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REVIEW ARTICLE

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Methods for subchoroidal implantation of Greene melanoma in rabbits

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Abstract
As an alternative to previously reported treatments, experimental animal models of uveal melanoma provide useful information for assessing the advantage of treatments designed to salvage eyes. Greene melanoma is the best-established model and has been used for the purpose of assessment of several treatments. Various other experimental models that implant melanoma cell lines into the anterior chamber of the rabbit eye have been reported. This article reviews some of the advantages and disadvantages of these experimental models of human uveal melanoma in rabbits and presents a modified subchoroidal implantation procedure for melanoma cells that is used in our laboratory. This procedure effectively, efficiently, and consistently enables us to provide animal models bearing large-sized choroidal melanomas. This modified technique may eventually be applicable to the development of new therapeutic options.

Key words Choroid · Malignant melanoma · Animal model · Greene melanoma · Rabbit

Introduction

Malignant uveal melanoma is the most common malignant primary intraocular tumor in adults.[1] This malignancy has a tendency to metastasize hematogenously to the liver, lung, and other sites. Although enucleation of the eye with intraocular tumor has been the traditional treatment for posterior uveal melanoma, the management of patients with uveal melanoma remains controversial.[1] Zimmerman et al.[2] speculated, a quarter of a century ago, that enucleation of a melanoma-containing eye might cause metastasis and increase the risk of mortality. Recently, a prospective, randomized, multicenter clinical trial (Collaborative Ocular Melanoma Study; COMS) of episcleral plaque radiotherapy for medium-sized choroidal melanoma documented that mortality rates following this therapy did not differ from mortality rates following enucleation.[3] To the contrary, large choroidal melanomas continue to be managed primarily by enucleation.[4] Also, no evidence of a survival difference between enucleation alone and pre-enucleation radiation of large choroidal melanoma has been demonstrated in COMS.[5]

Several eye-conserving treatments, such as photocoagulation, episcleral plaque radiotherapy, and local resection, are widely accepted in the management of posterior uveal melanoma.[1] However, because, in some respects, there are several limitations to these treatments, they have been used only in selected instances, such as for small or medium-sized melanoma. Therefore, in medium- or large-sized melanoma, many clinical and experimental studies have been conducted with new therapeutic options, i.e., transpupillary thermotherapy,[6] charged-particle radiotherapy,[7] episcleral plaque radiotherapy with $^{106}R_u/^{106}Rh$ applications,[8] chemotherapy,[9] immunotherapy,[10] and combinations of these therapies. As alternatives to previously reported treatments, experimental animal models provide useful information for assessing the advantages of these treatments that are used to try and salvage eyes.

The present article reviews some advantages and disadvantages of experimental models of human uveal melanoma in rabbits and compares previous techniques with a modified subchoroidal implantation procedure for melanoma cells that is used in our laboratory.

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Experimental models of human uveal melanoma in rabbits

The rabbit eye is a suitable model because of its relatively large size; ability for funduscopy, fundus photography, laser

therapy, and surgery; relatively low cost; the animal's relatively long life span; and its well-known anatomy and physiology.[11]

Various models that implant melanoma cell lines into the anterior chamber of rabbits have been studied. The anterior chamber in the eye is an immune-privileged site, and this unique feature of immune responses has been termed anterior chamber-associated immune deviation. This explains the long survival of the tumor cells in the anterior chamber.[12] The subretinal space and the vitreous cavity are also immune-privileged sites.[13]

Greene melanoma

Greene[14] reported spontaneous cutaneous melanotic melanoma in hamsters and the occurrence of amelanotic transformation during homologous transfer. Additionally, heterologous transfer was successfully accomplished with the eye, brain, testicle, muscle, or subcutaneous space of rabbits or guinea pigs.[15] Thus, the hamster amelanotic melanoma has been defined as Greene melanoma.

