Review Article

Preclinical models in ophthalmic oncology—a narrative review

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Objective: This review serves as a comprehensive description and summary of currently available preclinical models of three tumors in ophthalmic oncology: conjunctival melanoma (CM), uveal melanoma (UM), and retinoblastoma.

Background: Malignant melanomas are the most common tumors of the eye in adults, most often localized in the uvea and conjunctiva. Although the primary tumor can be successfully eliminated in many cases, nearly one in two UMs—and one in three CMs—are fatal to the patient due to metastasis. Effective therapies for metastatic uveal and CMs are unfortunately still not available, so there is an urgent need for new therapeutic strategies to improve prognosis quoad vitam and prolong the survival of melanoma patients. Another widely known tumor of the eye is retinoblastoma, which is the most common pediatric ocular malignancy, occurring in approximately 1 in 15,000–18,000 live births. Overall, it is considered well treatable, with a survival rate of approximately 90% at 3 years, although fatal if untreated. For a long time, enucleation was also considered the treatment of choice, with bilateral cases having one eye irradiated and the eye with the more advanced tumor removed. Since the 1990s, however, systemic chemotherapy has been predominantly used to preserve the quality of life and vision of young patients, although the cellular activity of the retinoblastoma often remains after treatment with chemotherapeutic agents. Prognosis of the disease is immensely depending on the stage and time of diagnosis and is varying between countries due to different developmental status of health care systems.

Methods: We review recent advances in the available literature on established preclinical models in CM, UM, and retinoblastoma. In addition, we discuss the advantages and limitations of these models and provide an overview of current alternatives to animal testing in preclinical studies.

Conclusions: In the case of all three diseases, further research is needed for improved therapeutic options. Animal models in particular are indispensable for cancer research in order to mimic the extremely complex processes of human carcinogenesis, physiology and progression. Certainly, animal studies do not easily translate to human diseases due to biological differences and limitations. However, they continue to serve as the primary source and link between *in vitro* testing and clinical studies in patients. In order to minimize animal experiments and possibly even replace them in the future, alternatives such as 3D cell cultures and in silico predictions are useful and insightful additions and require further development. Still, no currently available preclinical model can be fully translated to some of here described diseases. Nevertheless, they all provide essential insights and knowledge that should be of use in the future for better understanding and pursuit of new therapeutic strategies.

Keywords: Conjunctival melanoma (CM); uveal melanoma (UM); retinoblastoma; ocular tumor; preclinical models

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Introduction

Conjunctival melanoma (CM)

CM is the second most common ocular surface malignancy representing melanocytic lesions and occur specifically in the conjunctiva (1-3). CM arises from melanocytes. While CM arises from melanocytes, CM can form from three different precursors: primary acquired melanosis (PAM) in 74% of cases, nevus in 7%, and 19% arise spontaneously *de novo* (4). The prevalence is 0.5–1.0 cases/million in the US and Europe, with an increasing trend in recent years (5-7). The current treatment of choice is surgical excision in combination with adjuvant radio-, cryo-, chemo- and immunotherapies, with recurrence occurring in 30–60% of patients, leading to metastasis in approximately 12–15% of cases and not infrequently fatal (8-10). Metastases in general befall predominantly the adjacent lymph nodes, which suggests transmission of metastases via lymphangiogenic route (11-14). Treatment options for metastatic CM are very limited; therefore, a better understanding of the mechanisms is essential to develop novel successful therapeutic approaches. All melanomas arise originally from melanocytes, sharing the same origin but often have different disease progression (15,16). At genetic and clinical level, many CMs share similarities with cutaneous melanomas that include genetic aberrations, metastatic behavior, and clinical course (2,17-19). Mutations shared between cutaneous and CM include BRAF (V600E), NRAS, and NF-1 mutations (19-24). Often, the mutations involve cytosine-to-thymine transitions, which may indicate damage from UV exposure, once again showing parallels to cutaneous melanoma (25). Although there are new promising therapies in development, e.g., the application of systemic BRAF/MEK inhibitors (26,27), there is still a tremendous lack of knowledge and reliable preclinical testing possibilities.

Uveal melanoma (UM)

UM is the most common melanoma entity after cutaneous melanoma and account for approximately 5% of all melanoma (28-31). The incidence is approximately 5.1/1,000,000 cases in the Caucasian population, making them the most common intraocular tumors in adults (32-34). UMs most commonly manifest in the choroid, but could also affect the iris and/or the ciliary body (35-38). Risk factors for the development of UMs are light skin, blue iris color, nevi in the choroid/iris or also skin (39,40). In addition to conventional pathological features such as tumor size and location, the clinical prognosis is influenced by genetic factors, such as the presence of monosomy 3 (41) or gain on chromosome 8q (42), which generally correlate with a poorer prognosis. Also unfavorable are mutations in genes such as GNA11/GNAQ (43-46), BAP1 (41,47,48), SF3B1 (45,49), and EIF1AX (45). Although uveal and CMs both originate in ocular melanocytes, the clinical pathology and genetic features are very different (33,50). Therefore, the molecular understanding and therapeutic options of CMs cannot be easily transferred to uveal melanomas.

Therapeutic options range from transscleral resection, enucleation and radiotherapy, with distinctions between plaque brachytherapy with plaques loaded with iodine-125, ruthenium-106, palladium-103, cobalt-60, and radiotherapy with proton beam therapy, helium ion therapy or a stereotactic radiosurgery such as Cyber Knife, Gamma Knife or linear accelerator (51-56). None of the listed therapies, however, provides an evidence-based treatment option for metastatic uveal melanoma. Approximately 25% of all UMs develop metastases at 5 years and 34% at 10 years (53) after local treatment, which predominantly settle to the liver via the hematogenous route (57-59), which is one of the main distinction between CM and UM, as CM predominantly metastasizes via lymphatic routes. Long-term survival in metastatic melanoma has remained very low and unaffected by therapy.

Retinoblastoma

Retinoblastomas are the most common tumors of the eye in childhood and occur in about 1 in 15,000–18,000 births in European countries (60). Retinoblastomas are almost in all cases caused by biallelic loss of *Rb1* and develop after additional genetic alterations (61,62).

Retinoblastomas have a very good chance of cure if treated in time. In cases with late discovery of the tumor, which still occurs in developmental countries an appropriate treatment cannot be initiated. Subsequently the vision may be enormously affected and distant metastases may occur, leading to a fatal diagnosis. Therapy options range from classic chemotherapy alternative treatment options like cryo- and thermotherapy, plaque radiation, external beam radiation.

Even if retinoblastoma is one of the most well studied tumor entities, there are still a lot unanswered questions about the origin of the tumor making it difficult to find

new development approaches in the design of novel drugs. Late detection at advanced stages often makes enucleation unavoidable, decreasing the life quality of young patients making it a top priority to be able to reproduce the disease using different models and be able to develop more efficient and non-invasive therapy options.

Modelling in cancer

Preclinical models are a top priority to gain a better understanding of tumorigenesis and key mechanisms in tumor development, as well as for testing therapeutic approaches (63). *In vitro* testing with cell lines and primary tumor cells are popular in this respect. These are easy to perform, inexpensive and provide rapid results. In the case of cell lines, the resources are self-replicating and are well suited for a variety of tests, such as new drugs. On the other hand, pure two-dimensional cell cultures cannot reflect the heterogeneity of tumors and do not map the tumor microenvironment and its effects. Furthermore, it is not always easy to establish new cell lines, especially for rare tumors that are not frequently diagnosed. In this case, an improvement could be achieved by using organoids and 3D cell cultures, where the tumor tissue could be better remodeled (64-71). Clear advantage is the use of co-cultivation, as well as higher heterogeneity and cell-environment interaction. At this point, however, these models are still in their infancy, so further optimization is also needed and the tumor cannot be completely replicated and is not yet possible for every tumor type. In addition, optimization and establishment is more expensive and time-consuming compared to two-dimensional cell culture.

