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Invited critical review

# Differential expression of genes in retinoblastoma

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ABSTRACT

Retinoblastoma is a pediatric eye tumor that serves as a paradigm for understanding the genetic basis of cancer. Mutations and/or epigenetic alterations inactivating both alleles of the retinoblastoma gene (RB) are associated with retinoblastoma. There are many other genes which express differentially in the preneoplastic retinal cells after RB loss, as cells progress to form tumors. These genetic changes and the pathways involved can provide valuable insight into the development and progression of this cancer. Conventional molecular and genetic methods for studying cancer are limited to the analysis of one locus at a time. A cluster of genes that are regulated together can be identified by DNA microarray, and the functional relationships can uncover new aspects of cancer biology. Meta analysis is an important tool for the identification and validation of differentially expressed genes to increase power in clinical and biological studies across different sets of data. Recently, meta analysis approaches have been applied to large collections of microarray datasets to investigate molecular commonalities of multiple cancer types not only to find the common molecular pathways in tumor development but also to compare the individual datasets to other cancer datasets to identify new sets of genes. The outcome of these analyses might accelerate the application of basic research findings into daily clinical practice through translational research and may have an impact on foreseeing the clinical outcome, predicting tumor response to specific therapy, identification of new prognostic biomarkers, discovering targets for the development of novel therapies and providing further insights. These and related research efforts reveal novel data that enhance our understanding of the biology of retinoblastoma. These observations may facilitate new therapeutic approaches to further decrease the morbidity and mortality associated with retinoblastoma and other more common forms of cancer.



**Contents**



## 1. Introduction

The global estimate suggests that millions of people worldwide are diagnosed with cancer every year. A thorough understanding of the mechanism of tumorigenesis is essential for the prevention, diagnosis

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and treatment of human cancer. Cancer research is mainly focusing to identify the genomic alterations responsible for neoplastic transformation [1].

The details of any biological system can be studied using several tools of genomics, such as microarray, comparative genomic hybridization, serial analysis of gene expression (SAGE), proteomics and siRNA technology [2]. It is believed that functional genomics will be proved of great importance to increase our understanding of the mechanisms underlying cellular function, and in combination with system biology to accelerate the application of basic discoveries into clinical practice despite the natural cautions associated with the implementation of new technologies in the clinical arena.

The human genome sequence is now available and genomic complexities are being revealed with technological advances. The development of new techniques for the large scale analysis of the genome, transcriptome and proteome has enabled functional genomics to have a profound impact on personalized medicine. Astounding amounts of molecular data resulting from usage of these techniques have accumulated and a multitude of sophisticated methods and algorithms have been developed for comprehensive analysis of these data.

The application of genomics to the study of cancer is rapidly shifting towards the analysis of patient samples to discover new biomarkers for early detection of cancers. Since characteristic patterns of gene expression can be measured in parallel by using microarrays, gene expression profiling with DNA microarrays has emerged as a powerful approach to study the transcriptome of individual cancers. Molecular biologists work with clinicians and pathologists to obtain samples from patients with a known medical history, so that the molecular characteristics of samples can be correlated with the clinical presentation. This approach provides an insight into molecular mechanisms of the different cancer types, and also helps to find novel cancer biomarkers. Using pioneering work on differentially expressed genes in retinoblastoma (Rb) as an example, we have reviewed the studies and discuss the clinical usefulness of the findings. Rb can be used to provide a model to demonstrate the current approaches to study the differential expression of genes.

