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Ocular Oncology Update

Animal models in retinoblastoma research

Rohini M. Nair, MSc [a]; Swathi Kaliki, MD [b]; Geeta K. Vemuganti, MD [a,*]

Abstract

Advances in animal models of retinoblastoma have accelerated research in this field, aiding in understanding tumor progression and assessing therapeutic modalities. The distinct pattern of mutations and specific location of this unique intraocular tumor have paved the way for two types of models- those based on genetic mutations, and xenograft models. Retinoblastoma gene knockouts with an additional loss of p107, p130, p53 and using promoters of *Nestin*, *Chx10*, and *Pax6* genes show histological phenotypic changes close to the human form of retinoblastoma. Conditional knockout in specific layers of the developing retina has thrown light on the origin of this tumor. The use of xenograft models has overcome the obstacle of time delay in the presentation of symptoms, which remains a crucial drawback of genetic models. With the advances in molecular and imaging technologies, the current research aims to develop models that mimic all the features of retinoblastoma inclusive of its initiation, progression and metastasis. The combination of genetic and xenograft models in retinoblastoma research has and will help to pave way for better understanding of retinoblastoma tumor biology and also in designing and testing effective diagnostic and treatment modalities.

Keywords: Retinoblastoma, Knock-out genetic model, Xenograft models, Preclinical models

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Introduction to animal models in cancer research

Animal models are an integral part of preclinical research in the field of oncology. Non-human tumor models have helped to identify the course of tumorigenesis and evaluation of diagnostic and therapeutic protocols in several human cancers such as colon, breast, ovarian, and hepatocellular carcinomas, and ocular melanomas.[1–6] In vitro studies have their own limitations of being conducted devoid of the complex microenvironment that exists within the human body. Interrogation of the cellular mechanisms of tumor progression within the complexity of an organism can help expose the full extent of pathophysiological changes that take place in neoplasms.

Retinoblastoma

Retinoblastoma is the most common pediatric ocular malignant tumor occurring in 1 of every 15,000–20,000 live births.[7,8] This tumor is caused due to inactivation of both the alleles of the Retinoblastoma (Rb) gene resulting in the defective formation of pRB protein. pRB is a major tumor suppressor gene that is involved in cell cycle progression, terminal differentiation and DNA replication.[9] Loss of pRB activity in the retinal progenitor cells leads to impaired cell cycle and uncontrolled cell proliferation. Retinoblastoma manifests in both unilateral and bilateral forms depending on whether it is sporadic or familial.[10] The understanding of genetic inheritance and advances in diagnostic techniques have not only led to early diagnosis and genetic prediction but have paved way for the first of its kind successful preimplantation genetic diagnosis. Xu et al. reported that it was possible to screen embryos with RB1 mutations, implant a healthy embryo following in vitro fertilization and subsequently achieve a healthy pregnancy and delivery.[11] Also, accurate identification of RB1 mutation enables early diagnosis and management of family members at risk for developing retinoblastoma.[12]

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[a] School of Medical Sciences, University of Hyderabad, Hyderabad, India
[b] Ocular Oncology Service, L V Prasad Eye Institute, Hyderabad, India

* Corresponding author. Address: School of Medical Sciences, University of Hyderabad, Prof C R Rao Road, Gachibowli, Hyderabad 500046, India. Tel.: +91 40 23013279, +91 40 23134781.
e-mail addresses: deanmd@uohyd.ernet.in, gvmd@uohyd.ernet.in, gkvemuganti@gmail.com (G.K. Vemuganti).

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Retinoblastoma is currently considered highly treatable, with an overall 3-year survival rate of over 90%.[13] However, it is invariably fatal when left untreated. Prior to the 1990's, the standard treatment approach to unilateral RB was enucleation and in the bilateral cases, the treatment typically involved enucleation of the worst eye and external beam radiation of the other eye. Systemic chemotherapy is now the treatment of choice for retinoblastoma with an effort to salvage life, eye, and vision. However, it has been observed that most of the retinoblastoma cases show continued cellular activity even in eyes treated with primary systemic chemotherapy regimen.[14] The prognosis of the disease is affected by the time of diagnosis and tumor stage.