Still, there is no way to avoid *in vivo* testing in preclinical research. In this case, a distinction is made between different forms of applications, as well as between different host animals. There are many studies with non-mammalian animals, such as zebrafish embryos and chicken embryos, which are relatively easy to use and inexpensive and allow a high-test throughput, making them ideal for testing agents (72-81). Of course, biologically the differences to mammals are immense here, so that these systems are not always ideally suited. Xenografts using mammals, such as mice, remain quite popular as they are easy to establish and can provide rapid results using human cells (74,82-91). However, a disadvantage is the use of immunodeficient animals and the associated loss of microenvironment and tissue specificity in the tumor, as well as lack of heterogeneity when cell lines are used. Instead of cell lines, however, so-called patient-derived xenografts (PDX) can be used, whereby a tissue section can be utilized in order to map heterogeneity and epigenetic factors.

Another method is the use of syngeneic models, in which case species-derived cell lines are used to induce a tumor in the host animal. In this case, effects of the immune system and tumor microenvironment can be considered. However, there are not enough suitable murine or other syngeneic cell lines for every type of cancer available at date (92).

Through biotechnological progress in the field of gene engineering, genetically engineered models (GEM) are becoming increasingly common. Through the targeted regulation of oncogenes it is possible to study tumorigenesis and to investigate the effects with a fully functional immune system and microenvironment. However, these models are still relatively complex to handle, especially in multigenic diseases. Targeted spontaneous tumors are not available for every species and there are also discrepancies to actual disease in humans (63,93-96).

It is important to evaluate in advance which type of model is best suited for one's research, depending on what is to be studied. In this narrative review, we discuss the most current and promising models for three types of tumors in ophthalmic oncology: UM, CM, and retinoblastoma. We present the article in accordance with the Narrative Review reporting checklist (available at https://aes.amegroups.com/article/view/10.21037/aes-21-39/rc).

Methods

Using electronic bibliographic databases, PubMed, Embase, Web of Science and Google Scholar were searched for the following keywords with different combinations: "ocular melanoma", "retinoblastoma", "uveal melanoma", "conjunctival melanoma", "animal models", "preclinical testing", "syngeneic", "xenografts", "transgenic mice", "in vitro", "in vivo", "modeling" and "preclinical research". Searches were limited to English and German studies until May 30th, 2021.

Discussion

CM

Syngeneic models are very useful when investigating immune responses in addition to tumorigenesis in experiments designed for this purpose. A common model

in CM involves the use of cutaneous melanoma cell lines, most commonly using the B16 murine melanoma cell line derived from a spontaneously occurring melanoma in a C57Bl/6J mouse (97-99). In this process, to induce intraocular tumors, the cells previously cultured *in vitro* are introduced in murine conjunctiva by microinjection, in most cases resulting in solid tumors after a few weeks. In order to develop a more invasive model, some alterations were made by serial passaging, resulting in a more invasive B16LS9 subline (100), which actually showed occurrence of liver metastases after injections. Another approach for a syngeneic model with focus on metastasis used C57BL/6N-derived HGF-Cdk4^{R24C} melanoma cells (92), which also lead to solid tumors and metastases, due to the impairment of the cell cycle by Cdk4^{R24C} mutant and allowing maintenance of melanocytes in interfollicular epidermis through HGF overexpression.

Certainly, there were also numerous studies apart from mice, using other animals, such as the application of Greene melanoma cells (hamster origin) in rabbits (101,102). Rabbit models in eye diseases have the advantage that compared to mice, rabbits have larger eyes, making application and monitoring much easier. Recently, this approach is hardly used in basic research, but much more for testing treatment options, since rapid tumor growth and the absence of metastases, compromised the model (103).

However, syngeneic models have some advantages, providing the perfect basis for studying angiogenesis and metastasis, as well as immune responses and, consequently, a reasonably reliable assessment of treatment strategies. Unfortunately, there are no syngeneic CM cell lines, but only murine cutaneous melanoma cell lines available at date (92).

Besides syngeneic model, the use of xenografts in preclinical studies is one of the tools of choice. Human tumor cell lines are cultured *in vitro* and subsequently injected in conjunctiva of immune suppressed hosts, including mice, rabbits and zebrafish. These models are mainly used for drug screening, different therapeutic options and tumor growth in general (92,97,104-106). Permanent human cell lines have the advantage that they are already characterized immunohistologically and genetically, so that biological and pharmaceutical effects can thus be better viewed in context. Unfortunately, relatively few established cell lines are available (*Table 1*) from CM cells (107-110), so that the heterogeneity of a tumor population can only be mapped to a limited extent, due to testing variety limitations.

Another possibility for extensive investigation of the efficacy of new therapeutics is the use of so-called PDX. In this case, instead of cells from a cell line, biopsies from patients are transplanted into a model animal and subsequently investigated in further approaches. This offers the advantage that more consideration can be given to heterogeneity of a tumor population and also provides an opportunity for personalized medicine (89). To date, there are no current studies in CM with PDX but some with cutaneous melanoma that have been able to provide not only opportunities in drug discovery but also basic insights into metabolism and metastatic behavior of melanoma cells (84,111).

In addition to animal testing, there are also *in vitro* alternatives in preclinical testing. For example, there is an interesting study by Fiorentzis and colleagues from 2020 that uses 3D cell cultures with CRMM1 and CRMM2 cell lines to test an approach with electrochemotherapy (69,70). In contrast to standard cell cultures with cells grown in a two-dimensional environment, 3D cultures are much better able to reproduce the spatial complexity of tumor tissue and mimic the tumor microenvironment (68). Thus, clearly representative results on the efficacy of e.g., new compounds can be obtained. 3D spheroids consists of aggregates of tumor cells that provide more natural conditions in terms of metabolism and oxygen distribution than 2D cell cultures (112). They can be evaluated using different assays on tumor growth while testing therapeutic application and furthermore bear the possibility to be transplanted as a PDX in xenograft animal studies. Apart from spheroids, there are also carrier substrates such as Matrigel (113) widely used in *in vitro* preclinical testing investigating the migration and invasion potential of melanoma cells.

Certainly, any of these models have to be viewed with caution, as they only partially represent the systems, cells and tissues of human organisms. Nevertheless, they are an important tool for understanding basic biochemical processes of tumor biology, such as proliferation, expression of angiogenic factors, intra- and extravasation and migration.

Another increasingly important method is the use of *in silico* predictions. *In silico* modeling can be used to accurately test binding affinities and efficacies of chemical compounds, as well as predict protein-protein interactions. Thereby, costly testing can be reduced to truly promising therapeutic candidates and is an interesting possibility for high-throughput screening of drug libraries in the future (114). Currently, there are several studies in cutaneous melanoma,

Table 1 Overview of current preclinical experimental models with regard to advantages and limitations

Experimental model	Advantages	Limitations
Syngeneic mice models	• Immune-competent hosts	• In CM and UM no murine cell lines available at date
	• Ideal conditions for investigations on TME and immune system	• Many differences in tumor biology between mice and humans
Xenograft model	• Ideal for high throughput testing of novel chemotherapeutic agents	• Purchase and maintenance of immunodeficient animals is often very cost-intensive
	• Metastasis models possible	• Effects of immune system and TME interaction are disregarded
	• Many different hosts available—mice, chick embryos, zebrafish	• In the case of non-mammals very high differences in biology
Genetically engineered models	• Optimal for studies on cancerogenesis and fundamental research	• Generation very time and cost-intensive
	• Immunocompetent hosts	• One or several genes mostly not sufficient for exact representation of the disease
	• Metastatic models possible	• Manipulation of genes often leads to multiple tumors
3D culturing	• Time-saving alternative - relatively fast establishment	• Long-term studies in terms of tumorigenesis and relapses not possible
In vitro testing	• No living organisms needed	• Not all cell types spontaneously form spheroids
Stem cell culture	• Scientific progress enables simulation of real tumors in cell culture-making testing of compounds and therapy options easier	• Despite diverse cell cultures with different cell types no complete representation of a TME so far
In silico	• Fast and inexpensive method for pre-selection of chemotherapeutic candidates	• Calculation of binding affinities alone not indicative for actual effect—efficacy must always be additionally evaluated *in vitro* or *in vivo*
	• Prediction of molecular docking, protein-protein interactions and pathway analyses	• For a better prediction, an expansion of biobanks would be necessary to complement pathways and to be able to include interactions and side effects

CM, conjunctival melanoma; UM, uveal melanoma; TME, tumor microenvironment.

e.g., on the efficacies of BRAF inhibitors but also on proteomic profiles in tumors (115-117), implying that this techniques could also be applicable for CM.