## 2. Retinoblastoma

The retinoblastoma is an eye tumor of childhood that arises in the retina and represents the most common intraocular malignancy of infancy and childhood [3]. It affects the retina of one eye (unilateral Rb) or both (bilateral Rb) in children, and the surgical ablation of the affected eye still represents a common modality in the management of the disease, although more conservative approaches have become increasingly popular given the availability of newer techniques of local treatment [4]. Rb is commonly reported to be sporadic in 60% of cases and hereditary in the remaining 40% [5]. Retinoblastoma is the first disease for which a genetic etiology of cancer has been described and RB is also the first tumor suppressor gene identified. Studies on Rb have been at the heart of many of the landmark discoveries in cancer genetics over the past 35 years. However, these advances in the laboratory have had little effect on the treatment of children with retinoblastoma. The loss is high in Rb, in part because of the asymptomatic nature of the disease in early stages, a lack of sensitive and specific diagnostic tools, and limited progress in development of effective therapeutics. Studies suggest that differential gene expression analysis may help predict which individuals are at risk and warrant close follow up. Through a comparative analysis of gene expression, we can also gain insight into the pathophysiology of diseases that afflict specific tissues. The occurrence of retinoblastoma is the result of multiple gene mutations. The expression of many genes is altered significantly in Rb. The differentially expressed genes include components of immune system, signaling pathways, angiogenesis, cell structure, proliferation, apoptosis, cell adhesion, and cellular metabolism. Recent advances in multimodality therapy have not significantly improved the prevention management in retinoblastoma patients. The development of new treatment strategies for more effective management of retinoblastoma requires identification of novel biological targets.

## 3. Genetics

In 1971 Knudson [6] proposed that retinoblastoma was initiated by inactivation of a putative tumor suppressor gene, and this hypothesis was subsequently confirmed by demonstration of loss of heterozygosity at 13q14 in retinoblastomas and the identification of first tumor suppressor gene RB. A few years later, Dyer et al. [7] extended these findings to small cell lung cancer showing that the RB1 locus was disrupted in tumors other than retinoblastoma and osteosarcoma. Since then, it has been found that most, if not all, tumors have defects in their Rb pathway through genetic lesions in the RB gene itself or other genes in the pathway. The tumor suppressor retinoblastoma gene product, pRb, is a member of a family of proteins that includes p107 and p130, characterized by interaction with the adenovirus oncoprotein, E1A [8]. p107 and p130 share high homology with each other, but less with pRb [9]. Though p107 and p130 are localized in chromosomal regions which are frequently deleted in tumors, there is no evidence that mutations in these genes play any role in human cancer.

## 4. Clinical description and classification

Leukocoria (white reflection in the pupil) and strabismus (squint eyes) are the most frequent clinical manifestations of retinoblastoma. Leukocoria is initially inconstant, visible only at certain angles and under certain light conditions. Strabismus, when present, becomes rapidly constant, reflecting impairment of the vision. These signs are too often overlooked. Some other signs may be observed, including iris rubeosis, hypopyon, hyphema, buphthalmia, orbital cellulitis, and exophthalmia. Some children with retinoblastoma may have no symptoms. Screening in case of familial history or dysmorphic syndrome with a 13q14 deletion may lead to diagnosis of retinoblastoma [10]. Most affected children are diagnosed before the age of five years.

The International classification of retinoblastoma is a new classification system for retinoblastoma and is based on tumor size, location, and associated seeding:- group A: retinoblastoma up to 3 mm in size; group B: retinoblastoma more than 3 mm in size, macular location, or minor subretinal fluid; group C: retinoblastoma with localized seeds; group D: retinoblastoma with diffuse seeds; and group E: massive retinoblastoma necessitating enucleation. This classification was designed to simplify grouping and to assist in predicting treatment outcomes [11].

## 5. Differential diagnosis

A number of ocular disorders in infants and children can clinically resemble retinoblastoma. Consultation with ocular oncologists experienced with retinoblastoma may be helpful in confirming the clinical diagnosis of retinoblastoma and assisting in designing a management plan for this potentially fatal disease. Accurate diagnosis in a child with suspected Rb is accomplished by taking a detailed history, physical evaluation, external ocular examination, slit lamp biomicroscopy, and binocular indirect ophthalmoscopy with scleral indentation. Ancillary diagnostic studies can be helpful in confirming the diagnosis of retinoblastoma. Fluorescein angiography shows early vascularity and late hyperfluorescence of the tumor. Ultrasonography and computed tomography can demonstrate the intraocular tumor and possibly detect calcium within the mass.