Emerging Developments in the Understanding and Treatment of Retinoblastoma

Current treatment for retinoblastoma emphasizes globe-salvaging therapies with chemotherapy and complete laser ablation of tumors.

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Retinoblastoma is the most common intraocular malignancy in children, affecting approximately one in 15,000 children, for an incidence of 250 to 300 new diagnoses a year in the United States. Tumors can be either heritable and associated with a germline mutation of the RB1 gene, or nonheritable. Heritable mutations typically present in the first year of life with bilateral disease. In comparison, the nonheritable form typically presents slightly later and is primarily unilateral. Common presentations include leukocoria (Figure 1) and strabismus. Evaluation often includes echography, which can also be important in differentiating retinoblastoma from other diseases. Differential diagnoses of retinoblastoma include Coats disease, persistent fetal vasculature (PFV), and toxocariasis, as well as other pathologies. In a recent analysis of 111 cases referred for possible retinoblastoma, 68% were retinoblastoma, while 32% were other diseases. Of the 32% with an alternate diagnosis, 31% had PFV and 29% had Coats disease.

Treatment of retinoblastoma has undergone significant advancements over the past few decades, as globe-salvaging therapies with chemoreduction and focal consolidation have replaced external beam radiation and enucleation. Survival in the United States has climbed to almost 100%, with many children maintaining functional vision. Advanced disease, however, often requires enucleation, and new technologic advances and adjuvant treatments are needed to improve control rates of advanced tumors. This article discusses current developments regarding the pathophysiology and treatment of retinoblastoma.

EMERGING DEVELOPMENTS IN LASER TREATMENT

With the advent of chemotherapeutic regimens and laser ablation for retinoblastoma, tumor management has embraced globe-salvaging and vision-preserving therapies. Control rates with chemoreduction and local consolidation therapy approach 100% for eyes with Reese-Ellsworth (R-E) groups I-IV and International Classification of Retinoblastoma (ICRB) groups A-D. More advanced disease, however, with vitreous and subretinal seeds, often recurs or fails primary therapy,
ultimately requiring enucleation. In a recent study investigating the use of aggressive chemotherapy (six to 10 cycles with or without cyclosporine) and foveal and extrafoveal laser ablative treatment, tumor control rate for these advanced tumors was shown to be 83%, higher than any other prior report. Additionally, despite foveal diode laser therapy, visual acuity remained 20/80 or better in 57% of patients. These findings emphasize the importance of aggressive chemotherapy coupled with the uses of repetitive foveal and extrafoveal laser ablation (Figures 2 and 3).

**INTRAARTERIAL CHEMOTHERAPY**

Current chemotherapy protocols have resulted in significant advances in retinoblastoma tumor control, but are not without risks and systemic toxicities, including secondary malignancies and bone marrow suppression. Intraarterial chemotherapy has recently been investigated for the treatment of advanced retinoblastoma. By selective ophthalmic artery cannulation (Figure 4), local chemotherapy with melphalan (Alkeran, GlaxoSmithKline), can be administered, thus minimizing
systemic toxicities. In a phase 1/2 study, seven out of nine children with advanced tumors (R-E V) were spared enucleation secondary to regression of tumor and the vitreous and subretinal seeds. Importantly, no severe side effects were observed, and all but one patient had stabilization or improvement in vision. Intraarterial chemotherapy offers an exciting alternative treatment; however, further studies are needed to determine safety profiles and appropriate treatment protocols.

INTRAOPERATIVE OPTICAL COHERENCE TOMOGRAPHY (OCT)

Children with retinoblastoma periodically undergo exam under anesthesia to survey for progression of disease. Utilizing echography, wide-angle photography, and indirect ophthalmoscopy, ophthalmologists devise further treatment decisions. More recently, with spectral domain optical coherence tomography (SD-OCT), we have shown that retinoblastoma tumors in infants can be imaged reliably and without significant motion artifact (Figure 5). Using the Spectralis SD-OCT (Heidelberg Engineering, Heidelberg, Germany) adapted to an intraoperative platform, images can be obtained consistently over time secondary to the eye-tracking technology. Additionally, OCT imaging includes the ability to obtain autofluorescence imaging, although it is yet to be determined how this technology will contribute to our current understanding of retinoblastoma tumors. Despite a lack of current investigations comparing SD-OCT with other standard imaging technologies, OCT may provide more detailed analysis of areas that may be suspicious for subretinal seeds or active tumor growth, potentially allowing quantitative assessment of tumor progression or treatment effects. OCT may also provide detailed assessment of macular edema, subretinal fluid, and other retinal pathologies that may occur secondary to the primary tumor or treatment-related effects. Overall,

Figure 4. Intraarterial chemotherapy. Selective cannulation of the ophthalmic artery (A-C); Intraarterial chemotherapy delivered locally to the eye (D-E).

