the anterior chamber of SCID mice that lacked the ability to develop adaptive immunity. These data indicate that eye-derived tumors acquire the immune escape phenotype in the absence of selective T cell pressure. It is possible that the immune selection of eye-derived tumors was mediated via NK and/or NKT cells as described in the experiments by Schreiber and coworkers on immune surveillance and immunoediting (29). However, we feel that this is unlikely, because the work of Niederkorn and coworkers (30, 31) indicates that the ocular environment inhibits NK cell-mediated lysis and, therefore, inhibits immune surveillance and immune selection of tumors growing in the anterior chamber. We conclude that immune escape occurs within tumors that grow within the anterior chamber in the absence of immune surveillance and selective T cell pressure.

We were surprised to observe that the eye-derived immune escape phenotype was permanent and stable in vitro. Even when tumor cells were removed from the eye and cultured in vitro for as many as 20 passages, they still retained the ability to grow progressively when injected into the flanks of immunized mice. Therefore, although the ocular environment is required for the induction of the escape phenotype, once it is induced it becomes permanent and does not require the continued presence of the surrounding environment. The permanence of the escape phenotype suggested that eye-derived tumors were derived from a mutation that developed among the tumor cells that provided them with a selective advantage. However, this advantage neither increased the proliferation rate of the tumor cells in vitro nor increased the growth rate of tumors in vivo. Mutations induced through genomic modification regulate gene expression by a permanent and irreversible alteration of the genes at the nucleotide level. Genomic modifications either activate or silence genes through point mutations, frame-shift mutations, genomic translocations, insertions, or deletions (3, 4). However, we were puzzled by how the ocular environment could induce a permanent and stable change in the phenotype of eye-derived tumor cells.

Permanent changes in gene expression that are heritable and passed onto progeny cells during DNA replication can only occur via either genomic or epigenetic changes in the tumor cells. A common form of epigenetic change is mediated via DNA methyl-ation (32, 33). In recent experiments, we observed that the treatment of eye-derived tumor cells with a demethylating reagent restored the eye-derived tumor cells to their original state, where they were susceptible to immune elimination (our unpublished observation). If the eye-derived phenotype was due to a genomic mutation, it would not be possible to reverse the phenotype, because genomic mutations are not reversible. Future experiments will confirm that the eye-derived escape phenotype is due to an epigenetic change in the tumor cells that is induced by the ocular environment.

Our previous studies indicate that CD4 T cells and CD8 T cells were required to eliminate P815 cells from the flank of an immunized mouse (9). Our current data indicate that eye-derived tumors in vitro are not lysed by tumor-specific CTLs, fail to induce the differentiation of precursor CTLs, and fail to induce T cell proliferation. All of these results suggest that the eye-derived tumors lost their ability to present tumor Ags. We ruled out the simplest explanation that eye-derived tumor cells failed to express normal levels of either MHC class I Ags or ICAM-1. We also ruled out the possibility that eye-derived tumors were devoid of tumor Ags by generating an eye-derived tumor cell vaccine. The vaccine was produced by injecting P815 cells that were transfected with CD80 and IL-12 into the anterior chamber of naive DBA/2 mice. Ten days later these tumor cells were recovered and used as an “eye-derived” tumor cell vaccine. Naïve DBA/2 mice were immunized...
with the eye-derived vaccine and then challenged with either wild-type or eye-derived tumor cells in the contralateral flank. The eye-derived vaccine protected 60% of the mice from a challenge with eye-derived tumor cells. Because the original vaccine derived from wild-type P815 cells was never able to offer any protection from a flank challenge with eye-derived tumor cells (0% of the mice were protected), we conclude that the eye-derived vaccine displayed Ags that were not found on wild-type P815 cells.

Boon and coworkers (17) studied extensively the tumor Ags expressed on P815 cells and discovered five tumor specific Ags: A, B, C, D, and E. Of these five Ags, A, B, and E are recognized by specific CTL clones. Eye-derived P815 cells may down-regulate transcription of one or more of these tumor Ag genes as an escape mechanism. This may explain our results showing that eye-derived tumor cells are poor targets for P815-specific CTLs and fail to induce T cell differentiation and proliferation. Studies using cloned CTLs specific for the different tumor Ags are currently being performed to determine whether the eye-derived P815 cells lost expression of one or more of the P815 tumor Ags. Our data also indicate that eye-derived tumor cells display Ags that are not normally found on wild-type P815 cells. We predict that eye-derived tumor cells may display unique Ag(s) due to a change in the pattern of genes expressed by the tumor cells after they grow in the anterior chamber of the eye. This unique pattern of gene expression may be related to regulation of tissue and/or eye-specific genes by the ocular environment.

In summary, our data demonstrate that tumors growing within the immune-privileged anterior chamber of the eye develop a tumor escape phenotype that allows the eye-derived tumor cells to avoid elimination by specific T cells. The development of this eye-derived escape phenotype occurs rapidly within the anterior chamber and in the absence of selective T cell pressure. We predict that immune escape within the eye is driven by the unique ocular environment that permanently alters the genes expressed in eye-derived tumor cells.

Disclosures

The authors have no financial conflict of interest.

References