

New Hopes in Retinoblastoma Treatment

Semra A. TURHAN, Osman CEKIC

Marmara University School of Medicine, Department of Ophtalmology, Istanbul, TURKEY

ABSTRACT

Survival rates of retinoblastoma have increased dramatically during the last decade. The current major treatment modalities for retinoblastoma include enucleation, intravenous chemoreduction, external beam therapy, focal treatments (such as brachytherapy and transpupillary thermotherapy), subconjunctival chemotherapy and selective ophthalmic artery chemotherapy. Although current treatment modalities have improved survival rates as well as eye salvage, new treatment modalities are expected to arise in the near future, since above mentioned non-targeted treatments have extensive side effects. Recent advances in genetic analysis of retinoblastoma 1 (RB1) mutations and pathways leading to retinoblastoma may provide vital information for the development of new targeted therapies which are less toxic.

Keywords: Genetics, Retinoblastoma, RB1 mutation, Targeted treatments

ÖZET

Retinoblastom Tedavisinde Yeni Umutlar

Son on yılda retinoblastomda sağ kalım oranları dramatik olarak artmıştır. Enükleasyon, intravenöz kemoredüksiyon, dışsal ışın radyoterapi, fokal tedaviler (brakiterapi ve transpupiller termoterapi gibi), subkonjonktival kemoterapi, selektif oftalmik arter kemoterapi retinoblastomda kullanılan önemli tedavi modaliteleridir. Yeni tedavi seçenekleri göz küresinin kurtarılması ve sağkalım oranlarında iyileşme sağlamasına rağmen, özgül olmayan kemoterapi ajanlarının yaygın yan etkileri nedeniyle gelecekte yeni tedavi modalitelerine ihtiyaç duyulmaktadır. Retinoblastom 1RB1 mutasyonlarının gen analizi ve retinoblastom oluşumunu sağlayan yollardaki son gelişmeler; daha az toksitesi olan hedeflenmiş terapilerin gelişiminde önemli bilgiler sağlayacaktır.

Anahtar Kelimeler: Genetik, Retinoblastoma, RB1 mutasyonu, Hedeflenmiş tedaviler

INTRODUCTION

Retinoblastoma (RB) is the most common primary intraocular malignancy of childhood. The management of RB is challenging. Current treatments for intraocular RB include enucleation, systemic chemotherapy, external beam radiation therapy (EBRT), focal treatments, intra-arterial or subconjunctival chemotherapy.¹ The results of treatment depend on the stage of the disease. Early diagnosis and intensive treatment have been adopted in developed countries. Survival rates of RB exceeds 90-95% in USA² but the survival rate changes country by country according to availability of medical care. Early diagnosis and intervention is crucial for successful treatment of RB. The current important issues in RB research are the targeted approaches which are more effective and less toxic. This article reviews the new targeted treatment modalities for RB.

GENETICS

The disease is associated with RB gene which is a tumor suppressor gene. RB results from loss of retinoblastoma 1 gene (RB 1) or inactivation of both alleles. In the early 1970s, Knudson theorized the 'two hit' model. In this model, loss of both copies of the RB gene is required for the development of RB and it occurs in two forms: the hereditary and non-hereditary forms. Although it requires loss of both alleles, one allele is mutated in the germline and other mutation occurs somatically in the developing retina in hereditary form. In non-hereditary form, both mutations occur somatically. However, some authors recently reported that genomic instability and aneuploidy have impact on initiation of tumor formation.³⁻⁵ RB and retinoma cells have common genomic changes.^{6,7}

They showed that retinomas are homozygous null for RB1 and lack expression of retinoblastoma protein product of RB1 (pRB).⁵ Retinomas also have a low level of genomic copy number changes in tumor suppressor genes and oncogenes. However, RB tissue showed high levels of genomic copy number changes and a low level of senescence proteins. In addition, retinomas are rarely stable because loss of pRB causes genomic instability, leading to proliferative RB tumors.

In spite of aneuploidy and genomic instability, the importance of mutational events other than RB1 is accepted by authors. Mutations in the RB gene show a high degree of mutational heterogeneity. Valverde

et al.⁸ reported that 932 mutations in RB gene are available in a searchable database. These mutations have been consisted of inactivated pRB by deletions, nonsense mutations and RB1 gene mutations which mainly include twelve nonsense, two missense and three splicing mutations.⁸

The authors hypothesized that the P13K/AKT/mTOR (insulin signaling) pathway may show dysregulation and they used microarray analysis for gene expression analysis of RB.⁹ This hypothesis was confirmed by semi-quantitative RT-PCR for related genes. The inhibitors of this pathway could be another option for the treatment of RB.

All these data support that biallelic inactivation of the RB1 gene is important for formation of retinoma and RB tumors, but additional genetic mutations are acquired for progression to the malignant state.