UM

Similar as in studies with CM, cutaneous melanoma cells have been used for syngeneic models of UMs for decades. For this purpose, cell lines derived from different animal species are used, such as Greene melanoma cell lines in rabbits (101,103,118) and B16 melanoma cells in mice (100). Even though the cutaneous cell lines are not derived from the choroid and accordingly have different properties, these models are still suitable for studying the intraocular growth of melanoma cells, and many of these models, actually lead to metastasis in the liver. Thus, the metastatic process including intra- and extravasation and growth in other organs can be monitored. Besides studying metastatic behavior, it is obviously very advantageous that the experiments take place in immunocompetent animals, to date remaining the greatest strength of these models.

As with CM, there are many murine models of UM most commonly involve inoculation of C57BL/6 mice with the B16LS9 cell line, a derivative of the B16 skin melanoma line. Serial passaging induced the metastatic potential of this cell line to form hepatic metastases, which has led to valuable insights into the biology of metastatic melanoma (43,46,100).

Although the syngeneic models are very useful, they are not suitable for UM. Since the used melanoma cells are

from cutaneous origin, the molecular drivers differ from drivers in UM. There were efforts made to compensate this by introducing canonical UM mutations in some studies. For this purpose, e.g., immortalized melanocytes were transduced with specific mutations like $GNAQ^{Q209L}$, actually leading to solid tumors and metastasis as well (119).

The desirable outcome would be the use of murine UM cell lines, so eventually in the future it will be possible to establish a stable murine cell line from transgenic animals, allowing the investigation of interactions of an actual UM in an immunocompetent host.

Just as in cutaneous and CM, xenografts are also being used in UM. Commonly, permanent UM cell lines are used, whose genetic and histological profile is already known (88,120-124). The great advantage of human xenografts is that cells derived directly from patients display characteristics of UMs at molecular level. The models are therefore ideal for testing new drugs and for screening intraocular tumor growth. Hence, the biological and pharmacological aspects can be studied *in vivo* with a view to interaction with new compounds identifying potential candidates for clinical studies. The selection of cell lines is of utmost importance as very few UM cell lines are available, as some cell lines turned out to be of cutaneous origin, by exhibiting e.g., BRAF mutations (125), which usually are not present in UM. Furthermore, some could be identified as identical cell lines by short tandem repeat (STR) analysis, which further limits the choice of reliable cell lines (126). Orthotopic mouse models of UM basically result in inoculation to the iris, ciliary body, or choroid. Suprachoroidal injection models have been described (98) and rapidly demonstrated tumors in the ciliary body and choroid. There are also methods to perform the injection intravitreally. Although in humans UMs do not arise in the vitreous body, animal models showed similar invasive behavior to human melanoma, making it well suited for preclinical testing (127,128)

A major disadvantage of these models is that even if the characteristics of the cell lines are well described to date, cell lines yet evolve through frequent passaging and become increasingly distant from the tumor of origin. In this way, results obtained in animals are not readily translationally applicable with regard to original tumors (71,120,129).

PDX models are a suitable solution and are becoming increasingly popular in cancer research. This often involves implanting tumor samples into mice, with resulting in solid tumors in nearly one-third of cases. Often the studies are using severe-combined immunodeficient (SCID) (63,87,90,91,120,122,130) and next-generation sequencing (NGS) (87,131) mice.

This method enables producing heterogeneous tumors that share the same molecular and genetic abnormalities as tumors in patients, making them particularly good models for testing combination therapies.

Xenotransplantation is primarily performed in immunodeficient mice and less frequently in rabbits (118,132). Nevertheless, in both cases, these are very costly and time-consuming variants. For this reason, approaches that allow high-throughput testing of entire compound libraries in UMs are becoming increasingly common.

Another suitable model for preclinical screening is zebrafish, due to its low maintenance cost and ease of manipulation of zebrafish embryos, as the adaptive immune system of the animals is not formed until 4 weeks after fertilization. In addition, there are similarities between zebrafish and human tumors at the histopathological level, as well as there is the presence of tissue-specific transgenic zebrafish lines that facilitate imaging. In general, melanoma cells are implanted by injection into the embryos and then growth and migration are identified using various imaging methods (63,74,75,80,133). The studies showed that the zebrafish xenograft model is useful for preclinical testing of a variety of compounds and has the advantage over mouse models in terms of cost-efficiency and time-saving.

Another method for preclinical testing is the use of chicken embryos in so called chorioallantoic membrane (CAM) assays. UM cells are applied to the CAM on tenth day after fertilization and subsequently tumor growth, angiogenesis and metastasis can be observed and analyzed in this model. The immune advantage of inducing tumor cells without rejection is due to the lack of an immune system in chicken embryos to this point of embryonic development (72). Considering the accessibility and application diversity of this method, it could be as well a very helpful tool in the future.

Like all preclinical models, xenografts also have deficiencies. In addition to the aforementioned use of cell lines, some of which are not translatable to the tumors in patients, the use of immunodeficient hosts is also an extreme disadvantage. With the increasing importance of immunotherapies, it does not seem reasonable to work with models that cannot represent the immune interaction with tumor cells. Although response rates to PD-1 and CTLA4 inhibition have been low in UM (134), there are other aspects of the immune system that play important roles and fundamentally affect angiogenesis, metastasis, and

ultimately prognosis. In addition, xenografts, even PDX, are often very expensive and have a low transplantation rate or, as with CAM assays or zebrafish, are not performed in mammals, which also challenges the transferability of these models.

Besides xenografts and syngeneic models, there is another subclass of animal models—GEMs. In this case, GEMs allow the study of autochthonous tumorigenesis coupled with the influence of the immune system (85,93,95)—given that spontaneous tumors arise.

This method, in combination with the use of immunocompetent hosts, has the advantage that signal transduction of tumorigenesis can be studied at genetic level and fundamental understandings of the disease can be gained.

Older models of UM primarily used pigment-specific promoters such as tyrosinases and HRAS, which resulted in not only uveal tumors but also retinal and cutaneous malignancies. Apart from that, these were gene alterations that cannot be observed in UM patients, which limits the clinical applicability immensely (135).

The discovery of the oncogenic drivers GNAQ/GNA11 has been one of the most important contributions in recent years, allowing the development and study of several mouse models using $GNAQ^{Q209L}$ transgenic mice. These mice also did not develop UMs initially, but still showed some molecular similarity in the cutaneous lesions, such as activation of the YAP protein (133,136-138).

Another $GNAQ^{Q209L}$ model resulted in the formation of UMs within a few months, although intravasation and metastasis were also observed here. However, there were also dermal neoplasms derived from melanocytes in addition to lesions on the choroid. Other models combined, for example, BAP1 deletion and expression of $GNAQ^{Q209L}$, in which, unexpectedly, choroidal melanomas turned out smaller but with overall increased dermal tumor burden (119).

These models allow us to have a more detailed look on tumorigenesis than before, for example, the role of GNAQ and GNA11 as oncogenes could be verified by this kind of modelling (40,119,136,137).

By all means, like other models, GEMs are not without drawbacks. First, the introduction of $GNAQ^{Q209L}$ leads to melanocytic neoplasms in other organs leading to undesirable side effects and to a premature termination of the studies without the possibility to sufficiently observe the development of UMs. In addition to this, the time factor also plays a role, since the occurrence of spontaneous lesions certainly is more time-dependent than inoculation in xenografts and syngeneic models. Another factor is the lack of distinction between primary tumors and metastases, as the tumor burden of the transgenic mice is generally very high.

Finally, yet importantly, despite all the advances, the immense differences in the biology of mice and humans are not to be overlooked and always have to be considered in preclinical testing.

