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# Upregulation of a Metastasis Associated 37 LRP/p40 Gene in the Sclera of Chick Eye with Form Deprivation Myopia

**Chang-Hao Yang, Yung-Feng Shih. Luke L. K. Lin**

**Department of Ophthalmology**
**National Taiwan University Hospital**
**Taipei, Taiwan**

Correspondence to:
Yung-Feng Shih, M.D.
Department of Ophthalmology
National Taiwan University Hospital
7, Chung-Shan South Road
Taipei, Taiwan
Tel: 886-2-23123456 ext 5184
Fax: 886-2-23412875
E-mail: @ha.mc.ntu.edu.tw

## Abstract

**Purpose:** Myopia is characterized by excessive elongation of eyeball. Increased DNA synthesis and cellular proliferation had been shown by previous studies; however, the underlying mechanism was still unknown. In this experiment, subtraction hybridization technique was used to evaluated the differential gene expression between myopic and control eyes.

**Methods:** Form deprivation myopia was induced in neonatal chicks by occlusion their right eyes with goggle. The chicks were sacrificed 2 weeks later and the eyes were enucleated. Sclera was harvested and mRNA was extracted. The differential gene expression between myopic eyes and control eyes was examined by subtraction hybridization methods.

**Results:** With subtraction hybridization technique, 5 out of 400 differential expressed clones were picked up. The clones were sequenced and compared with Gene bank data. The candidate genes included angiopoietin gene, caspase 6 gene, prkdc gene for DNA-dependent proteinase kinase, myosin heavy chain gene, and alpha1 integrin gene.

**Conclusions:** Subtraction hybridization is a powerful tool to study gene expression in myopia. The significant of these genes in myopia development need further investigation.

## Introduction

The prevention and treatment of myopia are important issue of public health in many countries, especially in Taiwan where the prevalence rate of myopia is extremely high. The most important complication of extreme myopia is retinal degeneration affecting the posterior pole that is associated with elongation of ocular axial length. Changes in the extracellular matrix components of the sclera or in the molecules required for the synthesis and degradation of the scleral matrix may lead to significant changes in scleral biochemical properties, leading to changes in scleral shape, ocular size and therefore the refractive state of the eye.

Isolation and identification of genes expressed in form deprived ocular tissue may aid in the molecular evaluation and understanding of possible mechanisms involved in myopia. Several gene products have been shown to change their levels of expression in the sclera during the development of experimentally induced myopia in animal studies. The synthesis and accumulation of total protein, the scleral proteoglycans decorin, biglycan and aggrecan, the matrix metalloproteinase, gelatinase A, inhibitor of matrix metalloproteinase TIMP-1 and TIMP-2, and collagen have all been shown to be altered in the sclera during form deprivation myopia development in chicks, tree shrews, and primates.

In an attempt to elucidate roles of genes in the sclera of form-deprived chick

eyes, we isolated genes up-regulated in the sclera. A suppression substractive hybridization methods was used to compare mRNA expression in the sclera of form-deprived and control chick eyes. As a result, a metastasis associated 37 LRP/p40 gene was identified as an up-regulated gene in the sclera of myopic chick eyes. Possible mechanism and function explaining the upregulation of this gene were discussed.

## Materials and Methods

### Induction of form deprivation myopia in chick

Male white Leghorn chicks were used in the experiments. Form deprivation myopia was induced in the right of neonatal chicks since one day old. The right eye was covered with a semi-spherical translucent plastic goggle, and the left eye was uncovered for control. The chicks were maintained on 12 hours light-dark cycle for 2-21 days during the study, then they were sacrificed and both eyes enucleated at different time points.

### Sample preparation

Using a surgical trephine, tissue buttons with diameter of 8.5 mm was excised from part of each posterior hemisphere located temporal to the exit site of the optic nerve. Specimen of sclera was separated from these tissue samples.

Specimen from 5 eyes will be pooled together for further experiments.

**Preparation of RNA and cDNA**

Total RNA was extracted from the iris/ciliary body with Trizol reagent (Life, Gaithersburg, MD). One microgram of total RNA from each sample was annealed for 5 min at 65°C with 300-ng oligo(dT) (Promega, Madison, WI) and reverse transcribed to cDNA by using 80 U Moloney murine leukemia virus reverse transcriptase (MMLV-RT) (Gibco, Grand Island, NY) per 50 μg reaction for 1 h at 37°C. The reaction was stopped by heating for 5 min at 90°C.

**Subtraction-hybridization PCR method**

Subtraction-hybridization was performed using the PCR-Selected cDNA Subtraction Kit (Clontech) and procedures were carried out according to the manufacturers protocol. Tester and driver cDNA were synthesized as mentioned. Then tester and driver cDNA were digested randomly into blunt cDNA fragments with *Rsa I*. The digested tester cDNA fragments were divided into two samples and ligated with two different adapters using T4 DNA ligase. An excess of driver cDNA fragments was added to each divided sample with hybridization buffer. The mixed cDNA fragment were heat-denatured at 98 ℃ for 90 seconds and annealed at 68 ℃ for 10 hours. After the first hybridization, the two sample were combined with a fresh portion of the heated

-denatured driver cDNA fragments in hybridization buffer. The mixed samples were allowed to hybridize at 68 ℃ for 10 hours.

**Cloning and Sequencing of Difference Products**

The hybridized cDNA fragments was amplified by primary and nested PCR using the Advantage cDNA PCR Kit (Clontecch). The PCR products were cloned into PGEM-T vector (Promega) using T4 DNA ligase. Sequencing was performed with ABI PRISM dye terminator cycle sequencing core kit (Perkin Elmer). The resulting sequences were compared with the Genebank, EMBL and DDBJ DNA databases.

**Reverse Transcription Polymerase Chain Reaction**

Total RNA was reverse-transcribed into cDNA. Primer sequences and amplification condition were as follows: angiopoietin-2B ACT GAC TAA TCA GAA GCG CTA (sense), TAG AAA TCT GCT GGT CGA ATC (antisense); caspase 6 TGA AAA CGA TCA TGT CTA CGC (sense), AAA TAC AAT TTT TTC GTC AAC (antisense); Expression level of GAPDH, a housekeeping gene, was examined separately and used as control for each DNA sample concentration. PCR products were separated on a 1.5% agarose gel and photographed.

## Results

### Isolation of up-regulated genes in form deprivation myopic eyes

One out of 200 clones was isolated as a cDNA fragment of an up-regulated gene in the myopic eye samples. The cDNA was sequenced and subjected to homology search with the Genebank database. The size of insert was 306 bp and the sequence was 100% homologous to a fragment of the chick 37LRP/p40 cDNA sequence (*Clausse N, DNA Cell Biol 1996, 15:1009-1023*).

A 37LRP/p40 polypeptide is of major interest because it is consistently up-regulated in cancer cells in correlation with their invasive and metastatic phenotype. Furthermore, this polypeptide presents intriguing multifunctional proerties because it has been characterized as the precursor of the metastasis-associated 67-kD laminin receptor (67LR) and as a cytoplasmic ribosomal-associated protein. The significant role of this gene in the regulation of sclera cell proliferation and extracellular matrix remodeling needs further evaluation.

## Discussion

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