Genetics of retinoblastoma

The predisposition to retinoblastoma was predicted by Alfred Knudson following statistical analysis of occurrence in the early 1970's. Identification of retinoblastoma gene in 1987 confirmed his hypothesis. The bilateral form is hereditary while the unilateral form is generally non-hereditary.[15] In the hereditary form, the predisposition to tumor formation is inherited from a parent who is a carrier of one mutant allele of the RB1 gene. The presence of one copy of the mutant gene through germ line transmission predisposes the child to the loss of second copy at a rate 1000 times more likely than a spontaneous mutation. This form is more likely to be multifocal since a copy of mutant RB1 is present in all cells and mutation of the second allele could occur in several retinal cells. Lack of RB1 in non-retinal cells can also predispose patients to second malignant neoplasm like osteosarcomas. Unilateral retinoblastoma involves somatic mutation/loss of both copies of the RB1 gene in the developing retina and is generally unifocal.

Tumorigenesis is a multiple step process with a series of mutations that render cells capable of indefinite proliferation, resistance to cell death, inducing angiogenesis, evading growth suppressors, activation of metastasis, evading immune destruction and deregulating cellular energetics. These neoplastic cells have high predilection toward genomic instability and triggering inflammatory response.[16] In Retinoblastoma, the loss of function of the RB1 gene initiates retinoma leading to genomic instability.[17] Retinomas with non-proliferative areas have extra copies of genes on chromosome 1q, that includes KIF14 and MDM4. Retinoblastomas with increased mitotic activity have multiple copies of oncogenes such as KIF14, E2F3, DEK and MYCN; in conjunction to loss of CDH11, a tumor suppressor gene.[18,19] The complete sequence of events promoting retinoblastoma tumorigenesis is still unknown and techniques such as whole-genome sequencing of cancers would help unravel the transformation of benign retinoma to malignant retinoblastoma.[20]

The need for animal models in retinoblastoma research

Retinoblastoma cases that warrant enucleation are presented in an advanced stage and it has been practically impossible to study the origin of the tumor in human samples owing to its late presentation and lack of enough viable tissues. Hence, animal models are indispensable tools to help study retinoblastoma origin and tumorigenesis.[21,22] Spontaneous generation of this tumor seems to be limited only to humans and several challenges lie in creating an animal model that mimics retinoblastoma structurally and functionally.

Transgenic models of retinoblastoma

During the past three decades several genetic murine models of retinoblastoma have been developed with moderate to high similarity with human form of the tumor. With the identification of the role of p107 in mouse retina, the knockout of both the genes was necessary to generate retinoblastoma in murine eyes.[21,23]

The retinoblastoma transgenic animal models are detailed below:

LH-beta T-Ag models

This is one of the first and widely studied transgenic models that was developed in 1990 by Windle JJ et al. This model expresses the oncogenic SV40 early region under the control of luteinising hormone β sub-unit (LHβ) promoter in the gonadotrope cells of the anterior pituitary region.[24] The oncogenes present in both Large-T and small-t of the SV40 early region were hypothesized to cause transformation as these oncoproteins bind to the pRB family, p53 and phosphatase pp2A.[25] Following this model, retinoblastoma has also been developed using mice expressing T-Ag/t-Ag from the IRBP promoter.[26,27] These models showed neuronal characteristics of human retinoblastoma histologically but the question of cell of origin still remained unanswered until the last decade. Both Flexner-Wintersteiner and Homer-Wright rosettes were observed by light and electron microscopy by five months of age. However, Pajovic et al. have recently studied T-Ag protein expression in the retina to track tumor development from the earliest stages.[28] They reported that Tag expression starts by P8 in the nuclei within the inner nuclear layer of the developing retina and then an increase in number by P21 following which there is a decline until P28. This decline of TAg expression was coupled with increase in expression of activated Caspase-3 indicating apoptosis. Expression of Müller glial markers in these Tag positive cells was observed at P8 and P9 with the absence of amacrine, horizontal and bipolar cells. This study concluded that the cell of origin in these tumors belongs to a sub group of progenitor like Muller glial cells that undergoes transformation following Tag expression. Wadhwa et al. developed a Pax6 driven T-Ag model and established a Rb murine cell line using the tumors from P7 mice.[29] Upon characterization, they have identified the presence of a subpopulation of tumor initiating cells that express CD133, Nestin and Sox2. The CD133+ cells were observed to be capable of generating neurospheres in vitro and form transplantable tumors in vivo identical to their parent tumor.

The T-Ag model has been widely used in evaluating therapeutics such as local carboplatin therapy, radiation therapy, cryotherapy, and vascular targeting therapies.[30–32] Jockovich et al. showed that conventional therapies (local carboplatin chemotherapy and external beam radiotherapy (EBRT)) and vascular targeting chemicals, such as anecortave acetate, increase apoptotic cell death, while not having a significant effect on necrosis in this murine model of retinoblastoma.[32]