In addition to basic preclinical metastasis assays such as migration assays, ring assays, and chemotaxis assays (139), there are a number of approaches to overcome two-dimensional cell cultivation and to establish more realistic methods for the evaluation of preclinical tests in UM. Based on the success of PDX, it has been shown that three-dimensional cultures from tumor samples can grow in mice. Thus bears the possibility to represent the molecular phenotypes and possibly also include the role of tumor microenvironment with fibroblasts and lymphocytes (71,129). There are already successful approaches to cultivate cell culture lines with e.g., added macrophages as well as to cultivate patient cells (66), which result in 3D spheroids and could also be used successfully in first tests. If these 3D cell models become established, this will be a superior tool for testing cell-matrix interactions and tumor microenvironment and probably could be used for *in vitro* testing as well as act as a xenograft model.

Retinoblastoma

Although the extraction of live cells from retinoblastomas was initially very difficult, some cell lines are now available that allow the application of xenografts. Therefore, retinoblastoma cell lines could be transplanted into immunodeficient animals by microinjection, which also lead to formation of tumors (83,132,140,141).

Among the animals used were rabbits, in which immunohistochemistry was used to demonstrate that the tumors had indeed grown (132,142). Both stronger vascularization and persistent tumor growth could be detected, as well as the presence of necrotic areas and hypoxic conditions, which can also be observed in human retinoblastomas. However, these models were inaccurate since the subretinal space was affected rather than the retina.

Another approach used athymic nude mice including the injection of a primary cell line and freshly derived patient samples after surgery (143). The fresh cells were transplanted into the anterior chamber and grew in the eye, but failed to grow if injected subcutaneously. On the other

hand, cells from the Y-79 cell line invaded orbit, brain and optic nerve and showed severe tumor growth.

There are also several models using zebrafish in an orthotopic approach, showing promising results in terms of high throughput screening of drug libraries (76-79,81,144). Like the most xenograft models they are used to test new chemotherapeutic agents and photodynamic therapies and allows a high number of agents to be tested in short time.

Besides the preclinical testing of novel drugs, xenograft models delivered some diagnostic advances in non-invasive imaging, such as micro CT, MRI and fluorescence and bioluminescence imaging (145-147). By labeling cell lines with fluorescent proteins like green fluorescent protein (GFP), it is possible to study the metastatic behavior of retinoblastomas.

However, like all xenograft models, these models have the disadvantage that they only reflect the disease to a limited extent and the tumor microenvironment of tumors cannot be monitored due to immunodeficiency. In addition, there are also differences in the various biological species: for instance, the optimal body temperature for zebrafishes is 28 ℃, while tumor cells show the best growth performance at 37 ℃ degrees, so naturally, the conditions are not limitless comparable (77). This limitation has been addressed by many groups and the Langenau group actually succeeded in performing preclinical experiments with zebrafish at 37 ℃. For this purpose, adult zebrafish of the Casper strain prkdc–/–, il2rgα–/– with weakened immune systems were used, in which human cancer cells could actually be implanted over a period of more than 28 days (73).

In addition to xenografts, there are a number of approaches to knockout models of retinoblastoma. Even though the *Rb1* gene is considered an oncopromoter in human retinoblastomas, it was initially insufficient to generate a retinoblastoma in the mouse eye. Only with the identification of *p107* in the signal pathways and the knockout combination of both genes was it possible to generate successful transgenic models.

One of the first models is the LH-β T-Ag mouse model, in which the oncogenic unit of the SV40 protein is expressed and can be induced by an *LH*-β promoter, and is combined with T-Ag-expressing mice (148-151). On histological level, the resulting retinoblastomas were also very similar to human retinoblastoma samples and therefore became a first basis for better understanding of retinoblastoma. The model was primarily used to test local therapies, yet had the disadvantage of using viral oncoproteins, whose impact on tumor and experimental animal is not completely evaluated. Consequently, it was inevitable to develop other transgenic models that could mimic retinoblastoma tumorigenesis in humans.

Although the original model provided some important results on therapeutic testing, the advent of gene knock-out technology (152) first provided important tools for developing new translational retinoblastoma models. The first attempts resulted in the knockout of the *Rb1* gene in the retinal hat of mice (153). However, this alone didn't bring the desirable retinoblastoma. It was not until the discovery of another protein, *p107* (154-157) and its role in inhibiting retinoblastoma formation in *Rb*-deficient mice until multiple knockout mouse lines were developed from it actually getting retinoblastoma tumors. Unfortunately most of the animals died very early in development, since *Rb*, as well as p107, plays a major role in embryonic development (158). Hence, a *Cre-Lox* model was generated to facilitate viable mutants and still knock out the genes. This way, it was indeed possible to obtain a group of animals in which retinoblastoma developed (156,157). This model also showed high apoptotic sensitivity and cell death resistance, which is very similar to human retinoblastomas. Unfortunately, the delayed tumorigenesis was unfavorable; in addition, little penetrance and invasiveness could be observed in this model.

Inclusion of an additional mutation of *p130* in these models resulted in enhancement of several developmental phenotypes seen with loss of Rb in the retina (157,159,160). This indicated that there is a functional synergy between these family members. The α-Cre *Rb/p130* DKO mouse turned out as a suitable model to study advanced retinoblastoma with tumors rapidly progressing and also producing metastases. Rb/p130-DKO retinoblastomas appear similar to *Rb/p107*-DKO retinoblastomas on histologic examination, and both resemble human retinoblastomas with neuroblastic differentiation. Numerous variations of this murine model exist, also e.g., including p53 mutations as well, while a lot of them are considered genetically and histologically translatable to humans. Obviously, there is always the fact that tumor biology of mice and humans is distinctive and the findings on tumorigenesis can only be transferred to a limited degree.

While many mouse models rely on the deactivation of *Rb1* and *Rb1l*, there are other models that make use of this principle. For example, there is a promising study in *Xenopus tropicalis* where means CRISPR/Cas9 techniques using a combination of double mutation of *Rb1* and *Rbl1*

resulted in tumors, while knockout of Rb1 or Rbl1 alone did not result in tumor formation in the tadpoles (161).

Many approaches have tested the use of immortalized Rb cell lines for 3D cell culture for biological and preclinical testing, but failed due to self-assembled structures and formation of cell-cell matrices in three-dimensional space.

A successfully tested study instead uses human pluripotent stem cells (hPSCs) that could mimic retinogenesis *in vitro*. Consequently, human embryonic stem cells with biallelic *Rb1* mutation were generated and did grow stepwise into *Rb* organoids. Through this model, it was indeed possible to identify genetic signatures and successfully test potential therapeutics (162). The use of pluripotent stem cells could therefore become an interesting innovative way to screen retinoblastoma therapies, without the time and cost effort of mice models.

In summary, it is certain that the one perfect preclinical model does not exist for any of the three cancer entities described here. All animal models as well as *in vitro* testing have their advantages and disadvantages. Crucially, to realize the experimental potential, it is definitely necessary to create more molecular datasets for ocular melanomas in order to create successful therapies and treatment options despite the rarity of the respective diseases. Besides the optimization of the respective preclinical models, it is also a top priority to support the expansion of biological databases in order to consolidate the state of knowledge and to combine all known findings and use them well in future scientific approaches.

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References

1. Brownstein S. Malignant melanoma of the conjunctiva. Cancer Control 2004;11:310-6.
2. Chang AE, Karnell LH, Menck HR. The National Cancer Data Base report on cutaneous and noncutaneous melanoma: a summary of 84,836 cases from the past decade. The American College of Surgeons Commission on Cancer and the American Cancer Society. Cancer 1998;83:1664-78.
3. Grimes JM, Shah NV, Samie FH, et al. Conjunctival Melanoma: Current Treatments and Future Options. Am J Clin Dermatol 2020;21:371-81.
4. Pacheco RR, Yaghy A, Dalvin LA, et al. Conjunctival melanoma: outcomes based on tumour origin in 629 patients at a single ocular oncology centre. Eye (Lond) 2022;36:603-11.
5. Kaštelan S, Gverović Antunica A, Beketić Orešković L, et al. Conjunctival Melanoma - Epidemiological Trends and Features. Pathol Oncol Res 2018;24:787-96.
6. Triay E, Bergman L, Nilsson B, et al. Time trends in the incidence of conjunctival melanoma in Sweden. Br J Ophthalmol 2009;93:1524-8.