Light and electron microscopic aspects of Greene melanoma are similar to those of human uveal melanoma.[16,17] Additionally, Greene melanoma is comparable to human uveal melanoma in its immunohistochemical reaction pattern.[18] Therefore, Greene melanoma, implanted into the anterior chamber of a rabbit eye, has become the best-established model for uveal melanoma and has been used for the purpose of the assessment of several treatments, including hyperthermia,[19–22] transpupillary thermotherapy,[23] photodynamic therapy,[24–27] photocoagulation,[28,29] episcleral plaque radiotherapy,[30] boron neutron capture therapy,[31] and chemotherapy.[32–34]

Other models

Greene melanoma has been of some value as a laboratory model for the study of human uveal melanoma. However, a discrepancy between Greene melanoma in the eye and human uveal melanoma, concerning the clinical course, has been reported. Even without therapy, the tumor shows necrosis, and hemorrhages 8–10 days after inoculation.[12,35]

A number of animal models of uveal melanoma have been studied in an attempt to gain more information about therapeutic efficacy,[11] and several investigators have examined the pathological and clinical features of uveal melanoma using these models.

Greene melanoma is amelanotic. Animal models of pigmented choroidal melanoma, such as the B16F10 cell line from mouse cutaneous melanoma, which is heavily pigmented, are suitable for an evaluation of the effectiveness of laser and photodynamic therapy.[36,37] However, a major disadvantage of this model is the need for immunosuppression by daily cyclosporin injection. Because the growth pattern is extremely aggressive, this model has been used in studies of metastasis.

Human uveal melanoma cells can be successfully heterotransplanted into the eye only in immunosuppressed animals. However, these immunosuppressed animals are not suitable for studies of immunotherapy. Liggett et al.[38] used rabbits immunosuppressed with cyclosporin A and established a novel choroidal xenograft model from human uveal melanoma.

An experimental animal model for intraocularly transplanted human melanoma has been demonstrated in the athymic nude mouse, and the tumor has been serially transferred.[39] However, so far no large-size animals that are similar to the nude mouse have been used successfully, although there has been an attempt to induce primary uveal melanotic lesions by using a chemical carcinogen.[40]

Each of these models has both advantages and disadvantages.[11] To date, the only melanoma cell line successfully implanted in the rabbit eye without immunosuppression is the Greene melanoma.[36]

Subchoroidal implantation procedure for Greene melanoma

The development of new therapeutic techniques, including surgery, laser therapy, radiation, and hyperthermia for large choroidal melanoma requires animal models possessing large-sized eyes bearing large-sized melanomas in the choroid.

Previous methods

Transscleral approach

Krohn et al.[41] reported transplantation of Greene melanoma cells into the posterior ocular segment of rabbits. Cyclodialysis by spatula was performed between the sclera and the choroid after a posterior scleral incision. Then pieces of the tumor were delivered into the subchoroidal space, using a trocar and an obturator. A major shortcoming of this procedure is the spread of the tumor into the surrounding tissue through the scleral incision. Additionally, implantation of the tumor in the posterior part of the eye is difficult.

Transvitreal approach

Lambrou et al.[42] described a new technique involving the deposition of a tumor fragment directly into the subchoroidal space via a transvitreal approach. The advantages of this method are elimination of the extrascleral extension of the tumor, rapid performance, and implantation at any location. Conversely, this technique has the disadvantage of the requirement for a surgical microscope and the production of a retinotomy and choroidal rupture. Serious complications, such as the seeding of tumor cells into the vitreous, severe vitreous hemorrhage, total retinal detachment, and proliferative vitreoretinopathy may occur because of the relatively large retinotomy required.

Modified method

In this review we would like to present a new technique that is used in our laboratory. This modified procedure enables the implantation of the tumor in a precise site in the vicinity of the posterior pole of the eye.

Animals

All animals were treated in accordance with the Association for Research in Vision and Ophthalmology Resolution on the Use of Animals in Research. A total of 78 New Zealand white rabbits, weighing 2.0 to 3.0 kg, were used in this study. All animals were found to have normal eyes.

