Review Article

Animal Eye Models for Uveal Melanoma

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Uveal melanoma · Animal models · Tumor cells

Abstract

Animal models play an important role in understanding tumor growth and may be used to develop novel therapies against human malignancies. The significance of the results from animal experiments depends on the selection of the proper model. Many attempts have been made to create appropriate animal models for uveal melanoma and its characteristic metastatic behavior. One approach is to use transgenic animal models or to implant tumor cells. A variety of tumor types have been used for this purpose: tumor cells, such as Greene melanoma, murine B16 melanoma, and human uveal melanoma cells, may be implanted in the eyes of hamsters, rats, rabbits, and mice, among others. Various inoculation routes, including into the anterior chamber and posterior compartment, and retro-orbitally, have been applied to obtain tumor growth mimicking ocular uveal melanoma. However, when we choose animal models, we must be conscious of many disadvantages, such as variable tumor growth, or the need for immunosuppression in xenogeneic grafts. In this paper, we will discuss the various eye models.

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Introduction

Uveal melanoma (UM) is the most frequent primary intraocular tumor in adults [1] and differs from cutaneous melanoma in several aspects. Both are initiated by genetic mutations, but in the case of cutaneous melanoma, mutations are often found in the BRAF gene, while in many UM, mutations are found in either GNAQ or GNA11 [2, 3]. Cutaneous melanomas usually spread through the lymph vessels, while UM preferentially spread hematogenously, and liver metastases may develop in up to 50% of patients [4]. Despite many advances in diagnostic techniques, UM metastases carry a high mortality rate, and better approaches regarding the molecular pathogenesis and potential targets for therapy are needed. However, due to the

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comparatively low incidence of UM, human tumor tissues are quite difficult to obtain [5]. In addition, many tumors are treated by irradiation, and only a small amount of tumor material obtained by a biopsy may be available for diagnosis and prognostic testing. Moreover, growing cell lines from UM is not easy, and only a limited number of cell lines is available worldwide. Animal models that are able to imitate and reflect human disease may help to understand UM tumor growth and metastatic behavior as well as to test potential treatments.

The significance of results from animal experiments relies on the selection of the proper animal model. An animal model should ideally closely simulate the behavior and pathological process of human disease, and one should be able to extrapolate the outcome to human beings. Although there are many arguments that question the validity of animal models, using such models, so far, is considered the best way to mimic human disorders.

Animal models are mainly comprised of three types: spontaneous models, transgenic models, and induced models, and all three exist with regard to ocular melanoma. In 1876, Born discovered a sarcoma in an 18-year-old male horse that invaded the choroid, retina, and optic nerve and metastasized to the orbit and submaxillary lymph nodes [6]. Since then, spontaneous primary pigmented intraocular tumors have been reported in the eyes of horses, cattle, sheep, cats, dogs, fish, rabbits, rats, and chickens. These models present naturally growing tumors, which have a natural route of metastatic spread, but their shortcomings, such as limited numbers of animals, unpredictable occurrence, and a nonuniform pattern of metastasis, limit their use as a model in experimental UM research. Transgenic models, as their name indicates, have been developed thanks to the immense progress of genetic engineering and technology during the past 30 years. In 1991, Bradl et al. [7] reported that Tyr-SV40E transgenic mice harbored ocular melanoma. Subsequently, TySV40, Tyr-Tag, Tyr-RAS+ Ink4a/Arf^{-/-} and Tpras transgenic mice have been created, all of which have some ocular tumor characteristic [8–12]. Transgenic models provide a great opportunity to monitor the early stages of tumor growth and follow tumor progress. Unfortunately, most of the tumors in these models either grow from retinal pigment epithelium (RPE) or are mixed tumors of RPE and UM, with the exception of the tumors in Tyr-RAS+ Ink4a/Arf^{-/-} transgenic mice, which do not contain RPE cells [11]. Furthermore, none of the tumors in the transgenic models are able to metastasize to the liver unless cultured tumor cells are transferred and transplanted intracamerally [13]. A recent paper [14] has described Tg (Grm1) transgenic mice that are known to develop nodular melanomas on hairless skin areas. An analysis of the eyes of these mice showed the presence of thickening of the choroid with a ciliary body tumor, with numerous Ki-67-positive cells present in the choroid [14]. Further studies will have to show the metastatic capacity of the intraocular tumor cells.

