Overexpression of the Eyes Absent Gene, *EYA1*, in Retinoblastoma, A Potential Therapeutic Target

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Abstract

Retinoblastoma is one of the most common and deadly cancers in children under three years of age. Worldwide, 9,000 children are diagnosed annually with hereditary retinoblastoma, with a 70% mortality rate in the middle- and low-income countries. The treatments for retinoblastoma (chemotherapy, radiation, and removing the eye) all have serious side effects. At any stage of diagnosis, the goals of treatment should be saving the child's life, preserving as much vision as possible, and minimizing the damage to noncancerous cells. This could potentially be accomplished by targeting specific genes or gene products that are known to be overexpressed in many cancers. Some genes are upregulated during development; however, they are also upregulated in cancer cells leading to tumor progression and carcinogenesis. The *EYA1* gene is a critical developmental gene and its gene product functions as a protein phosphatase and as a coactivator of the SIX/EYA transcriptional complex; it has been shown to be overexpressed in several cancers. In this literature review, the upregulation of the *EYA1* gene and its gene product are explored as possible therapeutic targets specific to retinoblastoma cancer cells, opening the door to the possibility of developing targeted therapies that could prove a viable option that will be less invasive to young children and be more effective in treating retinoblastoma.

Hypothesis

The *EYA1* gene will be overexpressed in retinoblastoma cells, leading to the overproduction of mRNAs for the Eya1 protein. RNA extraction, RT-PCR, and agarose gel electrophoresis will be used to quantify the amount of expression in retinoblastoma cells in comparison with noncancerous cells (negative control) and a cancer cell-line known to overexpress the *EYA1* gene (positive control).

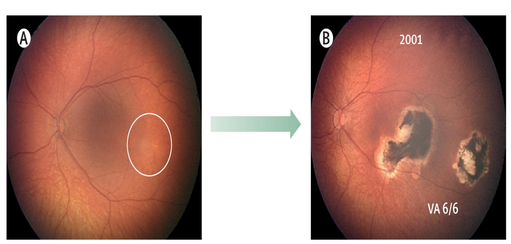


Figure 1: *Progression of Retinoblastoma* (1).

Background

Retinoblastoma

- Worldwide, most of the estimated 9,000 newly diagnosed patients every year will die (2).
- Retinoblastoma is an eye cancer that begins in the retina, the very back part of the eye, and occurs due to genetic mutations of the nerve cells in the retina.
- Retinoblastoma cells can metastasize to infect the brain and spine as well as other nearby structures (3).
- Hereditary retinoblastoma is the most common and aggressive intraocular cancer affecting infants and children. Parents can pass the gene to their children from an autosomal dominant pattern meaning only one parent has to possess a single copy of the mutated gene.
- Retinoblastoma is initiated by mutation of the *RB1* gene, which was the first tumor-suppressor gene to draw attention to the genetic origin of cancer (3). (**See Figure 1)**

Current Treatments

- Current management of retinoblastoma include chemotherapy combined with focal treatments, all which lead to long-term side effects.
- Drugs that can be used to treat retinoblastoma include carboplatin, cisplatin, vincristine, etoposide, cyclophosphamide, topotecan, and doxorubicin (4).
- Some children with retinoblastoma in only one eye may have the hereditary form, which means they are likely to develop the disease in the other eye.

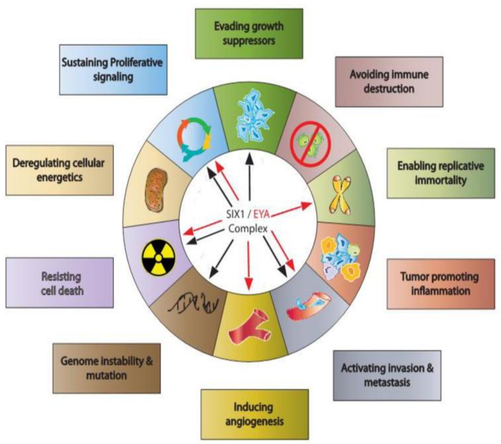


Figure 2: *The SixI/EYA Pathway (5).*

Background (continued)

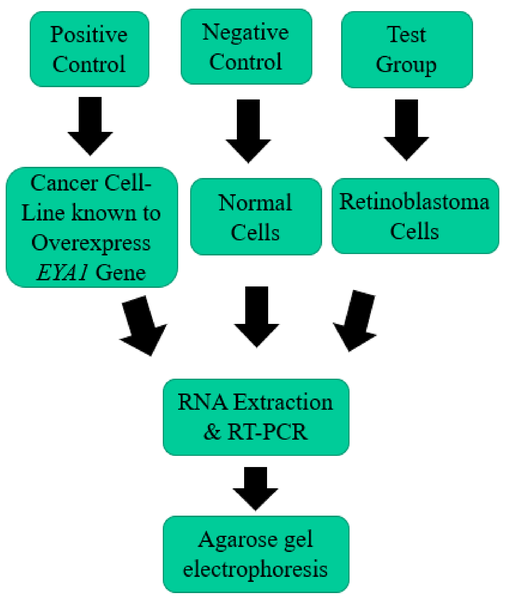
EYA1

- The *EYA1* gene encodes for a protein (eyes absent homolog 1) that functions both as a protein phosphatase and as a transcription co-activator to promote cell proliferation, cell migration, and angiogenesis. **(See Figure 2)**
- The *EYA1* gene is expressed during development and plays an important role in the development of many organ systems but is silenced in most adult somatic cells.
- In some cancers the *EYA1* gene is reactivated and overexpressed leading to several tumor-promoting properties.

Targeted Therapies

- Targeted therapy is a type of cancer treatment that uses drugs or other substances to precisely identify and attack certain types of cancer cells.
- A targeted therapy can be used by itself or in combination with other treatments.
- Targeted therapies are generally better tolerated than traditional chemotherapy (6).

Experimental Design



Methods

- Grow non-cancerous, breast cancer, and retinoblastoma cells in cell culture
- Extract and isolate RNA
- Amplify by reverse transcriptase PCR (RT-PCR) and use two gene specific primers
- Analyze cDNA, PCR product for overexpression using agarose gel electrophoresis **(Figure 3)**
  - Low expression (lane 3) = little or no band
  - High expression (lane1) = well-defined band

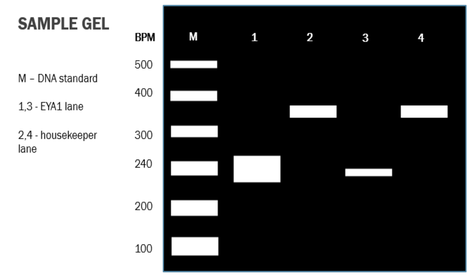


Figure 3: Sample agarose gel of expected results

Significance

- If the *EYA1* gene is overexpressed in retinoblastoma, then the possibility of developing targeted therapies to repress gene expression or inhibit Eya1 protein function would exist.

Future Directions

- Eya1 is a unique protein tyrosine phosphatase (PTP) that has already been identified as a target for novel anticancer drug development.
- Targeted gene therapies may be developed and applied to retinoblastoma or other cancers known to overexpress the *EYA1* gene.
- Reliance on traditional cancer treatments that are toxic to normal as well as cancer cells may by reduced or eliminated for some cancers, including retinoblastoma.

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