The genetic events have roles in RB tumorigenesis. Genetic alterations are common in RB tumors, including gains at chromosomal loci 1q32.1 (MDM+ gene), 6p22 (E2F3 and DEK) and 2p24 (MYCN) and losses at 16q22.¹⁰ pRB, acts primarily as a regulator of gene expression. These processes include cell proliferation, senescence, apoptosis, differentiation, and chromatin remodeling.¹¹ pRB also has important roles in the fate determination, differentiation, survival, and migration of developing neurons.¹² RB family proteins (pRB1/105, p107, pRB2/p130, referred to as pocket proteins) interact with E2F transcription factors to fulfill the functions.¹³ This interaction prevents transcription of genes required for DNA synthesis and effectively arrests the cell cycle at the G1/S interface in the non-phosphorylated state. Phosphorylation of pRB by cyclin-dependent kinases results in the release of E2F to provide progression of the cell cycle.¹⁴⁻¹⁶

Normal pRB binds to and inhibits the E2F target genes, by preventing the binding of transcriptional co-activators, and through recruitment of histone deacetylase 1 (HDAC1), ATPases, and DNA methyltransferases to the promoter of target genes which are responsible for cell progression.¹⁷ The E2F target gene MAD2 plays a role in encoding a mitotic spindle check point protein and deregulation of this protein promotes aneuploidy and tumor genesis in transgenic mice.¹⁸ Overexpression of MAD2 has a mechanism in the pathogenesis of many tumors, including RB.¹⁹ Chromatin condensation, cohesion, and stability by promoting the centromeric localization of the CAP-

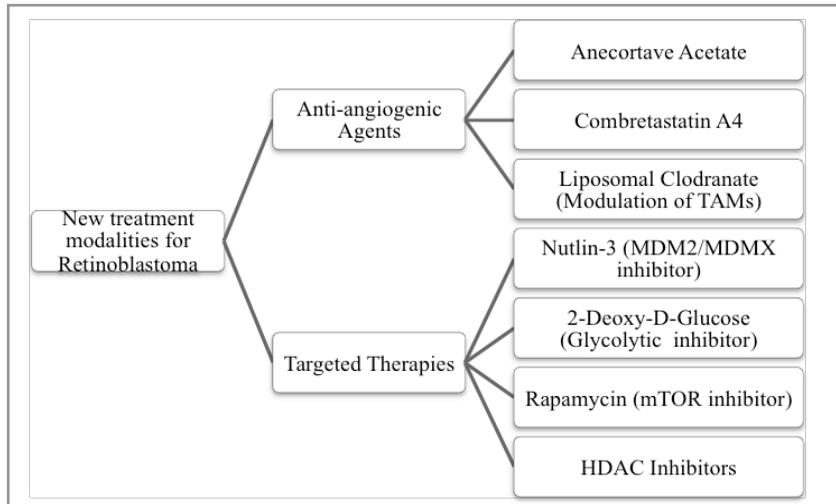


Figure 1. New treatment modalities for retinoblastoma

D3/condensing II protein complex are also regulated by pRB.²⁰

Although the development and progression of RB is not directly associated with p53 gene mutations, recent studies have shown that inactivation of the p53 pathway is also related with RB.²¹ MDM2 and MDM4 are two oncogenes associated with p53 regulation. Their amplification and polymorphism have been associated with RB development.²¹ p53 is activated after loss of pRB during retinogenesis and p53-mediated apoptosis is triggered in RB-deficient cells.²² Increased expression of MDM4 protein plays a role in tumor progression. Its mechanism is to suppress p53 response in RB1-deficient retinal cells and also MDM2/MDMX-p53 pathways must be inactivated for tumor progression. They suggested that MDMX is a specific chemotherapeutic target for treating RB.²¹

NEW TREATMENTS DERIVED FROM ANALYSES OF MOLECULAR PATHWAY

One of the important issues driving research in retinoblastoma is to identify targets of inactivated pRB and design targeted therapies. Despite the difficulty in generating accurate murine models of RB, recently mouse models showed that conditional deletion of RB1 on a p53-deficient, p107-deficient or p130-deficient genetic background gives rise to RB tumors that resemble the human disease.²²⁻²³ All new treatment modalities are listed in Figure 1.