Tumor growth in the anterior chamber

The right eye of each animal was inoculated with approximately 0.1 ml of culture medium, containing 5×10^5 cells of Greene melanoma, into the anterior chamber through the limbus, with a 27-gauge needle. The growth of Greene melanoma in the anterior chamber was observed at 1 week postinoculation (Fig. 1). Animals were killed with intravenous pentobarbital sodium 3 weeks postinoculation and before perforation of the globe due to extreme tumor growth. Under sterile conditions, the right eye was enucleated and the melanoma tissue was removed. A suspension

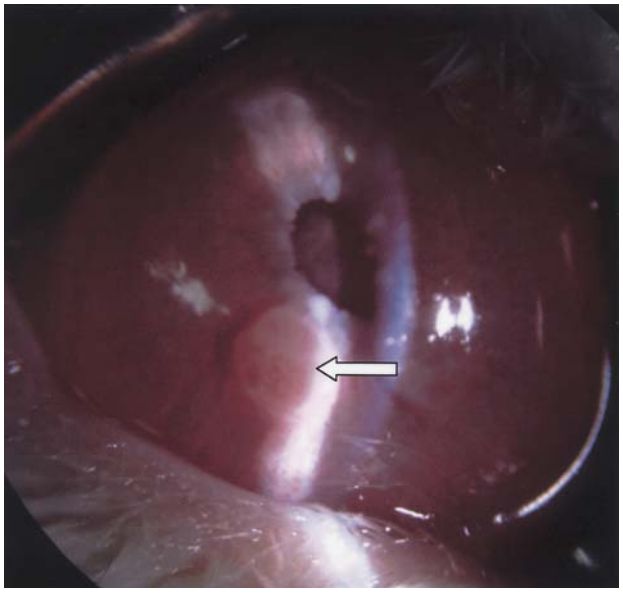

Fig. 1. Greene melanoma (*arrow*) growing in the anterior chamber

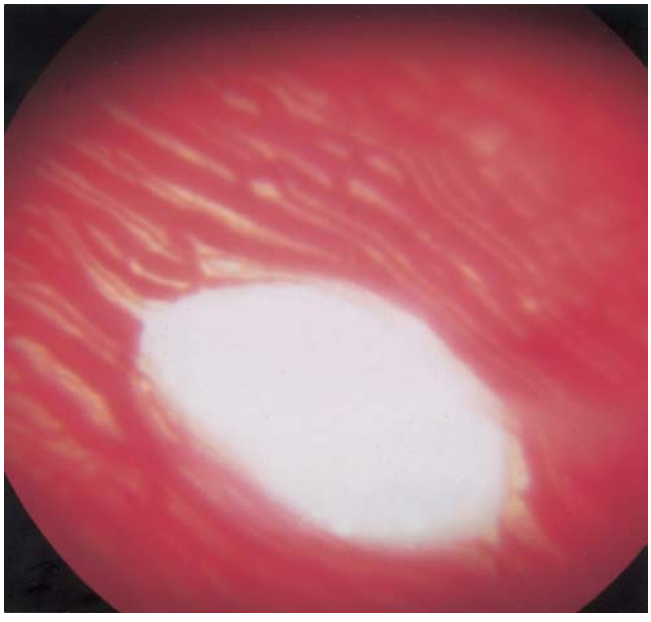

Fig. 3. Fundus photograph. Greene melanoma was deposited and grew to two disc diameters during the 2 weeks after implantation