OCT may provide more detailed analysis of areas that may be suspicious for subretinal seeds or active tumor growth.
intraoperative OCT imaging of retinoblastoma tumors offers a new ability to image children, aiding in our diagnosis and management of these tumors.

**TUMOR MICROENVIRONMENT**

The paradigm of cancer treatment has experienced tremendous advancements, as treatments are now targeting not only hyperproliferative neoplastic cells but also the tumor microenvironment. This unique cancer stromal tissue is made up of a milieu of cytokines, growth factors, extracellular proteins, tumor cells, endothelial cells, fibroblasts, and inflammatory cells. Together, these components serve as key modulators in tumor development, growth, resistance to treatment, and metastasis. A more thorough understanding of the mechanisms that drive tumorigenesis is imperative to develop more targeted treatments for tumors. Using the LHBETATAG (BETA and AG subscript) mouse model for retinoblastoma, we have investigated various constituents of the microenvironment and their effects on tumor progression.

With decreasing oxygen partial pressures, an angio-
genic switch occurs, as tumor cells outgrow their blood supply. Modulated through hypoxia-inducible factor (HIF) and growth factors such as vascular endothelial growth factor (VEGF), a complex vasculature arises to meet the needs of the growing tumor. In previous studies, retinoblastoma tumors were shown to consist of a heterogeneous population of vessels that were spatially distributed in the tumor (Figure 6). Mature vessels were concentrated primarily in the center of the tumor, while immature neovessels predominated in the periphery. Vascular targeting agents were able to decrease tumor burden, while also resulting in increased amounts of hypoxia. These agents show promise in animal models for improved tumor control rates and may serve as useful adjuvant treatments.

Hypoxia has been identified in solid tumors to contribute to more aggressive phenotypes, as these regions consist of slow-growing cells, rendering them resistant to current treatments with chemotherapy and radiation. Hypoxic stress leads to cellular adaptations, resulting in tumor cells that have altered their cellular machinery to survive in the harsh environment. Tumor cells adopt an anaerobic state, altering gene expression to favor an increase in glycolytic machinery required to survive. Utilizing the LHBETATAG (BETA and AG subscript) retinoblastoma model, hypoxic regions have been shown to be increased in more advanced tumors (Figure 7), with almost 26% of tumors being hypoxic. As a result, hypoxia and hypoxic cells may serve as important targets for adjuvant therapies. We have shown that utilizing a glycolytic inhibitor, 2-deoxy-glucose (2-DG), we were able to decrease hypoxia as well as tumor burden. Combining vascular targeting therapies that increase hypoxia with agents that target hypoxia may have a synergistic effect on tumor control. Other components of the tumor microenvironment...
associated with tumorigenesis include tumor-associated macrophages (TAMs) and matrix metalloproteinases (MMPs). MMPs have been shown to be involved in angiogenesis, tumor growth, and metastasis. We have shown that by decreasing the expression of MMPs in LHBETATAG (BETA and AG subscript) tumors with anti-vascular agents, there was a resultant decrease in tumor burden. Additionally, TAMs have been associated with increased levels of MMPs and mature vessels (Figure 8). With tumors depleted of macrophages, tumor burden decreased, highlighting a potential key role that macrophages may play in the modulation of the tumor microenvironment, leading to further tumor progression. Both MMPs and TAMs appear to be important therapeutic targets.

Recently, we have investigated the genomic expression of retinoblastoma tumors using the LHBETATAG (BETA and AG subscript) model. Utilizing microarray analysis, gene expression profiles were shown to be both regionally and temporally dependent. Identification of specific genes or gene pathways upregulated during tumor progression may lead to further novel targets for adjuvant therapy. Additionally, with a greater understanding of the time-dependent nature of tumor growth, the administration of adjuvant therapies may be timed more optimally to correspond to key time-points in tumor activity.

With a greater understanding of the pathogenesis of retinoblastoma tumors, novel drugs may be investigated to target important aspects of tumor progression. Combined with chemotherapy and focal consolidation with laser, adjuvant treatments that target tumor vascularity, hypoxia, macrophages, MMPs and specific genes and gene pathways, may lead to greater control rates for advanced tumors or less dependence on chemotherapy with systemic toxicities.

**CONCLUSION**

With advances in the current retinoblastoma treatment protocols, including aggressive chemotherapy and complete laser ablation, control rates for advanced tumors have improved significantly. Further progress in understanding the pathogenesis of retinoblastoma tumors has led to the investigation of adjuvant treatments. These new therapies may result in greater rates of globe-salvaging and a reduction in the systemic toxicities of treatment. Additionally, new imaging technologies and novel treatment techniques, such as intraarterial chemotherapy, may further increase control rates for retinoblastoma tumors.

In summary, current treatment for retinoblastoma emphasizes globe-salvaging therapies with chemotherapyp and complete laser ablation of tumors, including foveal involvement. On the horizon, we await improved imaging with SD-OCT, adjuvant therapies, and more precise and focally administered drug treatment.

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