Trends in Molecular Medicine

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Review

Lessons from Retinoblastoma: Implications for Cancer, Development, Evolution, and Regenerative Medicine

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Retinoblastoma is a rare childhood cancer of the developing retina, and studies on this orphan disease have led to fundamental discoveries in cancer biology. Retinoblastoma has also emerged as a model for translational research for pediatric solid tumors, which is particularly important as personalized medicine expands in oncology. Research on retinoblastomas has been combined with the exploration of retinal development and retinal degeneration to advance a new model of cell type-specific disease susceptibility termed 'cellular pliancy'. The concept can even be extended to species-specific regeneration. This review discusses the remarkable path of retinoblastoma research and how it has shaped the most current efforts in basic, translational, and clinical research in oncology and beyond.

Early Landmark Discoveries in Retinoblastoma Cancer Biology

Retinoblastoma is a rare childhood cancer of the developing retina, begins during fetal development, and is diagnosed at birth or during early childhood [1]. The first sign is often an abnormal white reflection in the eye called leukocoria. In developed countries, most children survive retinoblastoma because it is detected before it metastasizes and the eye can be removed if the tumor is not responding to treatment. The goal of retinoblastoma treatment in developed countries is to save eyes and functional vision. In developing countries, approximately half of the children who are diagnosed with retinoblastoma still die of the disease. In those regions, the goal of therapy is to diagnose the tumor early enough to save the child's life and save eyes and vision in children with early stage disease. With only 300 cases in the United States and 5–10 000 cases worldwide reported annually [2], prospective clinical trials for patients with retinoblastoma pose a challenge. Despite the rarity of retinoblastoma cases, many landmark discoveries have been made by studying this cancer because of several reasons. First, retinoblastoma exhibits little molecular or cellular heterogeneity across patients and is thus ideal for studying fundamental principles of human cancer genetics and biology [3–5]. Second, retinoblastoma is easy to detect, and long before researchers had access to sophisticated diagnostic imaging tools, they could identify patients with retinoblastoma and monitor disease progression. Third, retinoblastoma is one of the earliest diagnosed cancers, making it ideal for studying cancer genetics, because the inheritance pattern of disease-susceptibility mutations can be established in early childhood [1]. Finally, there is little evidence of environmental factors associated with retinoblastoma, making it easier to identify its molecular and cellular origins.

The first human tumor suppressor gene *RB1* was identified by studying the genomes of children with inherited retinoblastoma [6,7]. These data provided genetic validation of

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Basic and translational research on retinoblastomas has led to improved outcomes in patients. However, there have not been significant improvements in outcomes for other pediatric solid tumor patients in the past two decades. The approach used to advance cures in retinoblastoma is now being applied more broadly across childhood solid tumors.

Preclinical studies have predicted toxicity of intra-arterial chemotherapy for retinoblastoma and this supports the value of using preclinical models to anticipate therapeutic toxicity and exclude ineffective therapies from clinical development.

Cellular pliancy is a new model of cell type-specific disease susceptibility and begins to explain why children with *RB1* mutations develop retinoblastoma but not other tumors of the nervous system.

Standard transmission electron microscopy – retinae is a new method for scoring retinal differentiation from human and mouse stem cells. This can be used to directly measure the retinal epigenetic memory of induced pluripotent stem cells derived from different cell types. The cells with low pliancy are more likely to retain epigenetic memory of their cellular origins and this in turn relates to retinoblastoma susceptibility.

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Knudson's two-hit hypothesis (see Glossary) for the initiation of cancer by inactivation of tumor suppressors [8]. Besides, some initial attempts to model cancer in genetically engineered mouse models (GEMMs) were focused on retinoblastoma, because nearly all retinoblastoma patients present biallelic inactivation of *RB1* [9,10]. Interestingly, mice with a germline mutation in *RB1* do not develop retinoblastoma, and even biallelic inactivation fails to produce retinal tumors [11–13]. Subsequent work has also demonstrated the intrinsic species-specific genetic compensation and redundancy of Rb family members p107 and p130 in preventing retinoblastoma formation [13–18].

This review presents an update on what we have learned about the genomics of retinoblastoma since those early landmark discoveries. In addition, efforts to advance our understanding of retinoblastoma biology have led to the development of some of the first **orthotopic patient-derived xenografts** (O-PDXs) and this review discusses how preclinical testing using those models has improved outcomes for patients with retinoblastoma. This approach has now been extended to all pediatric solid tumors in a large-scale effort to validate 'druggable' mutations for personalized medicine in children with cancer. Finally, studies on the biology of retinal development, retinoblastoma, and retinal degeneration have led to new insights into cell type-specific susceptibility to malignant transformation and degeneration. The author outlines a new model to explain why some cells are intrinsically more susceptible to malignant transformation and others are more susceptible to degeneration.

GEMMS, Chromothripsis, and O-PDXs

Since these early discoveries, retinoblastoma has continued to provide new insights into cancer biology. Retinoblastoma was the first pediatric solid tumor grown as an O-PDXs, retaining molecular, cellular, and genetic features of a patient's tumor [3]. As shown in Figure 1, retinoblastoma O-PDX tumors grow as a disorganized mass with intercellular regions of neuronal plexus reflecting their retinal origins. This finding was important because, contrary to established dogma in the field, studies using O-PDX showed that retinoblastomas have remarkably stable genomes [3]. Another important recent advancement in cancer genetics has been the discovery of **chromothripsis** contributing to retinoblastoma initiation through inactivation of *RB1* in humans [5]. Chromothripsis was first described by Stratton's group as the shattering of a chromosome [19], and recently, Pellman's group suggested that its underlying mechanism involved uncoupled DNA replication in micronuclei [20]. Inactivation of *RB1* by chromothripsis in retinoblastoma was the first example of this process contributing to tumor initiation [5]. Subsequent studies demonstrated that epigenetic deregulation of genes such as *SYK* could contribute to tumor progression after *RB1* inactivation [3]. Specifically, in human retina, the *SYK* gene is not normally expressed but following *RB1* inactivation, it is epigenetically deregulated and expressed at high levels [3]. SYK protein expression is required in retinoblastoma to prevent programmed cell death through MCL1 [3]. This discovery provided an important mechanistic explanation as to why retinoblastomas progress very quickly after *RB1* inactivation. In particular, there are widespread epigenomic changes that lead to changes in cancer gene expression such as *SYK* that result from the loss of the RB1 protein. Unlike other types of tumors that rely on sequential accumulation of genetic lesions that alter cancer gene expression, loss of RB1 in retinoblastoma leads to rapid expression changes because the underlying mechanism is epigenetic. It also provided the impetus for many other studies to begin determining how epigenetics contribute to tumor progression in pediatric cancers, as in the case of **rhabdoid tumors** and **diffuse intrinsic pontine gliomas**. In addition, retinoblastoma was the first example of a cross-species comparison of O-PDXs and GEMMs reporting fundamental species-specific differences in their epigenomes [4]. For example, contrary to human tumors, *SYK* was not found to be epigenetically deregulated in murine retinoblastomas [4]. These findings were instrumental in shifting away from GEMMs for preclinical testing of pediatric solid tumors. Although GEMMs are still useful for testing genetic hypotheses and elucidating fundamental