Induced animal models are those in which a disease is artificially induced by chemical agents, radiation, virus, cells, or tissues. Compared to the others, induced models are easier to handle and more reproducible, which means they are more controllable and open to a standardized management. The most widely-used model in UM research is the inoculation model, in which tumor cells are implanted and which involves not only mice and rats, but also rabbits. In this review article, we will focus on various inoculation models in different animals as well as on the advantages and disadvantages of these models.

Hamster Model

Greene Melanoma Cell Line

In 1949, Greene [15] transplanted the Brown-Pearce tumor (an epidermoid carcinoma of the scrotal skin of a rabbit) into the anterior chamber (AC) of rabbits, and also into the AC, testicle, and subcutaneous space of hamsters and rats. Although, at the beginning, the trans-

plantation was successful in all sites, tumor growth failed and regressed in the end. Subsequently, in 1958, Greene [16] managed to grow out a spontaneous highly progressive cutaneous melanoma from Syrian golden hamsters. He found that after six serial passages, which took over 2 years, the melanotic melanoma in hamsters had transformed into an amelanotic melanoma. Continuous heterologous transfer of amelanotic hamster melanoma tissue was successful in the eye, brain, testicle, muscle, and subcutaneous space of rabbits and guinea pigs [17]. In addition, the spreading ability of this tumor was enhanced through serial passages. Journée-de Korver and colleagues [18, 19] successfully implanted around 1 mm³ of Greene melanoma tissue subcutaneously on both sides of a hamster's abdomen to evaluate the effectiveness of transpupillary thermotherapy (TTT), and they were able to demonstrate local necrosis 1 day after TTT treatment. This experimental work led to the clinical application of TTT as an adjunct treatment of UM. Rem and colleagues [20, 21] additionally explored transscleral thermotherapy in this hamster melanoma model by placing the Greene melanoma on the abdomen of a hamster and covering the surface of the tumor by a moistened specimen of human donor sclera prior to applying heat.

There are several advantages of the Greene melanoma hamster model. It can grow in a melanotic and an amelanotic version. The characteristics of a melanotic hamster melanoma, with high pigmentation and intense vascularization, are analogous to human choroidal melanoma, and the histopathologic appearance and dimensions of tumor necrosis after experimental TTT resemble the necrosis later found in patients after TTT treatment [20]. At the light and electron microscopic level, the amelanotic tumor is morphologically similar to human epithelioid UM [22]. A great advantage is that, although the naturally occurring Greene melanoma is of cutaneous rabbit origin, immunosuppressive drugs are not needed in the hamster. However, given these advantages, the fact that this is not an ocular tumor, and the limited availability of the cell lines, restrain the utilization of the Greene melanoma model as an ocular tumor model.

Bomirski Melanoma Cell Line

A spontaneous cutaneous melanotic hamster melanoma was used as an allograft transplant in several experiments (table 1) [23–25]. Small fragments of melanoma tissue were microsurgically implanted into the AC of hamsters, resulting in a rapidly growing, highly invasive, and vascularized tumor. Although it is useful that the implantation site and the size of the allograft can be controlled [25], the small size of the hamsters' eyes is a considerable problem. Furthermore, the lack of availability of the cell line provides a practical dilemma, although it can be obtained from laboratories in Poland.

Rat Model

Spontaneous UM is rarely found in rats [26], and many attempts of establishing melanoma cell lines from rat tumors have failed. For this reason, cell lines from other species are being used for experiments in rats. Braun et al. [27] developed an orthotopic xenograft rat model for choroidal melanoma (table 2) using the athymic albino mutant rat strain WAG/RijHs-rnu to permit xenotransplantation of human cell lines. The melanotic human OCM1 cell line was subcutaneously implanted in a donor rat, after which tumor tissue was removed and transplanted into the eye of another rat. A tiny scleral tunnel was made in the eye of the recipient rat, and a small piece (approx. 0.5 mm³) of minced OCM1 tumor was implanted through the tunnel. After 6–8 weeks, tumor growth was readily observed, with no sign of metastases. This model facilitated the observation of choroidal and tumor vessels with epifluorescence. Because the success rate was not as high as expected, Braun and colleagues [28–30] further