7. Yu GP, Hu DN, McCormick S, et al. Conjunctival melanoma: is it increasing in the United States? Am J Ophthalmol 2003;135:800-6.
8. Anastassiou G, Heiligenhaus A, Bechrakis N, et al. Prognostic value of clinical and histopathological parameters in conjunctival melanomas: a retrospective study. Br J Ophthalmol 2002;86:163-7.
9. Kenawy N, Lake SL, Coupland SE, et al. Conjunctival melanoma and melanocytic intra-epithelial neoplasia. Eye (Lond) 2013;27:142-52.
10. Norregaard JC, Gerner N, Jensen OA, et al. Malignant melanoma of the conjunctiva: occurrence and survival following surgery and radiotherapy in a Danish population. Graefes Arch Clin Exp Ophthalmol 1996;234:569-72.
11. Refaian N, Schlereth SL, Koch KR, et al. Comparing the Hem- and Lymphangiogenic Profile of Conjunctival and Uveal Melanoma Cell Lines. Invest Ophthalmol Vis Sci 2015;56:5691-7.
12. Zimmermann P, Dietrich T, Bock F, et al. Tumour-associated lymphangiogenesis in conjunctival malignant melanoma. Br J Ophthalmol 2009;93:1529-34.
13. Heindl LM, Hofmann-Rummelt C, Adler W, et al. Tumor-associated lymphangiogenesis in the development of conjunctival melanoma. Invest Ophthalmol Vis Sci 2011;52:7074-83.
14. Heindl LM, Hofmann-Rummelt C, Adler W, et al. Prognostic significance of tumor-associated lymphangiogenesis in malignant melanomas of the conjunctiva. Ophthalmology 2011;118:2351-60.
15. Iwamoto S, Burrows RC, Grossniklaus HE, et al. Immunophenotype of conjunctival melanomas: comparisons with uveal and cutaneous melanomas. Arch Ophthalmol 2002;120:1625-9.
16. Heindl LM, Platzl C, Wolfmeier H, et al. Choroidal melanocytes: subpopulations of different origin? Ann Anat 2021;238:151775.
17. Glitza IC, Davies MA. Genotyping of cutaneous melanoma. Chin Clin Oncol 2014;3:27.
18. Greaves WO, Verma S, Patel KP, et al. Frequency and spectrum of BRAF mutations in a retrospective, single-institution study of 1112 cases of melanoma. J Mol Diagn 2013;15:220-6.
19. Griewank KG, Westekemper H, Murali R, et al. Conjunctival melanomas harbor BRAF and NRAS mutations and copy number changes similar to cutaneous and mucosal melanomas. Clin Cancer Res 2013;19:3143-52.
20. Cao J, Heijkants RC, Jochemsen AG, et al. Targeting of the MAPK and AKT pathways in conjunctival melanoma shows potential synergy. Oncotarget 2016;8:58021-36.
21. Gear H, Williams H, Kemp EG, et al. BRAF mutations in conjunctival melanoma. Invest Ophthalmol Vis Sci 2004;45:2484-8.
22. Griewank KG, Westekemper H, Schilling B, et al. Conjunctival melanomas harbor BRAF and NRAS mutations--response. Clin Cancer Res 2013;19:6331-2.
23. Lake SL, Jmor F, Dopierala J, et al. Multiplex ligation-dependent probe amplification of conjunctival melanoma reveals common BRAF V600E gene mutation and gene copy number changes. Invest Ophthalmol Vis Sci 2011;52:5598-604.
24. Larsen AC, Dahl C, Dahmcke CM, et al. BRAF mutations in conjunctival melanoma: investigation of incidence, clinicopathological features, prognosis and paired premalignant lesions. Acta Ophthalmol 2016;94:463-70.
25. Stahl A, Riggi N, Nardou K, et al. 5-Hydroxymethylcytosine Loss in Conjunctival Melanoma. Dermatopathology (Basel) 2021;8:176-84.
26. Germann UA, Furey BF, Markland W, et al. Targeting the MAPK Signaling Pathway in Cancer: Promising Preclinical Activity with the Novel Selective ERK1/2 Inhibitor BVD-523 (Ulixertinib). Mol Cancer Ther 2017;16:2351-63.
27. Mor JM, Heindl LM. Systemic BRAF/MEK Inhibitors as a Potential Treatment Option in Metastatic Conjunctival Melanoma. Ocul Oncol Pathol 2017;3:133-41.
28. Amaro A, Gangemi R, Piaggio F, et al. The biology of uveal melanoma. Cancer Metastasis Rev 2017;36:109-40.
29. Khoja L, Atenafu EG, Suciu S, et al. Meta-analysis in metastatic uveal melanoma to determine progression free and overall survival benchmarks: an international rare cancers initiative (IRCI) ocular melanoma study. Ann Oncol 2019;30:1370-80.
30. Yousef YA, Alkilany M. Characterization, treatment, and outcome of uveal melanoma in the first two years of life. Hematol Oncol Stem Cell Ther 2015;8:1-5.
31. Mor JM, Rokohl AC, Mauch C, et al. Interdisciplinary Surveillance of Ocular Melanomas: Experiences in a German Tertiary Centre. Klin Monbl Augenheilkd 2021;238:85-91.
32. Singh AD, Topham A. Incidence of uveal melanoma in the United States: 1973-1997. Ophthalmology 2003;110:956-61.
33. Hilke FJ, Sinnberg T, Gschwind A, et al. Distinct Mutation Patterns Reveal Melanoma Subtypes and Influence Immunotherapy Response in Advanced