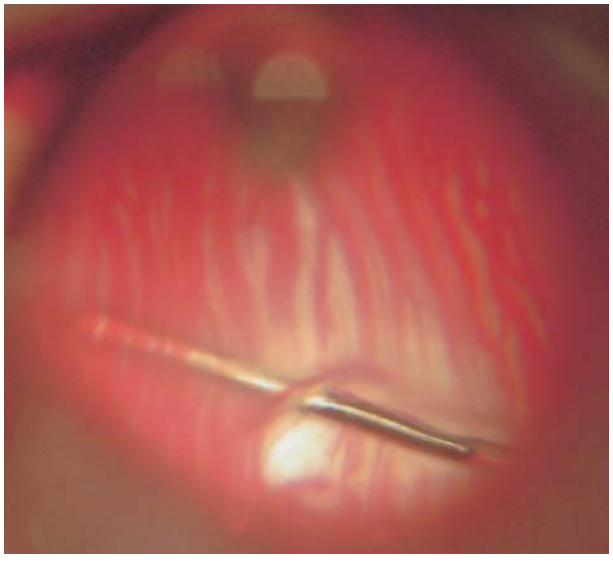

Fig. 2. A 33-gauge cannula is inserted under the choroid through the retinotomy

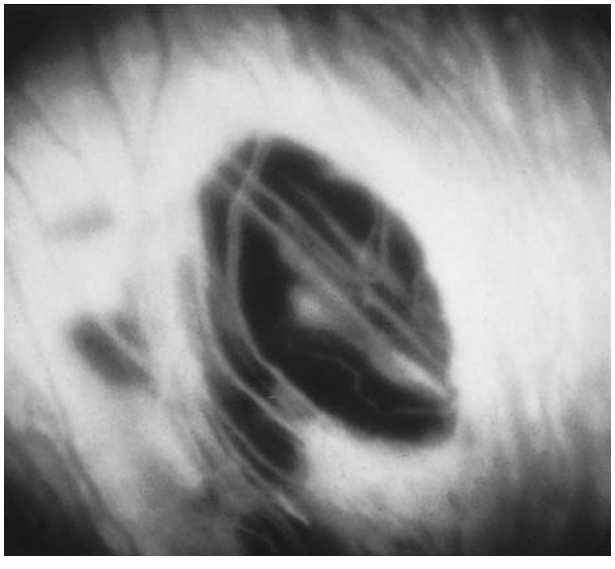

Fig. 4. Fluorescein angiography of the tumor shown in Fig. 3. Note that the tumor was lying under the choroidal vessels

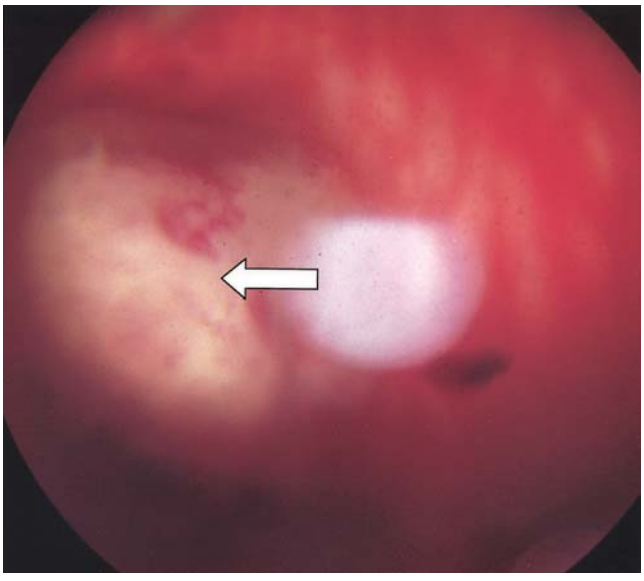

Fig. 5. Fundus photograph. Greene melanoma (*arrow*) at 1 month after implantation