Table 1. Hamster models

First author	Cell line	Inoculation site	Metastasis	Reference
Greene	Greene	Subcutis	Liver	16
		Vein and artery	Lung, liver, kidney, thymus, heart	17
Journée-de Korver	Greene	Subcutis	Nd	18, 19
Rem	Greene	Subcutis	Nd	20, 21
Bomirski	Bomirski	Subcutis	Lung, kidney, lymph node	23
Urbanska	Bomirski	AC	Lymph node	24
Romanowska-Dixon	Bomirski	AC	Lung, kidney	25

Nd = Not detected/not studied.

Table 2. Rat models

First author	Cell line	Inoculation site	Metastasis	Reference
Braun	OCM1, C918, M619	Suprachoroid	Nd	27–30

Nd = Not detected/not studied.

improved this model by injecting spheroids of human cell lines into the suprachoroidal space to obtain tumor growth. Rats were sacrificed 4–41 days after inoculation, and melanomas were found in 96% of the animals.

The athymic nude rat model provides us with a good opportunity to visualize and track the blood flow and tumor growth in comparatively large eyes using high-frequency ultrasound. Different cell lines (OCM1 and C918) showed different growth rates in nude rats, and the model is useful for assessing the effectiveness of tumor treatments [30]. Although this is a xenograft model, immunosuppressants are not necessary due to the natural lack of T and B cells in this rat. In addition, the implantation of tumor spheroids instead of smashed tumor tissue greatly reduced the requirement of donor rats and decreased the cost of experiments. Furthermore, the use of spheroids limited leakage of tumor cells, reducing extraocular tumor growth. For the first time, hemodynamic parameters could be quantified in angiogenesis research of UM [27]. The major deficiency of this model is that no hepatic metastasis could be detected, and thus the rat model can only be applied for studying primary intraocular tumors.

Rabbit Model

Rabbits have much larger eyes than rats and mice, which allows an easier examination of the retina and choroid by ophthalmoscopy and imaging with fundus photography; surgical procedures are comparatively simple. So far, three kinds of UM cell lines have been used in rabbits (table 3).

B16 Melanoma Cell Line

B16F10, a well-established cell line of murine cutaneous origin, has been widely applied in the rabbit [31–33]. Minced tumor fragments, obtained from established tumors of C57UBU6 or C57BL/6 donor mice, were transsclerally inoculated into the subchoroidal space of rabbits.

Table 3. Rabbit models

First author	Cell line	Inoculation site	Metastasis	Reference
Krause	B16F10	Subchoroidal	Nd	31–33
Hu	B16F10, OCM1	Subchoroidal	Nd	34
Mueller	OCM1	Suprachoroidal	Nd	43
Blanco	MKT-BR	Suprachoroidal	Lung	44
Bonice	IPC227	AC	Nd	46
López-Velasco	MKT-BR, OCM1, 92.1, SP6.5	Suprachoroidal	Lung	48
Blanco	92.1	Suprachoroidal	Lung, liver	49
Marshall	92.1	Suprachoroidal	Lung, liver	50, 51
Di Cesare	92.1	Suprachoroidal	Nd	52
Kang	92.1	Suprachoroidal	Nd	53
Lambrou	Greene	Subchoroidal	Kidney	37
Hill	Greene	Suprachoroidal	Nd	38
Römer	Greene	AC	Nd	40
De Waard-Siebinga	Greene	AC	Nd	41

Nd = Not detected/not studied.

Hu et al. [34] found that grafts from the B16F10 cell line showed a more aggressive growth pattern than OCM1 and were highly pigmented. Nonetheless, liver metastases could not consistently be observed in these studies. The major problem of this model is the necessity of cyclosporin A (CsA) injections, which reduces the possibility of tumor rejection but seriously decreases the immunity and life span of the rabbits. This makes this model unsuitable for immunological experiments and long-term (more than 12 weeks) studies.