Melanoma Patients. Cancers (Basel) 2020;12:2359.
34. Ortega MA, Fraile-Martínez O, García-Honduvilla N, et al. Update on uveal melanoma: Translational research from biology to clinical practice (Review). Int J Oncol 2020;57:1262-79.
35. Khan AM, Kagan DB, Gupta N, et al. Ciliary body lymphangiogenesis in uveal melanoma with and without extraocular extension. Ophthalmology 2013;120:306-10.
36. Heindl LM, Hofmann TN, Knorr HL, et al. Intraocular lymphangiogenesis in malignant melanomas of the ciliary body with extraocular extension. Invest Ophthalmol Vis Sci 2009;50:1988-95.
37. Heindl LM, Hofmann TN, Schrödl F, et al. Intraocular lymphatics in ciliary body melanomas with extraocular extension: functional for lymphatic spread? Arch Ophthalmol 2010;128:1001-8.
38. Heindl LM, Koch KR, Hermann MM, et al. Block Excision of Iridociliary Tumors Enables Molecular Profiling and Immune Vaccination. Ophthalmology 2017;124:268-70.
39. Harbour JW. Genomic, prognostic, and cell-signaling advances in uveal melanoma. Am Soc Clin Oncol Educ Book 2013;388-91.
40. Li Y, Shi J, Yang J, et al. Uveal melanoma: progress in molecular biology and therapeutics. Ther Adv Med Oncol 2020;12:1758835920965852.
41. Ewens KG, Kanetsky PA, Richards-Yutz J, et al. Chromosome 3 status combined with BAP1 and EIF1AX mutation profiles are associated with metastasis in uveal melanoma. Invest Ophthalmol Vis Sci 2014;55:5160-7.
42. Aalto Y, Eriksson L, Seregard S, et al. Concomitant loss of chromosome 3 and whole arm losses and gains of chromosome 1, 6, or 8 in metastasizing primary uveal melanoma. Invest Ophthalmol Vis Sci 2001;42:313-7.
43. Ambrosini G, Musi E, Ho AL, et al. Inhibition of mutant GNAQ signaling in uveal melanoma induces AMPK-dependent autophagic cell death. Mol Cancer Ther 2013;12:768-76.
44. Chen X, Wu Q, Tan L, et al. Combined PKC and MEK inhibition in uveal melanoma with GNAQ and GNA11 mutations. Oncogene 2014;33:4724-34.
45. Dono M, Angelini G, Cecconi M, et al. Mutation frequencies of GNAQ, GNA11, BAP1, SF3B1, EIF1AX and TERT in uveal melanoma: detection of an activating mutation in the TERT gene promoter in a single case of uveal melanoma. Br J Cancer 2014;110:1058-65.
46. Huang JL, Urtatiz O, Van Raamsdonk CD. Oncogenic G Protein GNAQ Induces Uveal Melanoma and Intravasation in Mice. Cancer Res 2015;75:3384-97.
47. Affar EB, Carbone M. BAP1 regulates different mechanisms of cell death. Cell Death Dis 2018;9:1151.
48. Szalai E, Wells JR, Ward L, et al. Uveal Melanoma Nuclear BRCA1-Associated Protein-1 Immunoreactivity Is an Indicator of Metastasis. Ophthalmology 2018;125:203-9.
49. Hou C, Xiao L, Ren X, et al. Mutations of GNAQ, GNA11, SF3B1, EIF1AX, PLCB4 and CYSLTR in Uveal Melanoma in Chinese Patients. Ophthalmic Res 2020;63:358-68.
50. van der Kooij MK, Speetjens FM, van der Burg SH, et al. Uveal Versus Cutaneous Melanoma; Same Origin, Very Distinct Tumor Types. Cancers (Basel) 2019;11:845.
51. Bhatia S, Moon J, Margolin KA, et al. Phase II trial of sorafenib in combination with carboplatin and paclitaxel in patients with metastatic uveal melanoma: SWOG S0512. PLoS One 2012;7:e48787.
52. Carvajal RD, Piperno-Neumann S, Kapiteijn E, et al. Selumetinib in Combination With Dacarbazine in Patients With Metastatic Uveal Melanoma: A Phase III, Multicenter, Randomized Trial (SUMIT). J Clin Oncol 2018;36:1232-9.
53. Carvajal RD, Schwartz GK, Tezel T, et al. Metastatic disease from uveal melanoma: treatment options and future prospects. Br J Ophthalmol 2017;101:38-44.
54. Chua V, Aplin AE. Novel therapeutic strategies and targets in advanced uveal melanoma. Curr Opin Oncol 2018;30:134-41.
55. Bosch JJ, Heindl LM. Novel Adjuvant Therapy for Ocular Melanoma. Klin Monbl Augenheilkd. 2017;234(5):670-3.
56. Heindl LM, Lotter M, Strnad V, et al. High-dose 106Ruthenium plaque brachytherapy for posterior uveal melanoma. A clinico-pathologic study. Ophthalmologe 2007;104:149-57.
57. Robertson AG, Shih J, Yau C, et al. Integrative Analysis Identifies Four Molecular and Clinical Subsets in Uveal Melanoma. Cancer Cell 2017;32:204-220.e15.
58. Shields CL, Furuta M, Thangappan A, et al. Metastasis of uveal melanoma millimeter-by-millimeter in 8033 consecutive eyes. Arch Ophthalmol 2009;127:989-98.
59. Tosi A, Cappellesso R, Dei Tos AP, et al. The immune cell landscape of metastatic uveal melanoma correlates with overall survival. J Exp Clin Cancer Res 2021;40:154.
60. Stacey AW, Bowman R, Foster A, et al. Incidence of Retinoblastoma Has Increased: Results from 40 European Countries. Ophthalmology 2021;128:1369-71.
61. McFall RC, Sery TW, Makadon M. Characterization

of a new continuous cell line derived from a human retinoblastoma. Cancer Res 1977;37:1003-10.
62. Tommerup N. Retinoblastoma: model for heredity and cancer. Ugeskr Laeger 1989;151:857-8.
63. Goldrick C, Palanga L, Tang B, et al. Hindsight: Review of Preclinical Disease Models for the Development of New Treatments for Uveal Melanoma. J Cancer 2021;12:4672-85.
64. Ravi M, Ramesh A, Pattabhi A. Contributions of 3D Cell Cultures for Cancer Research. J Cell Physiol 2017;232:2679-97.
65. Lv D, Hu Z, Lu L, et al.Three dimensional cell culture: A powerful tool in tumor research and drug discovery (Review). Oncol Lett 2017;14:6999-7010.
66. Linde N, Gutschalk CM, Hoffmann C, et al. Integrating macrophages into organotypic co-cultures: a 3D in vitro model to study tumor-associated macrophages. PLoS One 2012;7:e40058.
67. Jensen C, Teng Y. Is It Time to Start Transitioning From 2D to 3D Cell Culture? Front Mol Biosci 2020;7:33.
68. Imamura Y, Mukohara T, Shimono Y, et al. Comparison of 2D- and 3D-culture models as drug-testing platforms in breast cancer. Oncol Rep 2015;33:1837-43.
69. Fiorentzis M, Katopodis P, Kalirai H, et al. Image Analysis of 3D Conjunctival Melanoma Cell Cultures Following Electrochemotherapy. Biomedicines 2020;8:158.
70. Fiorentzis M, Katopodis P, Kalirai H, et al. Conjunctival melanoma and electrochemotherapy: preliminary results using 2D and 3D cell culture models in vitro. Acta Ophthalmol 2019;97:e632-40.
71. Aughton K, Shahidipour H, Djirackor L, et al. Characterization of Uveal Melanoma Cell Lines and Primary Tumor Samples in 3D Culture. Transl Vis Sci Technol 2020;9:39.
72. Kalirai H, Shahidipour H, Coupland SE, et al. Use of the Chick Embryo Model in Uveal Melanoma. Ocul Oncol Pathol 2015;1:133-40.
73. Yan C, Do D, Yang Q, et al. Single-cell imaging of human cancer xenografts using adult immunodeficient zebrafish. Nat Protoc 2020;15:3105-28.
74. van der Ent W, Burrello C, Teunisse AF, et al. Modeling of human uveal melanoma in zebrafish xenograft embryos. Invest Ophthalmol Vis Sci 2014;55:6612-22.
75. van der Ent W, Burrello C, de Lange MJ, et al. Embryonic Zebrafish: Different Phenotypes after Injection of Human Uveal Melanoma Cells. Ocul Oncol Pathol 2015;1:170-81.
76. Schultz LE, Haltom JA, Almeida MP, et al. Epigenetic regulators Rbbp4 and Hdac1 are overexpressed in a zebrafish model of RB1 embryonal brain tumor, and are required for neural progenitor survival and proliferation. Dis Model Mech 2018;11:dmm034124.
77. Jo DH, Son D, Na Y, et al. Orthotopic transplantation of retinoblastoma cells into vitreous cavity of zebrafish for screening of anticancer drugs. Mol Cancer 2013;12:71.
78. Duffy KT, McAleer MF, Davidson WR, et al. Coordinate control of cell cycle regulatory genes in zebrafish development tested by cyclin D1 knockdown with morpholino phosphorodiamidates and hydroxyprolyl-phosphono peptide nucleic acids. Nucleic Acids Res 2005;33:4914-21.
79. Chen X, Wang J, Cao Z, et al. Invasiveness and metastasis of retinoblastoma in an orthotopic zebrafish tumor model. Sci Rep 2015;5:10351.
80. Chen Q, Ramu V, Aydar Y, et al. TLD1433 Photosensitizer Inhibits Conjunctival Melanoma Cells in Zebrafish Ectopic and Orthotopic Tumour Models. Cancers (Basel) 2020;12:587.
81. Asnaghi L, White DT, Yoon L, et al. Downregulation of Nodal inhibits metastatic progression in retinoblastoma. Acta Neuropathol Commun 2019;7:137.
82. Zhang L, Smith KM, Chong AL, et al. In vivo antitumor and antimetastatic activity of sunitinib in preclinical neuroblastoma mouse model. Neoplasia 2009;11:426-35.
83. Zhang J, Benavente CA, McEvoy J, et al. A novel retinoblastoma therapy from genomic and epigenetic analyses. Nature 2012;481:329-34.
84. Xiao M, Rebecca VW, Herlyn M. A Melanoma Patient-Derived Xenograft Model. J Vis Exp 2019.
85. Rebecca VW, Somasundaram R, Herlyn M. Pre-clinical modeling of cutaneous melanoma. Nat Commun 2020;11:2858.
86. Krepler C, Sproesser K, Brafford P, et al. A Comprehensive Patient-Derived Xenograft Collection Representing the Heterogeneity of Melanoma. Cell Rep 2017;21:1953-67.
87. Kageyama K, Ohara M, Saito K, et al. Establishment of an orthotopic patient-derived xenograft mouse model using uveal melanoma hepatic metastasis. J Transl Med 2017;15:145.
88. Heegaard S, Spang-Thomsen M, Prause JU. Establishment and characterization of human uveal malignant melanoma xenografts in nude mice. Melanoma Res 2003;13:247-51.
89. Gao H, Korn JM, Ferretti S, et al. High-throughput screening using patient-derived tumor xenografts to predict clinical trial drug response. Nat Med 2015;21:1318-25.
90. Decaudin D, Frisch Dit Leitz E, Nemati F, et al. Preclinical evaluation of drug combinations identifies co-inhibition of