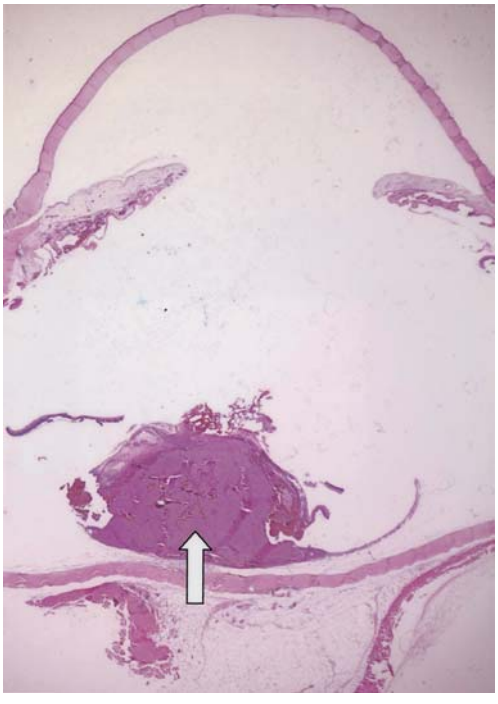

Fig. 6. Greene melanoma (*arrow*) in the choroid. H&E

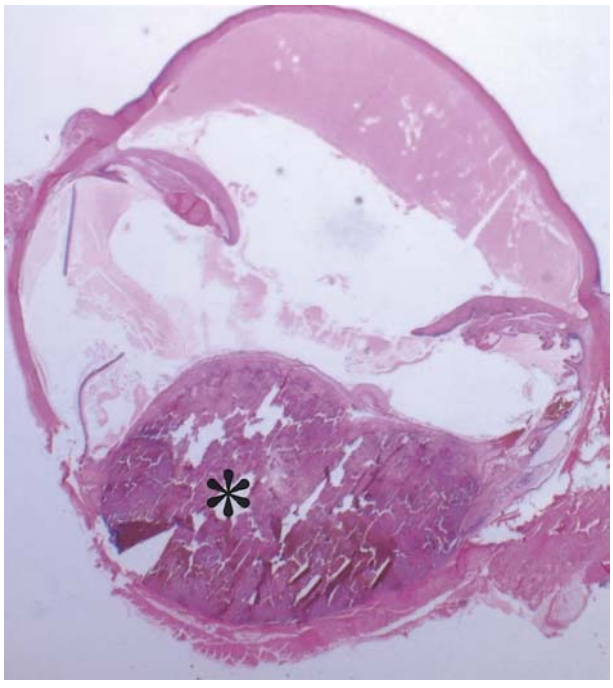

Fig. 7. Greene melanoma (*asterisk*) has grown to 1 cm at 1 month postinoculation. H&E

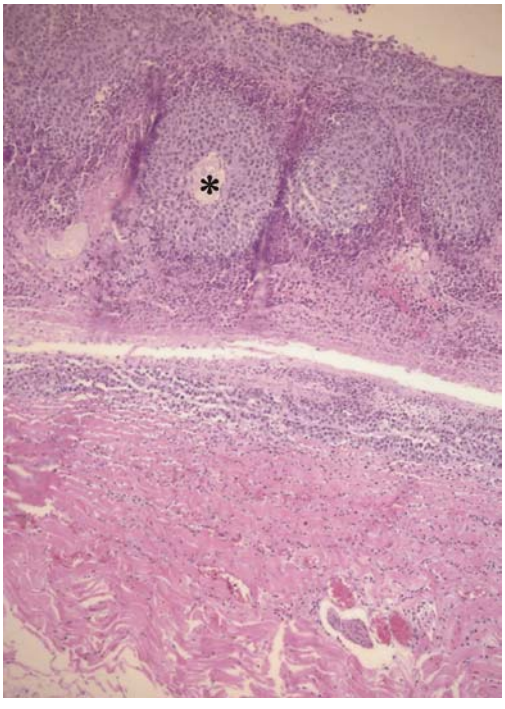

Fig. 8. Greene melanoma. An amelanotic tumor-cell cluster surrounds blood vessels (*asterisk*). H&E, ×40

containing viable and abundant tumor cells (500 mg/ml) in saline was obtained, using a loose-fitting (0.25-mm clearance) glass homogenizer with a Teflon pestle. Greene melanoma was maintained and subsequently grown by serial passage in the anterior chamber of another rabbit's eye.

Subchoroidal implantation

The recipient rabbits were anesthetized with an intramuscular injection of 35 mg/kg of ketamine and 5 mg/kg of xylazine. A sclerotomy was made 1 mm posterior to the limbus in the nasal site of the right eye. Under microscopic control, a small retinotomy and choroidal rupture was then performed in the appropriate position adjacent to the posterior pole of the eye, using a 33-gauge cannula, which was equipped for subretinal surgery. Subsequently, the same

cannula was carefully inserted under the choroid through the retinotomy (Fig. 2). A viscoelastic material was injected into the subchoroidal space, and artificial choroidal detachment was created. The prepared suspension (approximately 0.1 ml) was transplanted inside the space opposite the position of the retinotomy so as to not allow seeding into the vitreous cavity. Finally, the sclerotomy was closed.

The animals were followed for 10 to 40 days and examined by indirect ophthalmoscopy (Fig. 3). Fluorescein angiography defined the tumor lying under the choroidal vessels (Fig. 4). One month after transplantation, the tumor had grown to approximately five disc diameters (Fig. 5). Afterwards, eyes were sectioned and stained with hematoxylin-eosin (H&E) for light microscopy (Figs. 6–8).

This procedure effectively, efficiently, and consistently enables us to provide animal models bearing large-sized choroidal melanomas. Our procedure makes it possible to transplant Greene melanoma to an appropriate position adjacent to the posterior pole and to keep the architecture intact in rabbits. This technique offers some advantages over the previously used ones. This modified technique may eventually be applicable to the development of new therapeutic options.

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