Greene Melanoma Cell Line

The hamster Greene melanoma cell line is the only cell line that does not need immunosuppression in rabbits in order to grow [35]. Therefore, Greene melanoma cells were placed into the AC or subchoroidal space of rabbits as a model to obtain a better understanding of ocular tumors and to evaluate new treatments [36–38]. However, Greene melanoma shows very rapid growth, which usually restrains the follow-up to 7 days after inoculation [39]. According to another report, even without treatment, the tumor showed spontaneous necrosis and hemorrhages 8–10 days after inoculation [40]. The application of CsA did not decrease necrosis significantly [41].

Human UM Cell Lines

One can successfully inoculate human UM cell lines in CsA-treated rabbits. Liggett et al. [42] transplanted a human tumor cell suspension in the subchoroidal space of rabbits. In a subsequent study [43], solid tumor fragments were transplanted into the suprachoroidal space; this technique had a higher success rate (67%) than the transscleral choroidal injection of a cell suspension or surgically induced cyclodialysis cleft implantation. Blanco et al. [44] injected cells from the UM-derived MKT-BR cell line into the suprachoroidal space of albino rabbits and noticed the development of metastasis, which was not dependent on the CsA dose. MKT-BR cells are HMB positive and S100 negative [45]. This tumor gave rise to lung metastases but not hepatic metastases. Bonice et al. [46] obtained a 100% success rate of AC inoculation with the IPC 227 cell line, derived from a human ciliary body tumor [47], which was 4-fold more successful than subchoroidal implantation. López-Velasco et al. [48] suprachoroidally implanted four human UM cell lines (MKT-BR, OCM1, 92.1, and SP6.5), of which

Table 4. Mouse models

First author	Cell line	Inoculation site	Metastasis	Reference
Niederkorn	B16F10	AC	Lung	54
Knisely	B16F10	AC	Nd	56
Grossniklaus	B16F10, Queens	AC	None	57
	B16F10, Queens	PC	Lung	
Ly	B16F10	AC	Nd	58
de Lange	B16F10	AC	Nd	59
Diaz	B16LS9	PC	Lung, liver	63
Dithmar	B16LS9	PC	Lung, liver, lymph node	65
Yang	B16LS9	PC	Liver	66
Zhang	B16LS9	PC	Nd	67
Yang	B16LS9	PC, spleen	Liver	68
Niederkorn	B16LS9	PC, spleen	Liver	69
Ma	OCM1, OCM3, OCM8, EOM3, 92.1, OM431, Mel202	AC	Liver	70–72
van Ginkel	C918, MUM2B	SC	Nd	73
Trionzi	OCM1, MUM2B	SC	Nd	74
Surriga	OMM1.3	Retro-orbital	Lung, liver	76

SC = Subcutis; Nd = not detected/not studied.

92.1 and SP6.5 were the most aggressive human cell lines in rabbits as well as the most efficient in getting tumor metastases. In a study on circulating malignant cells, all of the 17 rabbits that were available for testing had metastases in the lungs [49], and 18% developed liver micrometastases. Subsequently, several experiments were undertaken based on the same rabbit model [50–53].

Mouse Model

For over 50 years, the laboratory mouse has been the most widely used species in cancer research. The small size of its eyes limits the possibility of using routine eye examination equipment and increases the difficulty of surgical manipulation. Due to its cost-effectiveness, rapid reproduction rate, and the fact that 95% of the mouse genome is similar to that of humans, many trials have been developed to replicate tumor growth and migration behavior of human UM in mice. B16 and human melanoma cell lines are commonly applied (table 4).

B16F10 Melanoma Cell Line

The murine melanoma B16F10 cell line has been successfully inoculated into the AC of syngeneic C57BL6 mice [54–57]. The Queens melanoma cell line, a subculture of the B16F10 cell line with a high metastatic rate, has also been applied [60, 61]. Considering the fact that the AC is an ideal immune-privileged site in the normal state and the B16 cell line is highly invasive, the tumor-bearing eye has to be enucleated at 7–14 days after inoculation. In order to determine the presence of metastases, mice usually have to be sacrificed at days 28–30 after tumor inoculation.

B16LS9 Melanoma Cell Line

B16LS9 is a cell line derived from B16 and is liver specific [62]; it grows well in the eye [63]. AC inoculation of B16LS9 cells in murine eyes, resulting in iris melanoma, is much less

likely to metastasize to the liver than posterior compartment (PC) inoculation [64]. PC tumors are more similar to human choroidal melanoma. To avoid tumor cell reflux, which can lead to subconjunctival melanoma, Dithmar et al. [65] created a new transcorneal technique for PC inoculation and found extraocular melanoma in 43% of mice, compared to 100% in the trans-subconjunctival inoculation group. Based on this model, several studies were performed regarding tumor angiogenesis and immunology [66–68].