Bcl-2/XL/W and MDM2 as a potential therapy in uveal melanoma. Eur J Cancer 2020;126:93-103.
91. Decaudin D, El Botty R, Diallo B, et al. Selumetinib-based therapy in uveal melanoma patient-derived xenografts. Oncotarget 2018;9:21674-86.
92. Schlereth SL, Iden S, Mescher M, et al. A Novel Model of Metastatic Conjunctival Melanoma in Immune-Competent Mice. Invest Ophthalmol Vis Sci 2015;56:5965-73.
93. Zitvogel L, Pitt JM, Daillère R, et al. Mouse models in oncoimmunology. Nat Rev Cancer 2016;16:759-73.
94. Kersten K, de Visser KE, van Miltenburg MH, et al. Genetically engineered mouse models in oncology research and cancer medicine. EMBO Mol Med 2017;9:137-53.
95. Hill W, Caswell DR, Swanton C. Capturing cancer evolution using genetically engineered mouse models (GEMMs). Trends Cell Biol 2021;31:1007-18.
96. Hayes SA, Hudson AL, Clarke SJ, et al. From mice to men: GEMMs as trial patients for new NSCLC therapies. Semin Cell Dev Biol 2014;27:118-27.
97. de Waard NE, Cao J, McGuire SP, et al. A Murine Model for Metastatic Conjunctival Melanoma. Invest Ophthalmol Vis Sci 2015;56:2325-33.
98. Dithmar S, Rusciano D, Grossniklaus HE. A new technique for implantation of tissue culture melanoma cells in a murine model of metastatic ocular melanoma. Melanoma Res 2000;10:2-8.
99. Jockovich ME, Suarez F, Alegret A, et al. Mechanism of retinoblastoma tumor cell death after focal chemotherapy, radiation, and vascular targeting therapy in a mouse model. Invest Ophthalmol Vis Sci 2007;48:5371-6.
100. Diaz CE, Rusciano D, Dithmar S, et al. B16LS9 melanoma cells spread to the liver from the murine ocular posterior compartment (PC). Curr Eye Res 1999;18:125-9.
101. Liu LH, Ni C. Rabbit model of uveal Greene melanoma: morphologic studies of metastatic lesions. Graefes Arch Clin Exp Ophthalmol 1983;220:179-83.
102. Olsen KR, Blumenkranz M, Hernandez E, et al. Fluorouracil therapy of intraocular Greene melanoma in the rabbit. Arch Ophthalmol 1988;106:812-5.
103. Römer TJ, van Delft JL, de Wolff-Rouendaal D, et al. Hamster Greene melanoma implanted in the anterior chamber of a rabbit eye: a reliable tumor model? Ophthalmic Res 1992;24:119-24.
104. Cao J, Brouwer NJ, Jordanova ES, et al. HLA Class I Antigen Expression in Conjunctival Melanoma Is Not Associated With PD-L1/PD-1 Status. Invest Ophthalmol Vis Sci 2018;59:1005-15.
105. de Waard NE, Kolovou PE, McGuire SP, et al. Expression of Multidrug Resistance Transporter ABCB5 in a Murine Model of Human Conjunctival Melanoma. Ocul Oncol Pathol 2015;1:182-9.
106. Mor JM, Rokohl AC, Koch KR, et al. Sentinel lymph node biopsy in the management of conjunctival melanoma: current insights. Clin Ophthalmol 2019;13:1297-302.
107. Aubert C, Rouge F, Reillaudou M, et al. Establishment and characterization of human ocular melanoma cell lines. Int J Cancer 1993;54:784-92.
108. Nareyeck G, Wuestemeyer H, von der Haar D, et al. Establishment of two cell lines derived from conjunctival melanomas. Exp Eye Res 2005;81:361-2.
109. Keijser S, Maat W, Missotten GS, et al. A new cell line from a recurrent conjunctival melanoma. Br J Ophthalmol 2007;91:1566-7.
110. Li Y, Shang Q, Li P, et al. Characterization of a conjunctival melanoma cell line CM-AS16, newly-established from a metastatic Han Chinese patient. Exp Eye Res 2018;173:51-63.
111. Booth L, Roberts JL, Poklepovic A, et al. HDAC inhibitors enhance the immunotherapy response of melanoma cells. Oncotarget 2017;8:83155-70.
112. Ishiguro T, Ohata H, Sato A, et al. Tumor-derived spheroids: Relevance to cancer stem cells and clinical applications. Cancer Sci 2017;108:283-9.
113. Wessels D, Lusche DF, Voss E, et al. Melanoma cells undergo aggressive coalescence in a 3D Matrigel model that is repressed by anti-CD44. PLoS One 2017;12:e0173400.
114. Duncavage EJ, Abel HJ, Pfeifer JD. In Silico Proficiency Testing for Clinical Next-Generation Sequencing. J Mol Diagn 2017;19:35-42.
115. D'Arcangelo D, Scatozza F, Giampietri C, et al. Ion Channel Expression in Human Melanoma Samples: In Silico Identification and Experimental Validation of Molecular Targets. Cancers (Basel) 2019;11:446.
116. Garrisi VM, Strippoli S, De Summa S, et al. Proteomic profile and in silico analysis in metastatic melanoma with and without BRAF mutation. PLoS One 2014;9:e112025.
117. Pereira ACL, Bezerra KS, Santos JLS, et al. In silico approach of modified melanoma peptides and their immunotherapeutic potential. Phys Chem Chem Phys 2021;23:2836-45.
118. Kan-Mitchell J, Mitchell MS, Rao N, et al. Characterization of uveal melanoma cell lines that grow as xenografts in rabbit eyes. Invest Ophthalmol Vis Sci 1989;30:829-34.
119. Moore AR, Ran L, Guan Y, et al. GNA11 Q209L Mouse