1. NUTLIN-3

Identification of MDM2/MDMX, a p53 inhibitor, has led to development of a potential targeted therapy using Nutlin-3.^{24-26,28} Nutlin-3 is a small-molecule inhibitor of the MDM2/MDMX-p53 pathway, which promotes the apoptosis pathway. Nutlin-3 can be used in combination with topotecan, a p53 inducer. Topotecan's pharmacologic effects have been studied in LHBetaTag murine model and subconjunctival administration of topotecan in fibrin sealant provided a clinical effect for 3 weeks after treatment.²¹ Treatment provided a bilateral reduction of tumor burden without a significant difference between treated and untreated eyes, suggesting that the drug was delivered predominantly through the hematogenous route. The combination Nutlin-3 and topotecan synergistically disrupt RB cells in vitro and promotes an 82-fold reduction in tumor burden following the subconjunctival injection in mouse model.^{22,28} No side effect was reported in the animal models. Nutlin-3 is currently in phase 1 clinical trial for treatment of RB and its effects will require further investigation in the near future.²⁹

2. COMBRETASTATIN A4 AND ANECORTAVE ACETATE

The effects of vascular targeting therapy has been studied in LHBetaTag murine model. Vascular targeting agents have an effect in decreasing tumor vasculature with subsequent enhancement in tumor control. Conventional therapies (focal treatment, external beam radiation) and the vascular targeting

agents (combretastatin A4 and anecortave acetate) induced apoptotic cell death with minimal necrosis.³⁰ Histopathologic analysis showed that external beam radiation and focal carboplatin treatment had effect in 1 week after treatment but the vascular targeting treatment had effect after only 1 day only. They suggested that this differential apoptosis time can have information in combined therapies for RB treatment.

3. 2-DEOXY-D-GLUCOSE (2-DG)

Other novel agents have also developed murine models. 2-Deoxy-D-Glucose (2-DG) can be used as an adjuvant agent for treatment of murine RB.³¹ 2-DG effectively reduces human lung tumor and bone tumor burden when combined with standard chemotherapeutic drugs. 2-DG is currently in phase 1 clinical trial for treatment of lung, breast, and bone cancers.³² They hypothesized that in advanced disease, the tumor has hypoxic cells and 2-DG can be used for this hypoxic portions of the tumor.³¹

4. ANECORTAVE ACETATE AND LIPOSOMAL CLODRONATE

The tumor microenvironment is also important for tumor genesis. Tumor-associated macrophages (TAMs) and matrix metalloproteinases (MMPs) have been associated with tumor genesis. MMPs have important roles, which are angiogenesis, tumor growth and metastasis. Anecortave acetate reduced the expression of MMPs in LHBetaTag murine tumors and resulted in a decrease in tumor burden.³³ TAMs have been associated with increased levels of MMPs and mature vessels in retinoblastoma models. Similarly, the TAMs were present during early disease and the density of these macrophages steadily increased with tumor progression.³⁴ Subconjunctival liposomal clodronate provided the depletion of intraocular macrophages. After depletion of macrophages, MMPs expression decreased and tumor control improved but modulation of the total TAMs population did not reduce tumor.³⁴

Combined with vascular targeting therapies that increase hypoxia, glycolytic inhibitors and anti-angiogenic agents may be used synergistically for a greater reduction in tumor burden.³⁵ Combination of 2-DG and Anecortave Acetate provided advanced tumor control and reduction in tumor burden compared to 2-DG alone.³⁶

5. RAPAMYCIN

The PI3K/Akt/mTOR pathway was recently demonstrated to be a potentially important dysregulated pathway in the genomic expression of human RB.⁹ This pathway is dependent on oxygen. Treatment with the mTOR inhibitor, rapamycin, may provide destruction of chemo-resistant hypoxic cell population in LHBetaTag retinal tumors.³⁷

6. HDAC INHIBITORS (HDACi)

Another option of targeted therapies currently in phase 1 clinical trials for RB is HDAC inhibitors (HDACi). pRB binds to HDAC and promotes silencing of transcription. When E2F1 activity is increased, proapoptotic factors are overexpressed and subsequently cells will be sensitive to HDACi.³⁸ They suggested that HDACi will be more effective in tumors with high E2F1 activity. HDACi reduced cell survival in human RB cell and reduced tumor burden.³⁹

CONCLUSION

If the origin of RB cell was exactly known, it would shed light on new treatments for the initial precursor cell. Some authors reported that RB tumors express markers specific to cone precursor cells, including RXR γ and TR β 2.⁴⁰ They also demonstrated that human cone precursors prominently express MDM2 and N-Myc, that RB cells require both of these proteins for proliferation and survival. The presence of a cone precursor to RB tumors might suggest that these tumors would be more concentrated in the fovea, the portion of the retina with the highest cone density; however, it was not observed clinically.⁴¹ Further studies will likely bring answers to many of these questions.

In conclusion, understanding of the genetic basis and the mechanism of underlying malignant transformation of retinal and other cell types will lead to the development of new therapies. This will provide a less toxic but more effective treatment.

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Correspondence

Dr. Semra AKKAYA TURHAN
Marmara Üniversitesi Tıp Fakültesi
Göz Hastalıkları Anabilim Dalı
Pendik
ISTANBUL / TURKEY

Tel: (+90.216) 625 45 45
e-mail: semraakkaya85@hotmail.com