Human Melanoma Cell Line and Tumor Material

Tumors derived from animal cell lines or tumor specimens do not have the same characteristics with regard to histopathology and genetic mutations as human tumors. Although the models described above are sometimes suitable to study immune responses, tumor behavior and treatment, nowadays human xenografts with human cell lines and human tumor fragments are being used in almost all types of anticancer drug tests and treatment strategies. Niederkorn and his group [69–72] published a series of studies using human cell lines in mice (table 4), in which several different human cell lines were successfully transplanted intracamerally in BALB/c (H-2d) athymic mice. Using the human cell lines, not only orthotopic xenografts were established, but also some ectopic xenografts: van Ginkel et al. [73] and Triozzi et al. [74] successfully placed human cell lines (C918 and MUM2B) subcutaneously into the flanks of athymic mice. Heegaard et al. [75] directly implanted UM specimens with the same method and observed a low success rate (13%). Given the fact that metastasis does not occur in this model, it is only suitable for studying primary tumors, unless one directly implants tissue from a metastasis (see below).

With the development of bioluminescence techniques, Surriga et al. [76] labeled the OMM1.3 cell line with EGFP-luciferase protein, and then implanted the cells into the retro-orbital area of SCID mice. Liver and lung metastases were detected 6–7 weeks after inoculation by bioluminescence imaging. This imaging skill greatly facilitates the monitoring of tumor migration and reduces the amount of mice needed for experiments. Other metastases models are described by Yang et al. [77].

The rapid accumulation of knowledge through new genomic technologies indicates that cancer is a genetic disease in which most mutations occur in somatic cells. In this case, the idea of developing drugs which target cells carrying specific mutations has become a hot topic. Inspiringly, dabrafenib, which effectively inhibits BRAF kinase, was approved by the Food and Drug Administration (FDA) for advanced cutaneous melanoma in 2013. This trend, thus, leads to the overwhelming interest of ‘human tumor xenografting’ in oncologic research. For tumor xenografting, tumors are obtained directly from a patient, chopped into fragments, and subsequently inoculated into immunocompromised mice without any in vitro culture, and the tumors show the original human gene expression profiles and mutations [78]. The primary use of this model is to evaluate drug candidates and predict therapeutic efficacy by screening molecular markers [79]. A leading example in UM is the panel of heterotransplanted UM xenografts that was established in Paris [80, 81]. Pieces of primary UM or metastases from patients were implanted into the interscapular fat pad of SCID mice, and a tumor take rate of 28% was obtained. The increase in the take rate compared to the study of Heegaard et al. [75] was probably due to the fact that a great number of metastases was used as xenografts, as these had a higher take rate than pieces from primary tumors. This novel French panel of xenografts in immunodeficient mice allows the study of drugs and is also useful for immunotherapeutic approaches, as one can infuse human T cells. Nevertheless, it is debatable whether research would benefit more from orthotopic transplantation to study the effect of the ocular environment. In addition, it will be interesting to have larger panels of tumors that represent the human diversity in chromosome aberrations and GNAQ or GNA11 mutations.

Conclusions

UM is a complicated and diverse disease, and there are as yet many unknowns. Why do so few tumors give rise to xenografts, either placed orthotopically in an immune-privileged environment, or heterotopically in an immunodeficient animal? Why do orthotopically placed UM cells hardly ever induce liver metastases in animals? In order to be able to study the mechanism of UM spreading and assess drug therapeutics, we still require suitable animal models as important tools. We may use cell lines to investigate the immune reaction of UM, while for preclinical drug testing, we should carefully consider the pros and cons of the various models that are available. One may argue that drugs which work effectively in xenografts do not always predict drug effects in humans. However, recent improvements have demonstrated promising humanized animal models which have less mutation drift and more stable tumor biology. When in vitro work does not provide the answer whether a new treatment is effective, it seems that animal studies are still the necessary step prior to the use in humans.

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