Model Reveals RasGRP3 as an Essential Signaling Node in Uveal Melanoma. Cell Rep 2020;33:108277.
120. Amirouchene-Angelozzi N, Nemati F, Gentien D, et al. Establishment of novel cell lines recapitulating the genetic landscape of uveal melanoma and preclinical validation of mTOR as a therapeutic target. Mol Oncol 2014;8:1508-20.
121. Cao J, Pontes KC, Heijkants RC, et al. Overexpression of EZH2 in conjunctival melanoma offers a new therapeutic target. J Pathol 2018;245:433-44.
122. Carita G, Némati F, Decaudin D. Uveal Melanoma Patient-Derived Xenografts. Ocul Oncol Pathol 2015;1:161-9.
123. Cassoux N, Thuleau A, Assayag F, et al. Establishment of an Orthotopic Xenograft Mice Model of Retinoblastoma Suitable for Preclinical Testing. Ocul Oncol Pathol 2015;1:200-6.
124. Forsberg EMV, Lindberg MF, Jespersen H, et al. HER2 CAR-T Cells Eradicate Uveal Melanoma and T-cell Therapy-Resistant Human Melanoma in IL2 Transgenic NOD/SCID IL2 Receptor Knockout Mice. Cancer Res 2019;79:899-904.
125. Cruz F 3rd, Rubin BP, Wilson D, et al. Absence of BRAF and NRAS mutations in uveal melanoma. Cancer Res 2003;63:5761-6.
126. Griewank KG, Yu X, Khalili J, et al. Genetic and molecular characterization of uveal melanoma cell lines. Pigment Cell Melanoma Res 2012;25:182-7.
127. Yoo JH, Shi DS, Grossmann AH, et al. ARF6 Is an Actionable Node that Orchestrates Oncogenic GNAQ Signaling in Uveal Melanoma. Cancer Cell 2016;29:889-904.
128. Kilian MM, Loeffler KU, Pfarrer C, et al. Intravitreally Injected HCmel12 Melanoma Cells Serve as a Mouse Model of Tumor Biology of Intraocular Melanoma. Curr Eye Res 2016;41:121-8.
129. Angi M, Versluis M, Kalirai H. Culturing Uveal Melanoma Cells. Ocul Oncol Pathol 2015;1:126-32.
130. Carita G, Frisch-Dit-Leitz E, Dahmani A, et al. Dual inhibition of protein kinase C and p53-MDM2 or PKC and mTORC1 are novel efficient therapeutic approaches for uveal melanoma. Oncotarget 2016;7:33542-56.
131. Onken MD, Makepeace CM, Kaltenbronn KM, Choi J, Hernandez-Aya L, Weilbaecher KN, et al. Targeting primary and metastatic uveal melanoma with a G protein inhibitor. Journal of Biological Chemistry. 2021;296.
132. Harbour JW, Worley L, Ma D, et al. Transducible peptide therapy for uveal melanoma and retinoblastoma. Arch Ophthalmol 2002;120:1341-6.
133. Mouti MA, Dee C, Coupland SE, et al. Minimal contribution of ERK1/2-MAPK signalling towards the maintenance of oncogenic GNAQQ209P-driven uveal melanomas in zebrafish. Oncotarget 2016;7:39654-70.
134. Singh L, Singh MK, Kenney MC, et al. Prognostic significance of PD-1/PD-L1 expression in uveal melanoma: correlation with tumor-infiltrating lymphocytes and clinicopathological parameters. Cancer Immunol Immunother 2021;70:1291-303.
135. Tolleson WH, Doss JC, Latendresse J, et al. Spontaneous uveal amelanotic melanoma in transgenic Tyr-RAS+ Ink4a/Arf-/- mice. Arch Ophthalmol 2005;123:1088-94.
136. Li H, Li Q, Dang K, et al. YAP/TAZ Activation Drives Uveal Melanoma Initiation and Progression. Cell Rep 2019;29:3200-3211.e4.
137. Perez DE, Henle AM, Amsterdam A, et al. Uveal melanoma driver mutations in GNAQ/11 yield numerous changes in melanocyte biology. Pigment Cell Melanoma Res 2018;31:604-13.
138. Vader MJC, Madigan MC, Versluis M, et al. GNAQ and GNA11 mutations and downstream YAP activation in choroidal nevi. Br J Cancer 2017;117:884-7.
139. Malandrino A, Kamm RD, Moeendarbary E. In Vitro Modeling of Mechanics in Cancer Metastasis. ACS Biomater Sci Eng 2018;4:294-301.
140. del Cerro M, Seigel GM, Lazar E, et al. Transplantation of Y79 cells into rat eyes: an in vivo model of human retinoblastomas. Invest Ophthalmol Vis Sci 1993;34:3336-46.
141. Aerts I, Leuraud P, Blais J, et al. In vivo efficacy of photodynamic therapy in three new xenograft models of human retinoblastoma. Photodiagnosis Photodyn Ther 2010;7:275-83.
142. Kang SJ, Grossniklaus HE. Rabbit model of retinoblastoma. J Biomed Biotechnol 2011;2011:394730.
143. Gallie BL, Albert DM, Wong JJ, et al. Heterotransplantation of retinoblastoma into the athymic "nude" mouse. Invest Ophthalmol Vis Sci 1977;16:256-9.
144. Asnaghi L, White DT, Key N, et al. ACVR1C/SMAD2 signaling promotes invasion and growth in retinoblastoma. Oncogene 2019;38:2056-75.
145. Murray TG, Roth DB, O'Brien JM, et al. Local carboplatin and radiation therapy in the treatment of murine transgenic retinoblastoma. Arch Ophthalmol 1996;114:1385-9.
146. Ruggeri M, Wehbe H, Jiao S, et al. In vivo three-dimensional high-resolution imaging of rodent retina with

spectral-domain optical coherence tomography. Invest Ophthalmol Vis Sci 2007;48:1808-14.
147. Dimaras H, Marchong MN, Gallie BL. Quantitative analysis of tumor size in a murine model of retinoblastoma. Ophthalmic Genet 2009;30:84-90.
148. al-Ubaidi MR, Font RL, Quiambao AB, et al. Bilateral retinal and brain tumors in transgenic mice expressing simian virus 40 large T antigen under control of the human interphotoreceptor retinoid-binding protein promoter. J Cell Biol 1992;119:1681-7.
149. Marcus DM, Lasudry JG, Carpenter JL, et al. Trilateral tumors in four different lines of transgenic mice expressing SV40 T-antigen. Invest Ophthalmol Vis Sci 1996;37:392-6.
150. Murray TG, Cicciarelli N, O'Brien JM, et al. Subconjunctival carboplatin therapy and cryotherapy in the treatment of transgenic murine retinoblastoma. Arch Ophthalmol 1997;115:1286-90.
151. Wadhwa L, Bond WS, Perlaky L, et al. Embryonic retinal tumors in SV40 T-Ag transgenic mice contain CD133+ tumor-initiating cells. Invest Ophthalmol Vis Sci 2012;53:3454-62.
152. Zhang J, Schweers B, Dyer MA. The first knockout mouse model of retinoblastoma. Cell Cycle 2004;3:952-9.
153. Williams BO, Schmitt EM, Remington L, et al. Extensive contribution of Rb-deficient cells to adult chimeric mice with limited histopathological consequences. EMBO J 1994;13:4251-9.
154. Zhu L, van den Heuvel S, Helin K, et al. Inhibition of cell proliferation by p107, a relative of the retinoblastoma protein. Genes Dev 1993;7:1111-25.
155. Robanus-Maandag E, Dekker M, van der Valk M, et al. p107 is a suppressor of retinoblastoma development in pRb-deficient mice. Genes Dev 1998;12:1599-609.
156. Vooijs M, te Riele H, van der Valk M, et al. Tumor formation in mice with somatic inactivation of the retinoblastoma gene in interphotoreceptor retinol binding protein-expressing cells. Oncogene 2002;21:4635-45.
157. Dannenberg JH, Schuijff L, Dekker M, et al. Tissue-specific tumor suppressor activity of retinoblastoma gene homologs p107 and p130. Genes Dev 2004;18:2952-62.
158. Maandag EC, van der Valk M, Vlaar M, et al. Developmental rescue of an embryonic-lethal mutation in the retinoblastoma gene in chimeric mice. EMBO J 1994;13:4260-8.
159. MacPherson D, Sage J, Kim T, et al. Cell type-specific effects of Rb deletion in the murine retina. Genes Dev 2004;18:1681-94.
160. Dimaras H, Khetan V, Halliday W, et al. Loss of RB1 induces non-proliferative retinoma: increasing genomic instability correlates with progression to retinoblastoma. Hum Mol Genet 2008;17:1363-72.
161. Naert T, Colpaert R, Van Nieuwenhuysen T, et al. CRISPR/Cas9 mediated knockout of rb1 and rbl1 leads to rapid and penetrant retinoblastoma development in Xenopus tropicalis. Sci Rep 2016;6:35264.
162. Wu X, Wang S, Li M, et al. Conditional reprogramming: next generation cell culture. Acta Pharm Sin B 2020;10:1360-81.

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